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BETA CELL DEVELOPMENT, DIFFERENTIATION & REGENERATION

1: Esra Karakose (Icahn School of Medicine at Mount Sinai)

Abstract Title

Epigenetic Regulation of Human Beta Cell Replication in Insulinomas

Authors

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

Purpose

Type 1 diabetes results from insufficient numbers of insulin-producing beta cells. We 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, TGFβ Inhibitors, KDM6A inhibitors, and GLP1 receptor agonists. Interestingly, the most common genetic alterations in insulinomas occur in epigenetic regulatory genes exemplified by MEN1, KDM6A, EZH2, YY1 and others.

Methods

Here, we performed ATACseq and histone ChIP-seq experiments on FACS-sorted pure human beta cells and compared these to human insulinomas, and integrated and correlated the ATACseq and ChIPseq results with RNAseq dataset on human beta cells and insulinomas. This is the first in-depth epigenetic analysis on human insulinomas.

Summary of Results

Insulinomas and beta cells share common accessible chromatin regions (identified by ATACseq), but also contain additional large and distinct regions of open chromatin. Similarly, both contain shared as well as distinct proximal and distal enhancer regions (identified by H3K27Ac ChIPseq). Integration of ATACseq and H3K27Ac ChIPseq datasets, together with transcription factor motif enrichment analysis, suggested distinct patterns of transcriptional networks in beta cells vs insulinomas. Finally, integration of accessible chromatin sites 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. Further bioinformatic analysis reveals that distinct biological pathways are enriched in each of these four clusters. Studies integrating bulk and single cell RNAseq into these analyses are ongoing.

Conclusions

Collectively, these studies indicate that while all insulinomas display mutations in different genes, almost all insulinomas contain variants in epigenetic regulatory genes. These, in turn, lead to altered ATACseq and histone ChIPseq patterns, and downstream differences in expression of genes that control cell cycle and beta cell function. Most importantly, these studies continue to provide clues to novel pathways to human beta cell regenerative drug pathways, and mechanisms of action for beta cell regenerative drugs such as the DYRK1A inhibitors, TGFβ inhibitors, and GLP1R agonists.

2: Hyo Jeong Yong (Florida State University)

Abstract Title

Identify cell cycle regulators of human beta cells by candidate genetic screening

Authors

Hyo Jeong Yong, Yue J. Wang

Purpose

Beta cell self-replication has been identified as a promising method to compensate for functional beta cell loss in diabetes. Several mitogens and growth factors that enhance beta cell proliferation have been identified in animal or various cell-line models. However, these factors fell short of promoting the proliferation of human beta cells. Only a handful of cell cycle regulators such as CDKN1A (p21), CDKN2C (p18), and DYRK1A were confirmed to be effective in human beta cells. Using the virus-mediated CRISPR/Cas9 system in human beta cells, we will directly compare the efficiencies of 27 top candidate cell cycle regulators in controlling human beta cell proliferation, cross-compare their efficiencies, and elucidate their roles in cell cycle machinery. Our study will help to develop innovative beta-cell regenerative treatments for diabetes.

Methods

Based on the literature review, we selected 27 candidates, which are known cell cycle inhibitors or associated with mendelian disorders that are associated with the over-proliferation of pancreatic cells. We designed guide RNA (gRNA) targeting the candidates, validated the efficiency of those gRNA, cloned gRNA sequences in lentiviral plasmid expressing Cas9 genes (lentiCRISPRv2), and produced lentivirus. After knock-out candidates in EndoC-βH1 cells by lentivirus, the proliferation rate will be measured by EdU labeling. In addition, a glucose-stimulated insulin secretion assay will be conducted to examine whether the candidates affect the endocrine function of human beta cells. Taking the results, we will decide on the most promising candidate and perform transcriptomic analysis to elucidate its role in the cell cycle of human beta cells.

Summary of Results

We already obtained some intriguing findings through our preliminary results. For example, the treatment of harmine increased the proliferation rates in EndoC-βH1 cells. However, the knock-out of DYRK1A did not increase the proliferation rates compared to the control. By far, the knock-out of GSK3B in EndoC-βH1 cells showed the highest proliferation response compared with other candidates.

Conclusions

In summary, we have established a lentivirus-mediated CRISPR/Cas9 screening system to directly read out the proliferative effects of different targets in human beta cells. We have found several candidates that elicited different proliferative responses in our system compared with the results of previous high-throughput screenings using siRNA and compounds in animals or other cell line. Because of the higher efficiency and increased specificity of CRISPR/Cas9 mediated genetic loss of function and the utilization of the highly relevant human beta cell line, we hypothesize that our system will yield the most relevant targets to promoting beta cell regeneration as diabetes treatments.

3: Isabella Altilio (University of Miami)

Abstract Title

Tracking Functional Beta Cell Regeneration in Human Pancreatic Slices Using Adenoviral Transduction

Authors

Isabella D Altilio Bove, Silvia Avarez-Cubela, Dagmar Klein, Joseph Schulz, Fahd Qadir, Helmut Hiller, Maria Beery, Irina Kusmartseva, Mark Atkinson, Chris Fraker, Alberto Pugliese, Camilo Ricordi, Ricardo Pastori, Juan Dominguez-Bendala

Purpose

The study of pancreatic regeneration would benefit greatly from the design and validation of robust human-based models. Human Pancreatic Slices (HPSs) are thin organotypic sections of live pancreatic tissue. The sectioning method preserves the overall histological structure of the organ, maintaining the integrity of the extracellular matrix and the natural interaction between the endocrine and exocrine compartments, as well as the local neural, vascular and immune milieu. Conditions for the long-term culture of HPSs, recently reported by our team, have enabled the real-time analysis of beta cell neogenesis using adenoviral (AV) co-transduction of a red-green reporter and an insulin tracer in human pancreatic slices. We report here a refinement of this system to test whether neogenic beta cells arising as a result of exposure to bone morphogenetic protein (BMP-7) or THR-123 (a cyclic peptide derived from the active form thereof) are also glucose-responsive.

Methods

To determine whether new INS+ cells respond to glucose, we designed an AV in which an insulin expression-dependent recombination event leads to the expression of a blue marker (moxBFP) and a Calcium Imaging Reporter (gcAMP6, green) whose intensity is proportional to glucose-dependent insulin secretion. This allowed us to monitor glucose stimulated calcium influx in the newly created cells using the time lapse function on the Keyence BZ-X800 all-in-one Fluorescence Microscope.

Summary of Results

Human slices from a type 1 diabetic donor showed an increase in insulin-producing cells when treated with THR-123. Further functional analysis with calcium time-lapse imaging demonstrates the ability of those beta cells to secrete insulin when treated with high levels of glucose.

Conclusions

This setting allows for functional analyses in precise experimental conditions. Our ability to study regeneration in a clinically meaningful model represents a groundbreaking advance that may fast-track the screening and preclinical development of therapeutic agents.

4: Jan Czyzyk (University of Minnesota)

Abstract Title

The dichotomic and timely regulation of serpinB13 plays a role in beta cell neogenesis and pancreatic islet expansion.

Authors

Yury Kryvalap, Rim Habte, Shawn Meng, Jan Czyzyk

Purpose

There has been recent emphasis on the potential role of a functional crosstalk between the exocrine and endocrine pancreas in the development of type 1 diabetes (T1D). However, exactly how and to what extent, the cues that arise in the exocrine pancreatic tissue can be exploited for the purpose of preventing T1D has been largely unexplored. Our interest in this was sparked by our observations that, (i) serpinB13, a protease inhibitor of cathepsin L, is expressed in mouse pancreatic cytokeratin-19+ cells, and (ii) an antibody response to serpinB13 is associated with better clinical outcomes in mice and

humans with T1D. The exact pattern of serpinB13 expression in the human pancreas has, to date, not been examined. Similarly, the exact role of serpinB13 in beta-cell biology and diabetes remains unclear.

Methods

We aimed to determine the distribution of serpinB13 expression using immunochemistry on pancreatic sections obtained from nPOD. In addition, we developed a mouse serpinB13 genetic knockout model to examine the role of this serpin in regulating pancreatic islet morphology. Visiopharm software with Author module was used for an unbiased quantitative image analysis of islet size and cellularity.

Summary of Results

We found that in nPOD samples from healthy donors, serpinB13 expression was confined to the exocrine pancreatic ducts, mimicking the pattern we observed previously in rodents. Studies of mice with a serpinB13 genetic deficiency revealed that early inhibition of serpinB13 increased the small pancreatic islet population (e.g., beta-cell clusters measuring less then 15 micrometers in diameter, and which may represent a beta-cell neogenesis event), while with time this trend reversed, and a drop in the number of pancreatic islets was observed.

Conclusions

Our results demonstrate that serpinB13 is expressed in the exocrine epithelial compartment of the human pancreas. In addition, quite unexpectedly, serpinB13 appears to play a dual role in regulating pancreatic islet mass. More specifically, we postulate that serpinB13 initially hampers the development of additional insulin-producing cells, but with time it promotes expansion of the already formed beta cells. Thus, we propose an approach that first impedes serpinB13, followed by later administration of this serpin, which may lead to an increase in beta-cell mass and improved resistance to T1D.

5: Kathrin Maedler (University of Bremen)

Abstract Title

Modulating the Hippo regulator YAP promotes beta-cell regeneration in a model of Type 1 Diabetes

Authors

Murali Krishna Madduri, Mohamed Elawour, Sqahar Rafizadeh, Amin Ardestani, Kathrin Maedler

Purpose

Loss of functional pancreatic beta-cells is a major hallmark of both type 1 diabetes (T1D) and type 2 diabetes (T2D). Modulating the pathways which faster beta-cell regeneration is urgently required for a beta-cell-targeted therapy of diabetes. The Hippo signaling pathway is a master regulator of organ size and tissue homeostasis. Its major downstream transcriptional regulator Yes Associated Protein (YAP) acts through TEA domain (TEAD) family transcription factors to promote the expression of genes targeting proliferation and survival. Highly expressed during embryogenesis in the pancreas, YAP is switched off in mature pancreatic beta-cells. Overexpression of active YAP induces robust beta-cell proliferation in human islets with full preservation of beta-cell function and identity. Here we identified robust beta-cell regeneration in mice in vivo by transient activation of YAP.

Methods

Doxycycline inducible active YAP overexpressing mice (Teto-YAPS127A) mice were cross-bred with mice carrying the tTA tetracycline transactivator under the control of the insulin promoter (RIP-rtTA) to achieve inducible beta-cell specific Rip-Ins2-TetO-hYAP1-S127A (beta-YAP) mice. We tested the impact of transient beta-cell specific YAP induction on glucose

homeostasis, beta-cell proliferation, apoptosis and gene expression at the physiological level and in the severe diabetes model of a single high dose streptozotocin (STZ) injection, which induces rapid beta-cell ablation in WT mice.

Summary of Results

Consistent with YAP overexpressing human islets, YAP profoundly induced beta-cell proliferation, seen by increased Ki67- as well as pHH3-positive beta-cells, compared to the Dox-untreated control group. Beta-specific YAP-overexpression in mice was confirmed by IHC and Western blot and showed a very robust, up to 12-fold increased beta-cell proliferation, compared to non-Dox treated littermates, together with a remarkable beta-cell mass expansion. Pancreatic islets looked morphologically normal with typical islet structure and cellular composition (physiological alpha-beta-cell distribution) as well as normal unchanged expression of beta-cell functional identity genes such as PDX1, GLUT2, and NKX6.1.
Two different experimental intervention strategies, namely YAP induction for 2 weeks one week after STZ injection and for 2 weeks with an interim pause, both showed robust reduction of glucose levels throughout the experiment and improvement of glucose tolerance by YAP induction. In both experimental approaches, beta-cell area, -mass, -survival and -proliferation were robustly improved.

Conclusions

Our results suggest that transient restoration of YAP fosters beta-cell regeneration and restores β-cell mass without showing major cellular or systemic metabolic deregulation. YAP has a strong pro-proliferative activity in human islets in vitro and in mice in vivo and might be a novel target for beta-cell regenerative therapy to prevent loss (rapid in T1D, progressive in T2D) of functional pancreatic beta-cell mass in diabetes.

6: Peng Wang (Mt. Sinai Icahn School of Medicine)

Abstract Title

All DYRK1A Inhibitor Class Small Molecule Induce Human Beta Cell Proliferation, But Most Have No Effect on Beta Cell Differentiation: Implications for Human Beta Cell Regenerative Drug Development for Diabetes.

Authors

Peng Wang, Kunai Kuma, Hongtao Liu, Olivia Wood, Esra Karakose, Lauryn Choleva, Luca Lambertini, Adolfo Garcia-Ocana, Robert DeVita, Andrew Stewart

Purpose

Small-molecule inhibitors of dual-specificity tyrosine-regulated kinase 1A (DYRK1A), such as harmine, induce therapeutic adult human β cell proliferation. Surprisingly, harmine also enhances differentiation of human beta cells in vitro in normal and T2D islet cells and enhances human beta cell function in vitro (GSIS) and in vivo (human islet transplant models). Here, we sought to explore the mechanism through which harmine and other DYRK1A inhibitors induce human beta cell differentiation.

Methods

We measured responses of human cadaveric islets to multiple members of the DYRK1A inhibitor family, including harmine, 2-2c, 5-iodotubericidin (5-IT), INDY, Leucettine-40, CC-401, and GNF4877. Readouts included canonical markers of mature beta cells at the qPCR, RNAseq and proteomic levels, including transcription factors (PDX1, NKX6.1, MAFA, MAFB, NeuroD1, NKX2.2, SIX2) and essential beta cell functional proteins and markers (GLUT2/SLC2A2, PCKK1,UCN3, SLC30A8, ENTPD3).

Summary of Results

As expected, harmine induced striking and reproducible increases in all of the transcription factors and beta cell functional markers listed above, and enhanced GSIS. In addition, 5-IT and 2-2c phenocopied harmine, yielding comparable increases in canonical beta cell transcription factors and markers. Remarkably, and in contrast to expectations, most other DYRK1A inhibitors had little or no beneficial effect on human beta cell differentiation. These included GNF4788, INDY, Leucettine-40, CC-401. Remarkably, silencing DYRK1A induced human beta cell proliferation, but had no effect on beta cell differentiation.

Conclusions

All pharmacologic DYRK1A inhibitors, and DYRK1A silencing, induce human beta cell replication. In contrast, and unexpectedly, most DYRK1A inhibitor class members do not enhance human beta cell differentiation and function: this important beneficial effect is restricted to harmine, 2-2c and 5-IT. The beneficial pro-differentiation effect of harmine is not mediated by DYRK1A inhibition, but instead is attributable to as yet unidentified molecular pathways and mechanisms.

7: Robert Whitener (Stanford University)

Abstract Title

Transcriptional profiling of human islet cells identifies transcription factors important to development of fully mature endocrine cells.

Authors

Robert L. Whitener, Yan Hang, Mario A. Miranda, Rita Bottino, Romina Bevacqua, Diane Saunders, Marcela Brissova, Mark A. Atkinson, Alvin C. Powers, Seung K. Kim.

Purpose

Autoantibodies (AAb) against beta cell antigens have long been used in longitudinal studies of children to identify persons at risk for the development of Type 1 Diabetes (T1D), markers of disease progression that often develop within the first years of life. However, knowledge as to why these AAb develop in early life remains poorly understood. We hypothesized that alterations in islet cell development may contribute to this process and and in support of this notion, recent studies note that islet cell dysfunction can be detected before T1D autoantibody development. Therefore, we sought to understand the unique features of human islet cell development in early life, as deepening our understanding of cellular events during these early developmental stages is critical to understanding the pathogenesis of human T1D.

Methods

Fetal and cadaveric human islets from organ donors aged from 1 day to 15 years old were dispersed into single cell suspension and stained with a custom antibody panel designed to maximum capture pancreatic cell types. Single, live cells were sorted into four populations by FACS based on surface marker expression. Each population was distributed to 96- or 384-well plates pre-loaded with cell lysis buffer using a second FACS sort. Libraries were then generated and sequenced using standard methods. Cells were clustered and identified based upon known marker gene expression. Islet cells were then organized into pseudotime trajectories using multi-trajectory temporal reconstruction and cell type-specific gene expression networks were identified.

Summary of Results

After careful quality control assessment, 10,589 libraries were analyzed at an average depth of 950,000 reads and 3,677 genes captured per cell. UMAP clustering generated clusters corresponding to islet, duct, acinus, stellate, immune, endothelial, neuronal, and mitotic cells. Sub-clustering of the islet cells identified all major islet cell types and a population of endocrine progenitor cells. Expression analysis confirmed enrichment of known markers, such as SOX4, IAPP, and HHEX, among others, in the appropriate cell types. Pseudotime trajectories originating at the progenitor population diverged into each of the five lineages. Highly variable genes were organized into modules with highly correlated expression patterns, and gene ontology analysis confirmed each module was enriched for unique cellular processes. Examples include a module in all endocrine cell

types comprised highlighting decreasing expression of pancreas and endocrine development related GO terms. Additionally, transcription factors within each gene module were identified for future study.

Conclusions

To our knowledge, this is the first report to cover such breadth of developmental stages and cell types within the pancreas with this depth. Our sorting strategy has enabled characterization of pancreatic cell subsets from these rare human samples. The breadth of stages covered in this dataset has enabled us to identify unique patterns of gene expression across developmental time. Using our novel sorting strategy and computational approach, we have identified transcription factors, surface proteins, and other gene products which are important to the development of pancreatic β, α, and δ cells. Many genes have been implicated in Type 2 Diabetes but have not been studied in the context of development and thus should be subject to further investigations for their potential impact on this metabolic disease. The data generated here will serve to anchor future studies using modern techniques in imaging (CODEX), human islet genetics (targeted gene modulation in primary human islet cells), or generation of mature human stem-cell derived β cells; each with the purpose of understanding of disease pathogenesis and potentially to aid in identifying a means to prevent T1D.

This work is supported by the Leona M. and Harry B. Helmsley Charitable Trust.

8: Saumadritaa Kar (University of British Columbia)

Abstract Title

Generating amyloid resistant human-embryonic stem cell-derived beta cells to improve islet transplant outcomes

Authors

Saumadritaa Kar, Paul C. Orban, Shugo Sasaki, Derek Dai, Galina Soukhatcheva, Heather C. Denroche, Francis C. Lynn, Bruce C. Verchere

Purpose

Type 1 diabetes (T1D) is a devastating autoimmune disease that leads to lifelong insulin dependence and affects over 9 million people worldwide. Transplanting insulin-producing β-cells of the islet can be a curative therapy; however, the lack of organ donors, need for lifelong immunosuppression, islet graft failure, and graft rejection hinder widespread clinical implementation. Lack of organ donors may be addressed by inducing human embryonic stem cells to produce mature β-cells (SC-β cells), thereby presenting an unlimited source of cells for transplantation. However long-term survival and function of these cells following transplantation still needs further optimization.
Islet transplant failure can be exacerbated by the aggregation of islet amyloid polypeptide (IAPP), a peptide hormone co-secreted with insulin from β-cells. Recent studies have found amyloid deposition in islets transplanted into T1D recipients – a pathological feature seen in islets from T2D individuals – which could be a contributor to graft loss and dysfunction. The amino acid sequence in IAPP lends it the propensity to form aggregates that contribute to islet inflammation and β-cell death. Pramlintide is a human IAPP analogue containing proline substitutions in the amyloidogenic region, rendering Pramlintide soluble and non-cytotoxic. We hypothesize that genetically engineered SC-β cells expressing a non-amyloidogenic form of IAPP, such as pramlintide, will lead to human β-cell sources with improved survival and function following transplantation in T1D.

Methods

SC lines, CRISPR modified and GFP tagged to produce a pramlintide analogue, along with wild-type IAPP (WT) SCs were differentiated to β-cells suitable for transplant. Glucose-stimulated insulin and IAPP secretion in aged SC-β cells and serum from transplanted animals were measured with a sensitive human C-peptide ELISA and in-house IAPP1-37 ELISAs respectively. Diabetic immunodeficient mice transplanted with 50 sorted SC-β cell clusters in the anterior chamber of the eye

(ACE) were monitored for blood glucose, fast-refeed measures, and engraftment of SC-β cell clusters with live in vivo imaging.

Summary of Results

SCs differentiated into insulin+ (and therefore GFP+) β-cells were either sorted and reaggregated for transplantation or cultured long-term in 'Balboa' media. While our SC-β cells resemble immature β-cells in initial stages, long-term culture yielded functionally mature SC-β cells that secrete insulin and IAPP in a glucose-responsive manner at 5 and 6 weeks of maturation. A human IAPP1-37 ELISA specific to the amyloidogenic region, indicated pramlintide SC-β cells secrete an altered, non-amyloidogenic IAPP form. Diabetic immunodeficient mice transplanted with WT or pramlintide SC-β cells in the ACE showed a higher C-peptide response in mice with pramlintide SC-β cells at 6-weeks with a fast-refeed test.

Conclusions

Our preliminary data suggest pramlintide expression does not adversely impact maturation of SCs into insulin-producing cells and that these cells may show higher C-peptide production post-transplant. Future studies will focus on evaluating efficacy of encapsulated and non-encapsulated pramlintide-expressing SC-β cell transplants in immune-deficient and immune competent diabetic mouse models.

BETA CELL PHYSIOLOGY AND DYSFUNCTION

9: GongXin Yu (AdventHealth)

Abstract submitted by:

Camilla Tondello

Authors

GongXin Yu, Alejandra Petrilli, Yury Nunez Lopez, Richard Pratley, Anna Casu

Purpose

The autoimmune-mediated loss of insulin-producing β-cells is central to the pathogenesis of type 1 diabetes (T1D). Growing evidence suggests that the pancreatic microenvironment in which β-cells reside and pancreatic resident cell populations may also play a role.
Pancreatic stellate cells (PSCs) are implicated in the pathobiology of major exocrine pancreatic disorders such as chronic pancreatitis and pancreatic cancer. PSCs can transition under various stimuli from a quiescent state to an activated state, losing their vitamin A content, expressing αSMA, producing collagen leading to extracellular matrix (ECM) deposition, releasing cytokines (CKs), and promoting inflammation. These effects are thought to interfere with acinar and β-cell function. The known effects of PSCs in exocrine pancreatic diseases lead us to hypothesize that they could also play a role in the pathogenesis of T1D. Activation of PSCs could also underlie the exocrine pancreas alterations seen in T1D. In pancreatic cancer, PSCs and macrophages bidirectionally interact, modulating each-other phenotypes and the microenvironment. As the role of PSCs in T1D has not been investigated, we compared the PSC transcriptome of a subgroup of donors with and without T1D by analyzing the single-cell RNA sequencing (scRN-seq) data produced by the NIH-sponsored Human Pancreas Analysis Program (HPAP) database (https://hpap.pmacs.upenn.edu) comparing PSCs.

Methods

A subgroup of published scRNA-seq data of human pancreatic islet preparations was downloaded in the format of molecule_info.h5 from NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148073). Available data were preprocessed with 10X Cellranger Aggregate. Subsequent analyses include: 1) doublet identification, ambient RNA

analysis, 2) Seurat analysis for sample integrating, clustering, and cell subtyping, and 3) differentially expressed gene and enriched function analyses to evaluate the differences between PSCs from donors with T1D and without pancreatic diseases.

Summary of Results

We analyzed the scRNA-sequencing data from 11 pancreatic islet preparations obtained from 5 donors with T1D and 6 donors without pancreatic disease. Transcriptomes of 26501 individual pancreatic cells were analyzed after doublet removal, data quality control, and harmonization. With this analysis, eleven cell clusters were identified, where cluster number 5 was characterized as having the highest expression of markers previously shown to be present in isolated and cultured PSCs and used to define mesenchymal cells (COL1A1, COL1A2, COL3A1 by Muraro et al and Blobner et al.), PSCs (REG10 by Fasolino et al., TIMP1, FN1, POSTN, ACTA2 by Segerstolpe et al., COL4A2, COL4A1, SPARCL by Tosti et al., COL1A1 by Lawlor et al. PDGFRB by Baron et al.), quiescent PSCs (PGS5 by Baron et al., and PDGFRB, ITAGA1 by Tosti et al.), activated PSCs (PDGFRA, COL5A2, COL6A3, LAMA2, LAMB1, SLIT2, LUM, DNC, NEGR1 by Baron et al and Tosti et al.), and vascular smooth muscle cells (MYL9, CALD1 by Blobner et al.). Some of these markers were used to define pericytes, while some of them are also expressed in endothelial cells, highlighting the heterogeneity of the population and the need to carefully chose the markers to define the subtypes of stellate/mesenchymal cells (STM, our preferred definition).
We then looked at differentially expressed PSC genes (DEGs) in samples from donors with T1D and controls. Among the highest upregulated DEGs in T1D samples were several chemokines and interleukins like CXCL1, CXCL2, CXCL3, CXCL8, CXCL12, and IL6 indicative of immune activation of PSCs in T1D, as proposed by Baron. The enrichment pathways analysis showed gap junction, tight junction, neurotropin signaling, cAMP signaling, B-cell receptor signaling, and COVID-19, salmonella, yersinia infection pathways among the top upregulated KEGG pathways. Among the Gene Ontology Biological Processes (GO-BP), the top upregulated processes were regulation of cell morphogenesis involved in differentiation, viral transcription and viral gene expression, and protein localization in ER, while myeloid, leucocyte neutrophil and granulocyte activation pathways were modestly downregulated.

Conclusions

Compared to control donors, pancreatic stellate/mesenchymal cells obtained from islet preparations from T1D donors show different scRNA-seq transcriptome and pathways analysis, indicative of pro-inflammatory and immunomodulatory activation. Future cell culture studies are needed to validate these preliminary data. Our analysis also identified the need for a rigorous standardized definition of the mesenchymal/stellate cells to guide further studies in T1D.

10: Arianna Harris-Kawano (Indiana University School of Medicine)

Abstract Title

Relative Proinsulin Secretion is Altered in Pancreas Slices from Individuals with Type 1 Diabetes

Authors

Arianna Harris-Kawano, Gabriela Monaco, Peristera-Ioanna Petropoulou, Paola Apaolaza, Helmut Hiller, Maria Beery, Carmella Evans-Molina, Irina Kusmartseva, Mark Atkinson, Teresa Rodriguez-Calvo, Emily K. Sims.

Purpose

Under conditions of β cell stress, processing of preproinsulin into mature insulin may be compromised. Consistent with this view, we previously demonstrated that islet proinsulin relative to mature insulin is increased and circulating proinsulin:C-peptide is elevated in many individuals before and after the onset of type 1 diabetes (T1D). We believe these elevations in the proinsulin:insulin and proinsulin:C-peptide ratios provide valuable insights into the role for β cell stress in T1D. However proinsulin secretion has never been described in ex-vivo β cells from donors with or at elevated risk for disease. To address this, we measured basal and glucose-stimulated proinsulin and insulin secretion in nPOD pancreas slice tissues from organ donors with T1D or islet autoantibody positivity in comparison to nondiabetic controls.

Methods

Slice perifusions were performed by the University of Florida nPOD laboratory, with Q1 minute samples collected after exposure to basal glucose (3mM) followed by high glucose (16 mM) stimulation. Perfusate samples from 3 perifusion timepoints were combined to allow for adequate volume for simultaneous testing of proinsulin (TECO intact proinsulin ELISA) and insulin (Mercodia ultrasensitive insulin ELISA). Proinsulin and insulin data were analyzed as absolute values and as an equimolar ratio of proinsulin/insulin (PI:I ratio). Slices were fixed and embedded in Optimal cutting temperature compound (OCT) for further tissue processing and cutting. Sections were stained for insulin, proinsulin, PC1/3, PC2 and CPE, and imaged using both slide scanning and confocal microscopy.

Summary of Results

Data were analyzed from 19 total slices including samples from 2 autoantibody positive donors (single GAD antibody positive), 6 donors with T1D, and 11 nondiabetic controls. Proinsulin and insulin were detected in perfusates from 18/19 slices (all autoantibody positive and control donors, and 5/6 donors with T1D). Although absolute values of insulin were significantly lower in stimulated samples from donors with T1D compared to controls, the average absolute proinsulin values were not significantly different between groups (mean proinsulin for controls: basal= 2.5 pmol/L, stimulated= 5.5 pmol/L; mean proinsulin for T1D: basal= 15.8 pmol/L, stimulated= 0.2 pmol/L; p=0.10 and p=0.31 for comparison of control vs. T1D groups). For control donors, insulin and proinsulin secretion significantly increased, 3.0-fold and 1.7-fold, respectively, from basal to high glucose stimulation. In contrast, only 2/5 donors with T1D and detectable insulin exhibited increases in insulin and proinsulin secretion with stimulation. Perfusates from donors with T1D showed significantly higher PI:I ratios at basal glucose ($p<0.05$) and tended to have higher PI:I ratios with glucose stimulation (p=0.0849). Samples from both autoantibody positive donors had insulin, proinsulin, and PI:I values in the range of the nondiabetic control samples. Analysis of insulin, proinsulin, PI:I ratios and the expression of the prohormone convertases in the tissue sections obtained from the slices are ongoing.

Conclusions

Compared to samples from nondiabetic control donors, pancreas slices from donors with T1D exhibited abnormalities in proinsulin secretion, with elevations in PI/I under basal and high glucose conditions. These findings suggest that abnormal islet prohormone processing persists in ex-vivo β cells independent of the in-vivo impacts of hyperglycemia, peripheral insulin resistance, or inadequate β cell mass for handling of whole-body insulin needs. Future work will expand testing to include additional cases and examine islet prohormone and processing enzyme expression within perfused slices.

11: Austin Stis (University of Florida)

Abstract Title

Mechanisms of GABA secretion from pancreatic islets

Authors

Austin Stis, Sandra Ferreira, Edward Phelps

Purpose

Gamma aminobutyric acid (GABA) is a neurotransmitter that is produced in the islet at levels as high as in the brain where it regulates secretion of insulin, glucagon and somatostatin. Beta cell GABA has been observed to co-release with insulin via glucose-induced vesicular fusion, but GABA is also released tonically from the cytosol via volume-regulated anion channels

(VRAC) in the plasma membrane. We are seeking to determine which of the two GABA release pathways predominantly maintains interstitial GABA levels and how the secretion is regulated. In GABA-producing neurons, the vesicular GABA transporter (VGAT) localizes to synaptic vesicles and mediates transport of GABA into the vesicle lumen. The existence of an analogous vesicular GABA-secreting system in beta cells would require expression of VGAT. However, VGAT is not detected in public RNA-sequencing databases of islet endocrine cells. Thus, we further investigated the expression of VGAT in beta cells to better understand the GABA secretion pathways in pancreatic islets.

Methods

Mouse islet monolayers were created by digesting mouse islets into single cell suspensions and plating on laminin coated glass 96-well plates. Cells were fixed, stained for VGAT, and imaged by confocal microscopy. INS-1 insulinoma cells were co-transfected with human GAD65-mCherry and VGAT-GFP. Cells were fixed, stained for GABA, and imaged by confocal microscopy. Lineage tracing was also performed to determine VGAT expression in mouse beta cells. Frozen pancreas sections from VGAT-IRES-Cre mice crossed with Ai14 tdTomato reporter mice were obtained from Dr. Sunil Gandhi's group (UC Irvine).

Summary of Results

Beta cell VGAT expression was below the detection limits of immunostaining in mouse islet monolayers. However, a negative immunostaining result could be due to alternatively spliced VGAT isoforms, as has been documented in rat islets, or poor antibody specificity. We used VGAT lineage tracing as an alternative approach to label VGAT expressing cells with a fluorescent reporter (VGAT-IRES-Cre x Ai14 tdTomato). No tdTomato positive cells were seen in islets while tdTomato positive neurons associated with pancreatic ducts were observed. Next, we asked whether ectopic expression of VGAT in beta cells would increase vesicular GABA loading. Transfection of INS-1 beta cells with VGAT-GFP resulted in unexpected cytosolic expression of VGAT that was not vesicle targeted and did not change GABA subcellular localization. This contrasts with neurons in which transfection of the same plasmids resulted in typical vesicular VGAT expression.

Conclusions

The combined data from single cell RNA-seq, immunostaining, ectopic VGAT expression, and lineage tracing indicate that VGAT is not detectable in beta cells. Based on the INS-1 cell transfection experiment, beta cells may not even have the appropriate structural machinery to direct VGAT into vesicles even when VGAT is expressed ectopically. This data provides evidence as to a lack of VGAT expression in beta cells and supports alternative mechanisms for GABA vesicular loading and non-vesicular secretion as being predominant in islets.

