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Abstracts for Annual Meeting

1: Alexia Carre (Cochin Institute)

Abstract Title

The antigenic visibility of beta cells as a driver of type 1 diabetes: insights from the HLA peptidome

Authors

Alexia Carre, Hanqing Liao, Annalisa Nicastri, Maikel L Colli, Raphael Scharfmann, Decio L. Eizirik, Nicola Ternette, Roberto Mallone

Purpose

In type 1 diabetes (T1D), beta cells are killed by autoimmune CD8+ T cells recognizing peptide-HLA Class I (pHLA-I) complexes on the beta-cell surface. However, these T cells circulate in all individuals, leading us to hypothesize that the switch from this "benign autoimmunity" to T1D may partly rely on beta cells themselves, which upon inflammation become more visible to T cells.
The current study aimed at identifying the HLA-I-bound peptides presented by human beta cells in vitro, exposed or not to the T1D early signature cytokine IFN-alpha (IFN-a).

Methods

The ECN90 human beta-cell line was exposed or not to IFN-a. pHLA-I complexes were immunoprecipitated and HLA-I-bound peptides retrieved by mass spectrometry. A complex bioinformatics pipeline was designed to identify canonical and post-translationally modified peptides, mRNA variants and neo-sequences corresponding to cis-splicing events (i.e. fusion of two non-adjacent sequences from the same protein).

Summary of Results

IFN-a increased HLA-I expression on beta-cell surface, thus yielding more peptides. IFN-a also led to an increased number of HLA-B ligands. Overall, 1,047 out of >30,000 peptides derived from beta-cell specific proteins. Granules proteins were largely overrepresented and some new source proteins were identified, namely secretogranin (SCG)2, SCG3 and chromogranin B. Some reported beta-cell antigenic proteins were absent (e.g. IGRP) or marginally represented (e.g. IAPP, GAD, ZnT8). The identity of all non-canonical peptides was confirmed by spectral matching. Mechanistic studies defined the role of the constitutive and immune proteasome in their generation.

Conclusions

Overall these results provide insights into beta-cell-specific antigen processing and presentation and how this pathway is altered upon IFN-a exposure. This will lead to a deeper understanding of the CD8+ T-cell immune response and will fuel the development of novel biomarkers.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

2: Julia Panzer (University of Miami)

Abstract Title

Targeting glutamate receptors in human alpha cells restores glucagon secretion in type 1 diabetes

Authors

Julia Panzer, Alejandro Tamayo, 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 defensive mechanism is lost, increasing the mortality risk. Thus, understanding the pathophysiological mechanisms that make alpha cells unresponsive to a drop in glycemia during disease progression is therefore of utmost is importance to improve the management of diabetes.

Methods

Studying alpha cell physiology in type 1 diabetes has met major technological roadblocks, as methods conventionally used are very difficult to apply to type 1 diabetic donors. We overcome these limitations by using living pancreas slices, which allow functional assessments of damaged and infiltrated islets within their native environment. We used slices from non-diabetic donors and donors with type 1 diabetes to determine alpha cell responses to (a) changes in glycemia, (b) agonists, antagonists, and positive allosteric modulators of glutamate receptors, and (c) reference stimuli such as adrenaline and KCl depolarization using functional recordings. 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

We found that alpha cells in slices from type 1 diabetic donors had normal glucagon content and responded to KCl depolarization but failed to respond to decreases in glucose concentration. Furthermore, we found severely dimished Ca2+ 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.

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. Our results further suggest that residual glutamate signaling can be potentiated with positive allosteric modulators to rescue glucagon secretion. These and other positive allosteric modulators are already approved to treat other conditions and could thus be repurposed to limit hypoglycemic episodes, allowing improved therapy of type 1 diabetes.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

3: Mathew Becker (University of Florida)

Abstract Title

Beta cell targeting of immune suppressive agonists in human pancreas tissue slices

Authors

Matthew Becker, Andrece Powell, Giovanna Bossi, Tara Mahon, Todd Brusko, Peter Weber, Edward Phelps

Purpose

This project investigates the application of a T cell receptor (TCR) technology to generate tissue-targeted immune suppressant bispecifics called ImmTAAI molecules. ImmTAAI molecules are comprised of a high affinity TCR-based targeting domain specific for a tissue-restricted peptide-HLA complex, fused to an immune suppressive effector domain. ImmTAAI molecules are designed to bind to target cells under autoimmune attack (eg, pancreatic beta cells) and protect them from T cell driven destruction, while not affecting the immune system elsewhere in the human body [1]. Here, we profiled the beta cell targeting specificity of ImmTAAI molecules using human pancreas slices. In addition, we characterized the ability of ImmTAAI molecules to inhibit beta cell destruction using T cell receptor (TCR) transduced T cell clones that recognize beta cell antigens.

Methods

An ImmTAAI bispecific molecule was engineered by fusing a PD-1 agonist to a high affinity TCR specific for PPI15-24, a well-characterized autoantigen found on beta cells and presented by HLA-A*02. Live human pancreas slices obtained through nPOD were incubated with CF647 labeled ImmTAAI at 2, 20, and 200 nM for one or two hours at 37°C together with AF568 labeled anti-ENTPD3 to label beta cells [2]. Pancreatic slices from HLA-A*02- donors and ImmTAAI specific for an off-target antigen were used as controls to assess non-specific binding.

Summary of Results

Confocal microscopy of live pancreas slices showed clear and specific labeling of islets with ImmTAAI at 20nM. The PPI15-24 specific ImmTAAI co-localized strongly with ENTPD3-labeled beta cells (Pearson's coefficient 0.7), whereas the off-target ImmTAAI showed poor co-localization (Pearson's coefficient 0.3). Additionally, ImmTAAI labeling in pancreas slices from HLA-A*02- donors showed minimal beta cell labeling, even at 200 nM. To test the immune suppressive function of the ImmTAAI, Jurkat T cells transduced with the diabetogenic 1E6 TCR [3] were co-cultured with K562 cells presenting PPI15-24 in HLA-A*02. Treatment of co-cultures with ImmTAAI reduced T cell IL-2 secretion by approximately 50%.

Conclusions

ImmTAAI molecules designed to inhibit T cell activation through the PD-1/PD-L1 pathway have been shown to inhibit primary T cell activation and cytotoxic functionality using in vitro cell systems [1]. Here, we provide evidence that ImmTAAI molecules maintain HLA and beta cell specificity within a complex tissue environment, paving the way for functional studies investigating T cell suppression in pancreatic tissue slices. These data strengthen the evidence that bispecific molecules have potential to limit T cell activity locally at a disease site, thus making it an attractive platform to treat T1D.
References: [1] Curnock, A. P., et al. JCI Insight, 2021. [2] Saunders, D. C., et al. Cell Metabolism, 2019. 29(3): p. 745-754.e4. [3] Skowera, A., et al. J Clin Invest, 2008. 118(10): p. 3390-3402

Preferred Presentation Format

Oral Presentation

Research Category: Novel Technologies

4: Herbert Gaisano (University of Toronto)

Abstract Title

Delta-cell exocytotic blockade by syntaxin deletion restores alpha-cell glucagon secretory response sufficient to counter insulin-induced hypoglycemia

Authors

Tairan Qin, Tao Liang, Li Xie

Purpose

The pancreatic islet is a complex micro-organ that regulates glucose homeostasis from the actions of major hormones secreted from three endocrine cell types (beta, alpha and delta cells) which become severely perturbed in type-1 diabetes (T1D), particularly in their cross-talk. A major complication in T1D is iatrogenic hypoglycemia wherein alpha-cells become insensitive to low glucose induced by insulin treatment. alpha-cell 'glucose blindness' has been attributed to delta-cell somatostatin (SST) inhibition of alpha-cell that becomes accentuated when beta-cells are destroyed in T1D, but the precise mechanism to explain these effects is not completely understood.

Methods

Syntaxin-1A is the key SNARE protein that forms the putative membrane-fusion SNARE complex mediating secretory granule exocytosis in neurons and neuroendocrine cells, including the 3 major islet cell types. We here induced delta-cell exocytosis blockade by treating Syntaxin(STX)-1A flox/flox mice with intraperitoneal injection of adeno-associated virus (AAV8)-SST promoter-Cre/RFP.

Summary of Results

Immunocytochemistry demonstrated STX-1A deletion only in the red-colored delta-cells as verified by SST and Cre antibody staining. delta-cell STX1A-KO, compared to WT mice, showed improved glucose homeostasis with larger biphasic rise in blood insulin levels during IPGTT; and ITT (insulin injection) showed improved insulin sensitivity with larger increases in glucagon release (60 min and 120 min). Whole pancreas perfusion performed on KO vs WT mice showed the following: 1) confirmed the reduction and flattening of stimulated SST secretion; 2) a higher increase in glucose (10 mM)-stimulated insulin secretion that was suppressible by exogenous SST to the same level as WT mouse pancreas; and 3) a much higher hypoglycemic (1 mM glucose) induced glucagon release that was also suppressible by exogenous SST to the same level as WT mouse pancreas. Initial exocytosis imaging with NPY-pHluorin that's specifically expressed in delta-cells in islets within pancreatic slices showed 10 mM glucose increased abundant exocytosis in WT delta-cells but with almost no exocytosis from STX1A-KO delta-cells.

Conclusions

Future studies. We plan to induce T1D in these WT and delta-cell STX-1A KO mice with streptozotocin (STZ) to assess whether the clinically-observed loss of hypoglycemia-induced glucagon release causing the 'hypoglycemic blindness' could be restored by the delta-cell STX1A deletion. We will then assess by exocytosis imaging (NPY-pHluorin) and electrophysiology of the islets within pancreatic slices prepared from these mice the effects of delta–cell exocytotic blockade caused by STX-1A deletion on whole islet alpha-cell (and also beta-cell) secretory responses and the corresponding changes in alpha-cell electrophysiology before and also after the STZ treatment. As well, we will recapitulate some of these findings in human T1D pancreatic slices vs normal pancreatic slices obtained from nPOD. This study when completed will elucidate the mechanistic basis by which delta-cell exocytotic blockade restores the alpha-cell secretory response to iatrogenic insulin-induced hypoglycemia.
Supported by Helmsley Charitable Trust and the Canadian Institute of Health Research

Preferred Presentation Format

No Preference

Research Category

Type 1 Diabetes Etiology & Environment

5: Gladys Teitelman (SUNY-Downstate Medical Center)

Abstract Title

Disruption of normal beta cell phenotypes precedes Type 1 diabetes diagnosis.