12: Chaitra Rao (University of Indiana)

Abstract Title

Protein death-ligand 1 (PD-L1) is present on the surface of β cell extracellular vesicles and is upregulated by INF exposure

Authors

Chaitra Rao, Dan Cater, Jerry Xu, Fei Huang, Arianna Harris, Andre DeOliveira, Charanya Muralidharan, Amelia Linnemann, Carmella Evans-Molina, Decio Eizirik, Raghavendra Mirmira, Emily Sims

Purpose

Extracellular vesicles (EVs) are membrane bound nanoparticles that carry bioactive molecules. Emerging data suggest that β cell derived EVs may act as paracrine effectors in the islet microenvironment. Expression of the transmembrane immune checkpoint protein death-ligand 1 (PD-L1), which inhibits immune cell activity via interactions with the PD-1 receptor, is

increased in β cells from residual insulin containing islets from donors with type 1 diabetes (T1D). In NOD mice, β cell PD-L1 overexpression reduces diabetes incidence while inhibition exacerbates insulitis. These findings suggest that β cell PD-L1 expression supports β cell survival in T1D. However, the presence of PD-L1 in β cell EVs has not been explored. Our hypothesis is that stressed β cells in the T1D microenvironment alter their EV contents to include protective PD-L1 cargo aimed at evading β cell destruction associated with autoimmunity.

Methods

Rat, mouse, and human β cell lines were treated with 2000 U/ml IFN-α or 1000 U/mL IFN-γ for 24 hours to model interferon signaling in the proinflammatory T1D islet microenvironment. EVs were isolated from cell culture media using sequential ultracentrifugation and size-exclusion chromatography. EV PD-L1 was analyzed via immunoblot, flow cytometry staining and ELISA. Fluorescence imaging/interferometry quantification (NanoView) of PD-L1 positivity for EV subpopulations based on presence of membrane tetraspanins (CD63, CD81, and CD9) was also performed. To query for an effect of human IFN-α on human islet release of PD-L1 in vivo, human islets were transplanted under the kidney capsule of NSG mice and allowed to engraft for 2 weeks. Mice were then intra-peritoneally injected with 2 µg recombinant human IFN-α or saline and euthanized 1 hour post-injection. Serum human PD-L1 was analyzed via ELISA (U-plex human PD-L1 assay, mesoscale diagnostics).

Summary of Results

PD-L1 protein was detected in small β cell EVs and was upregulated over 2-fold by IFN-α or IFN-γ treatment. Flow cytometry staining of intact EVs using an antibody that recognizes an extracellular epitope of PD-L1 also demonstrated PD-L1 upregulation with treatment, suggesting protein presence and upregulation on the EV surface, as well as potential for interaction with the PD-1 receptor on other cells. At baseline, CD63, CD81, and CD9 positive EVs showed similar percentages of PD-L1 positivity (~15-20%). 24-hour treatment with IFN-γ yielded significant increases in PD-L1 positive EVs within each subpopulation, but this was the most pronounced for CD81+ EVs (~3 fold increase) vs. ~2 fold increase for CD63 and CD9+ EVs. Human IFN-α treatment yielded significant upregulation of serum human PD-L1 compared to mice treated with saline alone. Ongoing testing is being performed to compare circulating EV PD-L1 levels in humans with or without T1D.

Conclusions

β cells increase PD-L1 protein expression on the surface of small EVs in response to IFN exposure, and these changes can be detected in circulation. Impacts of IFN signaling on EV PD-L1 cargo differ amongst EV subpopulations. Future work will test the idea that β cell EV PD-L1 may play an important role in islet intercellular communication and heterogeneity of β cell survival in T1D disease course. Furthermore, a beneficial effect of PD-L1+ EVs on β cell tolerance could ultimately be harnessed as an intervention to prevent autoimmune β cell destruction.

13: Dana Avrahami (Hebrew University)

Abstract Title

Cellular senescence, metabolic stress and SASP expression in human beta cells – linking aging and diabetes

Authors

Nathalie Groen, Eseye Feleke, Mati Mesenyashin, Adi Mazouz, Elisabetta Manduchi, Alexander van Oudenaarden, Eelco J P de Koning, Françoise Carlotti, Benjamin Glaser, Klaus H Kaestner, Dana Avrahami

Purpose

Cellular senescence, a hallmark of aging, is defined as stable cell cycle arrest accompanied by molecular and metabolic changes and frequently associated with secretion of pro-inflammatory substances known as SASP (Senescence Associated Secretory Pathway) that can negatively affect neighboring cells and cause tissue inflammation and damage. Senescent beta cells accumulate during aging while elimination of senescent cells improved glycemic outcome and prevented onset of

autoimmune diabetes in NOD mice, suggesting that this cellular state may contribute to pathogenesis of both T1D and T2D. Currently, however, the molecular mechanisms that lead to the senescence of beta cells are unknown. Achieving a better understanding of the interaction between age- and disease- related beta-cell senescence and disease progression could reveal a novel cellular basis linking aging and diabetes, and in doing so identify novel potential therapeutic targets.

Methods

To explore possible drivers of senescence and SASP in beta cells and to characterize the associated molecular and secretory profile, we cultured non-diabetic (ND) human islets under metabolic and inflammatory stress conditions for 24 hours and subjected them to secretome analysis by Luminex assay and transcriptome analysis with 10X single cell RNA sequencing.

Summary of Results

Whereas the Luminex assay confirmed that islets can be induced to secrete SASP substances under some stress conditions, primarily glucolipotoxic and IL-1B induced stress, single cell transcriptome analysis revealed that a subpopulation of beta cells which express CDKN2A (encoding p16) were mostly responsible for SASP-related gene expression and lost expression of identity markers under stress conditions. Importantly, CDKN2A positive beta cells are characterized by high expression levels of CDKN1A, encoding p21 and anti-apoptotic genes confirming their senescent state. These cells also express lower levels of genes involved in insulin processing and secretion such as INS, IAPP, SCGN and PCSK1 compared to CDKN2A negative beta cells which could indicate compromised function.

Conclusions

These findings reveal that the subpopulation of pre-existing, age-related senescent beta cells, are more susceptible to stress-induced SASP secretion and loss of beta-cell function markers compared to beta cells with undetectable levels of CDKN2A. Since the prevalence of p16 positive beta cells increase with age, these findings provide a mechanism linking aging with susceptibility to diabetes. Preventing or eliminating senescent beta cells could decrease susceptibility to stress-induced islet dysfunction and diabetes.

14: Denise Drotar (University of Florida)

Abstract Title

In Situ Study of Endocrine and Exocrine Cell Function in Recent-Onset T1D and AAb+ Organ Donors

Authors

Denise Drotar, Ana Karen Mojica-Avila, Drew Bloss, Amanda Posgai, Irina Kusmartseva, Mollie Huber, Edward Phelps, Clive Wasserfall, Stephan Speier, Mark Atkinson

Purpose

Beyond beta cell loss, recent efforts have demonstrated that both endocrine and exocrine compartments are affected in type 1 diabetes (T1D). While histological evidence of regional heterogeneity within the human pancreas is extensive, the functional evidence at the tissue level is scarce, especially in humans. Thus, here we aim to coregister possible differences in secretory function from endocrine (insulin, glucagon) and exocrine (amylase, lipase, trypsinogen) together with 3D morphology in recent-onset T1D and single autoantibody-positive (AAb+) donors using the pancreas tissue slice platform.

Methods

Islet and acinar cell functionality were assessed using freshly generated slices obtained from 14 non-diabetic (ND), 6 single AAb+ and 6 recent-onset T1D donors (0-24 months duration), acquired through the Network for Pancreatic Organ donors with

Diabetes (nPOD) program. Kinetics of hormone release and pancreatic enzyme secretion were evaluated in slices from the pancreas head (PH), body (PB) and tail (PT) regions, followed by whole slice 3D morphometrical analysis of insulin and glucagon positive volumes of the perifused slices.

Summary of Results

Insulin and glucagon secretion were measured in response to intermittent glucose stimulations of live pancreas tissue slices obtained from the PH, PB, and PT regions of 14 ND organ donors (age range 4-33 years). Our data suggests that insulin and glucagon secretory capacity (stimulation index over baseline) in response to changes in glucose concentrations is similar in slices obtained from the PH, PB and PT in ND individuals. Using this protocol, we also evaluated hormone responsiveness in slices from AAb+ and T1D pancreata. Our data shows reduced islet functionality in these T1D donors. We did not detect appreciable alterations in most single AAb+ donors evaluated, as insulin responses quantified by AUC were within the ND range. In terms of glucagon release, we observed reduced stimulatory capacity at low glucose in most T1D donors. Suppression of glucagon responsiveness at high glucose was similar across all three donor groups. Further studies are needed in pancreas slices from rare pre-T1D donors, defined as having two or more AAb, in order to appreciate the progressive loss of islet cell function across the disease stages.
Using slices from the same donors, we measured amylase, lipase and trypsinogen at baseline and upon stimulation with carbachol. Baseline enzyme release was comparable between the ND, AAb+, and T1D donors. When stimulated with carbachol, slices from ND donors exhibited highly heterogeneous responses across the PH, PB and PT; similar responses were observed in slices from AAb+ and T1D donors. Investigation of additional at-risk donors is needed to fully understand endocrine and exocrine cell dysfunction during the early stages of T1D.
Finally, we sought to correlate these functional observations with tissue 3D morphometry. To this end, slices were fixed and shipped to the Paul Langerhans Institute Dresden for 3D morphometrical analysis of insulin and glucagon positive cell volumes within whole slices. Thus far, we have not observed differences in the contribution of insulin and glucagon to the endocrine volume across the three pancreatic regions in ND slices. In terms of islet size, the PB seems to contain a higher frequency of smaller sized islets than PH or PT, nevertheless the functional relevance of this observation needs to be further investigated. Ongoing 3D morphometry analysis of the perifused tissue slices will allow us to better understand the distinct roles of endocrine cell mass and function during T1D pathogenesis.

Conclusions

This work provides proof that islet and acinar cell function can be simultaneously studied across the human pancreas using the tissue slice platform. Experiments are in progress to further evaluate the functional relationship between the endocrine-exocrine compartments in pancreas slices from T1D and at-risk AAb+ donors. In combination with 3D morphometry and live in situ imaging of calcium dynamics in islet and acinar cells within the same or neighboring lobules, this approach will additionally provide insight into possible mechanisms that lead to regional and lobular heterogeneity of insulitis and beta cell destruction in organ donors with T1D.

15: Diti Chatterjee Bhowmick (City of Hope Beckman Research Institute)

Abstract Title

DOC2b reduces beta-cell stress and inflammation via blocking chemokine ligand expression under pro-inflammatory stress conditions

Authors

Diti Chatterjee Bhowmick, Miwon Ahn, Debbie Thurmond

Purpose

The reduction of beta-cell stress, inflammation, and dysfunction hold promise for therapeutic intervention for type 1 diabetes. Previously, we have reported the beneficial role of DOC2b in preventing beta-cell dysfunction and beta-cell apoptosis. DOC2b

is a soluble protein that is highly expressed in beta-cells and is implicated in vesicle exocytosis as it relates to glucose-stimulated insulin secretion (GSIS). Mechanistically, DOC2b promotes healthy GSIS via the glucose-stimulated phosphorylation of DOC2b at tyrosine residue 301. However, the mechanism by which DOC2b prevents/mitigates proinflammatory stress-induced beta-cell apoptosis remains unknown.

Methods

To initiate studies of the underlying mechanism, we have used RNA-seq, RT-qPCR, and proteomics analyses using DOC2b enriched primary human islets and INS-1 832/13 beta-cells challenged with or without proinflammatory cytokine cocktail.

Summary of Results

Our RNAseq analysis using type 2 diabetic human islets showed a significant decline in chemokine ligand expression with DOC2b enrichment, and validations using RT-qPCR confirmed this observation. In line with this, our gene expression study revealed that enrichment of DOC2b significantly attenuated pro-inflammatory cytokine-induced CXCL9,10 gene expressions in primary non-diabetes human islets. To evaluate this exclusively in beta-cells, INS-1 832/13 beta-cells were proinflammatory cytokine-challenged, and the chemokine ligand CXCL10 was confirmed to be reduced in those cells overexpressing DOC2b. In beta-cells, proinflammatory cytokine-induced CXCL10 expression is known to be regulated via NFkb and STAT-1 mediated signaling pathways. Our subsequent proteomics study identified NFkb and JAK-STAT pathway members as binding partners of DOC2b in beta-cells.

Conclusions

These data support a model in which DOC2b protects beta-cells from proinflammatory cytokine stress via a mechanism involving attenuation of both NFkb and JAK-STAT signaling, reducing CXCL10 expression, consistent with the observation of reduced beta-cell apoptosis.

16: Ernesto Nakayasu (Pacific Northwest National Laboratory)

Abstract Title

Global and spatial lipidomics reveal a mechanism of omega-3 fatty acid-mediated β cell protection

Authors

Soumyadeep Sakar, Cailin Deiter, Jennifer Kyle, Michelle Guney, Dylan Sarbaugh, Ruichuan Yin, Xiangtang Li, Yi Cui, Mireia Ramos-Rodriguez, Carries Nicora, Farooq Syed, Jonas Juan-Mateu, Martha Campbell-Thompson, Lorenzo Pasquali, Martha Neuringer, Carmella Evans-Molina, Decio Eizirik, Bobbie-Jo Webb-Robertson, Kristin Burnum-Johnson, Galya Orr, Julia Laskin, Thomas Metz, Raghavendra Mirmira, Lor Sussel, Charles Ansong, Ernesto Nakayasu.

Purpose

Lipids have been implicated as regulators of insulitis and β-cell death during the development of type 1 diabetes, but the underlying mechanisms are poorly understood. In this study, we investigated how the islet lipidome and downstream signaling factors regulate β-cell death.

Methods

We performed lipidomic analyses in three models of insulitis: human islets and EndoC-βH1 β cells treated with the cytokines IL-1β and IFN-γ, and islets from non-obese diabetic mice. The spatial distribution of lipids and enzymes of interest were

determined by mass spectrometry imaging and microscopy, respectively. We also performed RNAi experiments combined with proteomics, biochemical assays, and western blots to study the downstream regulatory network of lipid signaling.

Summary of Results

All three insulitis models showed a consistent decrease of triacylglycerols and increase of lysophosphatidylcholines (LPC) and phosphatidylcholines with polyunsaturated fatty acids (PC-PUFA). Mass spectrometry imaging, fluorescence in-situ hybridization and immuno-histochemistry showed an enrichment of various LPCs, PC-PUFAs and their related metabolizing enzyme, namely inducible phospholipase A2 PLA2G6, in islets compared to surrounding tissue. To study downstream lipid signaling, we performed a global proteomic analysis of PLA2G6 deficient mouse MIN6 β cells, which showed that ADP-ribosylhydrolase ARH3 is regulated by cytokines in a PLA2G6-dependent manner. Ablating ARH3 expression by RNAi enhanced apoptosis of MIN6 cells treated with cytokines. We also found that ARH3-mediated protection of islet cells involves degradation of the histone methylation polycomb complex member SUZ12 by ω-3 fatty acids, which are released from PC-PUFAs by PLA2G6. This increases the expression of Arh3, which in turn reduces islet cell apoptosis.

Conclusions

Our data provide insights into alterations of the lipidome and related downstream signaling in T1D that influence loss of β cells and reveal a mechanism of β-cell protection by ω-3 fatty acids, which is mediated at least in part via the ADP-ribosylhydrolase ARH3.

17: Feroz Papa (University of California San Francisco)

Abstract Title

Next-generation kinase inhibitors of IRE1α allosterically tune its RNase to segregate physiological outputs in human β-cells through the unfolded protein response to endoplasmic reticulum stress

Authors

Luka Suzuki, Jaehong Kim, Mary Beth Moreno, Alina Olivier, Hannah Feldman, Vincent Auyeung, Zachary Potter, Venkata Vidadala, B. Gayani K. Perera, James Mueller, Julie Zikherman, Bradley Backes, Dustin Maly, Feroz R. Papa

Purpose

The unfolded protein response (UPR) homeostatically matches endoplasmic reticulum (ER) protein-folding capacity to cellular secretory needs; this signaling pathway is critical to determining the fate of professional secretory cells, such as pancreatic islet beta cells, which undergo apoptosis under high/chronic ER stress. In type 1 diabetes (T1D), autoimmune cell infiltration into islets and targeting of beta-cells leads to amplification of ER stress in beta-cells and conversion of an “adaptive” UPR to a “terminal” UPR. This binary switch occurs upon hyperactivation of the bifunctional ER transmembrane enzyme, IRE1α kinase/RNase, which promotes both adaptation though XBP1 mRNA splicing, and cell destruction though ER-localized mRNA endonucleolytic decay. Our labs at UCSF and Washington, Seattle have been studying the fundamental mechanisms underlying this binary life-death switch in order to modulate and preserve cytoprotective outcomes in beta-cells as disease-modifying therapies for T1D.

Methods

We previously found that IRE1 alpha’s RNase can be allosterically fully activated or fully inactivated by small molecule ATP-competitive kinase inhibitors we developed—with an intermediate state enforced by partially antagonizing IRE1alpha’s RNase at full kinase occupancy—the physiological ramifications of these therapeutic modulations were not clear. Here we will present data that a continuum of activation and inhibition steps is available to modulate IRE1alpha in reductionist systems (INS-1 chemical-genetic system lines), transplanted human islets in NSG mice, and in pancreata from human donors (obtained from

nPOD) exposed to small molecule compounds that differentially modulate movement of the helix alpha C in IRE1αalpha kinase, and thereby
effect oligomerization state and RNase activity—these differential modulation states have ramifications for therapy. Using structure activity relationships, we will show an IRE1α activator (G1749)—"AIRE", a partial antagonist (UC4)—"PAIR", and a full antagonist (KIRA8)—"KIRA" that predictably segregate distinct physiological outputs (UPR gene expression, apoptosis, and GSIS) due to differential effects on XBP1 mRNA splicing and ER-localized mRNA endonucleolytic decay.

Summary of Results

We propose that a gamut of modulation states using small molecule kinase inhibitors that allosterically enforce distinct IRE1's RNase outputs "closes a circle" going back to our original chemical-genetic manipulation of this bifunctional kinase/RNase using a bump-hole system and the designer kinase-inhibitor, 1NM-PP1. We are currently advancing nanomolar-potent, mono-selective, and orally-bioavailable kinase inhibitors from structure-based drug design in three distinct modes: activator, partial inhibitor, and full inhibitor, for efficacy testing in murine T1D models, human islets, and pancreatic slices obtained from nPOD.

Conclusions

In summary, our work may advance a novel mode of beta-cell sparing during ER stress and ultimately deliver next-generation kinase inhibitors as disease-modifying oral drugs to test in clinical trials for patients suffering from T1D (and potentially those at risk).

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

18: Gulcan Semra (University of Wisconsin-Madison)

Abstract Title

IRE1α modulates mitochondrial metabolism and morphology in the β-cells of NOD mice

Authors

Gulcan Semra Sahin, Hugo Lee, Vaibhav Sidarala, Hulya Zeynep Oktay, Varghese Chacko, Kevin Eliceiri, Emily Knuth, Matthew Merrins, Scott Soleimanpour, Feyza Engin

Purpose

β-Cell endoplasmic reticulum (ER) stress and dysregulated unfolded protein response (UPR) have been implicated in type 1 diabetes pathogenesis. Inositol-requiring enzyme-1 α (IRE1α) is a key UPR sensor and its deletion in β-cells (IRE1αβ-/-) of non-obese diabetic (NOD) mice leads to transient β-cell dedifferentiation and protection from T1D. However, whether genetic manipulation of IRE1α in β-cells of NOD mice alters intracellular organelle homeostasis remains unknown. In this study, we investigated ER-mitochondria crosstalk in dedifferentiated β-cells of IRE1αβ-/- mice.

Methods

We performed single-cell RNA-sequencing (scRNA-seq) in dissociated islets of IRE1αβ-/- mice at 5 weeks of age, a time point that β-cells exhibit dedifferentiation. We investigated metabolic alterations in these mice using multiphoton NAD(P)H fluorescence lifetime imaging microscopy (FLIM) and lactate reporter assay. We further analyzed mitochondrial morphological changes in IRE1α-deficient β-cells by using immunofluorescence (IF) staining of pancreata and performing transmission electron microscopy (TEM) imaging on isolated islets.

Summary of Results

Our scRNA-seq analysis reveals that expression levels of genes involved in oxidative phosphorylation, TCA cycle, pentose phosphate pathway, and fatty acid β-oxidation are significantly increased, while the ones involved in glycolysis are markedly decreased in β-cells of IRE1αβ-/- mice compared to those of control mice. NAD(P)H FLIM analysis and lactate reporter assay show that β-cells of IRE1αβ-/- mice undergo metabolic reprogramming. Assessment of mitochondrial morphology via IF staining using antibodies against succinate dehydrogenase A (SDHA) indicated that dedifferentiated β-cells of IRE1αβ-/- mice exhibit significantly more elongated and branched mitochondria. TEM imaging analysis of islets further revealed markedly dilated ER structures, elongated mitochondrial morphology and substantially less cristae number per mitochondria area in β-cells of these mice.

Conclusions

Our results suggest that loss of IRE1α in β-cells during early stages of disease progression triggers metabolic reprogramming and mitochondrial alterations which may contribute to β-cell fate decision and functionality.

19: Holger Russ (Diabetes Institute UF)

Abstract Title

CD9+ marks a human beta cell subpopulation with increased immunogenicity

Authors

Ali Shilleh, Amanda Anderson, Laurie Landry, Scott Beard, Jaeann Dwulet, Jessie Barra, Alexander Baker, Hubert Tse, Richard Benninger, Maki Nakayama, Holger Russ

Purpose

The destruction of insulin producing pancreatic β-cells by an autoimmune attack is a hallmark of Type 1 diabetes (T1D). Despite tremendous research efforts focusing on elucidating the underlying mechanisms resulting in human T1D, the field currently lacks detailed understanding on how and why the disease develops in individuals. While T1D has been classically viewed as a disease of the immune system, increasing evidence suggest a critical role for the pancreatic beta cells in attracting their own immune destruction. Indeed, subpopulations of pancreatic beta cells that can withstand or critically contribute to autoimmunity have been identified using mouse models prompting the intriguing concept that beta cell heterogeneity is central in T1D development. However, if a human beta cell subpopulation with differential immunogenicity exist has not been comprehensively investigated as of yet.

Methods

Using two complementary human beta cell models, cadaveric islets and stem cell derived beta cells (sBC), we identified a small beta subpopulation marked by CD9 that exhibits key markers of senescence, a senescence associated secretory

phenotype, reduced function and an enrichment in immune response genes. HLA matched human beta cell and autoreactive T cell co-cultures showed increased T cell stimulation by CD9/senescent beta cells in these novel, functional assay systems.

Summary of Results

Our findings highlight the first demonstration of differential immunogenicity of human beta cell subpopulations. Furthermore, transplantation of cadaveric islets or sBC results in a dramatic increase in CD9/senescent beta cells providing important insights for current cell therapy efforts.

Conclusions

In sum, our study offers important novel knowledge on beta cell heterogeneity and immunogenicity and carries critical implications for our current understanding of T1D development and cell replacement approaches.

20: Hugo Lee (University of Wisconsin-Madison)

Abstract Title

IRE1α and XBP1-dependent targets and regulatory networks in beta cells during early type 1 diabetes

Authors

Hugo Lee, Jiaxin Li, Julia Doris, Khagani Eynullazada, Brett Pontelandolfo, Maria Hatzoglou, Sushmita Roy, Feyza Engin

Purpose

Beta cell endoplasmic reticulum stress and a dysregulated unfolded protein response (UPR) have been implicated in type 1 diabetes (T1D) disease pathogenesis. IRE1α is a key UPR sensor that exhibits pleiotropic effects during ER stress through its kinase and endoribonuclease domains. With its kinase domain, IRE1α can phosphorylate c-Jun N-terminal Kinase (JNK) or IκB kinase (IKK) to induce inflammation and apoptosis. With its RNase domain, it degrades miRNAs and mRNAs through RIDD activity. IRE1α can also use its RNase activity to generate transcriptionally-active spliced XBP1 (sXBP1) that can regulate UPR target genes. Deletion of Ire1α in NOD mice prior to insulitis confers protection against T1D, but the specific downstream effector(s) mediating this protective phenotype remain unknown.

Methods

Using an inducible Cre system, we deleted Xbp1, one of Ire1α's downstream effectors, in beta cells of NOD mice prior to insulitis. We performed histological analyses on pancreatic sections to examine the composition and morphology of islet cells, along with proliferation and apoptosis of beta cells. We performed single cell transcriptomic analysis and revealed changes in markers of beta cell maturity, along with disallowed, dedifferentiation, and progenitor genes. Finally, we utilized comparative single cell transcriptome and gene regulatory network analyses on scRNA-seq data from Xbp1- and Ire1-deficient mice to identify unique and shared transcriptional networks.

Summary of Results

NOD mice with beta cell-specific deletion of Xbp1 (Xbp1β-/-) were protected from T1D following a transient period of hyperglycemia during which they exhibited significantly altered islet composition and morphology, with no changes in either apoptosis or proliferation. However, when the Xbp1β-/- mice recover from hyperglycemia, islet morphology and composition are restored. Single cell RNA-sequencing analysis reveals that beta cells from Xbp1β-/- mice are undergoing dedifferentiation during the period of temporary hyperglycemia. These dedifferentiated beta cells showed markedly decreased expression of maturity markers and autoantigens, while substantially increasing expression of disallowed genes, progenitor markers, and

markers of other non-beta islet cells, similar to what was observed in Ire1αβ-/- mice. Using gene regulatory network analysis with MERLIN, we also identified unique transcriptional networks in Xbp1β-/- mice.

Conclusions

Our findings define the role of beta cell IRE1α/XBP1 pathway early in disease progression and identify previously unrecognized transcriptional targets and regulatory nodes of IRE1α and XBP1 in a preclinical T1D model.

21: James Johnson (University of British Columbia)

Abstract Title

Proteomic determinants of nutrient-specific insulin secretion from human islets and stem-cell derived islet surrogates

Authors

Jelena Kolic, WenQing Grace Sun, Leanne Beet, Renata Moravcova, Jessica Ewald, Jason Rogalski, Shugo Sasaki, Haoning Cen, Han Sun, Varsha Rajesh, Søs Skovsø, Aliya Spigelman, Jocelyn Manning Fox, James Lyon, Jianguo Xia, Anna Gloyn, Francis Lynn, Leonard Foster, Patrick MacDonald, James Johnson.

Purpose

Insulin the body’s most important nutrient-responsive hormone. Insulin secretion, either deficiency or excess, plays a role in the pathogenesis of type 1 diabetes, type 2 diabetes, obesity and associated conditions, as well as some cancers. The variations and mechanisms behind insulin secretion from human islets in response to carbohydrate, protein, and fat remain uncharacterized despite important implications for the advancement of personalized therapeutic nutrition.

Methods

Insulin secretion dynamics at baseline (3 mM glucose) and in response to the carbohydrate (15 mM glucose or 6 mM glucose), amino acid (5 mM leucine), or fatty acid (1.5 mM oleate/palmitate mix) were measured by perifusion and radioimmunoassay from 138 cadaveric donors sampled from the population, including individuals with or without type 2 diabetes, as well as human stem cell derived islet-like clusters. Gene expression was assessed with RNA sequencing, with confirmation using Nanostring. The abundance of ~8000 proteins was assessed by DIA-PASEF mass-spectroscopy proteomic analysis. Insulin response heterogeneity, donor characteristics, and protein abundances were analyzed using co-expression analysis modelling.

Summary of Results

Our data define a prototypical (average) response to carbohydrate, protein, and fat, in islets isolated from healthy people. Islets from individuals living with type 2 diabetes had a 50% reduced insulin response to 15 mM glucose, a 65% reduced response to fatty acids, and a 50% reduced response to direct depolarization with 30 mM KCl. Using RNA sequencing, we identify 186 differentially expressed mRNAs in type 2 diabetes. More proteins (640) were identified as differentially abundant in type 2 diabetes using direct analysis of the proteome. Most importantly, we show that there is a large degree of intra-individual variation in the response to each macronutrient, and we leveraged this human variation to identify proteins and networks of proteins that are significantly associated with the response to each nutrient. Indeed, an unusual proteomic signature conferred hyper-responsiveness to fat in ~8% of donors. By comparing to human embryonic stem cell-derived islet clusters, we show that fat hyper-responsiveness is a characteristic of functionally immature islet cells. Substantial differences in the proteomes of stem cell-derived islet clusters and primary human islet provides a roadmap for generating more realistic islet replacements for diabetes cell therapy.

Conclusions

Our study represents the first comparison of dynamic multi-nutrient responses and multi-omics analysis in a large cohort of human islets and human embryonic stem cell derived insulin secreting clusters, laying the groundwork for personalized nutrition and providing insight into the development of improved cell therapies for diabetes.

22: Julia Panzer (University of Miami)

Abstract Title

Targeting alpha cells to treat hypoglycemia in type 1 diabetes

Authors

Alejandro Caicedo

Purpose

Increased glucagon secretion from the pancreatic alpha cell is the first and most important defense against hypoglycemia. In type 1 diabetes this defense mechanism is lost, increasing the mortality risk. The islet cytoarchitecture allows the alpha cell to coordinate its activity with the beta cell. We propose that during the progression of T1D it is the loss of the neighboring beta cells that causes the alpha cell to respond inappropriately to blood sugar levels. The inhibitory signals released from the beta cell (like insulin, GABA, serotonin, Zn^{2+}) contribute to a healthy glucagon response. The sudden cessation of this inhibitory input during hypoglycemia is a necessary signal for the glucagon response. We therefore hypothesize that reactivation of endogenous paracrine and autocrine signaling pathways might restore the alpha cell's ability to respond to hypoglycemia in T1D.

Methods

We use isolated human islets as well as pancreatic tissue slices from non-diabetic donors and donors with type 1 diabetes. We measure alpha cell responses to (a) changes in glycemia, (b) agonists and antagonists of autocrine and paracrine alpha cell regulation, and (c) reference stimuli such as adrenaline, kainate and KCl depolarization. We are using dynamic hormone secretion and calcium recordings as functional readouts. We further performed in vivo studies using mouse models with defective glucose counter regulation to determine whether alpha cell responses to hypoglycemia can be restored.

Summary of Results

Alpha cell responses are briefer than the sustained beta cell responses. If not reset by inhibitory input, the alpha cell is not able to respond again. Glucagon secretion can be recovered by activation of paracrine signals to inhibit the alpha cell or through positive allosteric modulators, reactivation the autocrine feedback loop. In tissues from type 1 diabetic donors, we found that alpha cells fail to respond to decreases in glucose concentration despite normal glucagon content and responses to KCl depolarization. Furthermore, we found severely dimished Ca^{2+} responses to both lowering in glucose concentration and glutamate receptor stimulation. By reactivating residual glutamate receptor function with the positive allosteric modulators cyclothiazide and aniracetam we could rescue glucagon secretion in response to hypoglycemia in human tissue slices from donors with T1D. In addition, reactivation of individual paracrine signals like serotonin, GABA or somatostatin also recovered glucagon secretion in tissues from donors with T1D.

Conclusions

We demonstrate that alpha cells in people with type 1 diabetes are not able to mount an efficient glucagon response due to deficient glutamate receptor signaling and loss of paracrine inhibitory input. Our results further suggest that restoring both, autocrine and paracrine signaling rescues glucagon secretion. These signals could be used as targets to limit hypoglycemic episodes, allowing improved therapy of type 1 diabetes.

23: Katie Coate (Vanderbilt University)

Abstract Title

LOSS OF MAFB COMPROMISES ALPHA CELL IDENTITY AND HORMONE SECRETION IN ADULT HUMAN ISLETS

Authors

Katie Coate, Xin Tong, Jeeyeon Cha, Jin-hua Liu, Chunhua Dai, Vy Nguyen, Regina Jenkins, Radhika Aramandla, Matthew Cottam, Jean-Phillippe Cartailler, Seung Kim, Marcela Brissova, Alvin Powers, Roland Stein

Purpose

Islet-enriched transcription factors (TFs) are critical regulators of alpha and beta cell development and function. One such TF, MAFB, exhibits a distinct expression pattern between species in that it is restricted to alpha cells postnatally in rodents, but produced in both developing and adult human alpha and beta cells. Recently, MAFB was shown to be essential for glucagon and insulin production and the acquisition of glucose-responsive insulin secretion in hES-derived islet-like cells. By contrast, MAFB deficiency merely delays rodent beta cell development with no impact on hormone production. Importantly, both type 1 and type 2 diabetes are associated with marked downregulation of MAFB in islets, implicating compromised MAFB activity with alpha and/or beta cell dysfunction in diabetes. However, the requirement for MAFB in the regulation of adult islet function is poorly understood. The purpose of this study was to test the hypothesis that MAFB is a critical regulator of alpha and beta cells, with its loss contributing to dysregulated hormone secretion.