Authors

Gladys Teitelman

Purpose

Purpose: The current dearth of successful immune therapies to prevent Type 1 Diabetes (T1D) is partly due to a lack of knowledge of the concurrent changes in the beta cell phenotype. We recently discovered that beta cells of adult human islets, but not those of postnatal human or of mouse islets, are populated by cells that express proinsulin (ProIN) and variable levels of proprotein convertase 1/3(1/3), the main enzyme involved in insulin production. Islets also contain ProIN+PC1/3- and ProIN-PC1/3+ cell types. We hypothesized that in normal adult pancreas, beta cells interconvert through a cycle comprising those three cell types and that this cell-type interconversion is interrupted during progression to T1D.

Methods

Methods: sections of human pancreas from donors control, AA+ and T1[provided by the network of pancreatic organ donors (nPOD)] were immunostained for ProIN, PC1/3 and insulin and were examined using Leica TCS SP5 confocal laser scanning microscope system. The same confocal microscope settings were used to obtain all images. Two regions of the pancreas per donor and 10-25 islets per region were evaluated.

Summary of Results

Summary of results: We found that a subset of pancreatic islets of auto-antibody-positive (AA+) donor pancreata do not contain the three beta cell types found in controls. Rather, they are populated instead by a single beta cell type (a “monotype”), defined by their identical expression levels of PC1/3 and proinsulin. In contrast to AA+, islets of T1D donors exhibit monotyping defect in all islets, suggesting the increased action of an abnormal process connecting these two cohorts

Conclusions

Conclusions- The replacement of the three beta cell types of normal islets by a monotype insulin cell indicates the presence of alterations in the normal beta cell-type-cycle and suggests that factors inducing this abnormality may play an important role in the pathobiology of T1D.

Preferred Presentation Format

No Preference

Research Category

Pathology

6: Maria Redondo (Baylor College 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

Maria J. Redondo, Sarah Richardson, Daniel Perry, Charles Minard, Alice Carr, Irina Kusmartseva, Alberto Pugliese, Mark Atkinson

Purpose

The type 2 diabetes (T2D)-linked TCF7L2 variants are associated with features atypical for type 1 diabetes (T1D) suggesting that autoimmunity and beta cell loss are less severe. Hence, we determined the influence of TCF7L2 SNPs on insulin containing islet (ICI)% in T1D pancreata.

Methods

We analyzed 111 nPOD donors (www.jdrfnpod.org) with T1D who had data on the presence or absence of residual beta cells. In those donors with remaining beta cells, formalin fixed paraffin embedded (FFPE) pancreas tissue sections from regions containing beta cells (as determined by the nPOD Pathology Core) were stained for the presence of insulin-positive and glucagon-positive cells. The ICI% was calculated by quantifying the number of islets containing insulin-positive beta cells, and expressing this as a percentage of the total islets analyzed within each donor. The observed distribution of ICI% was examined to determine an interesting cutoff value. The largest difference between consecutive observations under 10% occurred between 2.2 and 6.4. Therefore, high ICI% was defined as ≥5% for this study. Mean age at T1D onset was 12.2 years (sd=7.9) (range=0-36), diabetes duration was 15.2 years (sd=13.7) (range=0-74), BMI was 24.5 (sd=4.6), 53% were male, 80% non-Hispanic white, 13% African American, and 7% Hispanic.

Summary of Results

Mean ICI% was 9.7 (sd=21.5) (range=0-92.2). At the TCF7L2 rs7903146 locus, 45.5% (50/110) of donors carried the T2D-associated T allele (41.8% as TC and 3.6% as TT). Donors with high (≥5) (n=30, 27%) vs low ICI% (<5) were older at onset (15.3±6.9 vs 11.1±7.9 years, p=0.013), had shorter diabetes duration at procurement (7.0±7.4 vs 18.3±14.3 years, p<0.001), and had higher African ancestry score (0.2±0.3 vs 0.1±0.2, p=0.043) and lower European ancestry score (0.7±0.3 vs 0.9±0.3, p=0.023). There were no significant differences in sex, BMI or TCF7L2 SNPs allele distribution but African American race was more common among high ICI (23.3%) than low ICI (8.6%, p=0.038). In multivariable logistic regression predicting high ICI % with adjustment for age of onset (p=0.086), diabetes duration (p<0.001), BMI (p=0.796), sex (p=0.417) and African American race (p=0.050), donors with the TCF7L2 rs7903146 T allele (TC or TT), compared to those without it (CC) were 2.91 times (95%CI=1.02-8.3) more likely to have high ICI% (p=0.046). A similar model was also fit including the interaction between African American race and rs7903146 T allele (p=0.021). Among African American donors, the odds of high ICI were 146.6 (95%CI=4.1-5300) times greater among donors with the T allele compared to those without the T allele. Among non-African American donors, the odds ratio was 1.7 (95% CI=0.5-5).

Conclusions

Among nPOD donors with T1D, carriers of the T2D-linked TCF7L2 variant had a higher number of residual ICIs, possibly representing a disease endotype characterized by T2D-like features and less severe beta cell loss.

Preferred Presentation Format

Oral Presentation

Research Category

Type 1 Diabetes Etiology & Environment

7: Udi Ehud Knebel (The Hebrew University)

Abstract Title

RNA editing in pancreatic beta-cells prevents aberrant innate immune activation and diabetes

Authors

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

Purpose

RNA-editing is a fundamental cellular process that involves the deamination of adenosines in RNA to inosines by adenosine deaminases acting on RNA (ADAR). In recent years, this process has emerged as crucial for dismantling endogenous dsRNA structures produced by retroelements inserted in an inverted orientation in expressed genes. When RNA editing is impaired, endogenous dsRNA accumulates and is sensed by IFIH1, which activates an anti-viral gene expression program that may lead to pathogenic inflammation. RNA editing has been studied in several organs and in cancer, yet little is known about its importance in pancreatic beta-cell biology. Furthermore, since islet inflammation is a feature of type 1 diabetes (T1D) and likely type 2 diabetes, and since GWAS revealed protective variants of IFIH1 on T1D risk, alterations in RNA deamination might contribute to islet inflammation in diabetes.
We hypothesize that disrupted RNA editing in β-cells triggers an interferon response, causing islet inflammation and potentially contributing to autoimmune destruction of beta-cells.

Methods

To test this hypothesis, we used a transgenic system to specifically delete Adar in beta-cells of adult mice. We performed metabolic assays and used histological images and gene expression profiles to characterize the effect of disrupted RNA editing on beta-cell biology and islet inflammation.

Summary of Results

We found that reduced editing results in the induction of interferon-stimulated genes in mutant beta cells, triggering a massive immune response involving the recruitment of both innate and adaptive immune cells to islets. Islet inflammation led to a disruption of the beta-cell expression program, which strikingly took place only in Adar1-deficient beta-cell cells and spared their wild type neighbors. Around 40% of mutant mice develop diabetes and in diabetic mice, Adar-deficient beta-cells are eventually eliminated. Preliminary results suggest that T-cells are not required for beta-cell destruction and diabetes in this model, whereas immune checkpoint inhibition aggravates the diabetic phenotype of mutant mice. Surprisingly, we observed an inverse correlation between the age at Adar inactivation and the severity of insulitis and diabetes.

Conclusions

Our findings reveal the essential anti-inflammatory effect of RNA-editing in beta-cells. Moreover, we present a new model for the interferon response and the recruitment of innate immune cells to pancreatic islets, mimicking processes taking place in early stages of T1D. This model will be useful for the study of beta cell-immune cell interplay in T1D pathogenesis.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

8: Zeina Drawshy (The Hebrew University)

Abstract Title

Insulin-independent assessment of human beta-cell mass

Authors

Zeina Drawshy, Daniel Neiman, Ori Fridlich, Ruth Shemer, Desmond A Schatz, Clive Wasserfall, Martha Campbell-Stuart, Benjamin Glaser, Agnes Klochendler, Yuval Dor

Purpose

Both Type 1 and Type 2 diabetes (T1D and T2D) involve a combination of reduced beta-cell mass and beta cell dysfunction. However, the extent of these defects varies among patients and they may contribute differently to etiology. Immuno-detection of insulin is central for the measurement of beta-cell mass, and serves also to assess islet insulin content for normalization of glucose-stimulated insulin secretion (GSIS) assays ex-vivo. Recent studies have reported that metabolic stress, such as prolonged hyperglycemia, can cause beta-cell degranulation, loss of identity or dedifferentiation; such phenomena may result in insulin loss and subsequent underestimation of beta-cell mass and flawed assessment of beta cell function. Thus, there is a need to develop an accurate method for counting beta cells in islets and pancreatic sections which does not depend on insulin content. To address this need, we have developed an approach for quantifying beta cells based on measuring beta-cell DNA, using beta cell-specific DNA methylation markers.

Methods

We have identified 3 loci in the human genome, each containing 5-8 CpG sites that are unmethylated in beta cells while methylated in other cell types present in the pancreas (alpha, delta, acinar, duct, endothelial cells and leukocytes). We use PCR followed by Next Generation Sequencing to analyze amplicons of bisulfite converted human islet DNA and identify beta-cell DNA. Additionally, we have developed similar methylation markers for quantifying the DNA of alpha, delta and acinar cells.

Summary of Results

Calibration experiments showed that DNA methylation-based quantification of human beta cells, as well as alpha, delta and acinar in islets and tissues, is highly specific, accurate and sensitive.
DNA analysis of material from histological sections revealed no evidence for the presence of insulin-negative beta cells in T2D, arguing against a model of massive beta cell dedifferentiation. In material from T1D and T2D donors we found a dramatic increase in the fraction of alpha cells, compared with non-diabetic donors.

Conclusions

We describe a reliable method for the quantification of beta-cells, as well as additional islet cell types, in histological sections and islet preparations, which is independent of protein markers such as insulin. The method can aid studies of beta cell identity, and the mechanisms underlying beta-cell failure in diabetes.

Preferred Presentation Format

Oral Presentation

Research Category

Novel Biomarkers

9: Giovanna Bossi (Immunocore LTD)

Abstract Title

β-cell targeted Immune Suppressive PD-1 Bispecific Agonists - a Novel Approach to Treat Type 1 Diabetes

Authors

Adam Curnock, Emma Henderson, Rita Figueiredo, Katherine Wiseman, Ronan O'Dwyer, David Overton, Veronica Gonzalez, Nicola Smit, Lorraine Whaley, Hemza Ghadbane, Shephen Hearty, Tara Mahon, Peter Weber.