Methods

To model the decrease in MAFB seen in diabetic human islets, dispersed islet cells were infected with lentiviruses expressing scrambled (control) or MAFB knockdown (MAFBKD) shRNA sequences and allowed to re-aggregate for 6 days to form pseudoislets (N=6 adult normal donors [4 males, 2 females], ages 16-61 years). Static hormone secretion assays and bulk RNA-sequencing (RNA-seq) were performed to evaluate the functional and transcriptional consequences of MAFBKD.

Summary of Results

MAFBKD pseudoislets exhibited a 55.7 +/- 7.4% reduction ($P<0.001$) in MAFB mRNA compared to control, a finding that was confirmed at the protein level via immunofluorescence staining. In addition, glucagon mRNA and hormone content were reduced by 46.8 +/- 11.3% ($P<0.01$) and 24.0 +/- 6.4% ($P<0.05$), respectively, whereas insulin and somatostatin expression were unchanged. To evaluate the impact on hormone secretion, pseudoislets were exposed to low (1.7 mM) and high (16.7 mM) glucose +/- IBMX for 1 hour. While glucose regulated and IBMX-potentiated glucagon secretion were significantly blunted in MAFBKD pseudoislets (~30% lower than control across all conditions; $P<0.05$), insulin secretion was impacted only in the presence of high glucose + IBMX (~20% decrease; $P<0.05$). RNA-seq analysis of MAFBKD versus control pseudoislets illustrated that alpha cell identity genes were downregulated, selected beta and delta cell identity genes were upregulated, and the gut peptide gene, PYY, was misexpressed. Furthermore, GSEA showed that the upregulated genes were associated with pathways related to abnormal endocrine pancreas physiology, morphology, hormone secretion, and glucose tolerance, whereas pathways in the downregulated genes were linked to cell cycle regulation and DNA metabolic processes.

Conclusions

Our findings suggest that MAFB is a molecular “gatekeeper” of islet cell identity by activating the expression of genes associated with the maintenance of alpha cell identity and glucagon secretion while simultaneously repressing those associated with (for example) beta and delta cell identity.

24: Lisa Turk (University of Florida)

Abstract Title

Head trauma does not increase GADA titer in type 1 diabetes or autoantibody positive organ donors

Authors

Lisa Turk, Laura Jacobsen

Purpose

Glutamic acid decarboxylase (GAD) is expressed in the beta cells of pancreatic islets and in neurons, and is the rate limiting step in the synthesis of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. As such, GAD antibodies (GADA) are present in both type 1 diabetes (T1D) and in a variety of neurological disorders such as stiff-person syndrome. GADA are also an important predictor of T1D and are the most prevalent autoantibody at diagnosis in individuals with T1D. We aimed to determine if there is a relationship between cause of death (i.e., head injury) and GADA positivity or titer in T1D or autoantibody positive organ donors that might interfere with interpretation of autoantibody status.

Methods

We assessed 143 organ donors with T1D and 33 autoantibody positivity in the Network for Pancreatic Organ Donors with Diabetes (nPOD) cohort from 10/15/2006 to 2/28/2022. Of the 143 organ donors with T1D, 57 had GAD titers available. Data were analyzed using a non-parametric Mann-Whitney test comparing median GADA titers among all GADA-positive donors with and without head trauma as the cause of death. A sub-analysis among T1D donors used Fisher's Exact test to compare GADA positivity rates among those with and without head trauma. ICU time was also assessed; however, due to correlation with the incidence of head trauma, is not reported.

Summary of Results

While the rate of head trauma (n=44, 25%) was higher among T1D donors with GADA positivity compared to non-head trauma causes of death (n=132, 75%), this was not significant (p=0.36). Furthermore, head trauma was not a predictor of GADA titer among GADA-positive organ donors in nPOD (p=0.82); those whose cause of death was head trauma (n=32), the median [IQR] GADA titer was 131.2 [71.8, 582.5], compared to no head trauma (n=58) which was 175.9 [61.0, 327.7].

Conclusions

While limited by smaller sample sizes and GADA titer outliers, our results support that head trauma does not significantly affect GADA in nPOD organ donors, and therefore is not a hindrance to accurate donor classification.

25: Mark Mamula (Yale University School of Medicine)

Abstract Title

Protein Modifications Alter Both Autoimmunity and Glucose Metabolism in Type 1 Diabetes

Authors

Mei-Ling Yang, Cate Speake, Carmella Evans-Molina, Lut Overbergh, Li Wen, Kevan Herold, Eddie James, Mark Mamula

Purpose

Autoimmune B and T cell responses often arise to post-translational protein modifications (PTMs), contributing to chronic inflammation by infiltrating lymphocytes of target organs such as the pancreas in type 1 diabetes (T1D). The identification and assessment of autoimmunity to novel PTMs may serve as biomarkers of T1D onset and pathogenesis. Moreover, it is similarly critical to determine if PTM proteins alter key processes in beta-cell biology, namely glucose sensing and insulin production, folding and release. This study specifically addresses these latter biologic functions of PTM proteins associated to T1D.

Methods

Citrullination is mediated by calcium-dependent peptidylarginine deiminase (PAD) enzymes, which catalyze deimination, the conversion of arginine into the non-classical amino acid citrulline. Carbonylation is one of the major protein modifications in response to oxidative stress. We performed various proteomic analyses, including mass spectrometry, in both murine and human pancreatic islets to identify citrullinated and carbonylated beta cell proteins. Glucose-stimulated proinsulin/insulin secretion was examined in human islets stressed with inflammatory cytokines and physiologic oxidative conditions in the presence or absence of PAD inhibitors. In addition, we also examined the effect of citrullination on the enzyme kinetics of glucokinase (Vmax and Km), the first rate-limiting step of glycolysis in the liver and pancreas.

Summary of Results

We identify autoantibodies and T cells from T1D patients against glucokinase (GK; native and citrullinated forms) and to beta subunit of prolyl-4-hydroxylase (P4Hb; native and carbonylated form). By mass spectrometry, six carbonyl residues and sixteen citrulline residues were mapped in oxidative P4Hb and PAD-treated GK, respectively. In regards to glucose metabolism, the carbonylated-P4Hb is amplified in stressed human islets coincident with decreased glucose-stimulated insulin secretion and increased proinsulin to insulin ratios. Citrullination alters GK biologic activity (Km) and suppresses glucose-stimulated insulin secretion. Moreover, PAD2/4 inhibitor can partially correct IFNγ +IL-1β suppressed glucose stimulated insulin secretion in INS-1E beta cells.

Conclusions

The major function of pancreatic beta cells is to secret insulin in response to glucose uptake in order to maintain the blood glucose level. Glucokinase is expressed in hepatocytes to regulate glycogen synthesis, and in pancreatic beta cells as a glucose sensor to initiate glycolysis and insulin signaling. P4Hb is a critical macromolecule for the accurate folding of insulin. Our studies implicate the crucial enzymes, glucokinase and P4Hb, as the biomarkers, providing new insights into creating autoantigens and define the impact of PTMs on the aberrant beta cell functions of T1D. Targeting glucose metabolism by PTMs, such as PAD inhibitors, may lead to preventing diabetes autoimmunity and restoring beta cell function.

26: Mollie Huber (University of Florida)

Abstract Title

Declines in β Cell-Glucose Responsiveness in Live Pancreas Tissue Slices During T1D Pathogenesis

Authors

Mollie Huber, Adrienne Widener, Denise Drotar, Helmut Hiller, Maria Beery, Ellen Verney, Irina Kusmartseva, Marjan Slak Rupnik, Mark Atkinson, Clayton Matthews, Edward Phelps.

Purpose

Type 1 diabetes (T1D) is an autoimmune disease that results from destruction of the insulin-producing β cells of the pancreas. Established features of T1D include loss of first-phase insulin secretion in response to glucose, declining c-peptide, and glucose

intolerance. These are indicative of a period of β cell dysfunction prior to β cell death. We believe that live pancreas tissue slices represent a useful model to study this dysfunction since both β cell functionality and T cell infiltration can be evaluated during the different stages of T1D's natural history. Here, we explore the hypothesis that immune dysregulation during the early stages of T1D development impacts β cell dysfunction in pathways affecting glucose metabolism.

Methods

Live human pancreas tissue slices were prepared from nPOD organ donors without diabetes or T1D associated autoantibodies (ND, n=6), persons positive for one or more autoantibodies without a diagnosis of T1D (AAb+, n=7), and individuals with short duration T1D (within 4 years of diagnosis, n=5)). Imaging studies were performed using a Leica SP8 confocal microscope. Slices were stained with anti-CD3 to identify and track endogenous T cells and anti-ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3) to identify β cells. Dynamic responses to glucose and KCl were assessed through changes in intracellular Ca2+ using Fluo-4 or Calbryte 520. Area under curve (AUC) calculations were made from the average trace generated from regions of interest (ROIs) selected on individual β cells. Insulin secretion was measured in parallel using a Biorep perifusion system. To investigate possible mechanisms for the loss of glucose responsiveness, we also stained fixed pancreas tissue sections with antibodies for insulin, glucagon, ATP5B (a mitochondrial ATP synthase subunit) and ATPIF1 (ATP synthase inhibitor) in a ND donor and a donor with T1D (analyses using additional donors are ongoing).

Summary of Results

Islets within slices from ND and AAb+ donors had no insulitis (defined here by 15 or more CD3+ cells) and mobilized cytosolic Ca2+ in response to both high glucose (HG) and potassium chloride (KCl) stimulations. Infiltrating T cells were present in a majority of β cell positive islets in slices from donors with recent diagnosis T1D and several islets were insulitic. The simultaneous Ca2+and T cell recordings of these insulitic islets in situ allowed for the relationship between T cell infiltration and β cell function to be assessed. HG Ca2+ responses in remaining β cells for donors with recently diagnosed T1D were significantly lower than ND donors ($p<0.01$). There were no differences for β cell Ca2+ responses to low glucose or KCl between ND, AAb+, and donors with T1D. These results are consistent with persistence of viable but dysfunctional β cells in T1D. Furthermore, while preliminary, results of the fixed tissue staining demonstrated that ATP5B expression was significantly lower in the donor with T1D ($p=0.0005$), suggesting loss of mitochondrial mass as a mechanism of altered glucose metabolism.

Conclusions

The novel contribution of this work involved assessment of β cell functionality along with endogenous immune cell activity in living human pancreas tissue during the various stages of T1D. Our data show dysfunctional β cell responses to high glucose and a preliminary indication that dysfunction may be occurring in the mitochondria. Current studies are attempting to correlate β cell function with presence of CD3+ T cells and to determine the metabolic markers that differentiate dysfunctional β cells during T1D development. The determination of this mechanism of β cell dysfunction will be critical for the understanding of T1D pathogenesis.

27: Paola Apaolaza (Helmholtz Center München)

Abstract Title

Insulin, proinsulin and PC1/3 expression heterogeneity and beta cell function in type 1 diabetes

Authors

Paola Apaolaza, Yi-Chun Chen, Yannik Lurz, Kavi Grewal, Bruce Verchere, Teresa Rodriguez-Calvo

Purpose

The detection of proinsulin, insulin, or C-peptide in blood may help predict the decay in beta cell mass and function during diabetes progression. Here, we aimed to provide an in-depth characterization at the beta cell level of insulin (INS), proinsulin

(PI) and the prohormone convertase PC1/3 (PC1) in non-diabetic (ND), autoantibody-positive (AAb+), type 1 diabetic (T1D) and type 2 diabetic (T2D) donors using confocal imaging and state-of-the-art image analysis. We also correlated beta cell phenotype and islet morphology during disease progression.

Methods

FFPE-Pancreatic sections from 20 age-matched ND, 7 Aab+ (4 single (s) and 3 double (d)), 8 short-duration T1D (<5y), 9 long-duration T1D (>15y), and 6 T2D donors were analyzed (mean age 33.5± 13.2 years). Confocal microscopy images from islets stained for INS, PI, and PC1 (up to 30 islets/donor) were analyzed using QuPath. First, islets were detected. Among the different cell types contained in the islets, we selected just beta cells (defined as cells expressing either INS, PI, or both). Next, beta cell populations were defined as follows: 1) INS+PI+PC1+ as triple+, 2) INS+PI+PC1-, INS+PI-PC1+, INS-PI+PC1+ as double+, and 3) INS+PI-PC1-, INS-PI+PC1- as single+ cells. The proportion of each cell population as well as islet area and beta cell density were calculated. The sum of triple+, double+, and single+ cell populations was used to determine total INS+, PI+, and PC1+ beta cells.

Summary of Results

The proportion of total beta cells (INS+ or PI+ and INS+PI+PC1+) was lower in most T1D donors compared to ND and the majority of AAb+ donors. Total PC1+ beta cells tended to decrease in dAAb+ and T1D donors. Although it did not reach statistical significance, the percentage of INS+PI+PC1- beta cells was higher in 2 out of 3 dAAb+ donors (39.2 ± 33.7%) compared to T1D (>15y) (1.2 ± 3.7%), T2D (3.6± 2.1%) and ND (9.6± 12%) donors. A higher number of INS+PI-PC1+ cells was observed in T1D (<5y) (9 ± 13.8%), T1D (>15y) (27.4± 25.1%), and T2D (2.8± 2.8%) donors compared to ND (0.9± 0.8%) donors. In contrast, it was lower in dAAb+ (0.4 ± 0.2%). No differences were observed in INS-PI+PC1+ cells among groups, with the exception of T1D (>15y) donors (32.7 ± 33% vs 2.1± 1.7% in ND). INS-PI+PC1- cells were more abundant in some dAAb+ donors while INS+PI-PC1- were more frequently found in T1D donors. General morphological assessment showed that, as expected, the number of beta cells per islet decreased significantly in T1D (>5y) and T1D (>15y) (60.5±49.31 cells and 1.2±1 cells respectively) compared to ND donors (140.8±47.2 cells). Furthermore, islet area, as well as beta cell density, were significantly lower in T1D compared to ND donors.

Conclusions

Beta cells undergo functional and morphological changes in T1D. We observe a lower number of certain beta cell phenotypes expressing INS, PI, or PC1 during T1D progression. These alterations are already present in some dAAb+, where we detect increases in INS+ and PI+ beta cell populations, either alone or as double+ cells, reflecting possible alterations in insulin synthesis. Subtle changes in distinct double+ beta cell subpopulations may represent different stages of beta cell dysfunction. These changes continued after onset of T1D but were strongly influenced by beta cell loss. In conclusion, we detected functional and morphological changes in beta cell subpopulations in some AAb+ and T1D individuals. These findings contribute to our understanding of diabetes pathogenesis and progression at a beta cell level (in a predominant adult population). This could help us to identify functional defects that occur early in the disease. In light of recent developments on immunotherapies aimed to stop the immune attack, our study highlights the need for combination therapies that are also directed to recover beta cell function.

28: Raghavendra Mirmira (The University of Chicago)

Abstract Title

Activation of the Integrated Stress Response in β Cells Governs a Signaling Cascade that Contributes to Suppression of PD-L1 Production

Authors

Charanya Muralidharan, Fei Huang, Jennifer Nelson, Sarah Tersey, Raghavendra Mirmira

Purpose

The cellular pathogenesis of T1D involves a dialog among β cells, acinar cells, and cells of the innate and adaptive immune systems. This dialog may be initiated by environmental factors that impose stress on β cells, resulting in the production of posttranslationally modified proteins, MHC I molecules, immune checkpoint regulators, and other proteins that serve to trigger or modulate autoimmunity. The integrated stress response (ISR) is an evolutionarily conserved, central signaling network that senses environmental stress through four kinases: PERK (ER stress), PKR (viral infections), GCN2 (nutrient deprivation), and HRI (heme deprivation). These kinases phosphorylate eIF2α, which suppresses global RNA translation to alleviate stress. However, when cellular stress is unmitigated, the ISR becomes maladaptive and promotes apoptosis. In this study, we asked how inhibiting the β cell ISR in preclinical models and human cells impacts the cellular dialog with the immune system and T1D outcomes.

Methods

We utilized isolated human islets from the Integrated Islet Distribution Program and the Alberta Diabetes Institute Islet Core, EndoC-βH1 human β-cell line, and NOD mice. Human islets and EndoC-βH1 cells were pre-treated with vehicle or inhibitors and exposed to proinflammatory cytokines (PIC; 50 IU/mL IL-1β and 1000 IU/mL IFNγ). Afterwards, they were analyzed by immunoblotting/staining and/or polyribosomal profiling to assess mRNA translation. Female NOD mice were treated with vehicle or novel inhibitor of PERK (HC-PERKi) during the prediabetic phase (6-10 weeks) and then followed for insulitis and diabetes incidence. Pancreas from NOD mice were analyzed by Nanostring® spatial proteomics, and islets from NOD mice were subjected to single cell RNA sequencing.

Summary of Results

Treating human islets and human EndoC-βH1 cells with PIC for 24 h activated the ISR, as revealed by increased phosphorylation of the translation initiation factor eIF2α. Consistent with this finding, PIC-treated human islets showed a global block in mRNA translation initiation by polyribosome profiling. This effect was partially reversed by pre-treating human islets with either ISRIB, an inhibitor of p-eIF2α, or the PERK inhibitor HC-PERKi, suggesting that inflammation represses translation via the ISR. To test if ISR suppression delays or prevents T1D onset, we treated female prediabetic NOD mice with HC-PERKi for four weeks. In NOD mice receiving HC-PERKi, diabetes incidence was substantially delayed (vs age- and sex-matched vehicle controls), and insulitis was significantly reduced during the prediabetic phase. Single cell RNA-sequencing analysis of islets from HC-PERKi-treated NOD mice revealed alterations in pathways handling amino acid metabolism, a finding consistent with the interference by HC-PERKi of p-eIF2α and ATF4 (the master regulator of amino acid metabolism). In HC-PERKi treated mice, Nanostring® spatial proteomics analysis of the pancreatic β cell area revealed a striking upregulation of PD-L1, an immune checkpoint regulator that suppresses adaptive T-cell immunity. Based on immunostaining, PD-L1 was present primarily on the surface of β cells that abut the insulitic lesions. The upregulation of PD-L1 levels is a posttranscriptional event, since levels of its mRNA (Cd274) were unaltered in β cells of mice treated with HC-PERKi and in EndoC-βH1 cells treated with ISRIB. Further studies in EndoC-βH1 cells showed that PD-L1 colocalizes with and is stabilized by GOLM1, a Golgi membrane protein that is acutely upregulated by proinflammatory cytokines.

Conclusions

Our studies depict a scenario where extracellular inflammation triggers the β-cell ISR, which suppresses general mRNA translation, enhances immunogenicity via reduced PD-L1 production, and increases T1D risk. Although the ISR is an adaptive response to help cells recover from environmental stress, our work emphasizes how this process may become maladaptive under chronic inflammatory stress. These results suggest that manipulation of the ISR through inhibitors of upstream kinases may be a therapeutic strategy in T1D.

29: Samantha Crawford (University of Colorado Anschutz)

Abstract Title

Non-enzymatic Formation of Isoaspartic Hybrid Insulin Peptides

Authors

Samantha Crawford, Mylinh Dang, Aaron Wiles, Katie Haskins, Thomas Delong

Purpose

The recent findings of hybrid insulin peptides in beta cells have opened a new scope of research in the field of type 1 diabetes (T1D). Our lab has discovered the mechanism of formation of distinct hybrid insulin peptides (HIP) that form non-enzymatically at aspartic acid residues in the beta cell. This mechanism not only forms a HIP with a standard peptide bond, but also an isoaspartic HIP (isoHIP) where a peptide bond is formed on the side chain of an aspartic acid residue. We hypothesize that a standard HIP and an isoHIP form non-enzymatically in the beta cell via a cyclic anhydride intermediate. Our aim was to identify the mechanism of formation of this HIP and isoHIP and verify the presence in a biological sample. Using our highly vigorous validation method, P-VIS, we were able to fully validate the presence of these non-enzymatic HIPs and isoHIPs in NOD islets. It is unknown now whether this HIP has immunological relevance, however if there is a T cell response to the non-enzymatically formed HIP or isoHIP, then this information will add to the understanding of T1D disease onset. Discovery of the mechanism behind non-enzymatic hybrid insulin peptide formation can lead to the discovery of other hybrid insulin peptides in T1D or hybrid peptides in other autoimmune diseases.

Methods

To identify the non-enzymatic HIP and isoHIP forming potential, we incubated several in vitro reactions and prepared the samples for mass spectrometry analysis. The presence of the HIP/isoHIP pair in NOD islets were identified by mass spectrometry and validated with P-VIS methodology. Several parameters of the proposed non-enzymatic mechanism of HIP formation were tested such as timecourse and pH optimum.

Summary of Results

Through mass spectrometry analysis of in vitro reactions and NOD islets, we have identified the non-enzymatic formation of a HIP and isoHIP through a cyclic anhydride intermediate. The presence of HIPs and isoHIPs have been validated in NOD islets and through in vitro reactions, these HIPs/isoHIPs have been shown to increase overtime at an optimal pH of 5.0.

Conclusions

HIPs and isoHIPs form at aspartic acid residues through a non-enzymatic mechanism.

30: Sandra Blom (University of Iowa)

Abstract Title

Proinflammatory cytokine driven remodeling of pancreatic β-cell Golgi morphology

Authors

Sandra Blom, Samuel Stephens

Purpose

Pancreatic islet inflammation (insulitis) plays a key role in the loss of β-cell mass during the development of autoimmune, Type 1 diabetes (T1D). In addition, defects in β-cell function are thought to arise early in disease progression, prior to the loss of β-cell mass, and may directly contribute to disease development. Proinflammatory cytokines, IL-1β, TNF-α, and IFN-γ, blunt

metabolic-exocytic coupling of insulin secretion and are recently suggested to impair proinsulin processing. The specific impact of cytokines on proinsulin trafficking and secretory organelle function is not well understood, yet may directly contribute to early defects in autoimmune-mediated β-cell dysfunction.

Methods

In our studies, we used primary human and mouse β-cells and an insulinoma cell culture model (832/3 INS-1 cells) to explore the effects of proinflammatory cytokines, IL-1β, TNF-α, IFN-γ on β-cell secretory organelle function. Proinsulin trafficking was examined using an in situ fluorescent pulse-chase reporter. Golgi structure was investigated via confocal microscopy. Golgi protein expression was examined by immunoblot and density sedimentation was used to further explore protein subcellular localization.

Summary of Results

Using primary β-cells and insulinoma cells, we demonstrate that proinflammatory cytokines elicit a striking alteration in Golgi structure, which coincides with diminished insulin exocytosis. In insulinoma cells, the Golgi ribbon structure is lost upon cytokine exposure and results in fragmentation of the Golgi into multiple individual ministacks. This change was accompanied by a relocalization of the cis-Golgi structural protein, GRASP55, into non-Golgi regions. In contrast, mouse β-cells retained overall Golgi structure, albeit with reduced Golgi volume; however, similar to insulinoma cells, re-distribution of GRASP55 into non-Golgi cellular regions was also prominent. The effects on Golgi structure and GRASP55 relocalization could be phenocopied using the nitric oxide (NO) chemical donor, dipropylenetriamineNONOate, in insulinoma cells, suggesting a direct role for NO in the remodeling of the β-cell's Golgi structure in response to cytokine exposure.

Conclusions

Proinflammatory cytokine exposure results in marked remodeling of the β-cell's Golgi ribbon morphology that coincides with relocalization of the cis-Golgi protein GRASP55, a major regulator of Golgi structure. Potentially, this alteration in Golgi structure could have substantial effects on the β-cell's secretory organelle functions leading to dysregulation of proinsulin and other secretory cargo trafficking. Further studies will be necessary to investigate the effects of GRASP55 relocalization on β-cell Golgi function and how these alterations contribute to early defects in the development of T1D.

31: Sandra Ferreira (University of Florida)

Abstract Title

GABA modulates glucose response and Ca2+ oscillations in pancreatic β-cells

Authors

Sandra Mara Ferreira, Austin E Stis, Edward A Phelps.

Purpose

Fine control of insulin release is crucial to maintain glycemic homeostasis and not to develop metabolic diseases like diabetes. Several hormones and neurotransmitters are involved in this modulation. GABA (gamma-aminobutyric acid) is a neurotransmitter produced and secreted by beta-cells that modulate insulin secretion. Our previous results from human islets indicate that GABA affects insulin secretion dependent on glucose concentrations, stimulating insulin release in low glucose and inhibiting in high glucose. These effects of GABA could be through GABAA Receptor Cl- channels activity that in low glucose contribute to beta-cell depolarization and in high glucose contribute to beta-cell hyperpolarization. Here, we evaluated the effect of GABA and GABAA Receptor agonists and antagonists on islets' Ca2+ oscillations in high glucose.

Methods

Conditional knockout mice lacking the GABA-synthesizing enzymes GAD65 and GAD67, specifically in pancreatic beta-cells (GADKO) and Ins1-Cre mice 30 days old, were evaluated for 12 weeks. Weight and glycemia were assessed weekly, and glucose tolerance tests were performed at 60 and 90 days old. Ca2+ oscillations were analyzed in isolated islets incubated in different glucose concentrations (5.6, 11, 16.7, and 25 mM) and transiently with GABA, GABAA Receptor agonist (Muscimol), and GABAA Receptor antagonists (SR95531 and Picrotoxin). Stimulus incubations were done twice for 5 min with a 10 min interval between them. We measured the [Ca2+]i using Calbryte 520, and images were processed and quantified in ImageJ.

Summary of Results

GADKO resulted in islets completely depleted of GABA as measured by HPLC yet GADKO mice have normal islet architecture and no diabetes. Male GADKO mice gained more weight than Ins1-Cre mice; in female mice, the weight gain was similar between the groups. Glycemia between male groups was not different. On the other hand, in female GADKO mice, the glycemia was lower than for Ins1-Cre. We did not observe a difference in glucose tolerance in both male and female mice at 60 days old compared with their controls. However, at 90 days old, male GADKO was less glucose tolerant, and female GADKO mice were more glucose tolerant. Upon stimulation with 11 mM glucose, GADKO islets respond more rapidly to glucose than Ins1-Cre. However, GADKO islets also presented a long delay in initiating Ca2+ oscillations. The first application of GABA or Muscimol transiently reduced slow Ca2+ oscillations in both Ins1-Cre and GADKO islets. A second application after a short recovery period had no effect. Surprisingly, both SR95531 and Picrotoxin also similarly reduced slow Ca2+ oscillations. Fast Ca2+ oscillations were increased after the agonist and antagonist's stimulations in both groups.

Conclusions

We conclude that GABA is an important modulator of response time to glucose and of both the slow and fast components of Ca2+ oscillations. In addition, loss of GABA biosynthesis, specifically in beta-cells, leads to a different effect on glucose metabolism in male and female mice.

32: Soumyadeep Sarkar (Pacific Northwest National Laboratory)

Abstract Title

Inflammation-induced repression of insulin production by targeting its mRNA to stress granules via ADP-ribosylation machinery

Authors

Soumyadeep Sarkar

Purpose

ADP-ribosylation is a common modification that occurs in proteins and RNAs, regulating many cellular processes from DNA repair to inflammatory signaling. ADP-ribosylation has been shown to play an important role in cancer biology, infectious diseases, and obesity, but its role in the development of type 1 diabetes (T1D) is not well understood. Especially ADP-ribosyltransferase PARP12, which has been associated with stress granule formation, a characteristic feature of Integrative stress response (ISR) and unfolded protein response (UPR) in T1D patients, and could play a role in exacerbating the disease

progression. Here, we investigate the role of PARP12 in the β cell inflammatory response and its role in stress granule-mediated translational halting.

Methods

We performed bioinformatics on RNAseq, and proteomic data, followed by qPCR to study the expression regulation of ADP-ribosyltransferases in islets (human islets) and β cells (EndoC-βH1 and MIN 6) treated with the various combinations of pro-inflammatory cytokines IL-1β, IFN-γ and TNFα and omega-3 fatty acids. To study the function of PARP12, we performed PARP12 depletion experiments (using RNAi) in MIN6 cells and then undertook proteomics analysis to determine its downstream regulatory network. Transcripts and proteins were imaged by fluorescence in situ hybridization and immunofluorescence, respectively.

Summary of Results

Bioinformatics analysis of transcriptomics and proteomics data from human and mouse islets and β cells revealed that ADP-ribosyltransferases PARP1, 9, 10, 12, and 14 are regulated by pro-inflammatory cytokines. Among these ADP-ribosyltransferases, we found that PARP12 was not only upregulated by pro-inflammatory cytokines but also by protective omega-3 fatty acids. Our proteomics analysis of cytokine-treated MIN6 cells revealed upregulation of several stress granule proteins in a PARP12-dependent manner, which potentially participates in the translational halting of several proteins, including insulin 2. As stress granules are formed to sequester mRNAs and block their translation during the integrated stress response, as seen in response to pro-inflammatory cytokines, we investigated whether PARP12 is involved in targeting insulin mRNAs to stress granules. As a result, knocking down PARP12 expression with RNAi showed the targeting of insulin transcripts to stress granules is dependent on this protein.

Conclusions

Our data show that several ADP-ribosyltransferases are up or down-regulated in islets or β cells in response to cytokine treatment. In particular, PARP12 targets insulin mRNA to stress granules to block its translation in response to cytokine treatment. These results have implications in the context of insulitis and T1D development, where sudden halting of insulin translation could overload the insulin biosynthesis process leading to increased stress and immune response. Also, PARP12 being part of innate immunity, this pathway may play a role in T1D progression due to Coxsackievirus infections. Overall, this brings new insights into disease development and opens new opportunities for therapies targeting the improvement of insulin production.

33: Staci Weaver (Indiana University)

Abstract Title

Loss of SERCA2 and reduced endoplasmic reticulum calcium induces mitochondrial dysfunction, β-cell senescence, and accelerated type 1 diabetes development

Authors

Staci A. Weaver, Robert N Bone, Dominic J. Acri, Tatsuyoshi Kono, Farooq Syed, Carmella Evans-Molina

Purpose

Type 1 diabetes (T1D) results from immune-mediated destruction of pancreatic β-cells. Alterations in β-cell endoplasmic reticulum (ER) calcium (Ca2+) homeostasis leads to ER stress, diminished insulin secretion, increased β-cell death, and ER stress which has been linked with increased β-cell immunogenicity. Under normal conditions, high Ca2+ concentrations within the β-cell ER are maintained by ER Ca2+ uptake via the sarco-endoplasmic reticulum Ca2+ ATPase (SERCA2) pump. SERCA2 expression is reduced in islets from organ donors with T1D. Interestingly, humans with Darier White Disease, an autosomal dominant condition characterized by mutation(s) in the Atp2A2 gene encoding SERCA2, exhibit an increased incidence of T1D, suggesting that genetic loss of SERCA2 may pattern interactions between the immune system and the β-cell. We hypothesized that altered ER Ca2+ homeostasis via reduced SERCA2 expression may drive the development of β-cell dysfunction in T1D, and we aimed to test whether this phenotype was associated with increased ER stress, reduced mitochondrial function, or β-cell senescence.

Methods

We generated SERCA2 haploinsufficient mice on the non-obese diabetic (NOD) background (NOD-S2+/- mice). Diabetes incidence, insulitis, body weight was assessed, and the expression of ER stress markers in pancreatic islets was analyzed via RT-qPCR and immunoblot. Single cell RNA sequencing of isolated islets was performed, mitochondrial structure/function/content was investigated via electron microscopy, and oxygen consumption/mitochondrial membrane potential assays were performed in NOD-S2+/- and NOD-WT littermates prior to diabetes development. Additionally, islet ER and mitochondrial Ca2+ levels and oscillation patterns in response to stimuli were measured using ratiometric organelle targeted FRET biosensors. Lastly, markers of senescence and cytokine secretion were measured in isolated islets between ages 6-12 wks via RT-qPCR and multiplex ELISA.