Purpose

The success of immunosuppressive therapies to preserve pancreatic islets and treat Type I Diabetes (T1D) has, so far, been limited either due to safety concerns or lack of persistent efficacy. To overcome these issues, we designed a novel targeted immune-suppressive approach, called ImmTAAI, that binds specifically β-cell in pancreatic islets, engages autoreactive T cells and suppresses their effector functions only when bound to target cells. By restricting T cell inhibition to the pancreas, we aim to achieve potent localized immune suppression while avoiding systemic immunosuppression.

Methods

To create a β-cell specific PD-1 agonist ImmTAAI, an affinity-enhanced T cell receptor (TCR) specific for the peptide-HLA-A*02 complex of pre-pro-insulin PPI15-24, a well-characterized autoantigen found on pancreatic β-cells, was fused to an agonistic PD-1 antibody to mimic the activity of PD-L1, the endogenous ligand of the PD-1. To verify that the TCR targeting domain is able to bind its cellular target, β-cell lines and disaggregated β-islets derived from healthy donors were stained with biotinylated PPI15-24 TCR. Furthermore, the subcellular localisation of PPI PD-1 agonist ImmTAAI at the β-cell - T cell interface was studied by confocal microscopy. To evaluate the impact of the β-cell bound PD-1 agonist bispecific on TCR signalling, a β-cell line - Jurkat NFAT cellular reporter assay was used. Finally, inhibition of T cell functions by cell-bound ImmTAAI was measured in cytotoxicity co-culture imaging experiments using CD8+ T cell clones that recognize the PPI6-14 peptide-HLA presented by EndoH-BH2 target cells.

Summary of Results

Microscopy studies showed that the PPI TCR bound specifically to immortalized β-cell lines and primary β-cells from disaggregated pancreatic islets. In co-culture localization experiments we observed that, once bound to β-cells, the PPI PD-1 agonist ImmTAAI engaged the PD-1 receptor on attacking T cells, accumulated at the T cell synapse and inhibited TCR signaling. The engineered bispecific did not compete with PD-L1 binding to PD-1 and was additive with PD-L1 in inhibiting TCR signaling. At picomolar concentrations, the PPI PD-1 agonist ImmTAAI suppressed secretion of inflammatory cytokines and inhibited β-cell killing by autoreactive CD8+ T cell clones. Notably, this PD-1 agonist was unable to inhibit T cells when free in solution and not targeted to β-cells.

Conclusions

We have generated a TCR bispecific inhibitory ImmTAAI molecule that is targeted to β-cells to suppress autoreactive T cells and protect pancreatic islets. These data support the concept that this bispecific inhibitor with the potential to bind β-cells with high specificity, enhance natural immune suppression delivered by PD-L1 and inhibit attacking T cells. Importantly, the molecule is inactive when free in solution and therefore has the potential to deliver localized immune suppression in islets while avoiding systemic immunosuppression. These features make PPI PD-1 agonist ImmTAAI molecules an attractive and novel approach to potentially treat T1D.

Preferred Presentation Format

No Preference

Research Category

Novel Technologies

10: Kathrin Maedler (University of Bremen)

Abstract Title

MST1 inhibition restores normoglycemia in a patient with severe diabetes and in mice

Authors

Amin Ardestani, Vasileios Angelis, Stephen Johnston

Purpose

A critical decline of functional insulin-producing pancreatic beta-cells represents a central pathologic element of both type 1 and type 2 diabetes. The pro-apoptotic kinase Mammalian Sterile 20-like kinase 1 (MST1), a central component of the Hippo pathway, is a key regulator of organ size, stress response and tissue homeostasis; its aberrant hyperactivation is linked to multiple pathological disorders including diabetes. MST1 is a key inducer of beta-cell failure and its genetic and pharmacological inhibition restores beta-cell survival, function and normoglycemia and represents a promising approach for a causative diabetes therapy.

Methods

A 62-year-old patient was enrolled in the plasmaMATCH clinical trial and received 240 mg neratinib, a potent HER2/EGFR/MST1 kinase inhibitor once daily and her glycemia and HbA1c levels were closely monitored before, during and after the trial.
MST1 knockout (KO) mice were fed a high fat/ high sucrose diet (HFD) for 16 weeks and then received a single dose of 100 mg/kg BW streptozotocin (STZ) to induce beta-cell failure and insulin deficiency, HFD feeding was continued for 3 more weeks. Glycemia and insulin was monitored during the study and pancreas morphology analysed thereafter.

Summary of Results

Pharmacological as well as genetic inhibition of MST1 correlated with great improvement in glucose levels. Neratinib resulted in an average 57% reduction of random glucose and 44% reduction of glycated haemoglobin both to physiological levels during the whole treatment period. 18 months later, when neratinib was withdrawn, random glucose rapidly raised together with high blood glucose fluctuations, which reflected in elevated HbA1c levels 10 months later.
MST1 deletion in mice resulted in improved glucose tolerance and insulin secretion, and restored pancreatic β-cell mass as a result of improved beta-cell survival and proliferation in the combined high fat/high sucrose and streptozotocin (HFS/STZ) model of β-cell

destruction and diabetes. Importantly, the glucose-lowering effects in the MST1-knockout (KO) mice could be accounted to the enhanced beta-cell mass and improved insulin secretion without changes in insulin sensitivity. Metabolic and morphological data suggest that normalization of blood glucose and insulin secretion, islet architecture, and β-cell mass by MST1 deletion in response to diabetes-induced injury occurred as a result of improved β-cell survival and proliferation establishing MST1 as potent regulator of physiological β-cell turnover.

Conclusions

Previous findings as well as this additional mechanistic study of MST1 deletion in a mouse model of severe diabetes sets up the potent antidiabetic effect of MST1 inhibitory therapies, which restore normoglycemia, insulin secretion and β-cell survival. In confirmation, the combination of HER2/EGFR/MST1-inhibition by neratinib is a potent strategy for the pharmacological intervention to effectively restore normoglycemia in a patient with poorly controlled T2D and suggests neratinib as potent therapeutic regimen for the cancer-diabetes comorbidity.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

11: Aisha Callebaut (KULeuven)

Abstract Title

Deamidated peptides from human islet proteins elicit autoreactive CD4 T-cell responses in peripheral blood mononuclear cells from T1D patients

Authors

Aisha Callebaut, Rita Derua, Perrin Guyer, Mijke Buitinga, Fernanda M.C.Sodre, Saurabh Vig, Mara Suleiman, Piero Marchetti, Chantal Matthieu, Eddie James, Lut Overbergh

Purpose

In type 1 diabetes (T1D), β-cells play an active role in their own autoimmune-mediated destruction by the generation of post-translational modifications (PTMs). One such PTM is deamidation: the conversion of glutamine (Gln) to glutamic acid (Glu) by tissue transglutaminase 2 (TGM2) (enzymatic deamidation) or the biochemical conversion of asparagine (Asn) to aspartic acid (Asp) (non-enzymatic deamidation). In this study, we aimed to identify relevant enzymatic and non-enzymatic deamidated peptides in human islets with LC-MS/MS, and evaluate their potential for eliciting autoreactive CD4 T- cell responses in T1D patients.

Methods

Human islets were exposed in vitro to a mixture of cytokines (IL1β, IFNγ and TNFα) or IFNα. After stress was confirmed through microscopic assessment of apoptosis and/or qPCR analysis of stress markers, islet lysates were subjected to LC-MS/MS after trypsin digestion, under conditions shown to minimize irrelevant non-enzymatic deamidations (induced during sample digestion and processing) (n=4 for each). Deamidated peptides detected through LC-MS/MS with predicted HLA class II DRB1*0401 binding motifs were synthesized and tested using an in vitro binding assay. Finally, in vitro tetramer assays were performed on peripheral blood mononuclear cells of T1D patients to evaluate CD4 T-cell responses against deamidated peptides.

Summary of Results

Exposure to a cytokine mixture resulted in apoptosis (24.90% vs 5.84% in control islets, n=4) and upregulation of CHOP and ATF3 mRNA levels (1.65- and 2.21-fold respectively, n=3 for each). Although no apoptosis was seen after exposure to IFNα, CXCL10, MX1 and HLA class I mRNA levels were upregulated compared to control islets (233.9-, 34.18- and 2.39-fold respectively, n=3-4). Exposure to cytokines and IFNα resulted in a 5.34- and 2.17-fold increase in TGM2 mRNA, compared to control islets, respectively. By LC-MS/MS, a total of 16 Gln and 27 Asn deamidations were detected in 13 T1D related proteins, of which several were found in multiple donors. Examples are enzymatically deamidated chromogranin-A, glucose-regulated protein 78 (GRP78) and secretogranin-2 (SCG2) peptides, as well as non-enzymatically deamidated GRP78, glucose regulated protein 94 (GRP94) and heat shock 70kDa protein 9 (HSPA9) peptides. Out of the 43 deamidated peptides, 8 (4 Gln and 4 Asn) deamidated peptides bound to HLA class II DRB1*0401. In vitro tetramer assays showed responses against all 8 deamidated peptides in peripheral blood of T1D patients (n=15, at least 1 strong or 2 weak responses for each peptide), with particularly strong responses against an Asn deamidated HSPA9 peptide.

Conclusions

These results highlight that cytokine- and IFNα-exposed human islets are prone to deamidations and suggest that both Gln and Asn deamidated peptides may promote the activation of CD4 T-cells in T1D patients. Although further confirmation is needed by ex vivo tetramer assays, these findings add to the growing list of evidence that PTMs undermine tolerance in T1D and may open the road for the development of new diagnostic or therapeutic applications for patients with T1D.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

12: Louise Ganlund (Uppsala University)

Abstract Title

A simple dynamic culture system for maintaining islets of Langerhans

Authors

Louise Granlund, Sofie Ingvast, Oskar Skog, Olle Korsgren

Purpose

Isolated islets are valuable research material and there are several isolation clinics around the world providing isolated islets to research labs. Although the details of culturing and keeping the islets differ between labs, they usually have one thing in common – they are cultured statically, with changes of medium only every 48 hours. When maintained in culture, islets will secrete a variety of compounds, such as hormones and cytokines (Henquin, 2021). The islets are sensitive to oxidative and inflammatory stress, which is induced by cytokines such as TNFα, IL-1β and INFγ (Kanitkar et al., 2008). As these accumulate to supraphysiological levels in the medium, they likely influence the islets and alter the transcriptome profile. We therefor aimed to construct a dynamic culture system, with hormone- and cytokine levels that would more accurately mimic physiological conditions and hence provide more reliable results from downstream experiments.

Methods

Standard culture medium was used (CMRL-1066, with 5 mM glucose). Islets were cultured in 5 cm petri dishes containing 3 ml medium and 75 islets per dish, which were subsequently covered with 75 µl beads (Bio-Gel P-4 Media, Extra fine) to protect the islets from the flow of the medium. The dishes were kept inside a 37°C incubator. A metal inlet and outlet were attached to the petri dish lid, the inlet in turn being connected by tubing to fresh medium, also placed inside the 37°C incubator. The outlet was connected to a lambda fraction collector, especially built for this purpose, which dispensed the medium from the petri dish into a 96-well plate. The system was run by a peristaltic pump at a speed of 450 µl/min, hence changing all the medium in the petri dish every 6 min and 40 seconds. Three petri dishes containing 75 islets each were set up and run in parallel, collecting medium from all dishes. In addition to the dynamic cultures, three static cultures were set up at the same time, containing 3 ml medium and 75 islets covered with 75 µl beads per dish. Samples of 200 µl were taken twice a day from the static cultures, and subsequently replaced with 200 µl fresh medium to maintain the same volume in the dish, but no other media were exchanged. After 48 hours, the culturing was stopped and the islets were obtained for downstream quality tests and analysis. Islets were used in a perifusion instrument (Biorep) to test their glucose-stimulated insulin secretion (GSIS) with and without addition of a GLP-1 analogue. Additionally, islet morphology was examined by IF and gene expression of common islet genes were investigated by qPCR. The obtained medium from both static and dynamic cultures was analyzed by insulin and glucagon ELISA, and an Olink Inflammation panel was used to evaluate the accumulated concentrations of different inflammatory markers.

Summary of Results

The islets maintained in a dynamic culture display a preserved potentiating effect of incretins (GLP-1) when stimulated with glucose. They maintain a normal morphology as well as expression of both beta- and alpha cell markers. The obtained medium from islets in static culture show supraphysiological levels of the islet hormones insulin and glucagon as well as an accumulation of inflammatory markers, such as IL-8 and IL-6, compared with islets in dynamic culture.

Conclusions

When working with isolated islets of Langerhans, one should always strive after good experimental conditions that do not modify the function of the isolated islets in an unintended manner. Extreme culturing conditions could give misleading results that do not represent what is truly happening in vivo. In this paper, we wish to introduce a simple dynamic culture system, with the goal of achieving physiological concentrations of islet-secreted compounds, to give the best possible prerequisite for downstream experiments.

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Preferred Presentation Format

Poster Presentation

Research Category

Novel Technologies

13: Alexander Jonsson (Uppsala University)

Abstract Title

Transcriptional profiles of microvascular endothelial cells and macrophages in islet- and exocrine tissue isolates

Authors

Alexander Jonsson, Anders Hedin, Malin Muller, Oskar Skog, Olle Korsgren

Purpose

The pancreatic microenviroment contains various non-parenchymal cells that contribute to tissue homeostasis. The purpose of the current work is to investigate the gene expression profiles of microvascular endothelial cells and macrophages to generate hypotheses regarding their functions in health and diabetes.

Methods

Islet- and exocrine tissue isolates from brain-dead multiorgan donors were dissociated into single cells immediately following islet isolation. Microvascular endothelial cells (CD31+ CD34+ CD45-) were sorted from eight normoglycemic donors and five donors with impaired glucose metabolism using a microfluidic FACS device. Gene expression was measured through AmpliSeq. Differential gene expression analysis and gene set enrichment analysis was performed.

In a separate group of donors, two subgroups of macrophages (CD206+ or CD206-) are currently being sorted from each tissue compartment and will be processed similarly.

Summary of Results

Post-sorting analyses suggested that endothelial cells were highly purified in the sorted samples. The samples clustered according to tissue of origin. Islet samples were enriched in gene sets involved in angiogenesis and extracellular matrix remodeling. Exocrine samples were enriched in gene sets involved in biomolecular metabolism and ribosomal processes.
No differences were detected between normoglycemic donors and donors with impaired glucose metabolism at gene- or gene set level.

Conclusions

Highly purified microvascular endothelial cells were analyzed through bulk-RNASeq. The transcriptomic data from islet microvascular endothelial cells is consistent with potential intraislet angiogenesis and remodeling. We expect to also have bulk transcriptomic data from pancreatic macrophages in time for the conference.

Preferred Presentation Format

Poster Presentation

Research Category

Beta Cell Physiology and Dysfunction

14: Mark Mamula (Yale University)

Abstract Title

Regulation of glucose metabolism by posttranslational modifications in Type 1 Diabetes

Authors

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

Purpose

Emerging evidence indicates that autoimmunity often arises to post-translational protein modifications (PTMs), leading to chronic inflammation by infiltrating lymphocytes of target organs such as the pancreas in type 1 diabetes (T1D). While the identification and assessment of autoimmunity to novel PTM biomarkers is important to understanding pathogenic processes in T1D, 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 biologic functions of PTM proteins associated to T1D.

Methods

Carbonylation is the major protein modification in response to oxidative stress. Citrullination is mediated by calcium-dependent peptidylarginine deiminase (PAD) enzymes, which catalyze deimination, the conversion of arginine into the non-classical amino acid citrulline. Various proteomic analyses, including mass spectrometry, were performed in both murine and human pancreatic islets to identify carbonylated- and citrullinated-islet 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, 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, was also examined.

Summary of Results

We identify autoantibodies against to beta subunit of prolyl-4-hydroxylase (P4Hb; native and carbonylated form) and glucokinase (GK; native and citrullinated form) in both human T1D and murine models. 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 IFNgamma +IL-1beta 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.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

15: Paola S. Apaolaza (Helmholtz Center Munich)

Abstract Title

The proinsulin, insulin and PC1/3 trichotomy: understanding beta cell function and dysfunction through image analysis.

Authors

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

Purpose

Investigations in mice and/or human pancreas showed a general decay in beta cell mass, which may correlate with loss of beta cell function in type 1 diabetes (T1D). Detection of different biomarkers in blood such as proinsulin, insulin and/or C-peptide may help predict what happens to islet function and beta cell mass during disease progression. In this study, we aimed to provide an in-depth characterization of not only insulin and proinsulin expression but also of the prohormone processing enzyme PC1/3 in non-diabetic (ND), autoantibody-positive (AAb+), and T1D donors using high-resolution confocal imaging and state-of-the-art image analysis. In addition, we aimed to correlate islet beta cell phenotype and islet morphology to account for possible changes in cell size, number and islet area during disease progression.

Methods

Pancreatic sections from 20 age-matched ND, 7 AAb+ (4 single and 3 double), 8 short-duration (<5 years) T1D and 9 long-duration T1D (>15 years) donors were analyzed. Formalin-fixed paraffin-embedded sections were stained for insulin (INS), proinsulin (PI) and PC1/3 (PC1) by immunofluorescence staining. Confocal microscopy images (up to 30 islets per donor) were analyzed using the software QuPath. Beta cell populations were defined as follows: 1) INS+PI+PC1+, triple positive cells, 2) INS+PI+PC1-, INS+PI-PC1+, INS-PI+PC1+ double positive cells and 3) INS+PI-PC1-, INS-PI+PC1-, INS-PI-PC1+ single positive cells. The proportion and density of each cell population were then calculated as follows: Proportion was defined as total number of positive cells divided by the total number of endocrine cells (per islet), and density as the total number of positive cells divided by islet area. Islet cellularity and morphology were also calculated. The sum of triple, double and single cell populations was used to determine total INS+, total PI+ and total PC1+ cells.

Summary of Results

The proportion and density of total positive cells and triple positive cell (INS+PI+PC1+) population was lower in 2 out of 3 double AAb+ donors, in most short-duration T1D donors, and in all long-duration T1D donors. The number of PC1-negative beta cells (INS+PI+PC1-, 24.34 ± 17.44%) was increased in double AAb+ donors, although there was high variation. Conversely, the number of PC1-negative beta cells was decreased in long-duration T1D donors (0.01 ± 0.02%) compared to ND donors (8.46± 10.36%). A decrease in the proportion of PI-negative beta cells (INS+PI-PC1+) was observed in double AAb+ (0.27 ± 0.12%) and long-duration T1D (0.26 ± 0.35%), compared to ND donors (0.79± 0.61%). In contrast, an increase of PI-negative beta cells in short-duration T1D donors was observed (1.77 ± 1.94%). No differences were observed in cells negative for insulin (INS-PI+PC1+) in the majority of the groups, with the exception of T1D donors with long disease duration (0.16 ± 0.16% vs 1.75± 1.32% in ND). The density and proportion of cells positive for PC1/3 (INS-PI-PC1+) was increased in T1D donors (both short and long disease duration). The proportion of cells staining positive for PI only (INS-PI+PC1-) or INS only (INS+PI-PC1-) was very low in all groups. When islet cellularity and morphology were analyzed, the mean number of cells per islet was comparable in all groups (152.2 ± 41.9 cells/islet). Interestingly, cell density (number of cells/islet area) was reduced in double AAb+ donors (cell hypertrophy), yet increased in all T1D donors (cell atrophy) compared to ND donors.

Conclusions

During the development of T1D, it is known that beta cells undergo functional and morphological changes. Our data showed a general decrease of cells expressing insulin, proinsulin and PC1/3 in double AAb+, and T1D donors with short and long disease duration, which might reflect the ongoing autoimmune process. In addition, changes in distinct double-positive cell subpopulations may represent different stages of beta cell dysfunction. The increase in single PC1/3+ cells in T1D could be due to an increase in PC1/3+ alpha cells, or remaining beta cells that are no longer able to produce proinsulin and insulin. Lastly, islet morphological changes before and after onset

of the disease might reflect possible alterations in insulin/proinsulin production and cell function. These findings complement the current knowledge in beta cell pathophysiology, and contribute to our understanding of T1D pathogenesis.

Preferred Presentation Format

No Preference

Research Category

Beta Cell Physiology and Dysfunction

16: Shani Peleg (The Hebrew University)

Abstract Title

Investigating the role of impaired editing in beta-cells and alpha-cells

Authors

Shani Peleg, Ehud Knebel, Roni Cohen-Fultheim, Klaus Klaestner, Erez Levanon, Agnes Klochendler, Yuval Dor

Purpose

Extensive evidence indicates that type 1 diabetes (T1D) is preceded by an anti-viral response within islets, yet a causal virus has not been identified. We hypothesize that the anti-viral response may in fact result from an intrinsic process. RNA editing, involving adenosine deamination by ADAR1, serves to dismantle endogenous double-stranded RNA (dsRNA) structures that could potentially mimic viral infection. We suggest that impaired editing of endogenous RNA molecules in islets could result in an aberrant interferon response, which may explain the anti-viral signature observed in T1D.