Summary of Results

Female NOD-S2+/- mice displayed accelerated T1D onset compared to NOD-WT mice (median age of onset 14 wks vs 18 wks; $p \leq 0.0001$). While ER stress markers (Chop, BiP, s/uXBP1, IRE1α, and ATF6) were not different between genotypes at 6 or 10 wks of age, prediabetic NOD-S2+/- islets show decreased glucose-stimulated O2 consumption and reduced ATP production as well as reduced mitochondrial membrane potential. Immunoblots showed decreased expression of ATP synthase and Complex III in NOD-S2+/- islets; however, mitochondrial content and gene expression of mitochondrial fission or fusion markers were not different. ER Ca2+ levels were significantly reduced, and oscillation patterns were eradicated in NOD-S2+/- islets compared to NOD-WT islets. Interestingly, basal mitochondrial Ca2+ levels were significantly elevated in NOD-S2+/- islets compared to NOD-WT, while cytoplasmic Ca2+ levels remained unchanged between genotypes. Markers of senescence (tp53, cdkn2b, cdkn2d, cdkn1a, cdkn2a) were increased in NOD-S2+/- islets between 6 and 12 wks of age compared to NOD-WT islets. Because senescence is associated with immune activation via senescence associated secretory phenotype (SASP), Cell Chat analysis was conducted on islet scRNA sequencing data to assay communication between cell clusters. This analysis revealed increased cytokine signaling (Inf, Tnf, and Il-6) and increased senescence associated signaling (Galectin, Psap, and Cdh1) in NOD-S2+/- islets. Next, we assayed serum and conditioned media from 10 wk old mice and isolated islets via multiplex ELISA and found that IFN-γ and IL-6 were increased in NOD-S2+/- mouse serum; however, no significant changes were observed in the islet conditioned media.

Conclusions

Taken together, our results suggest that reduced ER Ca2+ is associated with mitochondrial dysfunction, cell senescence, and increased systemic levels of cytokines in the NOD-S2+/- mouse model. Future studies will test whether SERCA activation in the presence of senolytics can further delay diabetes onset and rescue mitochondrial health in models of T1D and SERCA2 deficiency.

34: Yi-Chun Chen (University of British Columbia)

Abstract Title

Deletion of carboxypeptidase E in beta cells disrupts proinsulin processing and alters beta cell identity in mice

Authors

Yi-Chun Chen, Austin Taylor, James Fulcher, Adam Swensen, Xiao-Qing Dai, Mitsushiro Komba, Kenzie Wrightson, Kenny Fok, Annette Patterson, Ramon Klein-Geltink, Patrick MacDonald, Wei-Jun Qian, Bruce Verchere

Purpose

Carboxypeptidase E (CPE) facilitates the conversion of prohormones into mature hormones, and is highly expressed in multiple neuroendocrine tissues. Carriers of CPE mutations have elevated plasma proinsulin and develop severe obesity and hyperglycemia. We aimed to determine whether loss of Cpe in pancreatic beta cells disrupts proinsulin processing and accelerates development of diabetes and obesity in mice.

Methods

We generated pancreatic beta cell-specific Cpe knockout (βCpeKO; Cpefl/fl x Ins1Cre/+) mice, and assessed their islet prohormone processing capacity using immunoassays, prohormone convertase enzyme activity assays, western blotting, and a mass spectrometry-based top-down proteomic assay. We challenged βCpeKO and Wt littermate mice with high fat diet, or multiple low does streptozotocin, and analyzed islet function and the development of hyperglycemia. We also performed transcriptomic and mitochondria morphological analyses, and evaluated beta cell proliferation rate, in islets from βCpeKO and Wt mice.

Summary of Results

βCpeKO mice lack mature insulin granules and have elevated proinsulin in plasma; however, glucose-and KCl-stimulated insulin secretion in βCpeKO islets remained intact. High fat diet-fed βCpeKO mice showed comparable weight gain and glucose tolerance compared to Wt littermates. Notably, beta-cell area was increased in chow-fed βCpeKO mice and beta-cell replication was elevated in βCpeKO islets. Transcriptomic analysis of βCpeKO beta cells revealed elevated glycolysis and Hif1α-target gene expression. Upon high glucose challenge, beta cells from βCpeKO mice showed reduced mitochondrial membrane potential, increased reactive oxygen species, reduced MafA, and elevated Aldh1a3 transcript levels. After multiple low-dose streptozotocin treatments, βCpeKO mice had accelerated hyperglycemia with reduced beta-cell insulin and Glut2 expression.

Conclusions

Our findings suggest that Cpe and proper proinsulin processing are critical in maintaining beta cell function during the development of diabetes.

35: Yue Wang (Florida State University)

Abstract Title

Sex Differences in the Molecular Programs of Pancreatic Cells Contribute to the Sex Differential Risks of Type 2 Diabetes

Authors

Hyo Jeong, Maria Pilar Toledo, Richard Nowakowski, Yue Wang

Purpose

Epidemiology studies demonstrate that females are at a significantly lower risk to develop type 2 diabetes (T2D) compared to males. However, the molecular basis of this risk difference is not well understood. In this study, we examined the sex differences in the genetic programs of pancreatic endocrine cells.

Methods

We explored open-source pancreas perifusion data and single-cell genomic data to investigate multiple axes of the sex differences in the human pancreas at the single-cell type and single-cell level. We systematically compared female and male islet secretion function, gene expression program, and epigenetic regulatory principles of pancreatic endocrine cells

Summary of Results

The perifusion data indicate that female endocrine cells have a higher secretion capacity than males. Single-cell RNA-seq analysis suggests that endocrine cells in male controls have molecular signatures that resemble T2D. Single-cell ATAC-seq data reveals that some genomic regions are differentially accessible between female and male endocrine cells. These genomic elements may play a sex-specific causal role in the pathogenesis of T2D.

Conclusions

Here, we provide molecular mechanisms that explain the differential risk of T2D between females and males. Knowledge gained from our study will accelerate the development of diagnostics and therapeutics in sex-aware precision medicine for diabetes.

IMMUNOLOGY

36: Allison Bayer (University of Miami Miller School of Medicine)

Abstract Title

Combinational Immune Therapies with Antigen-Specific Tregs for Tolerance Induction in Islet Transplantation

Authors

Cecilia Cabello, Natalia Arenas, Camilo Jaramillo, George Burke, Allison Bayer

Purpose

Immune based therapies for T1D need to focus on restoring immune tolerance which would avoid the use of chronic immunosuppression. Our previous work with T regulatory cells highlights the critical factors that need to be considered to improve the outcomes of Treg-based therapies for the clinical setting. Moreover, we proved that in a preclinical, immune replete T1D model that a Treg-based combinational immunomodulatory regimen can successfully reverse autoimmune diabetes without compromising the remainder of the immune system and without the need for chronic immunosuppression. The aim of the present study is to advance Treg-based therapies for the treatment of islet autoimmunity by inhibiting autoimmunity and inducing transplantation tolerance, which would alleviate the need for chronic immunosuppression making islet transplantation a real biological cure for T1D.

Methods

Young, female NOD mice (5-7 weeks of age) were treated with combinational regimens that consists of 5 day-course anti-CD3 (clone 2C11), single injection of cyclophosphamide (CyP) and followed by Treg infusion of allogeneic C57BL/6 Tregs (AlloB6-Treg) and short-course of IL-2 complex (IL-2/clone JES6-1). Mice were given rapamycin (0.2mg/kg) for 4-week

starting at the time of Treg infusion. Treg engraftment was examined in the circulation, spleen, lymph nodes, and isolated pancreatic islets by flow cytometry. In an allogeneic skin transplant model, young, female NOD mice received combination therapies with anti-CD3, CyP, AlloB6-Treg infusion and with or without rapamycin. Anti-CD3 was started 1-day prior to placement of 3 skin grafts (NOD, donor specific B6, and 3rd party C3H) on the backs of NOD mice. Rapamycin was started at the time of transplant and continued for 38 days. Skin rejection was monitored for 180 days. In the allogeneic islet transplant model, recent onset NOD mice were treated with anti-CD3, CyP, Treg infusion and with or without rapamycin. Treg inoculums consisted of cells purified from allogeneic B6 or BDC2.5 TCR transgenic NOD mice or in combination. Mice were transplanted with B6 islets (750 IEQ) under the kidney capsule. Anti-CD3 was started 1-day prior to islet transplant and rapamycin started at the time of transplant. Islet rejection was monitored for up to 180 days.

Summary of Results

Robust engraftment of alloB6-Treg was observed in young, prediabetic NOD mice following our combinational regimen of anti-CD3, CyP and IL-2 complex, but stable engraftment required the addition of a short-course of rapamycin. AlloB6-Tregs could be found in the circulation and throughout immune compartments and their engraftment was comparable to levels observed with equal numbers of infused polyclonal, syngeneic Tregs Therapeutic efficacy was observed in an allogenic B6 skin transplantation model in NOD mice with our combinational regimens. Tolerance to fully allogeneic B6 skin was observed ($p<0.0001$, Mantel-Cox, Log-rank) but required more rigorous immunomodulation compared to diabetes reversal. In this transplant setting, efficacy involved the addition of CyP to anti-CD3 prior to infusion of MHC-matched AlloB6Tregs, in which 67% of recipients were tolerant to B6 skin allografts during the 6-month follow-up. A short-course of rapamycin improved outcomes with 88% tolerant recipients. Importantly, these allografts were accepted long-term and without the need for chronic immunosuppression (Mantel-Cox, Log-rank, $P<0.001$). The antigen specificity of infused Tregs was critical as tolerance was not achieved to 3rd party C3H skin allografts transplanted. In the islet allograft transplant model, in which tolerance induction to allogeneic islet transplant is examined with underlying autoimmunity and more closely reflects the clinical setting, anti-CD3 and CyP treatment prior to infusion with AlloB6-Tregs resulted in significant allograft prolongation with median survival of 90-day with rapamycin compared to 57.5-days without rapamycin and 13-days in controls. Infusion of BDC2.5 Tregs following immunomodulation with anti-CD3 and CyP leads to allograft prolongation with median survival 77-days and 55-days with and without rapamycin, respectively. Importantly, long-term tolerance was achieved in 25-33% of mice that received a combination of BDC2.5 and AlloB6 Tregs mice following anti-CD3 and CyP.

Conclusions

This data confirms our previous work in diabetes reversal studies that combination regimens that create immunological space and lessen competition with host-Tregs facilitates significant Treg engraftment and that the antigen specificity of Treg inoculums is critical to induce tolerance without the need for chronic immunosuppression. These studies lay the foundation to investigate the mechanisms contributing to tolerance for the successful treatment of autoimmune diabetes through islet transplantation.

37: Camilla Tondello (Goethe University Frankfurt, Germany)

Abstract Submitted by

Urs Christen

Abstract Title

The XCL1-XCR1 chemokine axis as novel target for T1D therapy: XCL1 or XCR1-deficient mice display reduced islet infiltration by dendritic cells and T cells and are protected from T1D

Authors

Camilla Tondello, Christine Bender, Giulia Buchmann, Monika Bayer, Richard Kroczek, Wolfgang Katenmuller, Ralph Brandes, Edith Hintermann, Urs Christen

Purpose

Data obtained from gene arrays of mRNA isolated from laser-dissected islets of diabetic RIP-LCMV-GP mice revealed a considerable upregulation of the chemokine XCL1 and its receptor XCR1 during the chronic phase of T1D. XCR1 is uniquely expressed on DC with high T cell activation properties (cDC1), whereas XCL1 is mainly produced by activated T cells. It was the goal of this study to evaluate the role of the XCL1-XCR1 chemokine axes in T1D.

Methods

The RIP-LCMV-GP mouse model, in which T1D is induced by LCMV-infection of mice expressing the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) specifically in the β-cells of the islet of Langerhans, was used for this project. The expression of XCL1 and XCR1 at several times after T1D induction was confirmed with RT-PCR and RNAscope. To evaluate the role of the XCL1-XCR1 axis we crossed the RIP-LCMV-GP mice with either XCL1 or XCR1-deficient mice. T1D incidence of double transgenic and regular RIP-LCMV-GP littermates was evaluated by measuring the blood glucose levels at several times after infection. Functional β-cell content was assessed in 3D by light sheet fluorescence microscopy (LSFM) of the entire pancreas stained with a fluorescent an anti-insulin antibody. Insulitis and nature of infiltrating cells was evaluated by immunohistochemistry of pancreas sections. The frequencies of cDC1 and antigen-specific T cells were determined in spleen, pancreatic lymph node, and pancreatic islets by flow cytometry.

Summary of Results

XCL1 and XCR1 are not expressed in the islets of naïve mice. However, they both appear at day 7 after T1D initiation and stay chronically expressed up to at least day 28 post-infection. RIP-LCMV-GP x XCL1 KO mice as well as RIP-LCMV-GP x XCR1 KO mice show a massively reduced T1D incidence compared to regular RIP-LCMV-GP mice. Mechanistically, a lack of XCL1 results in a reduced attraction of cDC1 to the pancreas, which in turn causes an insufficient activation of aggressive antigen-specific T cells and a shift in the local T cell balance towards Treg.

Conclusions

The XCL1-XCR1 plays an important role in the development of T1D in the RIP-LCMV-GP model. Disruption of the axis results in a reduced attraction of cDC1 to the islet of Langerhans and a diminished T cell activation. Subsequently, the number of activated antigen-specific T cells seem to be insufficient to further perpetuate T1D pathogenesis. It will be important to confirm these data in other model systems, such as the NOD mouse, and in human pancreas samples. Finally, these data should form an encouragement for the generation of specific XCL1-XCR1 inhibitors.

38: Catherine Nicholas (University of Colorado)

Abstract Title

Molecular phenotypes measured by single cell sequencing in diabetogenic B cells

Authors

Catherine Nicholas, Mia Smith

Purpose

Pancreatic islet-reactive B cells are thought to contribute to Type 1 Diabetes (T1D) disease progression through antigen presentation to cognate T cells, as well as cytokine and autoantibody production. However, given the low frequency of islet-reactive B cells in the peripheral blood and tissues, it makes it difficult to study these rare cells among total B cells. In order to better understand the function and phenotype of diabetogenic B cells in T1D, we aim to enrich for pancreatic islet-reactive B cells from blood and tissue using magnetic beads in early onset (<100 days post diagnosis) T1D donors and controls and

sequence these cells to reveal their specific protein and mRNA gene expression phenotypes, as well as BCR clonotypes, in order to better understand the contribution of autoreactive B cells to the pathogenesis of T1D.

Methods

Recent developments in single cell RNA sequencing methods have enabled simultaneous collection of gene expression, B and T cell receptor (BCR/TCR) V(D)J clonotype, protein expression (CITE-seq), and functional data from single cells. Using biotinylated pancreatic islet antigens and adding them to PE and DNA barcoded streptavidin reagents (LIBRA-seq), we enrich for islet-reactive B cells of varying specificities (e.g. insulin, GAD65, and IA-2) from the blood of T1D and control donors to further understand the phenotype and function of these pathogenic B cells in T1D development.

Summary of Results

Our preliminary results indicate that insulin-binding B cells (IBCs) are enriched in new-onset T1D blood compared to healthy controls. Differential gene expression shows that T1D IBCs are more activated and express markers consistent with antigen-presenting cells compared to healthy control IBCs. Subpopulations of B cells enriched in autoreactivity have been identified and their V(D)J BCR sequences are enriched for characteristic use of autoreactive gene segments.

Conclusions

We have developed a method to deeply phenotype islet-antigen reactive B cells from blood and tissue from T1D and control donors using single-cell RNA sequencing. As more subjects are analyzed, future studies aim to determine the differences between various islet-reactive B cells (e.g. insulin, IA-2, GAD65) among T1D donors, differences between T1D and control donors, differences between blood and tissue (e.g. pLN and pancreas), as well determine whether a public BCR clonotype exists in T1D donors. The cumulative results from these studies will help inform the role of B cells in development of T1D and may provide insight for therapeutic targeting of pathogenic autoreactive B cells in T1D treatment strategies.

39: Eddie James (Benaroya Research Institute)

Abstract Title

Recognition of nucleus-associated autoantigens by CD4+ T Cells and autoantibodies in subjects with type 1 diabetes

Authors

Perrin Guyer, Marija Lugar, Kapitolina Seminova, Cate Speake, Ezio Bonifacio, Eddie James

Purpose

During the development type 1 diabetes (T1D), pancreatic islets are infiltrated by immune cells, leading to the generation of autoantibodies that serve as the best predictive markers of the disease. Autoantibody formation is thought to be preceded and promoted by autoreactive CD4+ T cells. A substantial body of work has focused on recognition of “primary” islet antigens such as proinsulin, GAD65, and IA-2. However, recent studies indicate that responses that target less conventional self-antigens are relevant to disease. For example, a broad array-based screening process identified antibodies that recognize nucleus-associated proteins in patients with T1D. A follow-up study confirmed an enrichment of antibodies against MutL homolog (MLH1) in DR4 positive subjects with T1D and against Nucleoporin 50 (NUP50) in DR3 positive subjects with T1D. Therefore, we sought to investigate linked T cell and autoantibody recognition of these antigens.

Methods

To investigate T cell responses against MLH1 and NUP50, we utilized a systematic epitope discovery process. First, we utilized a published algorithm to predict peptide sequences derived from MLH1 containing motifs likely to bind to

DRB1*04:01 (DR0401) and sequences derived from NUP50 containing motifs likely to bind to DRB1*03:01 (DR0301). We synthesized the corresponding peptides and performed competition assays to measure their binding to recombinant DR0401 or DR0301 protein. For peptides with detectable binding, we assessed their immunogenicity by stimulating PBMC from subjects with T1D in vitro and visualizing expanded peptide-specific T cells using HLA class II tetramers. Apparent positive results were further verified through T cell cloning. To investigate linked antibody and T cell recognition of MLH1 and NUP50, antibody responses against both antigens were measured in a total of 74 serum samples from HLA typed subjects with T1D using a previously published ELISA assay. For a subset of these subjects archived PBMC samples were available. These were stained with HLA class II tetramers to directly enumerate MLH1 or NUP50 reactive CD4+ T cells and characterize their cell surface phenotype.

Summary of Results

Our epitope discovery process revealed five DR0401-restricted MLH1 epitopes and three DR0301-restricted NUP50 epitopes that were readily detectable in subjects with T1D. A wide range of serum autoantibody levels was observed (reaching >3000 units for MLH1 and >550 units for NUP50). In agreement with prior studies, NUP50 titers were significantly higher in DR3 positive subjects and MLH1 titers trended higher in DR4 positive subjects. For both antigens, HLA class II tetramer staining revealed that T Cell frequencies were significantly higher in subjects with T1D than HLA matched controls. Observed cell surface phenotypes indicated a wide variety of cell states, including Th1-like, Th2-like, and Th17-like lineages. Notably, whereas MLH1 antibody levels were positively correlated with T cell frequencies, NUP50 antibody levels were inversely correlated T cell frequencies.

Conclusions

The observation that T cell and antibody responses in subjects with T1D can target nucleus-associated self-antigens confirms and extends previously published studies. Disease-associated recognition of a class of proteins that are not exclusively expressed in pancreatic islets suggests that a systemic autoimmune component to the disease process may be present in certain subsets of patients. The finding that antibody and T cell levels can exhibit either a direct or inverse relationship suggests a complex relationship between these two facets of autoreactive immune responses. The finding that both expected and unconventional specificities may play a role during various phases of T1D development highlights the importance of continued epitope discovery efforts and has specific implications for tolerance induction strategies.

40: Francesco Vendrame (University of Miami Miller School of Medicine)

Abstract Title

Impact of dimethyl fumarate on immunometabolism in NOD mice

Authors

Allison Bayer, Alejandro Bianco, Sahana Ramasamy, Cecilia Cabello, Natalia Arenas, Flavia Pecanha, Carmen Fotino, George Burke, Francesco Vendrame

Purpose

Immunotherapies for type 1 diabetes (T1D) so far have failed to achieve long-lasting effects on islet autoimmunity, so that prevention and reversal of T1D remain an unmet goal. Dimethyl fumarate (DMF) is an FDA-approved treatment for relapsing remitting multiple sclerosis (RRMS). Data in both animal models of RRMS and human subjects have shown that DMF targets the innate and adaptive immune responses through mechanisms which includes cell metabolism, therefore support testing DMF in autoimmune diabetes. The aim of the present study is to assess the effect of DMF in diabetes prevention and reversal in NOD mice.

Methods

Female NOD mice 10-week-old were treated with DMF (100 mg/Kg) or vehicle (0.6% methylcellulose) daily by oral gavage for 12-weeks. Mice were then followed for additional 24-weeks and monitored for diabetes development. At 8 and 12 weeks of treatment pancreata were retrieved from some mice for histology studies to assess insulitis and beta-cell mass. A separate cohort of NOD mice was treated with heptelidic acid (1 mg/Kg, intraperitoneal; PBS for control mice) a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inhibitor representing one of the major targets of DMF pathway for 12 weeks. At sacrifice, spleens were retrieved for GAPDH activity assay (Sigma). IPGTT was performed in both cohorts of mice. In-vitro studies were performed on splenocytes isolated from 10-week-old NOD mice or NOD mice treated with DMF or vehicle for 4 weeks. Splenocytes were stimulated in culture with anti-CD3 (clone 2C11) for 5h in the presence or absence of DMF (100µM) and 2-NBDG glucose analog (30µM, Life Technologies) added during the last 2 hours. CD69 expression and glucose uptake were analyzed by flow cytometry in CD4 and CD8 T cells. Experiments with DMF were replicated in reversal studies performed in NOD female mice with recent diabetes onset followed for 12-weeks after the end of treatment. Diabetes reversal was defined as the absence of glycosuria and a return to normal blood glucose concentrations. Mice in this group were also implanted with a subcutaneous insulin pellet lasting 3-4 weeks to mimic studies in humans. Statistics for diabetes-free survival were done using the log-rank Mantel-Cox and Hazard Ratio tests. In-vitro studies were analyzed using One- or Two-Way ANOVA corrected for Tukey multiple comparisons, $p<0.05$ was considered statistically significant. Beta-cell mass was assessed by Mann-Whitney, one-tailed test, CI 90%, $p<0.10$ was considered statistically significant.

Summary of Results

In the prevention study, none of the DMF-treated mice (n=28) developed diabetes after 12-weeks of treatment compared to 41% of control mice (n=26) ($p<0.0001$). During the 24-weeks of follow-up after the secession of treatment the median survival of DMF treated mice was 45-weeks versus 20-weeks of the vehicle treated mice. DMF treated mice at any time during study period were 85% less likely to develop diabetes compared to the control group (Hazard Ratio, logrank: 0.1480). Histology studies in pancreata obtained from 8 weeks treated mice revealed less aggressive insulitis in DMF treated mice compared to control mice. We observed preservation of beta-cell mass in DMF treated mice compared to vehicle treated mice at 12-weeks ($p=0.0919$). Treatment with heptelidic acid resulted in similar outcomes to the DMF study in diabetes prevention during the treatment period (Hazard Ratio, logrank: 0.1062) and demonstrated a reduction in GAPDH activity in spleen lysates. These heptelidic acid studies confirmed the importance of the GAPDH pathway targeted by DMF treatment in autoimmune diabetes. In-vitro studies on splenocytes from 10-week-old NOD mice, showed that DMF treatment inhibited CD69 expression on CD4 and CD8 T cell by 64% and 78%, respectively ($P<0.0001$). Similar reduction in glucose uptake was observed in unstimulated and stimulated CD4 with DMF treatment ($p<0.001$). In the CD8 population, significant reduction in glucose uptake was observed in stimulated CD8 T cells compared to unstimulated ($p=0.0032$). However, the addition of DMF to stimulated CD8 T cells did not result in further reduction of glucose uptake with 5h of stimulation. DMF treatment in vitro (100 µM) of stimulated splenocytes from 4 weeks treated mice significantly inhibited glucose uptake in CD4 T cells by 41.1% in Vehicle-treated ($p<0.0001$) and by 55.8% in DMF-treated mice ($p=0.0036$). Moreover, in-vitro DMF treatment significantly inhibited the upregulation of CD69 in CD4 T-cells by 32.5% in vehicle-treated mice ($p=0.0179$) and by 69.7% in the DMF-treated mice ($p= 0.0001$). Direct comparison between DMF and vehicle treated mice showed significantly more inhibition of CD69 upregulation ($P=0.0021$, Two-Way ANOVA). A similar trend was observed in CD8 T-cells. The administration of 2-NBDG glucose analog in-vivo showed that 4-weeks DMF treatment altered glucose uptake in both innate and adaptive immune cells. Such results were replicated in a diabetes reversal study showing that 12-week treatment with DMF resulted in 50% diabetes reversal (median survival 105 days) compared to control mice (median survival 30 days). This effect was sustained over time during the 12-week follow-up after cession of DMF treatment (Hazard Ratio, logrank: 0.1787).

Conclusions

Our preliminary data strongly suggests that DMF is a promising drug for the treatment of islet autoimmunity. Mechanistic studies point to a role of immune metabolism, which could impact functional immune subsets and in turn alter the immune environment and temper islet autoimmunity.

41: Gregory Golden (University of Pennsylvania)

Abstract Title

Immunological Atlas of Pancreatic Lymph Nodes in Type 1 Diabetes

Authors

Gregory Golden, Vincent Wu, Alberto Sada Japp, Jacob Hamilton, Chengyang Liu, Leticia Kuri-Cervantes, Jayme Nordin, Maria Betina Pampena, Jay Gardner, HPAP Consortium, Ali Naji, Michael Betts

Purpose

Type 1 diabetes (T1D) is caused by the autoimmune destruction of insulin-producing pancreatic beta cells, leading to life-long dependence on exogenous insulin. Profiling immune cells that infiltrate islets would be invaluable to understanding how beta cell destruction occurs. However, human pancreatic samples demonstrating active infiltration and beta cell destruction are rare. Alternatively, peri-pancreatic lymph nodes (pLNs) or other secondary lymphoid organs may harbor immune cells which participate in memory responses that drive T1D autoimmunity.

Methods

To study the immune response throughout T1D onset and disease, pLNs, non-pancreatic mesenteric LNs (mesLNs), spleen, and peripheral blood mononuclear cells (PBMCs) were collected from human T1D, auto-antibody positive (AAb+), and normal donors (NDs) enrolled in the Human Pancreas Analysis Program (HPAP). Tissue immune cell composition was analyzed using high-parameter flow cytometry that allows for identification of more than 24 cell populations. For a deeper analysis of immune populations, cellular indexing of transcriptomes and epitopes by sequencing (CITEseq) was run on pLN from 17 donors across ND, AAb+, and T1D disease states. In addition, 15 of those donors had adjoining spleen samples, of which 9 donors had mesLNs, run through the CITEseq pipeline, allowing for cross-tissue and cross-disease state comparisons between immune populations.

Summary of Results

High parameter flow cytometry analysis shows that relative percentages of major immune cell populations did not strongly differ between disease states. However, immune cell sub-populations had altered activation and differentiation markers between ND, AAb+, and T1D within the pLNs. Within the pLN of AAb+ and T1D donors, there is an increase in CD8+ effector memory T cell (Tem) frequency and a reciprocal drop in naïve T cell frequency. Further, CD4+ central memory (Tcm) frequency was increased in the pLN of T1D donors. These shifts suggest that pLN T cells are more likely to have encountered cognate antigen or are in an immunostimulatory environment that promotes memory T cell maintenance or expansion. Expression of CD69, a marker of recent activation or tissue residency, is increased in Tem and CD45RA+ Tem (Temra) CD8+ T cell populations in the pLN of AAb+ donors. Further, CD8+ Tem and Temra in T1D pLN have decreased expression of CD27 and CD127, indicating these populations are more differentiated and have lower proliferative capacity. These findings suggest that memory T cells in pLN of AAb+ and T1D+ organ donors may be expanded, have increased residency, and may be in a more differentiated state compared to normal donor controls. Other immune populations in the pLN and other secondary lymphoid tissues showed significant phenotypic changes. Both pLN and mesLNs from AAb+ and T1D donors exhibited increased frequencies of CD27+ memory B cells. Further, pLN and mesLN from AAb+ and T1D donors had decreased frequency of regulatory NK cells and reciprocal increased cytotoxic NK cell frequency. A deeper analysis of pLN, mesLNs, and spleen using CITEseq resulted in 650,000 cells comprising T, B, and NK cells, in addition to other immune populations. Further analysis will focus on immune modulations across disease states that are otherwise undetectable by flow cytometry.

Conclusions

Findings from high-parameter flow cytometry indicate that immune cell phenotypes in pLNs and other lymphoid tissues reflect a more activated or immunological aged state during T1D and T1D development. Interestingly, some immunophenotypic alterations appear to be tissue restricted, implying that inflammatory signals or immune cell trafficking differ not only by disease state but by proximity to the pancreas. Further, AAb+ individuals, including single AAb+ donors, had immune perturbations in the pLN and other LNs, demonstrating that immune responses in these tissues occur well before T1D diagnosis. Large scale CITEseq provides a unique opportunity for a fine dissection of immune perturbations found in pLN and other tissues. This initial characterization of the pLNs and other lymphoid tissues in ND, AAb+, and T1D donors gives an in-depth glimpse into the autoimmune response during T1D and T1D development.

42: Jamie Felton (Indiana University School of Medicine)

Abstract Title

Type 2 diabetes-associated TCF7L2 genetic variants and African American race protect from loss of insulin containing islets in type 1 diabetes

Authors

Holly Conway, Mugtaba Swar-Eldahab, Jamie Felton

Purpose

Abnormalities in B cell tolerance checkpoints have been identified in individuals with type 1 diabetes (T1D), resulting in expansion of autoreactive B cells and the development of autoantibodies to islet antigens in the blood. However, clinical trials using the B-cell depleting agent rituximab in individuals with new onset T1D only transiently preserved insulin secretion, which suggests that B cell fate and function in T1D progression remain incompletely understood. Cellular metabolism is a key modulator of immune cell function. Changes in tissue microenvironments prompt alterations in bioenergetic pathways, such as glycolysis and oxidative phosphorylation, which yield metabolites and biosynthetic intermediates that can affect cell signaling, regulate gene expression, and polarize cell development toward pro or anti-inflammatory phenotypes. Hypoxia-inducible factors (HIFs) are key mediators of these alterations and have therefore emerged as important modulators of immunity. Recently, regulation of HIF-1α was shown to be critical to both normal B cell development, as well as the expansion of protective, regulatory B cell subsets in animal models of autoimmune disease. In the non-obese diabetic (NOD) mouse, the development of pathogenic, anti-insulin B cells has been shown to be preferentially skewed toward late transitional and marginal zone compartments, which are uniquely sensitive to environmental and homeostatic signals, suggesting their potential to be modulated by microenvironmental factors to either promote or protect against autoimmune disease. However, the metabolic state of B cells in T1D throughout disease progression, which is marked by progressive islet infiltration and systemic hyperglycemia, remains largely unknown.

Methods

To assess B cell metabolic phenotypes throughout T1D disease progression, we used flow cytometric detection of fluorescence to assess glucose uptake, mitochondrial mass, and mitochondrial polarity in the spleen, pancreatic lymph nodes, islets in NOD mice early in disease progression, immediately prior to diagnosis, and after diabetes onset. In addition, we compared metabolic phenotypes in splenic B cells ex vivo and after anti-CD40 activation in NOD and non-autoimmune prone C57BL/6J (B6) mice. To evaluate roles for HIF-1α in T1D, immunofluorescence and flow cytometry were used to assess HIF-1α expression in the spleen, PLNs, and islets of NOD and B6 mice. RT-PCR was used to determine how HIF-1α expression changes after activation with B cell mitogens in NOD and B6 splenic B cells. To assess hypoxia-induced changes in cellular metabolism in B cells from NOD and B6 mice, glucose uptake, mitochondrial mass, and mitochondrial membrane potential were assessed before and after treatment with hypoxia-mimic cobalt chloride (CoCl2).

Summary of Results

Glucose uptake was lowest in B cells, compared to CD4+ and CD8+ T subsets throughout disease progression in the spleen, PLNs, and pancreas in NOD mice. Conversely, mitochondrial polarity (normalized to mitochondrial mass) was highest in B cells in young and pre-diabetic NOD mice. Over time, both glucose uptake and mitochondrial polarity were highest immediately prior to disease onset (9-16 weeks). HIF-1α expression was higher in islet-infiltrating B cells, compared to B cells in the spleen and PLNs in NOD mice. However, when activated with B cell mitogens, HIF-1α expression was decreased in NOD compared to B6 mice.

Conclusions

Together, these data demonstrate that T1D progression is characterized by lymphocyte subset and tissue-specific changes in cellular metabolism. Compared to T cell subsets, B cells are characterized by decreased glucose uptake and increased mitochondrial polarity, suggesting that mitochondrial metabolism, rather than glycolysis, is the predominant driver of autoreactive B cells in NOD mice. HIF-1α expression in activated B cells from NOD mice was significantly lower than HIF-1α expression in activated B cells from B6 mice, suggesting that dysregulation of HIF-1α-induced glycolytic expansion of regulatory B cell subsets may exacerbate tolerance loss in T1D, and that HIF-1α-mediated signaling pathways are attractive therapeutic targets for restoration of tolerance in T1D.

43: Jeong-Su Do (City of Hope)

Abstract Title

CD318, a CD6 ligand, attenuates islet autoantigen-specific T-cell responses

Authors

Jeong-Su Do, David Arribas-Layton, Jemily Juan, Isaac Garcia, Enrique Montero, Helena Reijonen

Purpose

CD6 is expressed on both CD4 and CD8+ T cells and has been shown to regulate T cell activation. It has two ligands, CD166 (ALCAM), which is widely expressed in several tissues and cell types, and more recently identified CD318 (CUB domain-containing protein 1; CDCP1) with a more selective expression. So far, studies on the role of CD318 in disease have been limited to the cancer field and experimental autoimmune models. In tumors, CD318 has been implicated in tumor resistance to cytotoxicity and higher metastatic potential. It is unknown which, if any, human immune cell populations in the peripheral blood express CD318, and what role it would play in the immune system. In our study, we investigated the expression profile of CD318 in the cells from peripheral blood and assessed the potential role of CD318 in T-cell activation.

Methods

CFSE-labeled PBMCs from healthy blood donors were stimulated with anti-CD3 mAb w/ or w/o plate-bound recombinant CD166 or CD318. T-cell proliferation and cytokine production were analyzed by flow cytometry. To assess the expression of CD318, subpopulations of the PBMC were analyzed. Monocyte-derived dendritic cells (mDC) were generated in vitro from bead-separated CD14+ monocytes (MN), pulsed with 10 ug/ml of GAD65 on day 6, and matured in the presence of a maturation cocktail for additional 48 hrs. CD318+ mDC were bead purified and used to stimulate autologous CSFE-stained CD4+ T cells. The frequency and phenotype (surface markers, cytokines) of dividing (CSFE low) GAD65 responsive cells were analyzed by flow cytometry.

Summary of Results

Recombinant CD318 inhibits the activation and proliferation of CD4 and CD8 T cells. Recombinant plate-bound CD318 significantly reduced anti-CD3 induced CD4 and CD8 T cell activation in a dose-dependent manner (control vs. CD318; *** $p < 0.001$). Furthermore, the presence of CD318 inhibits intracellular expression cytokines, IFNγ and TNF, even in the presence of co-stimulatory CD28 mAb or CD166 (control vs. CD318; **** $p < 0.0001$).

CD318 is expressed on a subpopulation of DC and activated MN. A subpopulation of CD318+ mDC (myeloid) in the PBMC was identified, whereas pDC (plasmacytoid), monocytes and B cells lacked expression of CD318. However, CD318 can be induced on peripheral monocytes, but not on B cells, by LPS and IFNg suggesting that a pro-inflammatory environment may trigger the expression of this novel co-inhibitory ligand. Furthermore, a distinct subpopulation of in vitro monocyte-derived mature DC also expressed CD318, as seen in the mDC from PBMC.

GAD65-specific CD4 T cell proliferation was significantly reduced by stimulation with CD318+ DCs. Sorted CD318+ mDCs inhibited the proliferation of GAD65-specific T cells (CD318- DC vs. CD318+ DC; *** $p < 0.001$), and proinflammatory

cytokine production (CD318- DC vs. CD318+ DC, IFNg; * $p < 0.05$, TNF; **** $p < 0.0001$), suggesting a novel immune regulatory role of CD318 in T1D autoimmunity.

Conclusions

CD318 mediates suppression of T cell activation resulting in the reduced proliferation of islet autoantigen-specific T cells that produce pro-inflammatory cytokines. The novel subset of CD318+ DC potentially has a crucial role in the priming and triggering autoreactive CD4+ T cell response in T1D immunopathology. The findings of this study suggest that CD318+ DC could play a role in altering the delicate balance between inflammation and regulation, which is critical in the maintenance of tissue-specific immune tolerance. Identification of this novel subpopulation of immune regulatory DC contributes to understanding the immunopathogenesis of T1D and has the potential to provide new therapeutic targets.

44: Johnny Ludvigsson (Linköping university)

Abstract Title

Intralymphatic Glutamic Acid Decarboxylase administration in Type 1 Diabetes patients induced a distinctive early immune response in patients with DR3DQ2 haplotype

Authors

Sara Puente-Marin, Fabrícia Dietrich, Peter Anchenbach, Johnny Ludvigsson, Rosaura Casas

Purpose

GAD-alum given into lymph nodes to Type 1 diabetes (T1D) patients participating in a multicenter, randomized, placebo-controlled double-blind study seemed to have a positive effect for patients with DR3DQ2 haplotype, who showed better preservation of C-peptide than the placebo group. Here we compared the immunomodulatory effect of GAD-alum administered into lymph nodes of patients with T1D versus placebo with focus on patients with DR3DQ2 haplotype

Methods

GAD autoantibodies, GADA subclasses, GAD65-induced cytokine secretion (Luminex panel) and proliferation of peripheral mononuclear cells were analyzed in T1D patients (n=109) who received either three intra-lymphatic injections (one month apart) with 4 µg GAD-alum and oral vitamin D supplementation (2000 IE daily for 120 days), or placebo.

Summary of Results

Higher GADA, GADA subclasses, GAD65-induced proliferation and cytokine secretion was observed in actively treated patients after the second injection of GAD-alum compared to the placebo group. Following the second injection of GAD-alum, actively treated subjects with DR3DQ2 haplotype had higher GAD65-induced secretion of several cytokine (IL4, IL5, IL7, IL10, IL13, IFNγ, GM-CSF and MIP1β) and proliferation compared to treated individuals without DR3DQ2. Stratification of samples from GAD-alum treated patients according to C-peptide preservation at 15 months revealed that patients with < 30% loss of C-peptide (GR) had increased GAD65-induced proliferation and IL13 secretion at 3 months, and a 2,5-fold increase of IL5 and IL10 as compared to who lost more C-peptide (PR). The second dose of GAD-alum also induced a more pronounced cytokine secretion in GR with DR3DQ2, compared to the few GR without DR3DQ2 haplotype

Conclusions

Patients with DR3DQ2 haplotype had a distinct early cellular immune response to GAD-alum injections into the lymph node, and predominant GAD65-induced IL-13 secretion and proliferation that seems to be associated to a better clinical outcome. If

confirmed in the ongoing larger randomized double-blind placebo-controlled clinical trial (DIAGNODE.3), including only patients carrying DR3DQ2 haplotype, these results might be used as early surrogate markers for clinical efficacy

45: Jorge Santini-Gonzalez (University of Florida)

Abstract Title

Human stem cell derived beta-like cells engineered to present PD-L1 improve transplant survival in NOD mice carrying human HLA class I

Authors

Jorge Santini-Gonzalez, Roberto Castro-Gutierrez, Matthew Becker, Chad Rancourt, Holger A. Russ, Edward A. Phelps.

Purpose

Beta cell replacement in type 1 diabetes (T1D) has provided some patients with a few years of exogenous insulin independence but is insufficient to permanently cure a T1D patient due to eventual graft failure. Thus, there is a critical need for approaches that combine renewable sources of replacement beta cells with immunomodulation to counter recurrence of autoimmunity. Recent advances in the generation of stem cell-derived beta-like cells (sBC) have raised the possibility of providing a renewable source of functional beta cells for transplantation, effectively overcoming the severe shortage of human donor islets. Further, sBC can be engineered to express negative regulators of immunity to provide local immunological tolerance. The purpose of this project is to explore immune tolerance toward transplanted human sBCs that are engineered to express cell surface PD-L1 (iP-sBC) in a humanized HLA mouse model of T1D.

Methods

We employed HLA-A2 positive iP-sBC and HLA-A2 negative (beta-2 microglobulin knockout) iP-BKO sBC for transplantation experiments in a human HLA-A2 matched diabetic NOD mice (NOD-cMHCI-/--A2). These iP-sBC were genetically engineered to express inducible +/- doxycycline (DOX) PD-L1 and constitutive luciferase for longitudinal monitoring of viability with IVIS bioluminescence imaging. The mouse strain NOD-cMHCI-/--A2 (Jax Stock No: 031856), is a humanized model that recapitulates aspects of human diabetogenic autoimmunity. The MHC class I genes, H2-D1 and H2-K1, are genetically knocked out and the human HLA Class I transgene HLA-A*02:01 (HLA-A2) is incorporated. Diabetogenic CD8+ T cells from NOD-cMHCI-/--A2 mice target and kill human beta cells from A2 donors in an antigen-specific manner. NOD-cMHCI-/--A2 mice underwent transplantation of 1,000 sBC under the kidney capsule and were separated in two groups, iP sBC and iP-BKO sBC, each with +/- DOX. The viability of the sBC transplants was longitudinally monitored by IVIS bioluminescence imaging at days 1, 3, 7 or 9, and 14 after surgery. After two weeks, the pancreas and kidney were retrieved for histology. Cryosections were stained with H&E and immunostained for insulin, PD-L1, CD45, CD3, CD4 and FoxP3 then imaged via confocal microscopy.

Summary of Results

A two-way ANOVA of the total photon flux detected longitudinally by IVIS showed a statistical difference (p-value <0.05) for mice transplanted with iP sBCs + DOX feed at day 1 and 3. The data suggest that sBCs expressing PD-L1 were more resistant to immune rejection in the first 3 days, but that this expression was insufficient for full graft survival. Results from the iP-BKO + DOX sBCs showed a significant improvement in beta cell survival later, at day 9. Although luciferase signal dropped below detection limits by day 14 for most mice, one mouse from the iP-BKO + DOX group showed detectable transdermal luminescence at day 14. In addition, IVIS scan of the kidney removed ex vivo at day 16 showed surviving sBC clusters.
To further elucidate the effects of PD-L1 overexpression and HLA Class I KO on the survival of the transplanted sBCs we performed detailed immunohistochemistry on the recovered graft-bearing kidneys. Cryosections of the graft area were stained for insulin, CD3, CD45, CD4, FOXP3 and PD-L1. Histological examination revealed significant numbers of surviving insulin+/PD-L1+ sBC beta cells for DOX-treated mice at 16 days after transplant despite extensive infiltration with high numbers of CD3+ and CD45+ immune cells in both the sBC graft sites and native islets of the mouse pancreas.

Conclusions

In future studies, we seek to investigate if first establishing the sBC graft in immunocompromised NSG-HLA-A2 mice, followed by adoptive transfer of diabetogenic splenocytes from NOD-cMHCI-/--A2 to challenge the graft allows better examination of the role of PD-L1 in countering autoimmunity in this model. This study represents one of the first in vivo studies recapitulating key aspects of human autoimmune diabetes. Success of the study would further advance the technology available for testing a renewable source of functional beta cells for transplantation, effectively overcoming the severe shortage of human donor islets.

46: Justin Spanier (University of Minnesota)

Abstract Title

Identification of a public InsC-IAPP-specific T cell receptor in patients with type 1 diabetes.

Authors

Justin Spanier, Laurie Landry, Aaron Michels, Maki Nakayama, Brian Fife

Purpose

Islet-infiltrating T cells targeting hybrid insulin peptides (HIP) have been isolated from individuals with type 1 diabetes (T1D), yet their role in disease pathogenesis has not been firmly established. The goal of this research is to gain a better understanding of the T cell responses to HIPs.

Methods

We determined the T cell receptor (TCR) sequence from in vitro expanded and fluorescent DQ8 tetramer sorted InsC-NPY (fragment of C-peptide fused to neuropeptide Y) and InsC-IAPP (fragment of C-peptide fused with a peptide from islet amyloid polypeptide) reactive CD4+ T cells from recently diagnosed individuals with type 1 diabetes.

Summary of Results

We identified 5 unique InsC-NPY clonotypes and 3 unique InsC-IAPP clonotypes from one individual. Expression of these InsC-NPY and InsC-IAPP reactive T cell receptors in a TCR null cell line confirmed their specificity for HIPs and revealed one TCR with dual specificity for both HIPs. Furthermore, one InsC-IAPP reactive TCR was identical to a TCR specific to the same HIP epitope reported in recent publications. To determine if any features of the TCR repertoire against HIPs is shared among people with T1D, we compared our HIP reactive TCRbeta chain CDR3 sequences to a database containing 46x106 total TCR beta chain CDR3 sequences obtained from peripheral blood of 162 individuals with new-onset T1D, 66 islet autoantibody positive individuals, and 90 non-diabetic controls. We found one InsC-IAPP reactive TCR that was present only in individuals with T1D and another InsC-NPY reactive TCR that was found in 10/162 individuals with T1D and only 1/90 non-diabetic controls.

Conclusions

These data indicate the presence of shared HIP reactive CDR3 sequences within the TCR repertoire and suggests that there may be shared features of the CD4+ T cell response to HIPs that exist among individuals with T1D.

47: Leeana Peters (University of Florida)

Abstract Title

Using integrated analysis of immune phenotype and T cell receptor sequences to identify genotype and disease driven signatures in tissue.

Authors

Leeana Peters, Maigan Brusko, Todd Brusko

Purpose

T Cell-mediated phenotypes are likely to play a key role in T1D pathogenesis and progression and may also serve as a predictive biomarker of therapeutic responses. In particular, circulating T1D-Ag MHC multimer positive T cells were shown to exhibit transitional memory and exhausted-like phenotypes in T1D subjects, while separate studies have shown circulating intermediate memory or exhausted-like CD8+ T cell phenotypes to correlate with response to alefacept and teplizumab therapy. Notably, the identification of these phenotypes required the use of high parameter technologies and unbiased analysis techniques. However, questions remain as to the relevance of these phenotypes in tissue, as well as their receptor specificity and the potential molecular drivers of these phenotypes. Thus, we sought to conduct multimodal analysis of pancreatic lymph node (pLN) derived from human organ donors using high dimensional mass cytometry and single cell RNA sequencing (scRNAseq) with combined proteomic (CITEseq) and single cell T cell receptor profiling (scTCRseq).

Methods

Cryopreserved cells from pLN of healthy controls (n=12) and T1D subjects (n=10), were stained with a panel (35 markers) of MAXPAR metal-chelating polymers and run on a CyTOF mass cytometer. Beads were excluded and data was normalized between batches using CytoNorm and live single CD45+ cells were used for downstream analysis in R by adapting scripts for pseudotime and Coordinated Gene Association in Pattern Sets (CoGAPS) analysis from “Dimitri-Sid/CyTOFpatterns”. scRNAseq data was generated from (n=3 T1D, n=3 healthy controls) cryopreserved pLN. Single cell suspensions were stained with a cocktail of 7 oligo-barcoded TotalSeq antibodies (Biolegend) to aid in clustering naïve and memory CD8+ and CD4+ T cell phenotypes, after which cells were loaded into the Chromium Controller (10X genomics) and libraries prepared according to the 5’ v1.1 kit protocol. Resulting scRNAseq/TCRseq data was processed using Cellranger, and weighted nearest neighbor analysis and clustering was initially performed in Seurat, with integrated TCR and gene expression analysis subsequently performed using Platypus in R.

Summary of Results

We performed pseudotemporal analysis on both the CD8+ and the CD4+ T cell compartments of our CyTOF data, and calculated a weighted measure of pseudotime by patient. Increasing pseudotime reflected the trajectory of naïve to memory to exhausted-like T cells, as evinced by increased expression of markers such as PD-1, TIGIT and CD57 across pseudotime. When stratified by high-risk HLA genotype (DR3 and/or DR4), we observed significantly higher pseudotime weight in those carrying high risk HLA alleles as compared to those without, with no difference in age between groups. Notably, this comparison remained statistically significant when we performed the same stratification in controls alone, indicative of a general impact of high-risk HLA on CD8+ T cell phenotype. We sought to investigate which phenotypes might underly the skewing in pseudotime observed, thus we performed unsupervised Coordinated Gene Association in Pattern Sets (CoGAPS) analysis to identify signatures of cells defined by marker co-expression. We observed an increase in the presence of two signatures in high-risk HLA individuals, a T central memory (TCM) phenotype with expression of chemokine receptors CXCR3 and CCR6, as well as costimulatory molecules CD226, CD27, and CD28, and an effector memory (TEM) phenotype characterized by co-expression of negative regulators TIGIT and PD1, as well as chemokine receptor CXCR3 and costimulatory molecules CD27 and CD28. In assessing the CD4+ T cell compartment we did not identify differences in pseudotime across HLA genotype, but after performing CoGAPS analysis we identified an increased prevalence of a signature corresponding to an activated TFH phenotype, characterized by expression of chemokine receptors CXCR5 and CCR7 and activation markers CD69 and CD38 in individuals with T1D. Integrated analysis of our scRNAseq/TCRseq data from PLN revealed that both these cell phenotypes were identifiable within these data, and these clusters were more clonally expanded within T1D subjects as compared to healthy control subjects. Notably, the TFH cluster expressed less IKZF1, a negative regulator of proinflammatory cytokine production, in T1D subjects relative to healthy controls, while the CD8 TEM cluster expressed less KLF2 in T1D subjects as compared to controls, indicative of increased chemotactic capacity to the pancreas, as

well as increased type I interferon stimulated genes (IFITM1, IFITM2). Moreover, several clones possessing a TCR motif recently found within circulating IGRP-specific T cells were noted within this cluster. The presence of clones possessing this motif and a similar phenotype was also confirmed in fresh pancreatic slices from an independent new onset T1D case with insulitis.

Conclusions

We were able to identify tissue effector CD8+ T cell signatures possessing an activated phenotype and expression of co-inhibitory receptors similar to those previously identified in circulation, which seems to be driven by HLA genotype, as well as a disease-associated TFH signature. These signatures were confirmed with complementary high-parameter methods (mass cytometry, scRNAseq/CITEseq/TCRseq). Moreover, we were able to identify that clusters representing these phenotypes are more expanded in T1D and notably, the CD8 T cell cluster of interest is enriched for reactivity to IGRP. We are able to extend our findings within the pancreatic lymph node to the pancreas, where we observed a CD8 T cell clone with similar signature and possessing an IGRP-associated CDR3a motif in pancreatic slices of a recent onset T1D donor. Thus, we offer evidence that circulating phenotypic signatures previously identified to correlate with immunotherapeutic response are relevant to tissue immune phenotypes and contain autoreactive specificities, namely IGRP. Work is ongoing to identify more autoreactive specificities within these data to better determine associations between receptor specificity and phenotype to aid in targeting these cells in therapeutic interventions targeting T cells.

48: MacKenzie Williams (University of Florida)

Abstract Title

Development of an enzyme-linked immunosorbent assay for the detection of SARS-CoV-2 antibodies in serum for discernment of prior infections and vaccinations among nPOD donors.

Authors

MacKenzie Williams, Amber Batie, C. Ramsey Grace, Sean McGrail, Maria Berry, Helmut Hiller, Irina Kusmartseva, Mark Atkinson, Clive Wasserfall

Purpose

We sought to develop an assay to differentiate nPOD donors who experienced COVID-19 infections versus those who had no such infection, including those prior to the COVID-19 pandemic.

Methods

To address this notion, we developed ELISAs for quantification of antibodies against SARS-CoV-2 spike and nucleocapsid proteins from a variety of SARS-CoV-2 variants (i.e., alpha, beta, delta, and omicron). This study cohort consisted of three groups: 1) subjects who were previously confirmed COVID-19+ and recovered (n=64), 2) subjects who were procured prior to 2019 (i.e., negative control, n=77), and 3) nPOD donors with various medical histories with respect to prior COVID-19 infection (n=19). High-binding 96-well flat-bottom plates were coated with 50 ul of either alpha spike protein, delta spike protein, omicron spike protein, beta nucleocapsid protein, delta nucleocapsid protein, or omicron nucleocapsid protein. Plates were then blocked, washed, and serum was added to each well. Biotinylated mouse anti-human IgG in combination with HRP-conjugated streptavidin was used for detection of SARS-CoV-2 antibodies. Optical density (OD) values were compared among the three cohorts and preliminary (i.e., using OD values) ROC curve analysis was performed using GraphPad Prism software.

Summary of Results

As expected, subjects with confirmed medical histories of COVID-19 infection/recovery had significantly elevated antibodies against alpha, delta, and omicron spike proteins as well as beta, delta, and omicron nucleocapsid proteins versus serum samples collected prior to 2019. Interestingly, although serum from the known COVID-19-recovered donors was collected early in the

pandemic (i.e., prior to April 2020 during the alpha wave), we observed cross-reactivity of antibodies that also recognized beta, delta, and omicron antigens. COVID-19-recovered and pre-2019 samples allowed us to perform ROC curve analyses, which revealed good sensitivity and specificity for alpha spike (AUC=0.975, p<0.0001), delta spike (AUC=0.927, p<0.0001), omicron spike (AUC=0.954, p<0.0001), beta nucleocapsid (AUC=1.000, p<0.0001), delta nucleocapsid (AUC=0.995, p<0.0001), and omicron nucleocapsid (AUC=0.989, p<0.0001).

Conclusions

Screening nPOD donors for prior exposure(s) to SARS-CoV-2 antigens via ELISA is feasible. Current efforts are underway to calculate adjusted relative concentrations of antibodies using a standard curve (using serially diluted serum collected from an individual who had recovered from COVID-19 infection during the alpha variant wave). Future directions include establishing a means to differentiate between subjects who have experienced natural infection(s) versus those who have been vaccinated. We envision this methodology as being most useful for nPOD cases where medical history of COVID-19 infection is incomplete, as the association between SARS-CoV-2 and T1D etiology/pathogenesis remain unclear.

49: Maki Nakayama (Barbara Davis Center, University of Colorado School of Medicine)

Abstract Title

Characterization of pancreatic T cell populations reveals T1D-associated TCR sequences and public TCRs directed to viral antigens

Authors

Kristen L. Wells, Amanda M. Anderson, Laurie G. Landry, Ali H. Shilleh, Alvin C. Powers, Mark A. Atkinson, Clayton E. Matthews, Alberto Pugliese, Holger A. Russ, Aaron W. Michels, Maki Nakayama

Purpose

In type 1 diabetes (T1D), pancreatic beta cells are selectively destroyed by immune cells. Identifying the antigenic specificity of T cells is critical to our understanding the disorder's pathogenesis and identifying disease-specific therapeutic targets. Early efforts by ourselves and others have partially characterized the antigens targeted by islet infiltrating cells as well as their T cell receptor (TCR) utilization, however such properties for a substantial proportion of these pancreas-residing T cells remains unknown. In this study, we sought to address this knowledge void by using two novel approaches.

Methods

We determined TCR usage of in CD8+ T cells derived from the islets and pancreas slices of 27 organ donors (14 T1D, 4 glutamic acid decarboxylase antibody [GADA]-positive, and 9 non-diabetic donors). Tissues (either islets or slices samples) were dispersed to single cells and stained with T cell markers, followed by single cell sorting into 96-well plates for TCR sequencing. TCR sequences obtained from the entire tissue samples (i.e., including exocrine compartments for slice samples) were included for analysis. Utilizing this TCR information, we first generated T-hybridoma cells expressing TCRs from ten T1D donors and evaluated for the responses to 7,250 truncated peptides derived from major islet proteins (e.g., glutamic acid decarboxylase-65 [GAD65], islet amyloid protein peptide [IAPP], chromogranin A [ChgA], zinc transprter-8 [ZnT8], glucose-6-phasphatase 2 [IGRP]). Subsequently, we analyzed the TCR sequences from all 27 donors with and without T1D to identify those identical to TCR clonotypes in a public TCR database curated by the Immune Epitope Database (IEDB) to predict their antigen specificity.

Summary of Results

Among 267 TCRs analyzed for the response to the major islet antigens, only three TCRs responded to peptides derived from IGRP or ChgA, while no TCRs responded to GAD65, IAPP, and ZnT8. The frequency of these IGRP or ChgA-reactive TCRs was significantly lower compared to that of preproinsulin-specific TCRs (1.1% vs 22%, p<0.0001). Interestingly, two IGRP-

reactive TCRs were also reactive to preproinsulin peptides, one of which was reacting to the IGRP and preproinsulin peptides presented by different HLA molecules. Thus, the two IGRP-reactive TCRs likely recognize promiscuous peptide-MHC complexes. Next, we analyzed shared TCR sequences (4,529 alpha and 4,320 beta) identified from 5,023 CD8 T cells in the islets or pancreas slices of all 27 donors with and without T1D. Among 2,696 alpha and 2,640 beta unique clonotypes, 63 alpha and 30 beta clonotypes were identical to those in the IEDB database (call such clonotypes "public" hereafter), while there was only one paired alpha and beta clonotype that was completely identical to an IEDB clone. Approximately 75% of public clonotypes were identified in donors who had the same HLA allele as the cognate IEDB clonotype. The majority (i.e., 91 out of the 93 public clonotypes) were specific to viral antigens, such as influenza A virus (51% of public clonotypes identified), Epstein-Barr virus (17%), and cytomegalovirus (16%). T cells expressing virus-specific clonotypes accounted for 4.7% of all CD8 T cells and were found in 22 out of 27 donors whether or not having T1D. Also, frequencies of cells expressing virus-specific public clonotypes were similar between the sample types (average frequency: islets 3.9% vs slices 4.1%, no statistical difference). Of note, one of GADA-positive donors had a high proportion (i.e., 23%) of CD8 T cells expressing cytomegalovirus-specific TCRs. Six out of 11 clonotypes, including the cytomegalovirus-specific TCR in the GADA-positive donor, responded to a cognate peptide when expressed in T-hybridoma cells. Lastly, we evaluated one influenza-specific TCR clonotypes for the response to beta-like cells differentiated from induced pluripotent stem cells expressing a cognate HLA molecule. While preproinsulin-specific TCRs responded to the beta-like cells, we did not detect activation of the influenza-specific TCR.

Conclusions

Only a limited portion of CD8 T cells in the pancreas were specific to the native form of peptides derived from major islet proteins other than preproinsulin. On the other hand, T cells expressing a TCR chain identical to virus-specific clonotypes were routinely detected in the pancreas regardless of disease status, while there was no evidence of cross-reactivity with beta cell antigens by the virus-specific TCR. Our study implicates the importance of differentiating pathological T cells from physiological T cells to understand T1D pathogenesis. Concurrently, it will be important to determine whether and how physiological or tissue-unspecific T cells are involved in the T1D development.

50: Mary A. Markiewicz (University of Kansas Medical Center)

Abstract Title

The impact of NKG2D signaling on NOD diabetes development is affected by the microbiota

Authors

Zoe Bedrosian, Elizabeth Ruark, Mary A Markiewicz

Purpose

The drivers of type 1 diabetes are islet-specific T cells. Understanding the mechanisms by which these cells are activated is critical to developing better strategies to inhibit the disease. Signaling through NKG2D is implicated in this process, but its role is unclear owing to conflicting results from studies published to date. This made clear that new experimental approaches and models were required to determine the importance, and mechanism of action, of NKG2D signaling in autoimmune diabetes. To do this, we developed multiple novel mouse models.

Methods

We used the NOD mouse model as a genetically tractable model of spontaneous autoimmune diabetes to determine the effect of NKG2D on autoimmune diabetes development. We generated mice (1) genetically deficient in the gene encoding NKG2D, Klrk1, (2) genetically deficient in the NKG2D ligand endogenously expressed in NOD islets, H60a, and (3) mice with constitutive expression of an NKG2D ligand on beta-islet cells, on the NOD genetic background; specifically, NKG2D KO, H60a KO and RIP-RAE1epsion NOD mice. All of these mouse strains, and their littermate controls, were housed under specific pathogen free (SPF) conditions, and NKG2D KO and H60a KO mice, along with littermate controls, were additionally

housed under germ-free conditions. We followed diabetes development in all these mouse strains, analyzed expression of NKG2D ligands on antigen presenting cells (APCs) within the pancreatic lymph nodes, and compared the ability of APCs from the pancreatic lymph nodes of these mice to activate naive islet-specific CD8+ T cells (8.3 TCR) in the presence or absence of NKG2D signaling blockade.

Summary of Results

We found that the effect of NKG2D signaling on NOD diabetes varies with housing conditions, with NKG2D signaling having the greatest diabetes promoting role in germ-free mice. We found that this signaling does not occur through interaction with ligands expressed within pancreatic islets, as we demonstrate that NKG2D signaling within the islets instead inhibits NOD diabetes development. Specifically, we found that under SPF conditions, H60a KO mice have enhanced diabetes and RIP-RAE1epsilon mice have reduced diabetes. Further, unlike the decrease in diabetes observed in germ-free NKG2D KO mice, there was no difference in diabetes development between germ-free H60a KO and WT mice. We further demonstrate that CD11c+ APCs in the pancreatic lymph nodes of both SPF and germ-free NOD mice express members of the NKG2D ligand family RAE1. Demonstrating this APC ligand expression has an effect on T cell differentiation, we show that NKG2D signaling blockade resulted in the recovery of a lower percentage of live, proliferated islet-specific CD8+ T cells after four days of co-culture with APCs from pancreatic lymph nodes, with the greatest impact of NKG2D blockade observed when germ-free APCs were used.

Conclusions

Taken together, these data indicate that pro-diabetic NKG2D signaling is present, but can be redundant, in NOD mice containing microbiota. Clinically, this suggests both NKG2D signaling and the redundant signaling induced by the microbiota need to be eliminated to stop the development of an islet-specific CD8+ T cell response.

51: Matthew Johnson (University of Exeter)

Abstract Title

Mutation of the Programmed Death – Ligand 1 (PD-L1) results in infancy-onset autoimmune diabetes and immune dysregulation through reduced PD-L1 expression.

Authors

Matthew Johnson, Clara Domingo-Vila, Elisa De Franco, Brittany Resnick, Michelle Hudson, Richard Caswell, Rebecca Dobbs, Zineb Imane, Sarah Flanagan, Andrew Hattersley, Timothy Tree, Richard Oram

Purpose

Autoimmune diabetes usually results from high polygenic risk (type 1 diabetes; T1D) but can be the result of a single pathogenic variant (monogenic autoimmune diabetes). Identifying novel genetic causes of monogenic autoimmune diabetes provides insights into autoimmune diabetes pathogenesis, can optimise treatment, and offers a unique opportunity to study critical human immune homeostasis genes. We aimed to identify novel genetic causes of monogenic autoimmune diabetes.

Methods

We undertook whole genome sequencing in 14 patients with infancy-onset diabetes (<12m) and additional early-onset immune dysregulation. We used RNA analysis, protein modelling and immune studies on patient samples to evaluate the impact if identified putative pathogenic variants on the immune system.

Summary of Results

We identified a homozygous splice site variant (c.682+2G>A) in CD274, encoding the critical immune checkpoint ligand PD-L1, in a proband and his affected sister who were diagnosed with infancy-onset diabetes (diagnosed aged 1 day and 7 weeks, respectively. The proband additionally developed antibody positive hypothyroidism aged 3 years. RNA analysis confirmed aberrant splicing resulting in an in-frame deletion of 50 amino acids. Protein modelling and flow cytometry showed that the variant results in disruption of an extracellular domain of PD-L1 and may result in degradation. Immune profiling showed significant immune dysregulation with proportionally reduced neutrophils increased CD3+ cells compared to controls, and reduced markers of proliferation and activation on patient Tregs. Functional immune studies on patient cells found a ~5-fold reduction in stimulated PD-L1 expression in patient immune cells compared to age matched controls.

Conclusions

This mutation underlies neonatal onset diabetes through reduced expression of PD-L1 and subsequent immune dysregulation. This work therefore demonstrates the critical role of PD-L1 in early-onset beta-cell autoimmunity.

52: Philippe Pagni (Novo Nordisk)

Abstract Title

Impact of liraglutide treatment on gene and pathway expression in peripheral T-cell populations from adults with recently diagnosed type 1 diabetes.

Authors

Philppe Pagni, Hadas Lewinksy, Pryanka Vijay, Nikole Perdue, Sara Murray, Alisa Greenberg, Marisa Mariani, Ramneek Gupta, Alena Jiraskova, Iveta Mrizova, Per Greisen, Chirag Sachar, Johnna Wesley

Purpose

Type 1 diabetes is an autoimmune disease characterized by attack from immune cells infiltrating the pancreas and progressive loss of functional beta-cell mass, requiring exogenous insulin treatment. To address the unmet needs of people with recently diagnosed type 1 diabetes, Novo Nordisk has conducted a randomized, double-blind, placebo-controlled, phase 2 trial assessing whether the combination of glucagon-like peptide (GLP)-1 receptor agonist liraglutide and anti-interleukin (IL)-21 antibody could preserve beta-cell function (von Herrath M. et al, Lancet Diabetes Endocrinol. 2021 Apr;9(4):212-224). Herein, to better understand how liraglutide perturbs patients' immune responses in type 1 diabetes, Novo Nordisk and Immunai entered a collaboration aimed at using computational and multiomic approaches in select peripheral blood mononuclear cells (PBMC) samples from the placebo (n=13) and liraglutide (n=11) arms of this trial, at time-points including baseline, i.e., week (WK)0 and end of treatment on WK54.