Methods

Disruption of ADAR1 specifically in beta-cells or alpha-cells of young mice.

Summary of Results

In preliminary studies, disruption of ADAR1 specifically in beta-cells of young mice caused massive islet inflammation, beta-cell destruction and diabetes. Despite the extensive immune response in and around islets, alpha-cells remain unscathed, reminiscent of alpha-cell persistence in T1D. To study the basis for alpha-cell sparing, we disrupted ADAR1 specifically in alpha-cells of young mice. Strikingly, in contrast to the phenotype of beta-cell ADAR1 disruption, neither an immune response nor a metabolic phenotype was observed. Ongoing experiments are aimed at understanding the unique resistance of alpha-cells to ADAR1 deficiency.

Conclusions

The response of islets to impaired RNA editing shows multiple intriguing similarities to T1D, potentially shedding light on islet-triggered inflammation prior to the development of autoimmunity.

Preferred Presentation Format

Poster Presentation

Research Category

Type 1 Diabetes Etiology & Environment

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

Abstract Title

Macrophage infiltration is increased in pancreata of patients with type 1 diabetes

Authors

Estefania Quesada-Masachs, Samuel Ziberman, Tiffany Chu, Sakthi Rajendran, Sara McArdle, Willaim Kiosses, Zbigniew Mikulski, Matthias von Herrath

Purpose

Type 1 diabetes (T1D) is characterized by autoimmune destruction of insulin-producing beta cells. Macrophages are the predominant immune cells in the pancreas of both non-diabetic and T1D individuals. Macrophages commonly infiltrate the islets of patients with T1D, and they may play a role in human T1D. In the present study we aimed to characterize and quantify the distribution of macrophage populations throughout whole pancreatic sections from non-diabetic, autoantibody positive (Aab+), and donors with T1D.

Methods

Antibodies for CD68, HLA class II (DP, DQ, DR), and insulin were optimized in formalin fixed paraffin embedded (FFPE) sections of human tonsils and pancreas. Human pancreatic sections from five non-diabetic, five Aab+, and five T1D donors were stained with CD68, HLA class II, and insulin. 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 machine learning algorithms in QuPath. Enhanced resolution images of selected regions of interest from the tissue sections were acquired with the confocal Zeiss LSM880 with an Airyscan detector to visualize cellular details of infiltrating macrophages.

Summary of Results

We observed increased CD68 expression in islet, peri-islet, and exocrine regions in T1D and in some Aab+ individuals. Non-diabetic donors had little CD68 expression, but it was detectable in all pancreatic regions. With the cellular analysis of the whole tissue sections, we retrieved the information of more than 24 million cells. Across the entire tissue, the number, density, and percentage of macrophages (CD68+ cells) was higher in donors with T1D when compared with the other groups. The mean percentage of CD68+ cells in the T1D group was 8.6±4.7% compared to 3.2±2.7% and 1.5±0.5% in the Aab+ and non-diabetic groups, respectively. Notably, an outlier in the T1D group, with little cellular CD68 expression, exhibited zero insulin-containing islets (ICIs) in the section we stained. Despite having a greater proportion of macrophages in the T1D group, the percentage of CD68+ macrophages expressing HLA-II was not significantly increased in the T1D group compared to either the Aab+ or non-diabetic groups. Peri-islet regions had the highest percentage and density of CD68+ cells across all the groups independently of the disease status. However, the largest differences were observed in T1D patients, who exhibited the highest percentage of CD68+ cells in peri-islet regions (19.7±14.3%) compared to islet (10.8±10.2%; $p<0.0001$) and exocrine (7.9±4.7%; $p<0.0001$) regions. In the T1D group, the percentage of CD68+ macrophages in the islet and peri-islet regions was weakly correlated ($r=0.24$; 95% CI [0.14, 0.35]; $p<0.0001$) with the percentage of insulin+ beta cells expressing HLA class II.

Conclusions

We observed the highest proportion of macrophages (CD68+ cells) in the peri-islet regions, regardless of the disease status. The close proximity of macrophages to the islets suggests that they communicate with the endocrine cells. We observed more macrophages in all pancreatic regions of patients with T1D when compared with Aab+ or non-diabetic donors, except for the one T1D donor who had no ICIs. These findings support a potential role for macrophages in T1D initiation and progression, since their involvement may be dependent on the presence of insulin producing beta cells.

Preferred Presentation Format

No Preference

Research Category

Pathology

18: Matti Nykter (Tampere University)

Abstract Title

Gene expression and antiviral response profiles distinguish children who develop insulin or GAD-driven autoimmunity - the TEDDY study

Authors

Jake Lin, Elaheh Moradi, Karoliina Salenius, Suvi Lehtipuro, Tomi Hakkinen, Jutta E. Laiho, Sami Oikarinen , Sofia Randelin, Hemang Parikh, Jeffrey Krischer, Anette-G. Ziegler, Jorma Toppani, Ake Lernmark, Joe Petrosino, Nadim Ajami, Jin-Ziong She, Beena Akolkar, William A. Hagopian, Marian J. Rewers, Richard E. Lloyd, Kirsi Granberg, Heikki Hyoty, Matti Nykter

Purpose

Genetic basis of type 1 diabetes has been characterized extensively and several non-genetic risk-modifying factors have been identified. While several studies have pointed out the role of environmental factors in the pathogenesis, possible contribution of host responses that are induced by these environmental factors is not known. The distinct genetic background of the two type 1 diabetes endotypes, that is children who initially develop autoantibodies against either insulin or GAD65, suggest that different gene-environment interactions may play a role in their pathogenesis. The purpose of this study was to elucidate these interactions in the emergence of the two divergent antibody patterns that predispose to type 1 diabetes.

Methods

We utilized 2376 matched longitudinal transcriptome sequencing data to characterize dynamic host responses during the prospective follow-up of children within the The Environmental Determinants of Diabetes in the Young (TEDDY) birth cohort study using nested case-control (NCC) design. Transcriptomics response profiles were analysed in children who later developed autoimmunity and in matched control children, and correlated them with IAA and GADA autoantibody patterns and with 4536 virome profiles in stool and plasma samples. DESeq2 and temporal filtering were applied for selecting of differentially expressed genes. Comprehensive immune cell type proportions were estimated by regression analysis with elastic net regularization. Conditional logistic regression was used to assess the associated odds of virome exposures, the expression of the selected genes, and cell type proportions to the IA outcome in the NCC setting.

Summary of Results

We identified distinct temporal gene expression patterns and proportions of immune cells in children with the first appearing autoantibody against either insulin or GAD65. Applying statistical testing between the cases and controls (adjusted p-value<0.05, LFC>0.5, 2+ timepoints), we identified 17 genes in GADA first and 3 genes in IAA first seroconversion cohorts. GAD65 linked genes

include ZBED6, associated with pancreatic beta cell survival and FABP5, fatty acid gene modulating inflammation. The integration with enterovirus infections diagnosed by metagenomic sequencing data from stool and serum samples showed that the enterovirus-induced host response was weaker in children who later developed islet autoimmunity compared to enterovirus-infected autoantibody negative children exhibiting enriched type 1 interferon production (p-value<0.003), signaling (p-value<3.35e-11) and response (p-value< 0.0001). Case children who developed IAA as the first autoantibody had an elevated monocyte component, associated with chronic inflammation, throughout the time course. We showed that transcriptomic data provides additional independent information on top of genetic and environmental markers that can be used for improved islet autoimmunity prediction.

Conclusions

In conclusion, our study showed immune related transcriptomic differences between cases and controls prior to islet autoimmunity. These are presented differently in children with the first appearing autoantibody against either insulin or GAD65. We also found that enterovirus infections lead to a stronger antiviral response in control children than in children who develop autoantibodies. A major strength of our study is the comprehensive integration of transcriptomic profiles, virome and genetics while incorporating immune cell type alterations prior to seroconversion. Taken together, our analysis provides transcriptomic and immunogenic characterization of host responses in the context of type 1 diabetes-related autoantibody patterns and environmental triggers of the disease.

Preferred Presentation Format

No Preference

Research Category

Immunology

19: Kristina Pedersen (The Bartholin Institute, Copenhagen)

Abstract Title

Dysregulation of the innate antiviral immune response in type 1 diabetes

Authors

Kristina Pedersen, Martin Haupt-Jorgensen, Lars Krogvold, Simranjeet Kaur, Ivan C. Gerling, Flemmin Pociot, Kut Dahl-Jorgensen, Karsten Buschard

Purpose

Type 1 diabetes (T1D) is a prevalent autoimmune disease characterized by T cell infiltration of the pancreatic islets leading to destruction of the insulin-producing beta cells, hypoinsulinemia and hyperglycemia. The incidence of T1D is increasing more rapidly than can be explained by genetic drift, which is why environmental factors and especially virus infections are believed to play important roles. It is known that pancreatic beta cells compared to alpha cells are more vulnerable to infection and during an infection show increased expression of antiviral 2′-5′-linked oligoadenylate (2′-5′A) pathway genes. Here we investigated if the innate antiviral immune response, hereunder the 2′-5′A pathway, was associated with development of T1D.

Methods

We used a genome-wide association study (GWAS) approach to search for T1D associated SNPs within ±250kb flanking regions of the transcription start site of 64 genes of the innate antiviral immune response. The RNA expression profiles of the same genes were analyzed in laser-dissected islets from two to five tissue sections per donor from the Diabetes Virus Detection (DiViD) study and the

network of Pancreatic Organ Donors (nPOD). The donors included autoantibody positive (Ab+) individuals, new-onset T1D patients and non-diabetic controls.

Summary of Results

Highly interestingly, toll-like receptor (TLR) 7 and its transcription factor interferon regulatory factor 7 (IRF7) were 22% and 16% downregulated, respectively, in islets from Ab+ individuals compared to controls. Contrary, TLR7 was 70% upregulated in new-onset T1D patients. IFN-stimulated genes (ISGs), which are activated through the antiviral TLR3 and TLR7 receptors, constituted the largest group of upregulated genes in new-onset T1D patients vs controls. Of those, OAS1 was upregulated by 111%, myxovirus resistance 1 (MX1) by 142% and ISG15 by 197%. Our GWAS study identified 27 novel T1D associated SNPs in genes from the innate antiviral immune response. Some of these were located upstream of the genes in the 2′-5′A pathway, namely SNP rs4767000, rs1034687 and rs739744.

Conclusions

We showed dysregulation of genes involved in the innate antiviral immune response in islets of both Ab+ and new-onset T1D patients, in which the pathways of TLR7, 2’-5’A, MX and ISG15 were of high interest. These findings indicate that the innate antiviral immune response is possibly not only immature in predisposed Ab+ individuals but is highly upregulated in new-onset T1D patients. We hypothesize that the immature innate antiviral immune response in islets from Ab+ individuals is not able to thoroughly clear environmental factors such as enterovirus that leak over the intestinal barrier and that this will lead to destruction of beta cells and T1D.

Preferred Presentation Format

Oral Presentation

Research Category

Type 1 Diabetes Etiology & Environment

20: John Virostko (UT Austin)

Presenter of Abstract

Yorick Sanders

Abstract Title

Pancreas Morphology is Altered in Type 1 Diabetes

Authors

Yorick Sanders, Soumil Chopra, Melissa Hilmes, Daniel Moore, Alvin Powers, Mohamed Abouhawwash, Chandrqjit Bajaj, John Virostko

Purpose

The pancreas is smaller in individuals with type 1 diabetes (T1D) and those at risk for the disease. This study assessed three-dimensional shape attributes of the pancreas in individuals with and without T1D to characterize changes in pancreas morphology accompanying T1D. Our goal is to better characterize T1D progression with advanced shape metrics capturing pancreas topology and geometry.

Methods

Individuals with recent onset T1D (n = 90) or controls without pancreas pathology (n = 85) received non-contrast enhanced abdominal MRI on a 3T scanner. Fifty-six individuals with T1D received at least 1 follow-up MRI approximately six months after diagnosis to assess changes in the pancreas after diagnosis. The pancreas was manually outlined by a radiologist blinded to the status of each study participant to define a contiguous volume of each pancreas. Using this three-dimensional pancreas volume, the surface area to volume ratio, bounding box, bounding convex hull, and principal axes of an equivalent major ellipsoid were calculated using MATLAB. Additionally, we are exploring correlations using level set topology and geometry based on multi-attributed contour trees to generate multi-output Gaussian process co-regionalization results.

Summary of Results

The surface area to volume ratio was higher in individuals with T1D, while the bounding box volume, percentage of the bounding box composed of pancreatic tissue, percentage of the bounding convex hull composed of pancreatic tissue, and equivalent major ellipsoid principal axes were all smaller in individuals with T1D (all $p < 0.001$). Follow-up imaging found further alterations in the surface area to volume ratio, bounding box volume, and the percentage of the bounding box and bounding hull composed of pancreatic tissue associated with duration of disease ($p < 0.05$).

Conclusions

Smaller pancreata in individuals with T1D are accompanied by changes in pancreas shape. Characterizing changes in pancreas shape may provide insight into pancreas pathology in T1D and provide a comprehensive assessment of pancreas dynamics.

Preferred Presentation Format

Poster Presentation

Research Category

Novel Technologies

21: Joanna Filipowska (City of Hope)

Abstract Title

Membrane-bound LGR4 and its soluble form (LGR4-ECD) as novel regulators of ß-cell survival and proliferation

Authors

Joanna Filipowska, Nagesha G. Kondegowda, Nancy Leon-Rivera, Rupangi C. Vasada

Purpose

Type 1 diabetes (T1D) is an autoimmune cell-mediated disease which is characterized by destruction
of the pancreatic beta cells, leading to insulin deficiency, hyperglycemia, and systemic health-related problems in the affected subjects. We still do not understand what causes T1D and how T1D
is manifested. There is a need for more effective diagnostic and therapeutic approaches. It is believed that a dual approach of suppressing autoimmunity and beta cell destruction together with strategies to replace or regenerate functional beta cells can reverse the progression of T1D, and this renders such approaches particularly attractive from a clinical perspective.
We have shown that RANK (Receptor activator of NF-κB)/RANK ligand (RANKL) interaction inhibits β-cell proliferation and survival, which is reversed by Osteoprotegerin. Recently, the G protein-coupled receptor LGR4 (leucine-rich repeat-containing G-protein-coupled receptor 4), was identified as a novel receptor for RANKL in osteoclast precursor cells. LGR4 classically binds to R-spondins to

potentiate Wnt signaling, and has two natural forms, the membrane bound LGR4, and the soluble form containing only the extracellular ligand-binding domain (ECD), LGR4-ECD. LGR4 is also expressed in rodent
and human beta cells, but its role in β-cells remains unknown. We postulated that LGR4, through its stoichiometry with RANKL and RANK, is involved in regulating β-cell survival and proliferation and that LGR4-ECD acts as a decoy receptor to inhibit this pathway.

Methods

To study the role of Lgr4 knockout (ko) in vitro, we used INS1 cells (rat insulinoma) treated with a control siRNA (Scramble siRNA) or Lgr4 siRNA, Lgr4 Fl/Fl islets, treated with control LacZ or Cre recombinase adenoviruses. We analyzed the effects of Lgr4 ko in basal conditions and in the presence of cytokines. We examined the effect of Lgr4 overexpression in INS1 cells and mouse islets using adenovirus carrying the Lgr4 sequence (AdLgr4). To study the effect of Lgr4 knockout in vivo, we generated β-cell-specific conditional knockout mice (cko) (Lgr4Fl/Fl; INS1Cre+). We assessed the effect of Lgr4 cko in males and females, under basal conditions and under stress [high fat diet (HFD), and multiple low dose streptozotocin (MLDS)].
To test the therapeutic potential of LGR4-ECD, we used dispersed mouse or human islets treated with
or without species-specific LGR4-ECDs, in basal conditions or under cytokine treatment. We analyzed β-cell proliferation with phospho-histone H3 (PHH3) or bromodeoxyuridine (BrdU) and insulin co-staining; and β-cell death with TUNEL and insulin co-staining, or cleaved caspase3 staining.

Summary of Results

Lgr4 mRNA levels are regulated by cytokines, in rodent and human islets. Knockdown of Lgr4 in vitro
in INS1 cells is detrimental for β-cell proliferation and survival in basal and cytokine-stimulated conditions. Basal cell death is increased also in mouse islets. In contrast, overexpression of Lgr4 in INS1 cells protects them against cytokine-induced cell death. β-cell-specific Lgr4 cko mice, under basal conditions, exhibit normal blood glucose homeostasis, but have significantly increased ß-cell death compared to wild-type (WT) controls, in both male and female mice. However, ß-cell proliferation was reduced only in female but not male cko mice in basal conditions. When exposed to stressors such as HFD, male cko mice had reduced ß-cell proliferation compared to WT mice; and with MLDS treatment, Lgr4 male cko mice exhibit increased β-cell death, compared to WT-MLDS-treated mice. We also hypothesized the LGR4-extracellular domain (LGR4-ECD), which is the soluble form of LGR4 that inhibits RANKL/RANK interaction in osteoclasts, will benefit β-cells. Indeed, LGR4-ECD significantly enhances β-cell proliferation in young (8-12-week) and aged (1-year) rodent islets, as well as in human islets from subjects without (46±16 years), and with Type 2 diabetes (51±8 years). Furthermore, LGR4-ECD significantly promotes mouse and human β-cell survival against cytokine-induced cell death.

Conclusions

LGR4 and LGR4-ECD are positive regulators of β-cell proliferation and survival. Future studies will determine the role of LGR4 and LGR4-ECD in regulating β-cell function and the mechanisms by which this pathway modulates β-cell homeostasis.

Preferred Presentation Format

No Preference

Research Category

Beta Cell Development, Differentiation & Regeneration

22: Samantha Crawford (UC Denver)

Abstract Title

Hybrid Insulin Peptide Mechanism of Formation in Type 1 Diabetes

Authors

Samantha Crawford, Aaron Wiles, Roger Powell, My Linh Dang, Jason Groegler, Anita Hohenstein, Janet Wenzlau, Kathryn Haskins, Thomas Delong

Purpose

In Type 1 Diabetes (T1D) autoreactive T cells mediate the destruction of insulin-producing beta cells. Recent findings from our lab point to hybrid insulin peptides (HIPs) as targets for autoreactive T cells in T1D. A HIP is the product of a transpeptidation reaction in which a new peptide bond is formed between a proinsulin fragment and another beta cell peptide. As a result, HIPs contain non-genomically encoded amino acid sequences, making them plausible targets for autoreactive T cells in T1D. Various diabetes-triggering CD4 T cells target HIPs in non-obese diabetic (NOD) mice. In human disease, HIP reactive T cells were identified not only in the residual pancreatic islets of T1D organ donors but also in the peripheral blood of recent-onset T1D patients. How HIPs form is currently poorly understood, however, it is well established that proteases can drive the formation of new peptide bonds in a side reaction during peptide bond hydrolysis. We hypothesized that a protease in beta cells is responsible for the formation of various disease-relevant HIPs. Our goal was to determine what specific protease may be responsible for the formation of a disease-relevant HIP that is targeted by the diabetes-triggering CD4 T cell clone BDC-2.5 in NOD mice.

Methods

To isolate such a protease with HIP forming potential, we fractionated HIP-containing beta-cell granule lysates by size exclusion chromatography. We then incubated fractions with precursor peptides that are required for the generation of the HIP recognized by BDC-2.5. Formation of new 2.5HIP in the fractions was monitored through T cell ELISA assays. Experiments were done in the presence and absence of various protease inhibitors, to block HIP formation.

Summary of Results

Our inhibition experiments allowed us to identify a protease family that is responsible for the formation of the 2.5HIP. Subsequent mass spectrometric analyses on the HIP-forming fractions led to the identification of the specific protease responsible for the formation of the 2.5HIP in beta cells.

Conclusions

Hybrid insulin peptides form via proteolytic transpeptidation in beta cells.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

23: Luciana Goncalves (University of Miami)

Abstract Title

Changes in the human islet microvascular anatomy and function during the progression of type 1 diabetes

Authors

Luciana Mateus Goncalves, Joana Almaca

Purpose

Endocrine cells in pancreatic islets are embedded in a network of capillaries that provide trophic support and regulate islet hormone secretion. Islet capillaries are made of a thin layer of endothelial cells covered by pericytes. Pericytes regulate islet blood flow and endothelial permeability. Although islet capillaries are the point of entry of immune cells that mediate the autoimmune attack of endocrine beta cells, it is still not known what happens to the islet microvasculature during the development of type 1 diabetes (T1D). The aim of this research project was to examine in detail the human islet microvascular anatomy and function in non-diabetic (control), single islet autoantibody positive (GADA+), and type 1 diabetic donors.