Methods

Immunai leveraged a single cell multiomic approach to assay mRNA, protein, and TCR sequences using the 10x genomics platform. A combination of public and proprietary computational techniques was used to process sequence data, integrate samples, annotate cell types, and compare cell type abundances and differential gene and pathway expression between pre- and post-treatment samples in the liraglutide and placebo arms. Cell type annotation was performed using a machine learning classifier that is trained on millions of high-quality cells from Immunai's AMICA database, followed by manual review and refinement by immunologists, and utilised mRNA, surface protein, and TCR information. Pseudo-bulk differential gene expression was performed using limma-voom by averaging counts at the sample and cell-type level, and subsequent pathway enrichment was performed using fgsea. Downstream analysis focused on genes and pathways that changed from WK0 to WK54 in the liraglutide arm but did not show statistically significant changes from WK0 to WK54 in the placebo arm.

Summary of Results

Comparing liraglutide and placebo groups, differentially expressed genes were identified in 32 immune cell types with an average of 388 genes per cell type significant at a nominal P value < 0.05. For example, multiple cell types, including Th1, Th2 and Th17 memory CD4 T-cell subsets, NK CD56dim cells, and CD8 T cells, all showed increased expression of anti-inflammatory cytokine TGFb1 in liraglutide-treated patients compared to placebo.
In Foxp3-expressing T regulatory (Treg) cells, Immunai's CITE-seq data confirmed Novo Nordisk's flow cytometry findings generated as part of the trial, which indicated that Treg percentages were stable at WK54 with liraglutide while decreasing in the placebo treatment. Furthermore, 315 genes were differentially expressed after liraglutide treatment. Increased Ki67 expression as well as markers of suppressive capabilities were observed by performing a pathway analysis, which, again, was in line with pre-existing flow cytometry results. Last, to further strengthen the link between liraglutide treatment and Tregs, a public repository database of bulk and scRNAseq data on sorted Tregs from T1D and healthy patients was used. GLP-1R expression was increased in Tregs compared to bulk PBMCs, and decreased in Tregs from T1D patients compared to healthy controls.

Conclusions

In conclusion, using PBMC samples from individuals recently diagnosed with type 1 diabetes and treated with liraglutide, computational and multiomic approaches allowed to uncover differentially expressed genes and pathways in multiple immune cell types such as NK, CD8 T cells, and CD4 T-cell subsets including Tregs. Compared to the WK0 baseline, In-depth analyses in liraglutide-treated patients at WK54 have indicated decreased proliferation, cytotoxicity, and other markers of effector functions in NK and CD8+ T cells while indicating increased proliferation and suppressive function in Tregs. Of note, public human data analyses of 13 T1D cohorts and 11 Treg cohorts further substantiated a novel role for the GLP-1 receptor in Treg function, supporting the notion that GLP-1R might play a role in Tregs involved in autoimmunity and thereby guiding future clinical trials assessing GLP-1R agonists in type 1 diabetes.

53: Rocky Baker (University of Colorado)

Abstract Title

A C-peptide/C-peptide Hybrid Insulin Peptide is a Potent Epitope in T1D

Authors

Thomas Delong, Maki Nakayama, Eddie James, Peter Gottlieb, Kristen Wells-Wrasman, Kathryn Haskins

Purpose

Hybrid Insulin Peptides (HIPs) are post-translationally modified islet epitopes formed by fusion of proinsulin fragments to natural cleavage products from secretory granule proteins. We previously screened new onset Type 1 Diabetes (T1D) patients for reactivity to a panel of 16 HIPs to determine whether T cell reactivity to HIPs could serve as a biomarker of disease. Using IFN-g ELISPOT we found that reactivity to HIP11, a peptide consisting of insC20-26 joined to insC1-7 , was significantly elevated in T1D patients but not in control subjects. Importantly, HIP11 was also found in human islets by mass spectrometry making it a possible target for potentially pathogenic HIP11-reactive T cells.

Methods

We evaluated the presence, phenotype and HLA restriction of HIP11-reactive T cells from patient PBMCs and islet infiltrating T cells from nPOD case #6323 using flow cytometry, dual IFN-g/IL-10 ELISPOT, CFSE assays and single cell RNA sequencing.

Summary of Results

Our results indicate that the islet-infiltrating TCR isolated from nPOD case #6323, an organ donor with T1D, recognizes HIP11 - but not the native C-peptide - in the context of DQ8 (DQA2*0301-DQB2*0302). Using PBMCs isolated from recent onset

T1D patient, we demonstrate that HIP11 can also be presented in the context of DQ2 (DQA1*05:01-DQB1*02:01). Using a dual IFN-g/IL-10 ELISPOT, our results indicate a proinflammatory signature in response to HIP11. We developed a new DQ2 HLA class II tetramer loaded with HIP11 and assessed for the presence of HIP11-reactive T cells in longitudinal samples from a T1D patient. Using tetramer analysis in combination with a CFSE assay, we determined that HIP11-reactive T cells were detected in the peripheral blood of a T1D patient for over a year after diagnosis. Finally, TCR usage analysis by scRNAseq of longitudinal samples indicated that a restricted repertoire is used by HIP11-reactive CD4 T cells.

Conclusions

HIP11-reactive T cells with a proinflammatory phenotype can be detected in the peripheral blood of T1D patients suggesting that CD4 T cells reactive to a C-peptide/C-peptide HIP are indicator of disease in T1D.

54: Sally Kent (University of Massachusetts Chan Medical School)

Abstract Title

Contrasting single cell transcriptomic profiles of islet-derived T cells from non-diabetic donors and donors with type 1 diabetes (T1D)

Authors

Sally C. Kent, Sambra D. Redick, Mason W. Tarpley, Kristen L. Wells, Rachel S. Friedman, David M. Harlan

Purpose

Islet-infiltrating T cell populations, along with other immune cell populations, are key players in T1D pathology resulting in destruction of insulin-producing beta cells. While much phenotypic information of islet-infiltrating T cell populations in humans with T1D has been derived by conventional on-tissue detection methods, a more comprehensive profile of their transcriptome is necessary to understand the functions of these T cell populations.

Methods

Isolated islets were received from 9 donors with T1D (disease duration: 0.42 to 22 years) from nPOD, Dr. Alvin C. Powers (Vanderbilt University), and from the Alberta IsletCore. Isolated islets from 15 non-diabetic donors were received from IIDP, Proto Labs, and from the Alberta IsletCore. Upon receipt, Islets from each isolation were doubly handpicked to exclude acinar and ductal tissue, then islets (180-500 islets) were immediately processed for transcriptome analyses. Two platforms for microfluidic single cell capture and scRNA-Seq library construction were used: inDrops and 10x Genomics. Major considerations for analyzing these data sets include (1) the infrequency of the immune cell types as compared to the abundance of the islet hormone-producing cells, and (2) that immune infiltrated islets are generally rare and analyses from each donor is limited to 3,000 (inDrops) or 10,000 (10x Genomics) cells. Thus, the relative frequency of immune cells will be low. In addition, our methods were optimized for recovery of beta cells. To isolate the immune cells from the isolated islets of the non-diabetic donors and donors with T1D, we processed each sample dataset individually to remove doublets, empty droplets, and dead cells. All samples were then combined, and cell types were identified using a pancreatic islet reference dataset. From here we isolated all cells that were identified as immune or unassigned cells and re-clustered these cells to obtain a more detailed look at different immune cell subtypes in this large, combined dataset. A reference cell type annotation for scRNA-seq datasets (celltypist.org) for immune cells was then used to determine the cell types associated with each cluster.

Summary of Results

From an analysis combining the datasets from non-diabetic donors and donors with T1D, 19 cell clusters were identified. Eight of these clusters were identified as macrophage/myeloid cell populations, 3 clusters were identified as mast cells, 7 clusters were from non-immune cell types and 1 cluster was identified as T cells expressing multiple T cell specific genes. Eighty T cells were from the non-diabetic islets and thirty-eight were from the islets from donors with T1D (118 T cells total: 97.5%

CD8 and 2.5% CD4). Surprisingly, CD8 T cells from both non-diabetic donor islets and islet donors with T1D expressed similar amounts and combinations of GZMA, GZMB, and PRF1 and activation markers mRNA species. However, islet derived CD8 T cells from donors with T1D expressed significantly more mRNA associated with T cell signaling (CD3D mRNA) and numerous mRNA species associated with ribosomal assembly and protein synthesis as compared to those from non-diabetic donors (> 2-fold changes). Interestingly, a loss of GZMK mRNA was observed in CD8 T cells derived from islets of donors with T1D as compared to those from non-diabetic donors (-5.06-fold change).

Conclusions

In the context of the much more abundant islet cells with hormone mRNA species, analyses of the relatively infrequent immune cells using scRNA-Seq poses several technical and analytic challenges. In addition, all isolated T1D donor islets were obtained months to years after clinical onset of T1D. We observed CD8+ T cells in the islets of both from non-diabetic donors and from donors with T1D. In both donor groups, CD8 T cells had similar expression of mRNA for cytotoxicity markers (GMZA, GZMB, PRF1) and for activation markers. However, CD8 T cells from the islets of donors with T1D expressed significantly more CD3D mRNA, suggesting signaling through the TCR, and significantly more mRNA species associated with ribosomal assembly and potentially protein syntheses, suggesting CD8 T cell activity. Granzyme K is associated with cytotoxicity and also with promotion of a pro-inflammatory microenvironment by induction of cytokines (IL-1beta, IL-6). However, a significant loss of GZMK mRNA, was observed in CD8 T cells from the islets of donors with T1D. These data suggest (1) that non-diabetic islet donors, islet-derived CD8 T cells possess cytolytic potential and (2) that islet-derived CD8 T cells from donors with T1D showed cytotoxic potential along with evidence of TCR signaling and loss of granzyme K. This may suggest a dampening of their cytolytic functioning from donors with T1D. Analyses of CD8 T cells from islets of autoantibody+ donors may help elucidate the complex roles of these cells during the development of T1D.
These studies were supported by grants UC4DK116284 and DK104218-04.

55: Teresa Rodriguez-Calvo (Helmholtz Munich)

Abstract Title

The extent and magnitude of islet T cell infiltration as powerful tools to define the progression to type 1 diabetes

Authors

Paola Stephanie Apaolaza, Diana Balcacean, Jose Zapardiel-Gonzalo, Teresa Rodriguez-Calvo

Purpose

Insulitis is a hallmark of T1D. However, it is not present in all islets and it is elusive in humans. Although earlier studies focused on islets that fulfilled certain criteria (e.g., at least 15 CD45+ cells or 6 CD3+ cells), there is a fundamental lack of understanding of the infiltration dynamics in terms of its magnitude (i.e., how much) and extent (i.e., where). Here, we performed an in-depth characterization of T cell infiltration by investigating islets with mild and high infiltration. Furthermore, we proposed a new T cell density reference value able to distinguish non-diabetic from type 1 diabetes donors that additionally classifies autoantibody positive individuals as non-diabetic or type 1 diabetic-like.

Methods

Pancreatic tissue sections from 15 non-diabetic, 8 double autoantibody positive and 10 type 1 diabetic donors (0-2 years of disease duration) were obtained from nPOD, and stained for insulin, glucagon, CD3 and CD8 by immunofluorescence. T cell infiltration was quantified in a total of 8661 islets using the software QuPath. The percentage of infiltrated islets and islet T cell density were calculated. The validity of islet T cell density to differentiate between non-diabetic and T1D donors was tested in a training dataset. Further computational analysis was performed in a validation dataset.

Summary of Results

Our analysis revealed that CD3+ cells infiltrated 17.1% of the islets in non-diabetic donors, compared to 33% in autoantibody positive, and 32.5% in type 1 diabetic donors. Furthermore, the proportion of islets that would be considered to have insulitis, defined as islets infiltrated by at least 6 CD3+ cells, was 0.4% in non-diabetic, 4.5% in autoantibody positive, and 8.2% in type 1 diabetic donors. CD8+ and CD8- populations followed similar trends. While T cell density increased during the course of the disease (17.3 CD3+ cells/mm2 in non-diabetic vs 55.4 CD3+ cells/mm2 in autoantibody positive vs 74.8 CD3+ cells/mm2 in type 1 diabetes), only a small but significant number of islets had a high density of T cells, which likely reflected active beta cell destruction. To help standardize the analysis of T cell infiltration, we used T cell density data to developed a new threshold, and proposed the analysis of a minimum of 30 islets and a reference mean value for T cell density of 30 CD3+ cells/mm2 (the 30-30 rule), which can differentiate between non-diabetic and type 1 diabetic donors with high specificity and sensitivity. Furthermore, it can classify autoantibody-positive individuals as non-diabetic or type 1 diabetic-like. In our cohort, 1 out of 8 double AAb+ was classified as non-diabetic in more than 90% of the iterations, whereas five were classified as T1D-like in more than 95% of the iterations. Only two donors were classified as T1D-like in about half of the iterations. The 30-30 rule was further evaluated in two single AAb+ individuals. As opposed to the double, the single AAb+ donors were classified as T1D-like only in 14.5% and 17.8% of the iterations, further demonstrating the validity of the 30-30 rule as a possible tool to characterize the early stages of T1D.

Conclusions

Our data indicates that it is the proportion of infiltrated islets rather than the number of infiltrating cells what changes dramatically during the course of T1D. This suggests that mild infiltration extends throughout the pancreas as disease progresses, predominantly targeting insulin-containing islets, whereas large accumulations of cells are rare. Furthermore, we present new analytical tools for analyzing T cell infiltration. By applying the 30-30 rule to the study of more single and double AAb+ donors, we will investigate multiple disease features in those individuals identified as T1D-like and what makes them different to those with non-diabetic features. Our study fulfills the need to further understand T cell infiltration, not only after diagnosis but also in pre-diabetic individuals.

56: Urs Christen (Idorsia Pharmaceuticals Ltd.)

Abstract Submitted by:

Marianne Martinic (Idorsia Pharmaceuticals Ltd)

Abstract Title

Combination treatment of the novel CXCR3 antagonist ACT-777991 with an anti-CD3 antibody synergistically increases T1D remission in two mouse models of recent-onset T1D

Authors

Urs Christen, Laetitia Pouzol, Camilla Tondello, Monika Bayer, Edith Hintermann, Daniel Strasser, Sabrina Schuldes, Marianne Martinic

Purpose

Treatment of patients with recent-onset Type 1 diabetes (T1D) with an anti-CD3 antibody significantly preserves pancreatic β-cell function. However, this clinical benefit wanes over time and not all patients respond to the anti-CD3 monotherapy. This lack or escape of efficacy is not well understood and may rely on inflammatory mediators such as chemokines. The CXCR3/CXCL10 axis has been proposed as a key player in T1D pathogenesis, resulting in the migration of autoreactive CXCR3+ T-cells into the pancreatic islets and subsequent destruction of β-cells. Blocking the CXCR3/CXCL10 axis with the CXCR3 antagonist ACT-777991 in addition to anti-CD3 antibody treatment, may therefore prevent further β-cell damage and preserve insulin production.

Methods

The combination treatment was tested in the virally-induced RIP-LCMV-GP and the spontaneous non-obese diabetic (NOD) T1D model. Therapeutic treatments were initiated at T1D onset, defined as a blood glucose value (BGV) > 300 mg/dL. Mice were treated for three days with an anti-CD3 antibody in combination with ACT-777991 treatment as food admix until the end of the study. The effect on insulitis, blood glucose levels, plasma C-peptide (biomarker of endogenous insulin production), and T1D remission rate was evaluated for the combination treatment and the respective monotherapies.

Summary of Results

In both mouse models, while ACT-777991 monotherapy did not impact T1D remission, anti-CD3/ACT-777991 combination treatment synergistically and significantly reduced blood glucose values, resulting in an increased rate of T1D remission compared to the anti-CD3 monotherapy. Further, at the end of the study, mice in T1D remission demonstrated reduced insulitis and measurable plasma C-peptide levels. When treatments were initiated in non-severely diabetic mice at T1D onset (BGV between 300-400 mg/dL), the combination treatment fully reversed T1D.

Conclusions

In two mouse models of T1D, anti-CD3/ACT-777991 combination treatment showed significant enhancement of β-cell function compared to both monotherapies. In non-severe diabetic mice, the combination treatment led to the cure of T1D, providing rationale to investigate this combination in a clinical setting for the treatment of patients with recent-onset T1D.

CORE LAB

57: Data Portal Presentation

58: Melanie Shapiro (University of Florida)

Abstract Title

A genomic data archive from the Network for Pancreatic Organ donors with Diabetes

Authors

Melanie Shapiro, Daniel Perry, Sonya Chamberlain, Irina Kusmartseva, Srikar Chamala, Leandro Batzano-Nogeira, Mingder Yang, Jason Brant, Maigan Brusko, MacKenzie Williams, Kieran McGrail, James McNichols, Leeana Peters, Amanda Posgai, John Kaddis, Clayton Mathews, Clive Wasserfall, Bobbie-Jo Webb-Robertson, Martha Campbell-Thompson, Desmond Schatz, Carmella Evans-Molina, Aberto Pugliese, Patrick Concannon, Mark Anderson, Michael German, Chester Chamberlain, Mark Atkinson, Todd Brusko

Purpose

The Network for Pancreatic Organ donors with Diabetes (nPOD) is the largest biorepository of human pancreata and associated immune organs from donors with type 1 diabetes (T1D), maturity-onset diabetes of the young (MODY), cystic fibrosis-related diabetes (CFRD), type 2 diabetes (T2D), gestational diabetes, islet autoantibody positivity (AAb+), and without diabetes. nPOD recovers, processes, analyzes, and distributes high-quality biospecimens, collected using optimized standard operating procedures, and associated de-identified data/metadata to researchers around the world. Herein describes the release of high-parameter genotyping data from this collection.

Methods

372 donors were genotyped using a custom precision medicine single nucleotide polymorphism (SNP) microarray. Additionally, 207 donors were assessed for rare known and novel coding region variants via whole exome sequencing (WES).

Summary of Results

Data were technically validated using published algorithms to evaluate donor relatedness, ancestry, imputed HLA, and T1D genetic risk score.

Conclusions

These data are publicly-available to enable genotype-specific sample requests and the study of novel genotype:phenotype associations, aiding in the mission of nPOD to enhance understanding of diabetes pathogenesis to promote the development of novel therapies.

NOVEL BIOMARKERS

59: Farooq Syed (Indiana University)

Abstract Title

β Cell miRNAs Function as Molecular Hubs of Type 1 Diabetes Risk and Pathogenesis

Authors

Preethi Krishnan, Garrick Chang, Sarah R Langlais, Sumon Hati, Kentaro Yamada, Ivan A Restrepo, Raghavendra G Mirmira, Rajesh Sardar, Jing Liu, Carmella Evans-Molina

Purpose

Biomarkers capable of monitoring β cell stress during the evolution of type 1 diabetes (T1D) are currently lacking. MicroRNAs (miRNAs) are a class of small non-coding RNAs ~22 nucleotides in length that modulate gene expression by binding to the 3' untranslated region of target mRNAs. Given their stability in biological fluids and enrichment in cell-derived extracellular vesicles (EVs), we hypothesized that changes in miRNA content in human islets and islet-derived EVs could identify early β cell stress/death and be leveraged in T1D biomarker strategies.

Methods

Human pancreatic islets obtained from 10 cadaveric donors (5M/5F) were treated with or without pro-inflammatory cytokines (IL-1β and IFN-γ) to mimic in vivo T1D conditions. Exosomes were isolated using either an ExoQuick precipitation method or by serial ultracentrifugation and characterized using nanoparticle tracking analysis (NTA) and electron microscopy (EM and SEM). Small RNA sequencing was performed on islets and islet-derived exosomes using an Ion Proton System. Partek Flow and DESeq2 package in the R statistical program was utilized for data analysis. Human mature miRNAs were annotated using miRBase v 20, and those with a total read count of less than 10 were removed from further analysis. miRNAs with a fold change ≥ 1.5 and $p < 0.05$ were considered as differentially expressed (DE). DE miRNAs were validated using independent sets of islets and islet-derived exosomes using qRT-PCR. To identify the clinical utility of this technique, exosomes were isolated from plasma samples of individuals with new-onset T1D, AAb+ but normoglycemic individuals, and healthy controls, and droplet digital PCR was performed to determine the expression of select miRNAs. To further validate these miRNA signatures, we developed an ultrasensitive, nanoparticle-based sensor ("nanoplasmonic") that can detect and quantify EV-derived microRNAs. To identify the spatial expression of miRNAs, single-molecule FISH (smFISH) was applied to human pancreatic tissue sections obtained from controls, AAb+, and T1D individuals from the nPOD Biorepository

Summary of Results

Small RNA sequencing identified 1110 and 890 miRNAs in total and 20 and 14 DE miRNAs (FC ≥1.5 and p<0.05 control versus cytokine) from islets and islet-derived EVs, respectively. Islet-derived EV miRNAs were largely distinct from those in islets, suggesting selective sorting of miRNAs into EVs in response to extrinsic cues. Interestingly, miRNA expression patterns were strikingly different between male and female donors, with only <10% overlap among the DE miRNAs. Only two miRNAs, miR-155-5p and miR-146a-5p, were upregulated in both sexes in cytokine-treated islet and islet-derived EV samples. Functional enrichment analysis of a signature set of DE miRNAs from islet-derived EVs (155-5p, 146a-5p, 124-3p, 802, and 30c-1-3p) identified numerous pathways relevant to β cell function, including “insulin signaling,” “ER stress,” and “apoptosis.” smFISH revealed increased expression of miR-155 in insulin-positive cells in pancreatic tissue sections from nPOD donors with T1D or AAb+, further confirming results from our in vitro human islet model. To explore the biomarker potential of these miRNAs, plasma EVs were isolated from pre-diabetic NOD mice and analyzed using ddPCR. This analysis revealed increased expression of miR-155-5p and miR-146a-5p in EVs from 11-13-week old NOD mice. Similar trends in the expression of this miRNA signature were detected in plasma-derived EVs of individuals with AAb+ or newly diagnosed T1D in comparison to healthy controls. Finally, data from our custom-made ultrasensitive nanoplasmonic (LSR) biosensor further confirmed our findings, implying that these EV miRNA signatures may have clinical utility in T1D diagnosis and risk stratification.

Conclusions

Taken together, our analysis identified two miRNAs (miR-155-5p and miR-146a-5p) that were coordinately upregulated in both islets and islet-derived exosomes in response to pro-inflammatory cytokine treatment in both male and female donors. Additionally, we identified a strong sexual dimorphism in miRNA regulation in human pancreatic islets, which highlights the importance of considering sex as a biological variable when defining new biomarker strategies for T1D. Furthermore, we showed that plasma-derived EV miRNA signatures are modulated in individuals with AAb+ and new-onset T1D, supporting the idea that evaluation of miRNAs could aid in the early detection of T1D.

60: Fatoumata Samassa (INSERM Institut Cochin)

Abstract Title

Interferon-α skews the immunopeptidome of β cells towards the presentation of HLA-B ligands

Authors

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Purpose

Interferon (IFN)-α is the earliest cytokine signature observed in individuals at-risk of developing type 1 diabetes (T1D). Although IFN-α induces ER stress and HLA Class I (HLA-I) upregulation in β cells, its effect on the repertoire of HLA-I-bound peptides is unknown. Using immunopeptidomics, we characterized the differences in HLA-I peptide presentation between in-vitro resting and inflamed β-cell states.

Methods

The ECN90 β-cell line was exposed or not to IFN-a, followed by HLA-I immunoprecipitation, peptide elution and mass spectrometry identification. The peptides identified were tested for recognition by circulating CD8+ T cells using HLA-I multimers and by reporter 5KC cells transduced with T-cell receptors sequenced from islet infiltrates of T1D patients.

Summary of Results

In the basal state, the β-cell immunopeptidome was dominated by HLA-A-restricted ligands. IFN-α upregulated HLA-I expression and peptide presentation. However, this upregulation almost exclusively affected HLA-B and, to a lesser extent, HLA-C, while it was negligible for HLA-A. Accordingly, this biased HLA Class I induction translated into a larger increase in peptide display, notably from granule proteins, for HLA-B. Neo-epitopes derived from alternative mRNA splicing, post-translational modifications and protein cis-splicing were also identified. A subset of the β-cell peptides identified was recognized by circulating CD8+ T cells with a variable effector/memory phenotype. Some HLA-B-restricted β-cell epitopes were targeted by islet-infiltrating CD8+ T cells of T1D donors.

Conclusions

We conclude that the inflammatory milieu of insulitis may skew the autoimmune response toward epitopes presented by HLA-B, thus recruiting a different T-cell repertoire. In view of the paucity of HLA-B-restricted islet epitopes identified to date, this repertoire may be relevant to T1D pathogenesis and deserves further investigation.

61: Guido Sebastiani (University of Siena)

Abstract Submitted by:

Giuseppina Emanuela Grieco

Abstract Title

Circulating miRNome stratifies T1D subjects in two subgroups with different immunomic profile and insulin requirement at follow-up.

Authors

Giuseppina Emanuela Grieco, Guido Sebastiani, Stefano Auddino, Marco Bruttini, Alessia Mori, Daniela Fignani, Giada Licata, Elena Aiello, Laura Nigi, Caterina Formichi, Carmella Evans-Molina, Alberto Pugliese, Francesco Dotta

Purpose

Circulating miRNAs have been proposed as potential biomarkers in type 1 diabetes being altered in plasma of T1D subjects and associated with beta-cell functional decline. However, the ability of circulating miRNome to stratify T1D individuals in different subgroups based on their clinical progression and pathogenetic characteristics of the disease has not been described yet. Therefore, the aim of our study was to analyse circulating miRNAs in plasma of T1D subjects to verify whether they can distinguish different groups of T1D subjects by using two different sequencing platforms and a computational strategy of data cross-validation.

Methods

In the INNODIA consortium, human plasma samples were collected in different European sites through a highly standardised protocol from n=115 newly diagnosed T1D subjects followed-up with programmed visits at 3-, 6- and 12-months post-diagnosis. RNA was extracted from plasma samples at baseline (v1, <6 weeks from diagnosis) from n=115 patients. A high-throughput sequencing analysis by using two different platforms (HTG miRNA Edge-seq and Small RNA-seq) was performed. Following low-counts filtering step, only miRNAs consistently detected and positively correlated between the two platforms were considered for further analyses. Hierarchical clustering analysis was performed on miRNAs expression; then, miRNAs expression was correlated with clinical parameters at diagnosis and follow-up. Moreover, Weighted Gene Co-expression Network analysis (WGCNA) was also performed in order to potentially identify modules of co-expressed miRNAs. On n=67 out of n=115 T1D patients, immunomic profile was analysed through flow Cytek Aurora cytofluorometer. HLA typisation was performed through Affimetrix SNP array for n=107 out of n=115 T1D patients. Finally, miRNAs differential expression was

validated through two different reverse transcription strategies (miRCury and Custom Taqman) and subsequent droplet digital PCR (ddPCR) detection.

Summary of Results

We identified 248 miRNAs commonly and concordantly detected by both sequencing platforms. Following clustering analysis, we observed that the expression of miRNAs is able to distinguish two different groups of T1D patients (namely Cluster-A and Cluster-B) consisting of n=87 (Cluster-A) and n=22 patients (Cluster-B), respectively. In details, a group of miRNAs located in 14q32 T1D susceptibility locus (miR-409-3p, miR-127-3p, miR-382-5p, etc.) resulted highly upregulated in Cluster-B vs Cluster-A, and able to distinguish the two clusters. Furthermore, using a WGCNA statistical approach, we identified two modules of miRNAs (containing the miRNAs located in 14q32 locus previously cited and miR-375) correlated with insulin dose and beta-cell functional decline at 3- and 6- months post-diagnosis.
At baseline (v1) Cluster-A and Cluster-B T1D subjects did not differ in age, BMI, sex, DKA and collection sites. However, Cluster-A showed lower HbA1c (A: 75.1 mmol/mol, B: 8.94 mmol/mol, p=0.0093) and a higher titre anti-IAA antibody vs Cluster-B at v1 (A: 36.0 A.U., B: 7.1 A.U., p=0.0093). The proportion of HLA DR3-DQ2 T1D risk haplotype was higher in patients belonging to Cluster-A vs Cluster-B (A: 51.76%, B: 28.57%, p=0.05) In addition, circulating immunomic analysis showed different frequencies of circulating pro-inflammatory MAIT CD8+ T cells (A: 1.27%, B: 0.56%, p=0.041) and of TSCM CD8+ T cells (A: 0.39%, B: 0.74%, p=0.004) in Cluster-A vs Cluster-B.
At follow-up, Cluster-A was characterised by a higher insulin requirement at 3 months (A: 0.52 U/kg/day vs B: 0.19 U/kg/day, p=0.002) and 6 months (A: 0.5 U/kg/day vs B: 0.35 U/kg/day, p=0.03) and higher IDAA1c at 3 months (A: 8.59 (HbA1c+ 4 x insulin dose) vs B: 7.95 (HbA1c+ 4 x insulin dose), p=0.038) after diagnosis, compared with patients of Cluster-B. Finally, we validated through ddPCR the upregulation of 14q32 located miRNAs, i.e. miR-127-3p, miR-409-3p and miR-382-5p (in ratio with miR-375 expression, downregulated in Cluster-B vs Cluster-A) in Cluster-B vs Cluster-A.

Conclusions

Through standardised analysis of circulating miRNAs in T1D patients, we have demonstrated, by a cross-validation strategy of sequencing and ddPCR, that a group of miRNAs, importantly located in 14q32 T1D susceptibility locus, are able to stratify T1D patients into 2 groups clinically different for immunomic profile and HLA DR3-DQ2 T1D risk haplotype at diagnosis and for insulin need at follow-up.

62: Guido Sebastini (University of Siena)

Abstract Title

Identification of circulating isomiRNAs as predictive biomarkers of beta cell functional decline in type 1 diabetes

Authors

Stefano Auddino, Giuseppina Emanuela Grieco, Marco Bruttini, Daniela Fignani, Alessia Mori, Elena Aiello, Giada Licata, Guido Sebastiani, Francesco Dotta

Purpose

Circulating biomarkers in type 1 diabetes (T1D) remain urgently needed to stratify T1D patients in the early stages of the disease and to predict their progression. Iso-microRNAs (isomiRs) are post-transcriptional sequence variants of microRNAs (miRNAs) and represent an underestimated proportion of circulating RNAs in plasma. Standard bioinformatic sequencing pipelines and qPCR methods cannot efficiently distinguish circulating isomiRs from canonical miRNAs. Therefore, the purpose of the study is to implement bioinformatic pipelines for the analysis of circulating isomiRs of small RNA seq data from plasma from T1D patients and control subjects to discover novel potential biomarkers associated with baseline or follow-up clinical conditions.

Methods

RNA was extracted from plasma of n=16 non diabetic control subjects (age=28±5.4 yrs; BMI=21.3±2.3 Kg/m2) and n=16 newly diagnosed (< 6 weeks) T1D individuals (age=28.4±7.2 yrs; BMI=23.1±2.8 Kg/m2; ≥2 Aab+) followed-up for 12 months, from n=3 human β-cell line EndoCβH1 samples and from laser-captured microdissected pancreatic human islets deriving from n=13 patients with normal glucose tolerance. Small RNA seq was performed using QIAseq miRNA library kit and sequenced using Illumina NOVASeq6000. FastQ files were processed with the sRNAbench pipeline for the quantification of canonical miRNAs and isomiRs. Reads were filtered to remove low quality sequences, low expressed sequences, and possible sequencing errors. Remaining reads were then normalized with DESeq2's median of the ratios method. Differential expression analysis with DESeq2 was performed to identify sequences differentially expressed between T1D patients and control subjects (absolute log2FoldChange>1 and FDR<0.05). Correlation analysis of sequences' expression with clinical outcomes at baseline and follow-up was performed for T1D patients with Spearman's test (p<0.05).

Summary of Results

Differential expression analysis between T1D individuals and the control group revealed as differentially expressed 66 canonical miRNAs and 61 isomiRs sequences not differentially expressed in the canonical form. Among these 61 isomiRs, correlation with clinical data showed that isomiRs -222-3p (extension of 1nt at 3') and 320a-3p (extension of 1nt at 3' + 1 non-template addition of T at 3') (log2FoldChange: 1.3 , FDR: 0,00045 ; log2FoldChange:1.7 , FDR: 0.00005, respectively), but not the corresponding canonical miRNAs, are negatively associated with fasting c-peptide and MMTT c-peptide at the follow up (3; 6 and 12 months), potentially predicting a decreased β cell function. The presence of these isomiRs sequences was also confirmed in two independent small RNA seq datasets deriving from EndoCβH1 cell line and from laser capture microdissected pancreatic human islets.