Methods

We compared the density of islet endothelial cells and pericytes by immunohistochemistry and recorded the activity of these vascular cells by confocal imaging of living human pancreas slices. To determine whether pericytes were functional in these early stages of the disease, we conducted calcium imaging experiments as pericyte contractile activity requires changes in intracellular calcium levels ([Ca2+]i). We incubated living human pancreas slices with a membrane permeant calcium indicator (Fluo-4) and visualized pericytes with a fluorescent antibody that specifically labels their plasma membrane and islet capillaries with fluorescent lectins. We examined pericyte responses to substances representing endogenous signaling molecules of their native microenvironment. These included secretory products released from endocrine cells in response to changes in glucose concentration, the sympathetic agonist norepinephrine, and endothelium-derived endothelin-1.

Summary of Results

We found that islets were enriched in pericytes, with a density in the endocrine compartment that was ~10-fold higher than in the surrounding exocrine tissue. In donors that had T1D for longer than a year, islet endothelial cell density was not different, but islet pericyte density decreased by 30% compared to control. Interestingly, the density of islet pericytes was significantly higher in islets from GADA+ positive individuals and not different in recent T1D onset cases. Norepinephrine and endothelin-1 increased [Ca2+]i in pericytes in islets from non-diabetic donors, while high glucose had an inhibitory effect. Interestingly, pericyte [Ca2+]i responses to high glucose and norepinephrine were impaired in islets from GADA+ individuals and in recent T1D onset cases. By contrast, pericytes were responsive to endothelin-1 at all stages.

Conclusions

We conclude that islet pericytes are dysfunctional very early on in the disease process. Because pericytes control important microvascular functions, such as permeability and local blood flow, impaired pericyte activity may exacerbate immune cell infiltration and endocrine cell dysfunction, thus contributing to the pathogenesis of T1D.

Preferred Presentation Format

No Preference

Research Category

Type 1 Diabetes Etiology & Environment

24: Emily Sims (Indiana University)

Abstract Title

α-Difluoromethylornithine (DFMO) is safe, well-tolerated, and associated with preserved residual C-peptide in children and adults with recent onset type 1 diabetes (T1D)

Authors

Emily K. Sims, Audrey Hull, Stephanie Woemer, Susanne Cabrera, Lucy D. Mastrandrea, Fanqian Ouyang, Susan Perkins, Carmella Evans-Molina, Eugene Gerner, Raghavendra G. Mirmira, Linda A. DiMeglio

Purpose

The ornithine decarboxylase inhibitor DFMO reduces β cell stress and diabetes incidence in preclinical T1D models by inhibiting polyamine biosynthesis. We asked if oral DFMO at escalating doses is well tolerated in children and adults with T1D.

Methods

At 3 sites, we performed a randomized controlled, double masked dose-ranging study to test the safety of 3 months oral DFMO administration in persons with recent-onset T1D (within 100 days). Mixed meal tolerance testing was performed at baseline, 3, and 6 months post-randomization.

Summary of Results

Adverse event (AE) profiles were determined for daily oral doses of 125, 250, 500, 750, and 1000 mg/m2 in 41 participants (12-34 YO, 59% male, mean HbA1c 7.3%) with 6-9 participants treated with drug or placebo at each dose. Mild and moderate AEs were noted, including two persons who withdrew, one due to an allergic reaction (diffuse urticaria) and one due to IV access problems. Possible drug-related expected AEs included mild-moderate nausea/vomiting/abdominal pain and diarrhea, moderate headache, upper respiratory infections, a pump site infection, and mild anemia. No unexpected effects judged related to drug occurred in individuals on active drug. Compared to the placebo group, individuals receiving the 750 and 1000 mg/m2 doses had significantly higher C-peptide area under the curve values at the 6-month post-randomization visit.

Conclusions

A 3-month course of oral DFMO was well tolerated with a favorable AE profile in children and adults with recent-onset T1D and, at higher doses, was associated with greater β cell function compared to placebo.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

25: Gisele Silva Boos (Helmholtz Zentrum Munchen)

Abstract Title

The cross-talk between the duodenum and the pancreas – profiling the immune system to identify the role of the gut in the pathogenesis and prevention of Type 1 Diabetes

Authors

Gisele Silva Boos, Maria Teresa Rodriguez-Calvo

Purpose

The role of the gut in the pathogenesis of Type 1 Diabetes (T1D) is still not well understood despite extensive research in animal models and studies of the gut microbiome in humans. Similar to other autoimmune diseases, a connection between T1D and an altered intestinal microbiota has been reported in several geographically diverse cohorts. Alterations of the intestinal barrier and increased gut permeability could enhance the exposure of the immune system to pathogens, which is likely to contribute to an already defective tolerance in the context of T1D.
We propose that the underlying genetic predisposition to T1D promotes the disruption of immune tolerance mechanisms leading to the dysregulation of the mucosal barrier in the gut and the development of antigen-specific cells. Here, we provide preliminary data from our ongoing project, which is currently focused on determining the spatial nature and phenotype of the gut-associated lymphoid tissue (GALT) and epithelial layer in the duodenum of non-diabetic, autoantibody-positive (AAb+), and T1D donors.

Methods

To understand the role of the gut in the pathogenesis of T1D, we initially characterized the gut immune system by optimizing our staining panels and pipeline. CaCo-2 and PBMCs were used as initial staining controls followed by tests in formalin-fixed paraffin-embedded (FFPE) spleen and duodenum slides. CaCo-2 cells, a human adenocarcinoma-derived cell line that mimics a small intestinal enterocyte phenotype, were chosen to further support our study based on their expression of intestinal epithelial markers. PBMCs from healthy volunteers were collected, processed, and fixed following a standard protocol, and used as controls for immune cell markers in immunofluorescence (IF) staining. Expected staining pattern, cell type stained, and approximate frequency of positive cells were controlled after each round of staining and compared to the respective markers available at the Human Atlas Protein and literature. After all markers showed satisfactory preliminary results in cells, we optimized multiplex immunofluorescence assays using human non-diabetic spleen (donor 6014) and duodenum test slides from donor 6482 kindly provided by nPOD. Similar to the immunocytochemistry approach, we compared the stainings obtained to the literature available.

Summary of Results

Cells and tissue sections were stained with a panel of markers to identify 1) epithelial cells (Cytokeratin 18, E-cadherin, Mucin, and PDX1), 2) dendritic cells (CD11c, CD103, CD80, HLA-II), and 3) macrophages (CD14, CD68, HLA-II, CD64). Given the important role of T cells in T1D, we additionally defined T cell subpopulations and their phenotype (CD3, CD4, TCRαβ, RORγt, CD45RO, CD103, CD45RA, CD8α, CD69, Foxp3, CD25, CD127).
Tissue slides were scanned with an Axio Scan.Z1 slide scanner (Zeiss). QuPath (version 0.3.0), a specialized software for digital pathology, was used to extract information from more than 30 cellular features including area, location, perimeter, and intensity parameters for each marker and cell. We are currently optimizing a manual training detection classifier to apply machine learning to automatically assign cell classifications. We will then identify different cell populations and quantify their relative abundance in the tissue. The use of consecutive sections will allow us to correlate the data from every slide and provide an accurate overview of the immune environment of the duodenum.

Conclusions

Here we present an experimental overview from the first steps of our project. By its completion, we will provide information about the integrity and composition of the duodenum, and we will also determine if there is an abnormal innate and/or adaptive immune cellular repertoire before and after T1D onset. This is the first attempt to define the spatial and cell-surface cellular phenotype within the duodenum in the context of human T1D. Furthermore, as the project progresses, our goal will be to understand and define the relationship between the dysregulation of T cell tolerance mechanisms in the duodenum and the destruction of beta cells in the pancreas. This will open new opportunities for therapeutic intervention by nutritional and/or pharmacological means, as well as for the development of therapies that target antigen-specific cells aimed at re-educating the immune system and stopping the attack on beta cells.

Preferred Presentation Format

Poster Presentation

Research Category

Immunology

26: Jan Czyzyk (University of Minnesota)

Abstract Title

Anti-serpinB13 antibodies stimulate beta-cell development via Notch signaling pathway and delay progression to insulin-dependent diabetes

Authors

Yury Kryvalap, Jan Czyzyk

Purpose

Methods for repopulating the pancreas with new insulin-producing cells have strong potential for therapy in diabetes. Recently, we have found that inhibition of serpinB13 – a protease inhibitor of cathepsin L (catL) – with a monoclonal antibody (mAb) in mouse embryos leads to a robust increase in the number of pancreatic Ngn3+ progenitor cells, significant expansion of islet mass, and improved resistance to severe diabetes in adulthood. To unveil the molecular mechanism of the augmented Ngn3+ cell response following inhibition of serpinB13 during gestation, we focused on the Notch communication system – a critical signaling pathway for pancreatic development. In addition we examined autoantibodies (AA) to serpinB13 in the human sera for their potential functional characteristics in type 1 diabetes (T1D).

Methods

We used a mouse mAb and several recombinant human antibodies to stimulate mouse embryonic pancreas explants (E12.5), and examined Notch expression in these in vitro cultures by flow cytometry and Western blotting. Visiopharm software for quantitative image analysis was used to count all cells with Ngn3 expression per pancreas as a readout of early endocrine lineage. The human AA to serpinB13 in subjects previously enrolled in a DPT-1 clinical trial were measured using Luminex methodology.

Summary of Results

We found that serpinB13 is expressed and secreted by epithelial cells in murine embryonic pancreases. Moreover, inhibition of serpinB13 during embryogenesis caused protease-dependent cleavage of the extracellular domain of Notch1 receptor in the pancreas ($p<0.0001$). On the other hand, embryonic pancreases of mice with genetic deficiency of catL had significantly fewer Ngn3+ cells compared with wild type controls. The partial loss of the extracellular Notch was followed by decreased presence of active Notch intracellular domain (aNICD), a fragment of Notch that is critical for restraining endocrine cell development. Finally, our screening of children enrolled in DPT-1, for serpin B13 autoantibody (AA) revealed an inverse correlation of this AA with risk level for T1D, as well as a positive association with longer diabetes-free interval. Importantly dialyzed sera from serpinB13 AA-positive subjects stimulated the output of Ngn3+ cells in the pancreas suggesting that this AA is functional. This effect was dependent on serpinB13AA rather than other serum factors as positive serum samples immunodepleted of this AA were no longer able to stimulate development of additional Ngn3+ cells.