Conclusions

The implementation of bioinformatic pipelines for the distinction between isomiRs and canonical miRNAs represents a novel field for biomarker discovery. The analysis of circulating isomiRs revealed the presence of two isomiRs (isomiR -222-3p and -320a-3p) negatively associated with β-cell function at follow-up visits and up-regulated in T1D patients. Furthermore, the presence of these sequences was confirmed in human β-cell line and in pancreatic islets. The hyper-expression of these two isomiRs in T1D patients compared to control group suggests an error in the cleavage of the pre-miRNA performed by DICER, potentially related to cellular stress. Furthermore, the negative association with β cell function at follow-up may indicate a failure of β cells due to the previously stated stressogenic stimulus. The different expression pattern of these two isomiRs sequences and the corresponding canonical miRNAs corroborates the importance of the distinction between canonical miRNAs and isomiRNAs for the analysis of small RNA seq data.

NOVEL TECHNOLOGIES

63: Barbara Ehall (Medical University of Graz, Austria)

Abstract Title

Elucidating expression of beta and alpha cell transcription markers before, during and after insulitis in NOD mice

Authors

Lilli Bonstingl, Kaddour Bounab, Joakim Franz, Laurin Herbsthofer, Barbara Prietl, Amin El-Heliebi, Thomas R. Pieber

Purpose

The process of autoimmune destruction of beta cells - characteristic for type 1 diabetes - can be classified into different stages based on immunohistochemistry staining. Each stage of this process exemplifies the changes in endocrine and immune cell composition found in the islets of Langerhans. This process is often studied in the T1D model of non-obese-diabetic (NOD) mice due to the difficulties in studying pre-diabetes in humans. This model exhibits the same autoimmune reaction to beta cells as human T1D and can be studied in a more controlled manner. Using the mentioned stages of autoimmune destruction in NOD mice, we investigated the effects of insulitis on a transcriptional level and what distinguishes endocrine cells during this process. We did this using padlock probe technology- a highly specific approach to detect RNA directly within tissue samples while preserving its original spatial location.

Methods

Non-diabetic NOD mouse pancreata were collected at varying blood glucose levels (110-150 mg/dl) and embedded in paraffin. Adjacent sections were stained with fm-IHC and padlock probe technology respectively. Enough pancreatic sections were chosen to represent all islet stages of autoimmune destruction.
Fm-IHC staining is achieved using the BOND RX Fully Automated Research Stainer from Leica Biosystems. For primary antibodies we use Insulin (1:60000, Abcam ab181547), Glucagon (1:10000, Abcam ab92517), Somatostatin (1:12000, Abcam ab111912), CD45 (1:5000, Abcam ab208022), CD4 (1:500, Abcam ab183685) and CD8 (1:500, Cell Signalling Technology Europe 98941S). In addition to the Dako EnVision+ System-HRP 2nd anti-rabbit antibody we use reagents contained in the Opal 7-Color Automation IHC Kit (Akoya Biosciences SKU NEL821001KT). The required microscope scanning platform are Vectra3® and Vectra® Polaris™, spectral unmixing is done in Phenochart™ all from Akoya Biosciences.
In situ hybridisation with padlock probe technology was used to localize Ins1, Ins2, Gcg, Brn4 (Pou3f4), Pdx1 and Arx mRNA transcripts. In situ padlock probe- based mRNA detection procedures were performed as described previously. In summary, the target-mRNA is reverse transcribed to cDNA and linear oligonucleotides (i.e. padlock probes) bind with their 5´- and 3´parts to the complementary cDNA sequence forming a circular DNA structure. Upon exact base pair hybridisation, the padlock probe is ligated forming a closed circular DNA structure. The DNA structure is amplified by a rolling circle amplification, forming micron sized DNA structures which can be targeted by fluorescent probes. Reference sequences were retrieved from the National Centre for Biotechnology Information using the following GenBank accession numbers: NM_008386.4 Mus musculus insulin I (Ins1), NM_008387.5 Mus musculus insulin II (Ins2), NM_008100.4 Mus musculus glucagon (Gcg), NM_008901.2 Mus musculus POU domain (Pou3f4), NM_008814.4 Mus musculus pancreatic and duodenal homeobox 1 (Pdx1) and NM_001305940.1 Mus musculus aristaless related homeobox (Arx). Padlock probes were designed using an open-source Python software package (https://github.com/Moldia/multi_padlock_design) as described by Gyllborg et al. 2020. For reverse transcription, primers in close proximity to the padlock probe binding sites were designed using CLC Main Workbench software (CLC Bio Workbench version 7.6, Qiagen). In addition to the target-specific ends, padlock probe backbones contain a unique reporter sequence for each RNA transcript and share a common “anchor” sequence for dual-colour staining. The in- situ hybridisation probes were validated on positive and negative controls of tissue samples of mice.
Visual analysis was done in OlyVIA 2.4 (Olympus Soft Imaging Solutions).

Summary of Results

We found that padlock probe technology is a promising novel method for analysing islets of Langerhans during the autoimmune process. The comparison of fm-IHC and padlock probe technology elucidates characteristics of endocrine cells on a proteome and transcriptome level, which seems to indicate that cells close to immune cell infiltrates initially contain more glucagon mRNA, while insulin mRNA appears less abundant in these areas. In islets exhibiting insulitis, BRN4 signals are detectable in immune cell infiltrates and surrounding cells in addition to small numbers inside of islets. Interestingly, we also found that a considerable number of cells in the exocrine pancreas stain positive for typical endocrine mRNA, particularly PDX1, BRN4, or insulin.

Conclusions

Padlock probe technology is a promising novel method of determining the transcriptional signature of endocrine cells, especially when compared to staining on protein level by fm-IHC for example. Comparison of mRNA and protein signatures in the pancreas could elucidate the effects immune cell infiltration has on cells found in islets of Langerhans as well as indicate exocrine cells containing RNA usually associated with endocrine cells.

64: Ben Giepmans (UMC Groningen, The Netherlands)

Abstract Title

Microscopy of ultrastructure and in vivo pancreas to address if exocrine stress leads to beta cell stress

Authors

Peter Duinkerken, Noura Faraj, Ahmad Alsahaf, Anouk Wolters, Wilem Hoogaars, Elizabeth Carroll, Jacob Hoogenboom, Ben Giepmans

Purpose

The trigger(s) of T1D are not known, although several potential causes have been named. The past decade several nPOD studies showed that the exocrine pancreas might play a role prior to T1D onset. The nPOD data has been shown both at the whole organ level (Campbell-Thompson and coworkers), down to the ultrastructural level by our lab. Manual analysis of large-scale electron microscopy (EM) data from nPOD donors suggest that exocrine / endocrine cell stress is more prevalent in T1D. However, manual data analysis of gigabyte-size datasets might suffer from bias. Moreover, the donor tissue does not allow a cause consequence relationship. Today we introduce (1) IDENTIFY for automated segmentation of large-scale EM (nanotomy) datasets and (2) an in vivo model to address if exocrine cell stress leads to beta cell stress.

Methods

IDENTIFY combines nanotomy with analytical EM (ColorEM) in which the latter is used for identifying subcellular features such as organelles and vesicle content on the near-molecular level. The cause/ consequence dynamics are studied in the zebrafish larvae pancreas that contains a primordial Islet within 2 days after fertilization in a well vascularized, innervated pancreas. Transgene fish that (a) contain a controllable inducible stressor in the exocrine pancreas that can be crossed with (b) fish that express a read-out for beta cell function. Analysis is done using confocal and light sheet microscopy following modulation in living larvae, as well as histology and electron microscopy at end points.

Summary of Results

IDENTIFY is an automated method to segment granules in nanotomy datasets through data analysis and machine learning. The developed algorithm allows feeding of traditional EM data that will then be automatically processed to identify organelles in large scale maps. Our focus is on the different secretory granules in the Islets of Langerhans and surrounding exocrine tissue. Ongoing further training networks will lead to a generic algorithm to analyze high-throughput grey scale EM pancreas data. The cause/ consequence relation is now studied in a zebrafish model where a pro-drug to a cytotoxic drug conversion only takes place in exocrine cells. As indicated by a fluorescence reporter, these cells undergo apoptosis. At endpoint assays (histology, EM) exocrine failure is obvious after 12 hours of treatment and Islets appear affected as well. We will present our next approach inducing more subtle stress by variation in dose, time and pulse, as well as combining the exocrine stressor with live read outs for beta cell presence (GFP expression) and stress (fluorescent reporters).

Conclusions

Finding the trigger(s) of T1D needs novel approaches and techniques, like has been demonstrated by the human nPOD program itself. Quantitative analysis of ultrastructural anomalies has now been automated by elemental analysis (ColorEM) for automated analysis at the ultrastructural level. Dynamics are studied in an in vivo system where exocrine cells can be manipulated (stressed) followed by analysis of Islet (patho)physiology. Next, these new approaches will help to answer whether exocrine cell stress on its own might trigger beta cell stress leading to T1D.

65: Charles Lazimi (University of Florida)

Abstract Title

Adenoviral gene delivery to human pancreas slices

Authors

Charles Lazimi, Amelia Linnemann, Clayton Matthews, Edward Phelps

Purpose

Whole isolated islets retrieved from enzymatically digested pancreas have been a widely studied and useful model for understanding fundamental beta cell properties. However, isolated islets cannot always address questions of relevance to the in vivo islet niche. Because living tissue slices retain islets in the native 3D tissue, other cell types and signals contributing to pancreas physiology can be studied including nerves, vasculature, pancreatic ductal cells, exocrine cells, extracellular matrix, and immune cells. Furthermore, slices make it possible to monitor islet and immune cell behavior in the pancreas parenchyma under pathophysiological states such as T1D, which results in compromised islet morphology and thus makes isolation of whole islets challenging. One limitation of pancreas slices is that it has been difficult to deliver transgenes to express functional biosensors or perform genetic manipulations. Here, we demonstrate robust adenovirus gene delivery to human pancreas slices using both targeted and universal promoters while maintaining slice viability and function for up to five days.

Methods

Live human pancreas tissue slices were prepared by nPOD from organ donors without diabetes and transferred to our lab. Slices were allowed to recover for 24 hours and then transduced via adenovirus at 37°C. Viral media was replaced after 24 hours, and slices were cultured for another 1-3 days. We tested adenoviral vectors encoding for CMV-GCaMP6m, a Ca2+ biosensor, and INS-Grx1-RoGFP, a redox potential biosensor. Transduced slices were imaged using a confocal microscope and analyzed for relative transduction efficiency through ImageJ.

Summary of Results

We achieved excellent transduction efficiency for the Ad-CMV-GCaMP6m. We estimate >90% pancreatic cells expressed the transgene after 24 hours. Dynamic imaging experiments demonstrated that transduced slices retained strong glucose stimulated Ca2+ responses in islets and carbachol responses in exocrine tissue for up to five days after slice generation. Targeted expression of Grx1-RoGFP to beta cells using the INS promoter was also successful. Grx1-RoGFP responded correctly to changes in redox potential induced by oxidizing (H2O2) and reducing agents (DTT).

Conclusions

We have demonstrated the feasibility for robust general and targeted expression of transgenes in human pancreas tissues via ex vivo adenoviral transduction. Though this transduction method the use of genetic tools is now possible to efficiently investigate islet physiology and pancreatic disease progression in real time for several days. Without the need for any complex culture system or equipment, this technique is easily implemented.

66: Heather Cornnell (AdventHealth Translational Research Institute)

Abstract Title

Ex vivo human pancreas magnetic resonance imaging (MRI)

Authors

Heather H. Cornnell, Carley E. Glass, Yury O. Nunez Lopez, Charles Rowe, Alejandra Petrilli, Richard E. Pratley, Anna Casu

Purpose

Magnetic Resonance Imaging (MRI) is increasingly used to study exocrine-endocrine interactions in the pathogenesis of diabetes. The extent to which MRI indices reflect the tissue composition of the pancreas in humans is not well understood, as there have been relatively few studies comparing MRI to histology and these have largely been confined to surgically resected diseased organs. Here, we used MRI to estimate intrapancreatic fat in organs from donors and compared these indices with histological measures from the same organs. The ultimate goal is to use this methodology to translate the histological and biochemical characteristics to in vivo measurements in benign pancreatic diseases not requiring surgery (for example diabetes mellitus).

Methods

Nineteen human (8F/11M) pancreata were procured from donors ranging in age from 24-74 years and with BMIs between 21.8-60.3. These pancreata underwent ex vivo imaging in a 3T Philips Achieva followed by histological analysis. Organs were placed in a sealed bag submerged in 4˚C sodium polyacrylate gel on a supporting grid during MR imaging. T1 and T2 weighted images, diffusion, and T1, and T2*, and PDFF (proton density fat fraction) maps were acquired using acquisition parameters similar to clinical scans to allow future translation into in vivo studies of pancreatic structure and function. PDFF values were measured within three 100mm3 elliptical regions of interest (ROIs) within the head, body and tail of each pancreas. H&E sections of the head, body, and tail were used to quantify the area occupied by adipose tissue using images captured on a Nikon EclipseT at 4X magnification. Six random images were chosen on each slide and the area of adipose tissue infiltration and total area were drawn and measured using ImageJ software. The %fat was calculated as the (adipocyte area / total area) x 100. Measurements by MRI and histological analysis were both performed by independent single users to minimize any inter-user variability or bias.

Summary of Results

The fat fractions measured by MRI in three regions per pancreas segment (head, body, tail) were highly correlated to the average within the segment (all p<0.0001, R2 from 0.9604-0.9913). Additionally, the average fat fraction from each segment (head, body, tail) was correlated with the overall average of the pancreatic PDFF (all p<0.0001, R2 head 0.9141, body 0.8734, tail 0.7237). The overall average PDFF values were correlated with average fat fraction by histology (p<0.0001, R2 0.8518). The individual segments were also correlated between MR and histology (head p=0.0022, R2 0.4983, body p <0.0001, R2 0.8692, tail p=0.0004, R2 0.5589). The individual MR segments were also correlated to the average histological fat fraction (head p <0.0001, R2 0.7414, body p <0.0001, R2 0.7613, tail p<0.0001, R2 0.6811).
In this small dataset, pancreatic fat content did not correlate with BMI (R = 0.3628 95% C.I. -0.1094 to 0.7014).
There was no significant difference between the fat fraction values (either MR or histological) between pancreata from donors with or without diabetes, however this analysis may be limited by sample size as only 6 pancreata were obtained from donors with T2D.

Conclusions

Ex vivo MRI imaging is feasible and reliable as demonstrated by the good imaging quality and the high correlation between MRI fat estimates and histological estimates, generally considered to be the gold standard. The individual regions drawn within each pancreatic segment (head, body, tail) showed close agreement to the average value within that segment, indicating that it will likely be sufficient to measure only one region within a segment. Although the agreement between the individual segments and the overall average is significant, the R2 values are lower, which may indicate that measuring each region is preferable. Future studies will focus on the degree of fibrosis and correlation of the MRI metrics with ex vivo measured pancreas function.

67: Jessie Barra (Barbara Davis Center for Diabetes, University of Colorado Anschutz Medical Campus)

Abstract Title

Genetically engineered stem cell-derived beta cells and CAR-Tregs to induce localized suppression of effector T cells

Authors

Jessie Barra, Rob Robino, Roberto Castro-Gutierrez, Amanda Anderson, Maki Nakayama, Leonardo Ferreira, Holger Russ.

Purpose

The loss of functional β-cell mass is a hallmark of Type 1 diabetes (T1D). Exogenous insulin therapies reduce glucose fluctuations, yet effective glycemic control is only partially restored. Beta-cell replacement strategies such as islet transplantation represent a promising alternative approach, but eventual immune-mediated graft destruction remains a major clinical hurdle. Human stem cell-derived β-like cells (sBC) can solve the problem of low availability of high-quality human islets. However, like primary islets, sBC are still susceptible to both allogeneic and recurrent autoimmune attack. Unlike primary islets, sBCs can easily be genetically modified to provide localized immune suppression. Chimeric antigen receptor (CAR)-regulatory T cell therapies represent a unique way of suppressing the response toward a specific antigen. By harnessing the suppressive power of regulatory CAR-T cells (CAR-Tregs) at the site of β-cell engraftment, we predict that sBCs will be protected from, both allo- and auto-immune rejection.

Methods

Taking advantage of the previously published CAR against epidermal growth factor receptor (EGFR), we engineered our sBCs to constitutively overexpress a novel truncated form of this receptor (EGFRt). By transducing the EGFR CAR into purified polyclonal human CD4+CD25hiCD127low Tregs, we predict that we should induce localized suppression of effector T cell responses at the graft site through engagement with EGFRt overexpressing sBCs.

Summary of Results

We demonstrate uniform overexpression of EGFRt within stem cells while maintaining normal expression of pluripotent cell markers compared to wild type control cells (WT). Directed differentiation of WT and EGFRt hPSCs into beta-like cells displayed comparable efficiency in the generation of insulin positive cells (40-60%). We can successfully transduce the EGFR CAR with upwards of 70% efficiency. These CAR-Tregs are stable and still express key Treg markers such as FOXP3, HELIOS, and CTLA-4. EGFR CAR-Tregs demonstrate increased activation when co-cultured with EGFRt hPSCs and can suppress activation of effector T cell proliferation. Current experiments include assessing the ability of EGFRt CAR-Tregs to suppress the cytotoxicity of human allogeneic CD8 T cells or autoreactive CD8 T cells during in vitro co-cultures with WT versus EGFRt cells.

Conclusions

We have successfully integrated a truncated form of EGFR into our human stem cell line to serve as a unique target for CAR-Treg activity at the site of beta cell engraftment. We observe efficient transduction of EGFR CAR into our human Treg population, and these CAR-Tregs are robustly activated when co-cultured with our EGFRt overexpressing stem cell line. Based on these promising preliminary results, we predict that the addition of regulatory CAR-T cells will suppress autoreactive and alloreactive T cell activation in a highly localized manner thus ultimately provide long term sBC graft survival without systemic immunosuppression.

68: Michael Guyot (University of Florida)

Abstract Title

Development of a MACSima imaging cyclic staining (MICS) panel to evaluate exocrine pancreatic pathology in type 1 diabetes

Authors

Michael Guyot, MacKenzie Williams, Benjamin Bumgarner, Maigan Brusko, Martha Campbell-Thompson, Clive Wasserfall, Brittany Bruggeman

Purpose

Exocrine pancreatic mass and function are reduced by 20-50% in type 1 diabetes (T1D). However, mechanisms for these changes are not well understood. MACSima imaging cyclic staining (MICS) technology allows for the immunofluorescent imaging of 100+ target proteins within a single sample of interest. We sought to develop an immunofluorescent protein panel that will allow us to evaluate exocrine, endocrine, vascular, and immune-associated pancreatic protein expression and their interaction within T1D donors.

Methods

Based on prior studies, we developed a staining panel and procedure to evaluate 25 markers of exocrine and endocrine protein expression, their relation to each other, and to vascular supply and adaptive immune responses. These have been tested in two control and one T1D formalin-fixed paraffin embedded (FFPE) tissue sections.

Summary of Results

Preliminary images have been collected with successful imaging of several exocrine and endocrine pancreatic proteins. However, continued optimization is needed to minimize autofluorescence, image focus and concentration on regions of interest, and antibody staining. Further studies will be completed to optimize the protein staining and processing pipeline.

Conclusions

While it is still in its infancy, high content imaging using MICS technology seems to be a valuable tool to examine exocrine pathology in T1D. Further studies will be completed to employ this novel technology to its fullest potential in tissue analysis.

69: Roberto Castro-Gutierrez (Barbara Davis Center for Diabetes, University of Colorado Anschutz Medical Campus)

Abstract Title

Discovery of novel autoimmune beta cell antigens using ribosome profiling

Authors

Roberto Castro-Gutierrez, Kathryn Walters, Amber Baldwin, Neelanjan Mukherjee, Holger Russ

Purpose

The discovery of novel autoreactive T cells that target β cells during type 1 diabetes (T1D) has been challenging due to our limited knowledge in autoreactive β cell antigens. Most of these antigens are derived from canonical open reading frames (ORFs) of protein coding genes such as preproinsulin and chromogranin A. However, a defective ribosomal product (DRiP) was recently identified from the insulin mRNA (INS-DRiP). This antigen is the result from the usage of an alternative translation start site. The purpose of this study is to use an unbiased approach to discover similar autoreactive β cell antigens. This study aims to close the knowledge gap between novel β cell antigens and the discovery of autoreactive T cells.

Methods

In order to identify novel autoreactive β cell antigens we performed ribosome profiling in stem cell derived β cells (sBCs). Human pluripotent stem cells (hPSC) were differentiated into sBCs in order to generate an abundant source of human β cells. sBCs were then infected with a flag-tagged ribosomal protein (RPL22-Flag) driven by the rat insulin promoter. This resulted in enrichment of β cell specific expression of RPL22-Flag. We then performed IP-ribosome profiling and RNA-seq on sBC and found that the data was of excellent quality exhibiting 3-nucleotide periodicity consistent with translated ORFs and high correlation within biological replicates. Next, we performed de novo annotation of the translatome for each biological condition using RiboCode. Finally, we filtered these results to retain only high-confidence non-canonical ORFs (i.e. not known protein coding ORFs) specific to sBC cells.

Summary of Results

Preliminary results from ribosome profiling show that sBCs are enriched for bona fide β cell genes such as PDX1, NKX6.1 and Insulin, increasing the confidence of this method. β cell specific ribosome profiling revealed translation of the previously described INS-DRiP antigen encoded in the insulin mRNA. We confirmed that the DRiP antigen was indeed translated using immunofluorescence staining. Furthermore, we show that T cells expressing the INS-DRiP TCR become activated when co-cultured with our sBCs, demonstrating that the DRiP antigen can also be presented and activate T cells. In addition to the INS-DRiP, we identified at least 275 additionally novel translated ORFs found only in β cells that may represent novel antigens. This opens the door for the identification of novel autoreactive T cells that might be involved in a break in tolerance and T1D pathogenesis.

Conclusions

Ribosome profiling identified at least 275 novel translated ORFs in human β cells. We validated the identification of INS-DRiP as a β cell antigen that is translated and can activate autoreactive T cells. Our data represents a valuable peptide database for the search of novel autoreactive antigens in β cells. We believe that our data will improve our knowledge in β cell biology and accelerate the discovery of novel autoreactive T cells implicated in T1D pathogenesis.

70: Wei-Jun Qian (Pacific Northwest National Laboratory)

Abstract Title

Single Islet Proteomics Profiling of Intra-donor Heterogeneity in Presymptomatic Autoantibody Positive Subjects Reveals an Immune Signature of Progressive Islet dysfunction

Authors

Ying Zhu, An Fu, Sarah Williams, Elizabeth Butterworth, Tyler Sagendorf, Yumi Kwon, Adam Swensen, Jing Chen, Rohit Kulkarni, Clayton Mathews, Martha Campbell-Thompson, Wei-Jun Qian

Purpose

Type 1 diabetes (T1D) is a complex autoimmune disease, and its clinical diagnosis is preceded by an asymptomatic phase where autoimmunity and progressive β-cell dysfunction occur. As it is not ethical to obtain longitudinal human pancreas tissue samples, recent advances in single cell 'omics open novel opportunities to study the "pseudo-time" of disease progression by analyzing cellular heterogeneity using samples from organ donors with pre-diabetes. Herein, we explore the intra-donor islet heterogeneity in presymptomatic, autoantibody positive (AAb+) donors by applying single islet proteomics with the goal of revealing a detailed mechanistic understanding of early β-cell dysfunction in T1D.

Methods

Human fresh frozen islet sections from presymptomatic, multiple AAb+ cases from nPOD program were isolated by laser microdissection (LMD) following islet phenotyping on adjacent sections by immunofluorescence for insulin and CD3. Each islet section was collected into separate wells for single islet proteomics analysis. 102 and 104 islet sections were collected from two multiple AAb+ donors (nPOD case IDs 6450 and 6521), respectively. Single islet proteomics based on NanoPOTS-LC-MS/MS technology was performed on all islet sections to explore intra-donor islet heterogeneity on a Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer. Label-free quantification data were analyzed using the FragPipe data analysis pipeline.

Summary of Results

To identify the molecular determinants of early β-cell inflammation and stress, we have conducted highly sensitive single islet proteomics analysis of ~100 islet sections per donor isolated by LMD from two multiple AAb+ donors with islet subpopulations phenotyped by immunofluorescence. The large-scale single islet proteomics analyses using label-free quantification resulted in quantification of 4500-5000 proteins across all islet samples. UMAP (Uniform Manifold Approximation and Projection) clustering analysis reveals multiple islet clusters for each donor with several islet clusters displaying loss of pancreatic beta cell and delta cell markers, indicative of beta cell dysfunction. Moreover, the weighted correlation network analysis (WGCNA) clearly identifies an immune-related protein cluster (immune signature), which correlates the CD3 phenotyping data. Based on this "immune signature", the single islets from each donor can be clustered into three "subtypes" of immune-low, immune-moderate, immune-high, respectively, reflecting a "pseudo-time" of progression to beta cell dysfunction in presymptomatic cases. While this work is still preliminary in that two donors were profiled at the single islet levels, it is noteworthy that the patterns of 'immune signature" and islet subtyping are consistent between both donors. This work demonstrates the potential of in situ near-single-cell proteomics for exploring cellular heterogeneity to obtain mechanistic information related to disease progression. Further work in validating the discovery results in additional mAAb+ donors is still ongoing in conjunction with matched control donors.

Conclusions

Single islet proteomics analysis of intra-donor heterogeneity in presymptomatic AAb+ donors revealed interesting molecular level information related to the early progression of autoimmune mediated islet dysfunction in human T1D

PATHOLOGY

71: Camila Sarcone (University of Florida)

Abstract Title

Chronic pancreatitis and acinar atrophy by histopathology characterize young nPOD donors with reduced pancreas organ weight and may precede this finding in the progression to type 1 diabetes

Authors

Camila Sarcone, Lisa Turk, Laura Jacobsen, Martha Campbell-Thompson, Brittany Buggeman

Purpose

Prior studies have found reduced relative pancreas weight (RPW; PW [g] / body weight [kg]) in type 1 diabetes (T1D) and islet autoantibody positive (AAb+) organ donors and increased rates of acute and chronic pancreatitis by histopathology in T1D donors. However, the relationship between these findings is not known. We aimed to determine the prevalence of exocrine pathology (chronic pancreatitis, acute pancreatitis, acinar atrophy, fatty infiltrate, and interstitial fibrosis) in T1D, single AAb+, multiple AAb+, and control subjects and its association with RPW. We hypothesized that exocrine pathology would be increased in multiple AAb+ and T1D but not in single AAb+ donors and that findings would be associated with reduced RPW.

Methods

All nPOD subjects with T1D (n=116), single AAb+ (n=17), multiple AAb+ (n=7), and controls (n=139) available through June 2022 whose age at death was 25 years or younger were included. Donor demographics and RPW were obtained from the nPOD DataShare portal. Histopathologic images were reviewed on the nPOD online pathology database for the presence of insulin positivity, insulitis, acute (acinar cell loss, polymorphonuclear cell infiltrate, fat necrosis, and hemorrhage) and chronic (acinar cell loss, mononuclear cell infiltrate, and fibrosis) pancreatitis, acinar atrophy, fatty infiltrate, and interstitial fibrosis. Fisher's exact tests, t-tests, and Pearson correlation coefficients were used for categorical and continuous data, respectively, to compare donor category, histopathologic results, RPW, age at T1D onset, duration of T1D, and C-peptide levels. Data were analyzed and graphed using SAS 9.4 (Cary, NC).

Summary of Results

Subjects with chronic pancreatitis ($p=0.016$) and acinar atrophy ($p<0.0001$) were more likely to have a smaller RPW both generally and within T1D donors ($p=0.0012$ and $p=0.04$, respectively). Donors with a single AAb+ did not have any significant differences in RPW or exocrine histopathology. Donors with multiple AAb+ were more likely to have chronic pancreatitis ($p=0.01$) and acinar atrophy ($p=0.03$) but did not have significant differences in RPW. T1D donors were more likely to have histologic findings of acute pancreatitis ($p=0.0005$), chronic pancreatitis ($p<0.0001$), acinar atrophy ($p<0.0001$), and smaller RPW ($p<0.001$); with a longer diabetes duration correlated with smaller RPW ($r=-0.43$, $p<0.0001$).

Conclusions

In this young cohort, RPW was significantly smaller in donors with acinar atrophy or chronic pancreatitis, both in the overall cohort and among T1D donors, indicating that the smaller pancreatic size seen in T1D may be a biomarker for these histopathologic changes. Acinar pathology was present in donors with multiple AAb+ despite a lack of difference in RPW (though the sample size was small), suggesting that inflammatory changes within acinar tissue may precede loss of pancreatic volume.

72: Estefania Quesada-Masachs (La Jolla Institute for Immunology)

Abstract Title

Novel highly multiplexed platform unravels a potential role for S100 proteins in type 1 diabetes

Authors

Estefania Quesada-Masachs, Katharina Eschelbach, Samuel Ziberman, Niyati Banka, Sara McArdle, Ericka Castillo, Zbigniew Mikulski, Matthias von Herrath

Purpose

Type 1 diabetes (T1D) is characterized by autoimmune destruction of insulin-producing beta cells, but the events that drive this destruction have not been fully understood yet. In the present study we use a highly multiplexed novel platform that allows the staining of a single slide of human tissue with up to 18 markers (Orion, RareCyte), to characterize and quantify the distribution of individual immune cell populations and other proteins in whole pancreatic tissue sections to delineate the pancreatic microenvironment.

Methods

Formalin fixed paraffin embedded (FFPE) pancreatic tissue sections from a non-diabetic and three donors with T1D were stained with an IF 16-marker panel with antibodies for nuclei, CD31, CD45, CD4, CD8a, FOXP3, CD45RO, CD68, CD163, CD20, CD11c, PD-1, Ki67, PD-L1, S100 (alpha) and LAG3. Whole tissue sections were scanned in high resolution with Orion (Rarecyte). Previously, consecutive pancreatic sections were stained with Insulin, CD68 and HLA class II using conventional IF, and whole tissue sections were scanned with the Zeiss AxioScan Z1 slide scanner. Quantification of fluorescent signal and cells throughout the entire tissue section and in regions of interest (islet, peri-islet, and exocrine) was performed with a semi-automated approach using supervised machine learning algorithms in QuPath. Additionally, spatial analyses are being conducted and, with unsupervised machine learning algorithms in CytoMap, we are analyzing cellular clusters and cellular neighborhoods in the whole tissue and in the regions of interest described above.

Summary of Results

Whole tissue data containing 1073 islets and more than 5 million cells was retrieved. Overall, T1D tissues had a higher density of T cells and APCs in the islet, peri-islet and exocrine areas. The proportion of T cells, specially CD4 T cells, exhibiting a memory and/or exhausted profile was also higher in the T1D cases in comparison to the healthy control. We found no cells expressing LAG3 in the pancreatic sections studied. A striking observation was the high expression of S100 proteins in the peri-islet and exocrine regions of the T1D donors (also increased but to a lesser extend in the islets). In the T1D patients, the percentage of S100 positive cells in the peri-islet regions correlated moderately with a higher percentage of Insulin+ cells and with a lower percentage of beta cells expressing HLA class II in those islets, suggesting a potentially protective role for these cells in the diabetic islets. So far, the unsupervised spatial analysis has identified nine cellular clusters in the islet and peri-islet regions. The distribution of the cellular clusters in the T1D donors is relatively homogeneous in the IDIs (insulin-deficient islets) but very heterogeneous in the ICIs (insulin-containing islets) even from the same individual. The analysis of cellular neighborhoods is currently ongoing.

Conclusions

Even though our results are still preliminary this data suggest a role for S100 proteins in T1D. We have not fully elucidated its mechanism yet, but this preliminary data suggests a protective role. S100 proteins can be released in the context of tissue damage as endogenous damage-associated molecular patterns (DAMPs), bridging responses between the nervous and the innate immune systems and triggering adaptive immune responses. The results from the spatial analysis of cellular neighborhoods will provide more insight about the S100 cellular networks in health and disease. Highly multiplexed technologies are contributing to better understand the mechanisms involved in T1D. The high quality of data obtained with the Orion technology allowed for a complex and quantitative analysis, proving that this technology is efficient and suitable to study diabetic pancreata. Further studies are highly needed in the T1D field in order to better understand the cross-talk dynamics of immune, endocrine, and neural cells in T1D and those new approaches along with the novel image analysis strategies will aid in providing more knowledge

73: Kathrin Maedler (University of Bemen)

Abstract Title

Enteroviral infections are not associated with Type 2 Diabetes

Authors

Shirin Geravandi, Huan Liu, Alberto Pugliese, Kathrin Maedler

Purpose

For more than a century, enteroviral infections have been associated with autoimmunity and Type 1 Diabetes (T1D). The increased presence of enteroviral RNA in the pancreas from organ donors with T1D has just been confirmed in a large meta-analysis. Virus responses evoke chemokines and cytokines, the "cytokine storm" circulating through the body and attack cells especially vulnerable to inflammatory destruction. Uncontrolled viral response pathways repeatedly presented during childhood highly correlate with autoimmunity and T1D.
Whole intra-islet inflammation is a major trigger of beta-cell failure in both T1D and T2D. The genetic contribution of islet inflammation pathways is apparent in T1D, with several mutations in the interferon system. In contrast, in T2D, gene mutations are related to glucose homeostasis in beta-cells and insulin target tissue and have not been identified within viral response pathways.
Therefore, the current study evaluated whether enteroviral RNA can be found in organ donors with T2D and its association with disease progression.

Methods

Pancreases from well-characterized 29 organ donors with T2D and 16 age- and BMI matched controls were obtained from the network for Pancreatic Organ Donors with diabetes (nPOD) and were analyzed in duplicates. Results were confirmed in a second cohort of pancreases from the National Disease Research Interchange (NDRI; 15 controls and 18 T2D). Single molecule FISH analyses were performed using three probe sets to detect positive strand enteroviral RNA; pancreas sections were co-stained by classical immunostaining for insulin and glucagon.

Summary of Results

There was no difference in the presence or localization of enteroviral RNA in control nondiabetic and T2D pancreases; viral infiltration showed large variation in both groups ranging from 0-94 virus+ cells scattered throughout the pancreas, most of them in the exocrine pancreas. Very rarely, a single virus+ cell was found within islets or co-stained with CD45+ immune cells. Only one single T2D donor presented an exceptionally high number of viruses, similarly as seen previously in T1D, which correlated with a highly reduced number of beta-cells.

Conclusions

Based on the analysis of enteroviral RNA by smFISH of currently available organ donors from the nPOD cohort and its confirmation with pancreases from the NDRI cohort, no association of enteroviral infection and T2D diabetes could be found. Despite great similarities in inflammatory markers in islets in T1D and T2D, long-term enteroviral infiltration is a distinct pathological feature of T1D-associated autoimmunity and in T1D pancreases.

74: Melinee Dsilva (New York Medical College)

Abstract Title

12-Lipoxygenase Expression in Pancreatic Islets from SARS-CoV2 Infected Decedents

Authors

Melinee Dsilva

Purpose

SARS-CoV2 infection can have direct and indirect effects on human pancreatic islets leading to hyperglycemia and onset of diabetes. Data indicate that new diabetes diagnosis increased 1.7-fold during acute COVID-19 and this increased risk remained elevated during both post-acute and one year after having the infection. Recent studies also show specific SARS-CoV2 viral RNA with the presence of immature insulin granules in postmortem pancreatic tissues from patients who had COVID-19. 12-Lipoxygenase (12-LOX) is an established inflammatory pathway leading to pancreatic beta cell dysfunction and death in both Type 1 and Type 2 diabetes mellitus (DM). This study investigated the expression of 12-LOX in pancreatic sections from decedents with COVID-19 compared to decedents without COVID-19.

Methods

We evaluated 12-LOX protein expression using high resolution confocal microscopy of islets from 4 postmortem samples of decedents with well characterized COVID-19 and SARS-CoV2 positive lung tissue. We compared this with 12-LOX expression in 3 postmortem pancreatic samples from decedents without COVID-19. We also evaluated 15-Lipoxygenase(15-LOX) protein expression using a specific antibody to determine the selectivity for 12-LOX.

Summary of Results

The data showed positive expression of 12-LOX protein in islets from decedents who had COVID-19. The staining was specific for 12-LOX since no 15-LOX staining was seen in these samples. 12-LOX expression was absent in islets of postmortem samples from decedents without COVID-19. The data Indicates that 12-LOX expression is increased in islets in autopsy samples from decedents who had SARS-CoV2 infection. 12-LOX expression is specific since 15-LOX staining was absent.

Conclusions

The data suggests that 12-LOX may lead to pancreatic beta cell damage and metabolic decline in people with COVID-19 infection. If confirmed by more direct studies in vitro and in vivo, this could indicate a new therapeutic target to reduce risk of diabetes development in people with from SARS-CoV-2 infection.

75: Noel Morgan (University of Exeter)

Abstract Title

Selective sparing of the head region during type 1 diabetes-associated loss of pancreatic mass

Authors

Sarah Richardson, Noel Morgan

Purpose

A considerable weight of evidence has accumulated suggesting that pancreatic mass and volume are decreased in subjects with type 1 diabetes relative to people of equivalent age. This does not appear to be due simply to the associated loss of beta-cell mass since the overall decline is much greater than can be accounted for by changes in islet cell number. Rather, it appears that both islet cells and the surrounding acinar tissue are altered morphologically in type 1 diabetes, consistent with a more fundamental variation in pancreatic architecture. Consistent with the decline in pancreatic weight is evidence for reduced zymogen secretion by pancreatic acini in type 1 diabetes coupled with an increase in parenchymal inflammation. A similar trend has also been reported in the first-degree relatives of subjects with type 1 diabetes but it remains unclear whether loss of factors that regulate pancreatic weight are causative for type 1 diabetes or if they occur as a secondary consequence of inflammation and beta cell loss. In the present study, we have considered these questions and have examined whether the decline in pancreas mass and volume occurs uniformly across the gland in type 1 diabetes or if certain regions are affected differentially.

Methods

Data on regional pancreas weight were analysed for a wide range of organs from the nPOD and where available the EADB collection and the comparative pancreatic weights compared in relation to age at organ donation for control subjects (n=178), autoantibody positive (AAb+) individuals (n=33) and those with type 1 diabetes (n=128). The ratio of the respective weights of the head vs tail regions were calculated and examined in relation to the age of each subject and, where relevant, to disease duration and the age at disease onset. The acinar cell density was also calculated in the head and tail regions and compared between control subjects and those with type 1 diabetes.

Summary of Results

Cross-sectional analysis revealed that, in control subjects, pancreas weight increased almost linearly with age during the first 10 years of life. It continued to increase beyond this period, albeit at a decreasing rate, for a further 10 years, after which pancreas weight reached a plateau. A similar pattern was seen in individuals who were AAb+ at the time of death and no discernible differences were seen relative to controls. By contrast, among people with type 1 diabetes, pancreas weight was reduced relative to controls at all ages, including in children diagnosed within the first 5 years of life. For those with type 1 diabetes who had reached beyond the age of 20 years at their death, the pancreas weight was typically reduced by at least 50% relative to similarly aged controls. Visual inspection of organs revealed, however, that this decrease was not uniform across the gland but that the PP-rich head region was spared selectively. To evaluate this more formally, the ratio of the weights of each region (head vs tail) in donors >4y in age was calculated and it was clear that this ratio was increased relative to control and AAb+/++ subjects in those with type 1 diabetes (Median (IQR) ratio PH to PT – ND (n=124): 1.10 (0.92, 1.38); AAb+/++ (n=29): 1.07 (0.87, 1.34); T1D (n=93):1.46 (1.13, 2.00). ND v T1D, $p<0.0001$; AAb+/++ v T1D $p=0.0007$). In controls, the ratio between the weights of the head and tail regions was unchanged with age over a period of 60 years from early childhood (despite the overall growth seen in the early years) consistent with a parallel increase in relative size during growth and development. By contrast, in subjects with type 1 diabetes the ratio between the head and tail regions increased with age ($p<0.001$). A trend towards an increase in relative weight of the pancreas head was also evident with increasing disease duration in subjects with type 1 diabetes and this was independent of age at onset.

Conclusions

We confirm that pancreas weight is reduced dramatically in people with type 1 diabetes compared to controls and that this is seen at all ages, irrespective of the age at onset or disease duration. However, the loss of pancreatic weight occurs selectively, with a much greater loss in the tail of the organ while the head region is relatively spared. As a result, the ratio of the weight of these regions is increased in type 1 diabetes and this becomes more evident with increasing age and disease duration. Taken together, the results imply that a selective trophic effect occurs in the pancreas head during diabetes which leads to preservation of the acinar cells in this region. This is not mediated by the selective preservation of beta-cells since these are lost across all regions of the gland. We hypothesise that a trophic factor is present selectively in the head region of the pancreas which promotes this preferential preservation in subjects with type 1 diabetes.

76: Peristera Petropoulou (Helmholtz Center Munich)

Abstract Title

Dissecting the role of HLA-I elevation and hyperexpression before and after Type 1 Diabetes onset

Authors

Peristera-Ioanna Petropolou, José Zapardiel-Gonzalo, Teresa Rodriguez-Calvo

Purpose

HLA-I hyperexpression is one of the hallmarks of Type 1 Diabetes (T1D). Previous studies aiming to decipher the role of HLA-I hyperexpression were performed on a limited number of islets of autoantibody-positive (AAb+) and T1D donors and

were focused mainly on its expression in alpha and beta cells. Abrogated proinsulin processing is a clear marker of beta cell stress and a potential pathogenetic mechanism, yet its role in T1D remains obscure. Here, we propose a rigorous analysis workflow using mean islet HLA-I fluorescence intensity as surrogate for protein expression. We correlate HLA-I expression with insulin and proinsulin expression to elucidate if certain islets with abnormal expression are a preferential target for immune cells.

Methods

Formalin-fixed paraffin-embedded (FFPE) sections from the pancreatic tail of 24 donors were obtained through nPOD. All disease stages were represented in the analyzed samples by including 6 non-diabetic (ND), 4 single auto-antibody positive (sAAb+), 4 double auto-antibody positive (dAAb+), 1 T1D with recent-onset (<1 year disease duration), 4 T1D with short duration (1-5 years disease duration) and 2 T1D with long duration (6-7 years disease duration). We stained sections for Insulin (INS), Proinsulin (PI), Glucagon and CD45+. A consecutive section was stained for HLA-ABC, Chromogranin A, CD3 and CD8. Slides were digitalized in an Axio Scan.Z1 slide scanner (Zeiss). Whole-slide images (WSI) were analyzed with QuPath and the mean fluorescence intensity per islet was quantified. To avoid introducing bias by handpicking islets, we categorized the islets into hyperexpressing (H), elevated (E) and normal (N) by setting distinctive intensity thresholds. We analyzed all the hyperexpressing and elevated islets, as well as 30 randomly selected normal islets per donor. We extracted data regarding the % of beta cells, % of alpha cells, %INS+ cells, %PI+ cells, PI:INS, % and density of CD45+ cells, and islet cell density. Analysis of more donors is currently ongoing.

Summary of Results

Out of the 6164 detected islets from 24 donors, 306 (5,0%) had elevated HLA-I and 112 (1,8%) had hyperexpression. An additional 720 islets were randomly selected for the normal HLA-I category. We saw a tendency for decreased HLA-I expression in all the analyzed sAAb+ donors, whereas a subset of islets from dAAb+ and T1D donors had increased expression. By plotting intensity histograms, we observed that a larger number of islets have increased HLA-I intensity in dAAb+ and T1D donors, compared to ND. Interestingly, the HLA-I intensity histogram of one dAAb+ donor (nPOD 6267) resembled that of the T1D donors (T1D-like histogram). In general, we found more elevated than hyperexpressing islets (ratio 5:1 in dAAb+ and 5:2 in T1D) and higher proportions of elevated and hyperexpressing islets in dAAb+ (3.8% E and 0.9% H) and T1D (18.5% E and 7.8% H) donors, compared to the ND donors (2.1% E and 0% H). The proportion of infiltrated islets with elevated and HLA-I hyperexpression was significantly increased in T1D and was especially high for donors with recent onset (35% of E islets, 60% of H islets) and short duration of the disease (25% of E islets, 62% of H islets). HLA-I intensity positively correlated with the proportion and density of CD45+ cells per islet. Furthermore, preliminary results show that elevated and hyperexpressing islets of T1D donors have a reduction in % of beta cells, % of INS+ cells, % of PI+ cells and in the PI:INS, while the % of alpha cells and % CD45+ intra-islet cells are increased, compared to normal ND islets. The same results are observed in a smaller scale in the dAAb+ donors except for the PI:INS that shows a tendency for increase.

Conclusions

Our data shows that HLA-I elevation and hyperexpression start at the dAAb+ stage and peak shortly after T1D onset. We hypothesize that dysregulation of HLA-I expression might start already at the sAAb+ stage, where we observe lower HLA-I expression. This could result in reduced T-cell education and breech of self-tolerance. HLA-I downregulation could be a marker of progression to multiple AAb and the shift in the intensity distribution (histogram) of dAAb+ donors a marker of progression to disease onset. Furthermore, elevated and hyperexpressing islets differ in almost all the analyzed parameters in the dAAb+ and T1D stage, revealing different islet states. Elevated HLA-I in dAAb+ might show an advanced dysregulated state where the islet starts to present more antigens and attract immune cells. Hyperexpression in dAAb+ marks the beginning of islet destruction, since we observe a noticeable reduction in the proportion of beta cells. Elevated HLA-I in T1D represents either: 1) the beginning of islet destruction (in shorter duration of the disease, similar to dAAb+) or 2) a state where beta cells are mostly destroyed, and islets consist mainly of alpha cells (longer duration of the disease). Based on the higher proportion of infiltrated islets observed in T1D donors and the correlation with CD45+ infiltration, HLA-I hyperexpression is a state of “active and intense beta cell destruction” that peaks shortly after onset (0-5 years). This information is critical to understand disease pathogenesis and progression, and to inform mechanistic studies and clinical trials aiming to preserve beta cell function and to stop the immune attack.

77: Peristera Petropoulou (Helmholtz Center Munich)

Abstract Title

The human pancreas unveiled: digital pathology quantifies alterations in islet composition, hormone and prohormone convertase expression and immune infiltration before and after T1D onset

Authors

Peristera-Ioanna Petropolou, Severin Boulassel, Teresa Rodriguez-Calvo

Purpose

The interaction between beta cells and the immune system has recently become a major research focus in type 1 diabetes (T1D). Beta cells might be triggering their own demise, in part due to increased cellular stress, aberrant processing and accumulation of proteins, which could trigger or potentiate beta cell specific immune responses. Our aim is to fill the knowledge gap regarding the expression and localization of proinsulin, insulin and its processing enzymes, how it changes with disease progression, and if it might correlate with immune infiltration.

Methods

Formalin-fixed paraffin-embedded (FFPE) sections from the pancreatic tail of 24 donors were obtained through nPOD. All disease stages were represented in the analyzed samples by including 6 non-diabetic (ND), 4 single auto-antibody positive (sAAb+), 4 double auto-antibody positive (dAAb+), 1 T1D with recent-onset (<1 year disease duration), 4 T1D with short duration (1-5 years disease duration) and 2 T1D with long duration (6-7 years disease duration).Three individuals with T2D were also included for comparison. Sections were stained for insulin (INS), proinsulin (PI), PC1/3, PC2, CPE, glucagon, and CD45. Whole tissue sections were scanned using an Axio Scan.Z1 (Zeiss) slide scanner and images were analyzed with QuPath.

Summary of Results

Islet density decreases with disease progression, while the mean islet area tends to be larger in T1D. In dAAb+ individuals, the proportion and density of beta cells shows a tendency for increase, while the proportion and density of alpha cells a tendency for decrease, compared to ND. This is followed by a significant increase in both, proportion and density of alpha cells in T1D. Following a similar trend, INS- and PI-positive cells, and PI:INS increase with disease progression, but decrease in T1D. Furthermore, we classified the islets into 3 major islet types: insulin-deficient (IDIs, 0% of beta cells), poor insulin-containing (pICIs, >0 - ≤10 % beta cells), and insulin-containing islets (ICIs, >10% beta cells). IDIs and pICIs are more frequent in dAAb+ and T1D individuals and as expected, ICI density dramatically decreases in T1D. Islet cellularity tends to increase in ICIs compared to IDIs in all donor categories. Interestingly, T1D individuals have the highest cellularity in all islet types, compared to the rest of the donor groups. A preliminary analysis of prohormone convertases expression showed that all three enzymes show a tendency for increased expression before T1D onset. In T1D, PC1/3 expression is reduced and PC2 is increased, whereas CPE shows only a tendency for increase. We are currently investigating if these changes in expression correlate with beta or alpha cell proportion. Analysis of immune infiltration revealed high CD45+ cell density in endocrine and exocrine compartments during disease progression, being ICIs the islet type with the highest degree of infiltration. Of note, some dAAb+ individuals had the highest endocrine CD45+ density, indicating that immune infiltration is already present at early stages of the disease.

Conclusions

Analysis of more than 6500 islets from 24 donors at all disease stages showed distinct differences in islet composition, hormone and prohormone convertases expression and immune infiltration before and after T1D onset. The increase in the mean islet area in T1D donors is probably due to the increase in islet cellularity of these donors. dAAb+ donors show tendencies towards higher proinsulin, PC1/3, PC2, CPE and insulin expression, while only PC2 and CPE upregulation persists after onset.

The increase in PI+ cells is more pronounced than that of INS+ and this results in an increase of the islet PI:INS in dAAb+ donors. By making correlations among PI, INS and the prohormone convertases, as well as with immune infiltration, we will be able to elucidate the association between alterations in the proinsulin processing pathway and one of the hallmarks of T1D. But above all, we strive to quantify through digital pathology all these changes that are evident in the beta cells of AAb+ donors before disease onset.

TYPE 1 DIABETES ETIOLOGY & ENVIRONMENT

78: Agnes Klochendler (Hebrew University of Jerusalem)

Abstract Title

RNA Editing Deficiency in Pancreatic Beta Cells Induces Heterogeneous Interferon Response, Islet Inflammation and Diabetes

Authors

Shani Peleg, Udi Ehud Knebel, Roni Cohen-Fultheim, Erez Levanon, Roy Novoselsky, Shalev Itzkovitz, Agnes Klochendler, Yuval Dor

Purpose

Genetic and experimental studies support a role for an anti-viral type I interferon (IFN-I) response to double-stranded RNA (dsRNA) in the early stages of Type I diabetes (T1D). However, a viral etiology has not been established, raising the possibility that the interferon response results from an endogenous source of dsRNA. Indeed, a recent genetic study implicated reduced editing levels of double-stranded RNA (dsRNA) in the development of autoimmune diseases, including T1D. Thus, we hypothesized that defects in RNA editing in pancreatic islet cells may cause the accumulation of endogenous dsRNA and initiate islet inflammation, a hallmark of early-stage T1D.

Methods

We have generated a mouse model for deficient RNA editing, using knockout of the RNA editing gene Adar in pancreatic beta and alpha-cells. We have used fluorescent immunostaining of pancreatic sections to characterize islet inflammation and the phenotype of beta-cells following Adar disruption. To elucidate the molecular basis for islet inflammation in mutant mice, we have performed bulk RNA-sequencing on FACS-sorted beta and alpha cells. Finally, we have used single-cell RNA sequencing and single-mRNA molecule fluorescent in situ hybridization to reveal cellular heterogeneity during IFN-I responses.

Summary of Results

Inactivation of Adar in mouse beta-cells strikingly recapitulates key features of early T1D: a strong interferon response, causing massive insulitis; disrupted expression programs in beta-cells culminating in beta-cell death and diabetes; and preservation of alpha-cells. Moreover, Adar inactivation in alpha-cells caused only a minor interferon response without inflammation, and Adar-null alpha-cells were spared even in the context of inflammation triggered by Adar-knockout in beta-cells. To better understand the molecular basis for the heterogeneous interferon response in islet cells, we have manipulated the metabolic milieu of pancreatic islets. We found that calcium influx, triggered by glucose metabolism in beta cells and palmitate metabolism in alpha cells, is essential for the interferon response in Adar-null beta and alpha-cells. Surprisingly, single-cell RNA-sequencing and single-mRNA molecule fluorescent in situ hybridization revealed that only a small subset of islet cells mounted an IFN-I response at any given time after Adar deletion, leading to a mosaic pattern of islet inflammation, as seen in T1D.

Conclusions

Our findings indicate that deficient editing of dsRNA in islets leads to heterogenous islet inflammation and destruction, resembling features of early stage T1D. The results also suggest that metabolic stress in islets can enhance the interferon response to boost inflammation and cause further islet cell dysfunction towards diabetes.

79: Federica Vecchio (Université de Paris, Institut Cochin)

Abstract Title

Coxsackievirus infection induces direct beta-cell killing and poor CD8+ T-cell responses

Authors

Alexia Carré, Daniil Krenkov, Zhicheng Zhou, Paola Apaolaza, Soile Tuomela, Isaac Snowhite, Orlando Burgos-Morales, Javier Perez-Hernandez, Barbara Brandao, Georgia Afonso, Clémentine Halliez, John Kaddis, Sally C. Kent, Maki Nakayama, Joelle Vinh, Yann Verdier, Raphael Scharfmann, Michele Solimena, Zuzana Marinicova, Elisa Bismuth, Nadine Lucidarme, Janine Sanchez, Carmen Bustamante, Patricia Gomez, Soren Buus, the nPOD-Virus Working Group, Sylvaine You, Alberto Pugliese, Heikki Hyoty, Teresa Rodriguez-Calvo Malin Flodstrom-Tullberg, Roberto Mallone

Purpose

Infections by Enteroviruses such as Coxsackieviruses B (CVBs) are candidate triggers for T1D. However, very little is known on how CVB impacts beta cells and viral epitope recognition by T cells. Considering the CVB vaccination trials under development, it is critical to understand the mechanisms behind the primary (CVB-mediated) and secondary beta-cell killing by anti-CVB T cells, as the latter may boost islet autoimmunity upon vaccination. We therefore identified the HLA Class I (HLA-I) viral peptidome of CVB-infected beta cells, we characterized the CD8+ T-cell response against these peptides in various tissues from healthy and T1D donors, and we explored how CVB and T cells kill beta cells.

Methods

HLA-A2- and HLA-A3-bound peptides presented by a human beta-cell line infected with different CVB serotypes were identified by mass spectrometry. We tested their recognition by circulating CD8+ T cells from CVB-seropositive healthy donors using combinatorial HLA-I multimer assays, and we defined the frequency and phenotype of cytotoxic CD8+ T-cells against CVB peptides in the blood, spleen and pancreatic lymph nodes of nPOD donors. CD8+ T-cell transductants were generated using immunodominant T-cell receptors (TCRs), and used in real-time imaging cytotoxic assays to evaluate the primary killing of infected beta cells by CVB and the secondary cytotoxic killing by CD8+ T cells.

Summary of Results

CVB infection downregulated HLA-I expression on beta cells, which led to the presentation of only few, selected HLA-bound viral peptides from both structural and non-structural proteins. Circulating CD8+ T cells recognized only a fraction of these peptides, and only another sub-fraction was targeted by effector/memory T cells. A CVB epitope fully cross-reactive with GAD was identified. The immunodominant peptides were also recognized in nPOD spleen and pancreatic lymph node T cells, with the latter expressing the exhaustion marker PD-1 and sharing expanded yet private TCRs with peripheral tissues. Using anti-CVB TCR-transduced CD8+ T cells, we observed that infected beta cells were more efficiently killed by CVB than by T cells and used filopodia to propagate infection.

Conclusions

CVB infection generates a limited CD8+ T-cell memory in terms of epitope coverage and T-cell frequency. Together with the immune-shielded CVB transfer occurring through filopodia, this may predispose to the persistent infections associated with islet auto-antibody seroconversion. The predominant CVB-mediated beta-cell killing suggests that the triggering of islet autoimmunity relies primarily on the virus releasing islet antigens rather than on the ensuing anti-viral cytotoxic T-cell

responses, possibly in the context of limited immune protection. These results lend rationale to boost such protection through CVB vaccines and provide biomarkers to follow response to infection and vaccination along the natural history of disease.

80: Hervé Perron (Geneuro-Innovation)

Abstract Title

Unrecorded chromosomal insertion sites of Human endogenous Retroviral sequences in DNA from type-1 diabetes pancreata: possible somatic DNA modification in affected tissue only?

Authors

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Purpose

T1D is an autoimmune disease whose etiology is not clearly understood, but lies at crossroads between genetic predisposition and environmental factors. Research on the human genome has so far identified numerous candidate sequences as genetic risk factors, most notably within the HLA (Human Leucocyte Antigen) region. However, an important part of the human genome has been neglected in studies conducted so far, mostly including the so-called "mobile genetic elements", which represent about half of the human genome. HERV (Human Endogenous Retroviruses) belong to this group as retro-transposable elements, and the envelope protein from HERV-W family (HERV-W ENV) has recently been associated with T1D (Levet et al. 2017; JCI insights).
Our goal was to identify genetic risk factors belonging to mobile genetic elements, with an emphasis on HERV and HERV-W-ENV genes sequences encoding HERV-W envelope protein (ENV). A long-read whole genome sequencing (WGS) approach on DNA from T1D cells using a reference assembly free approach had generated de novo assembled genomes for each sample. But, when examined for the presence of HERV-W-ENV insertions using homology-based searches (RepeatMasker) we could only identify copies from the known HERV-W copies present in the reference genome. Thus, to allow the identification of potential somatic DNA recombinations or rearrangements in certain cells of affected tissue only, we used a dedicated inverse PCR-based protocol. This allows to isolate non-inherited sequences present in affected cells only, which cannot be detected from global sequencing of total DNA extracts from multiple cells, and can hardly be addressed with appropriate area selection from frozen tissue sections for single cell sequencing.

Methods

Inverse PCR used specific HERV-W-ENV primers on DNA from pancreatic samples obtained from nPOD biobank, FL, USA. PCR products were cloned in bacterial plasmid and isolated clones were sequenced with an automated platform (Yumab, Braunschweig, Germany). Clones encompassing 3'end HERV-W ENV sequences were selected. They were analyzed with a processing pipeline designed to successively instruct a mapping algorithm and the BLAST online vehicle to analyze the available sequences and (i) compare them to the human genome database, (ii) list those clones whose (artificial) paired end reads map > 1 kb from each other (or on different chromosomes), and (iii) secure that one of the two ends overlaps with a reported HERVW element (LTR17, or homologous). The pipeline then produces a list of non-canonical HERVW elements per sample and the approximate coordinates of insertion sites, all confirmed by automated BLAST searches.

Summary of Results

Applying the procedure outlined above to pancreatic DNA of T1D patients samples from nPOD biobank, we have now identified two clones of interest, which each contain a variant HERVW copy not represented in the human genome database. Interestingly, these were found not to be associated with the regular flanking sequences (long terminal repeats, LTR) of referenced HERV-W family. These findings confirm that a pipeline has been developed that allows for the identification of patient-specific HERVW sequences.
The genomic regions of interest will also be further analyzed for disease-specific content. We will discuss the genomic regions and ORFs whose transcription may be triggered by insertion of these HERVW elements. The possibility to compare control

and patient samples, and the efficiency of the method will be discussed and compared to alternative approaches to identify disease-associated HERV copies in patients otherwise than in post-mortem affected pancreas tissue.

Conclusions

Insertion of provirus must now be confirmed by PCR using DNA from the same samples. If confirmed to be HERV somatic DNA rearrangements in affected tissues, this would explain the failure to detect specific copies in other cells, e.g., peripheral blood cells or total tissue extracts with many different cells from pancreas samples. The concept of a pathogenic HERV protein expression induced by more-or-less transient infection of pancreas tissue, e.g., by enteroviruses (Levet et al. 2019 Curr. Diab. Rep.), could then be understood as the occurrence of HERV retro-transposition or rearrangement triggered by a virus within or around infected cells.

81: Klaus Kaestner (University of Pennsylvania)

Abstract Title

How do residual beta cells escape the immune attack? Insights from single cell analysis

Authors

Elisabetta Manduchi, Michelle Lee, Helene Descamp, The Human Pancreas Analysis Program, Klaus Kaestner

Purpose

The fact that there are residual pancreatic beta cells and insulin production even in patients with long-standing type 1 diabetes has been known for decades. However, the critical question of why cytotoxic T cells do not eliminate these beta cells has not been answered. Here, our goal was to identify the transcriptomic and epigenetic changes that enable survivor beta cells to escape the immune attack.

Methods

We employed single cell RNAseq and single cell ATACseq on dozens of islet preparations of organ donors in the Human Pancreas Analysis Program (HPAP). After careful filtering for cell doublets and other artifacts, we determined differential gene expression and chromatin accessibility between survivor beta cells from organ donors with T1D to those never diagnosed with diabetes.

Summary of Results

After careful filtering, we identified more than 300 beta cells from three organ donors with T1D among the HPAP data sets. Differential gene expression analysis in a comparison to beta cells from non-diabetic organ donors confirmed the previously documented up-regulation of the HLA class I gene HLA-A, HLA-B, and HLA-C, which render beta cells more visible to T cells. However, survivor beta cells also express multiple immune-modulatory genes at high levels.

Conclusions

While surviving beta cells show activation of HLA class I genes as expected, they also exhibit increased expression of multiple immune-modulatory genes, which could render them resistent to cytotoxic T cells and explain their survival. These findings raise the tantalizing possibility that activation of immune-dampening gene programs could be used in the future to protect beta cells from immune destruction in early onset T1D or even in high risk individuals.

82: Matthew Poy (Johns Hopkins All Children's Hospital)

Abstract Title

Therapeutic and Diagnostic Strategies Targeting CADM1 during T1D pathogenesis

Authors

Chandan Sona, Andreas Patsalos, Laszlo Halasz, Krisztian Csomos, Jolan Walter, Laszlo Nagy, Matthew Poy

Purpose

Given the established role of autoreactive T-cells in mediating beta-cell destruction during Type 1 diabetes (T1D), continued emphasis remains on identifying the islet proteins that mediate immune cell infiltration that could potentially be targeted to both preserve pancreatic beta-cell mass and function, as well as prevent the immune response that initiates the pathogenesis of T1D. Cell adhesion molecule 1 (referred to as human CADM1 and mouse Cadm1) is a cell surface protein of the Ig superfamily that mediates intercellular contact between dissimilar cell types including immune, neuronal, and endocrine cells. Notably, Cadm1 (also known as Necl2) has been shown to act as a ligand for the receptor Class I–restricted T cell–associated molecule (CRTAM) present in CD8+ T-cells. Single cell RNA sequencing of immune cell populations isolated from pancreatic islets of non-obese diabetic (NOD) mice, a model of autoimmune diabetes, revealed enrichment of Cadm1 expression in islet myeloid cells (including mature macrophages) prior to disease onset. Moreover, we observed the number of CADM1+ myeloid cells was increased in islets of auto-antibody-positive (aAb+) and T1D human subjects. Here we hypothesize that inflammatory pathways provoke increased CADM1 expression in myeloid cells in islets leading to increased cell:cell contact with cytotoxic T-cells. We further hypothesize that preventing these interactions can attenuate the onset of autoimmune diabetes. In this study, we tested whether administration of an anti-CADM1 monoclonal antibody (mAb) to NOD mice reduces both immune cell number within the pancreas in addition to direct contact with islet endocrine cells thereby preventing the inflammatory response and cell death that leads to T1D.

Methods

We tested whether weekly treatment with the CADM1 mAb prevented immune cell infiltration driving beta-cell destruction and hyperglycemia in female NOD/ShiLtJ mice. We evaluated whether the CADM1 mAb treatment reduces immune cell numbers through their decreased interaction with islet cells. We also tested whether treating NOD mice weekly after diabetes onset with the CADM1 mAb could halt beta-cell destruction and restore normoglycemia. FACS analysis was performed on pancreatic cell populations to evaluate whether CADM1 mAb treatment altered immune cell numbers and/or expression of activation markers. These studies aimed to address whether treatment of the CADM1 mAb can constitute a prevention/protective measure versus a curative one from pancreatic immune cell presence in NOD mice.

Summary of Results

Our results showed that treatment of NOD mice with the CADM1 mAb antibody prevented cytotoxic destruction of beta-cells and attenuated autoimmune diabetes onset compared to female NOD mice treated with a control antibody. Furthermore, treatment of NOD mice with the CADM1 mAb antibody after onset of hyperglycemia attenuated the escalation of blood glucose levels further suggesting the antibody treatment is effective in preventing beta-cell destruction in this model. Lastly, FACS analysis of pancreatic immune cell populations isolated from NOD mice revealed treatment with the CADM1 may reduce T cell numbers and activation.

Conclusions

Together these results suggest that the CADM1 'blocking' mAb binds Cadm1 in islet cells and prevents their interaction with cytotoxic T cells leading to reduced T cell activation and beta-cell destruction. Our results illustrate the potential for pursuing

CADM1 as a therapeutic target for either preventing disease in individuals at high risk or to possibly treat individuals already living with diabetes.