Conclusions

Our data point to a novel function of serpinB13 in maintaining Notch receptor-mediated repression of pancreatic endocrine progenitors. Consequently, the perturbation of this effect of serpinB13 enables protease activity to partially dismantle Notch signaling, thereby allowing for more efficient development of Ngn3+ progenitors cells and a subsequent increase in islet mass. Our data also demonstrate that blocking this serpin has the potential to partially prevent, or at least slow down, the development of T1D both in the mouse and human.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Development, Differentiation & Regeneration

27: Peristera Petropoulou (Helmholtz Center Munich)

Abstract Title

Changes in insulin and proinsulin expression, beta and alpha cell islet composition, islet cellularity, and endocrine infiltration are evident before T1D onset

Authors

Peristera-Ioanna Petropoulou, Teresa Rodriguez-Calvo

Purpose

Type 1 diabetes (T1D) is a complex disease in which genetic and environmental factors play an important role. The interplay between beta cells and the immune system has recently become a major research focus. Beta cells might be triggering their own demise, in part due to increased cellular stress, which could contribute to the aberrant processing and accumulation of proteins, triggering or potentiating beta cell specific immune responses. Individuals at risk of developing T1D, such as autoantibody positive donors (AAb+), have an increase in proinsulin levels in the serum, months to years before clinical diagnosis. Moreover, recent studies have shown an increase in pancreatic insulin and proinsulin content and increased proinsulin-to-insulin ratio in these individuals. Despite intensive research in the field, there are still unanswered questions regarding the expression and localization of proinsulin and insulin, how it changes with disease progression, and if it might correlate with immune infiltration. For this purpose, we employed our recently published whole-slide image analysis workflow, to extract quantitative data about the endocrine and exocrine cellular composition and immune infiltration of the pancreas during type 1 diabetes progression.

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+), and 7 T1D individuals (1 long duration-7 years, and 6 short duration-average 3.7 years). Individuals with T2D were also included for comparison. FFPE sections were stained for insulin, proinsulin, glucagon, chromogranin A, HLA-I, CD45, CD3 and CD8 by immunofluorescence. Whole tissue sections were scanned using an Axio Scan.Z1 (Zeiss) slide scanner. Images were analyzed with QuPath version 0.2.3, an open-source software for digital pathology.

Summary of Results

We generated quantitative data for islet density, cellularity (number of cells per islet), cellular composition, islet phenotype and immune cell infiltration during the development of T1D. Specifically, we found that islet density decreases with disease progression. In dAAb+ individuals, the proportion and density of beta cells increases, while the proportion and density of alpha cells decreases. This is followed by a significant increase in both, proportion and density of alpha cells in T1D. Following a similar trend, insulin- and proinsulin-positive cells and Proinsulin-to-Insulin ratio 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). We found IDIs, pICIs, and ICIs in all disease stages. However, IDIs and pICIs are more frequent in dAAb+ and T1D individuals. As expected, ICI density dramatically decreases in T1D. The number of cells per islet (islet cellularity) tends to decrease 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. Analysis of immune infiltration revealed that high CD45+ cell density in endocrine and

exocrine compartments is a defining feature 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

Our results show distinct differences in insulin and proinsulin expression, beta and alpha cell islet composition, islet cellularity, and endocrine infiltration during T1D progression. These changes are already observed in dAAb+, indicating that alterations in beta cell function (insulin and proinsulin expression) and immune infiltration (CD45+ cell density) occur before clinical onset. This information is critical to understand disease pathogenesis and progression, and to inform clinical trials aiming to preserve beta cell function and to stop the immune attack. Ongoing analysis on the same donors will allow us to further categorize islets according to their HLA-I content, and CD3- and CD8-positive infiltration, providing a better understanding of the events leading to T1D.

Preferred Presentation Format

No Preference

Research Category

Pathology

28: Farooq Syed (Indiana University)

Abstract Title

Pre-clinical Evaluation of TYK2 Inhibitors as a Therapy for Type 1 Diabetes

Authors

Farooq Syed, Olivia Ballew, Chi0Chun Lee, Jyoti Rana, Angela Castela, Stephane Demine, Maria Ines Alvelos, Kara Orr, Garrick Chang, Staci A. Weaver, Jacqueline Del Carmen Aquino, Kentaro Yamada, Jing Liu, Donalyn Scheuner, Decio L. Eizirik, Carmella Evans-Molina

Purpose

T1D results from a complex interaction that occurs between the invading immune cells, which release a variety of chemokines and cytokines, and immunogenic signals produced by injured or dying β cells. Multiple lines of evidence suggest a prominent role for type 1 interferons, particularly interferon-α (IFNα), in T1D pathogenesis. A type I IFN-inducible transcriptional signature is present in the blood of children with a high genetic risk of T1D, even prior to the development of T1D-associated autoantibodies, and interferon-stimulated gene signatures are present in biopsies of pancreatic islets from individuals with recent-onset T1D. IFNα has been shown to induce endoplasmic reticulum stress, chemokine production, and HLA class I overexpression in human β-cells - three histological hallmarks of islets in human T1D. The effects of IFNα are mediated via its receptor (IFNAR1) and the protein tyrosine kinases JAK1 and TYK2 that phosphorylate and activate the signal transducers and activators of transcription (STAT1 and STAT2) proteins. JAK inhibitors have shown promise in mouse and in vitro models of T1D and are being tested in a clinical trial in adolescents and adults with recent-onset T1D. Notably, polymorphisms that decrease TYK2 activity are protective against T1D, and TYK2 inhibitors (TYKi) are being tested in other autoimmune conditions such as psoriasis. However, whether strategies to inhibit TYK2 have similar efficacy in models of T1D has not been tested.

Methods

We tested two small molecule inhibitors of TYK2 (BMS-986165 and BMS-986202). Both compounds target the pseudokinase Janus homology 2 regulatory domains (JH2) of TYK2, leading to inhibition of receptor-mediated TYK2 activation. Isolated human islets from

cadaveric organ donors and EndoC-βH1 cells were pre-incubated with TYKis for 2hrs followed by exposure to IFNα alone or in combination with IL-1β or TNFα for 24hrs or 48hrs. Expression of HLA class I, CXCL10, and MX1 were evaluated by RT-qPCR; STAT phosphorylation was assessed by immunoblot. Apoptosis was evaluated by Hoechst/Propidium Iodide staining. To determine the in vivo efficacy of TYK2 inhibition, we utilized two mouse models: 1) the RIP-LCMV-GP model, which is a transgenic and virally-induced model of T1D, and 2) the NOD mouse model of spontaneous T1D development. RIP-LCMV mice were pre-treated with BMS-986202 for 2 days prior to the LCMV injection (0.5x105 PFU), and NOD mice were treated with BMS-986202 from 6-12 weeks of age. In both models, vehicle and drug-treated mice were monitored for diabetes incidence. Immunostaining for insulitis and β cell mass and single-molecule RNA Fish (smRNA FISH) was performed in pancreatic sections from treated mice. Flow cytometry was used to profile immune cell populations in the blood, pancreatic lymph node (PLN), and spleen.

Summary of Results

Results from the in vitro studies showed that BMS-986165 and BMS-986202 pre-treatment prevent IFNα-induced upregulation of CXCL10, MX1, and HLA-ABC in EndoC- βH1 and human islets at 24hrs and 48hrs ($p<0.05$). TYK2 inhibition also decreased STAT1/2 phosphorylation and prevented cytokine (i.e. IFNα + IL1-β) -induced apoptosis in dispersed human islets. BMS-986202 reduced diabetes incidence by 80% in RIP-LCMV mice (n=18 vehicle/18 TYK2i; $p<0.001$). Flow cytometry analysis of blood, spleen, and PLN 3 days after LCMV injection revealed a significant decrease in the percentage of CD11b+F4/80+ macrophages (M1) and CD11b+CD49+ NK cells in the PLN and blood and increased circulating CD11b-CD49+ tolerogenic-NK cells in TYK2i treated RIP-LCMV mice. At day 7 and 14 post LCMV induction, PDI+CD8+T-cells in the blood, spleen, and PLN and PDI+FOXP3+ Treg cells were increased in the spleen of BMS-986202-treated RIP-LCMV mice. Treatment with BMS-986202 resulted in a 44% decrease in diabetes incidence in NOD mice (n=32 vehicle/ 34 TYK2i; $p=0.0075$) and significantly reduced insulitis ($p<0.05$). smFISH analysis revealed decreased β cell expression of STAT1 and MX1 expression in TYK2i-treated RIP-LCMV and NOD mice compared to vehicle-treated mice.

Conclusions

Treatment with TYK2 inhibitors protected human islets in vitro, reduced diabetes incidence in two mouse models of T1D, and was associated with early changes in innate immune and late changes in adaptive immune signatures, most notably leading to increased T regulatory cells and increased PDI expressing CD8+ T cells, a phenotype which has been linked T cell exhaustion, Taken together, these data highlight the potential role for TYK2 inhibitors as a novel disease-modifying therapy in T1D, with beneficial effects in both the immune and β cell compartments.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

29: Isaac Snowhite (University of Miami)

Abstract Title

Circulating miRNAs reflect enterovirus infection in the DIPP Study

Authors

Isaac Snowhite, Jay Sosenko, Ricardo Pastori, Mikael Knip, Jorma Toppari, Sami Oikarinen, Jussi Lehtonen, Keikki Hyoty, Riitta Veijola, Shari Messinger, Alberto Pugliese

Purpose

Enterovirus (EV) infections are linked type 1 diabetes (T1D). We hypothesized the EV infections may induce changes in circulating miRNAs. To test this hypothesis, we conducted a pilot study to examine circulating miRNA profiles in longitudinal samples from children with increased HLA risk for T1D enrolled in the Finnish population-based DIPP (T1D Prediction and Prevention) study.

Methods

We studied 10 DIPP participants (5 males, 5 females, mean age 1.0 + SD 0.9 years) with acute EV infection demonstrated by EV RNA detection in the serum using PCR. We obtained 3 longitudinal serum samples from each subject, one corresponding to the EV infection, another obtained on average 6 months prior to the infection, and the last obtained on average 6 months after the infection.

Levels of 2,083 miRNAs were assessed with the HTG Molecular EdgeSeq assay in all serum samples. We used the Reveal software 4.0.0 in paired analysis to identify miRNAs that were differentially expressed comparing levels from the 1st sample to the sample corresponding to the EV infection (sample 2) and to the last follow-up sample (sample 3). Statistical significance differences are reported with adjustment for multiple comparisons using the Benjamini and Holberg method to control false discovery rate.

The Overall Chi-Square was used to test for differences between groups, similar to ANOVA, and the differential expression was assessed through estimated fold changes between each of the two groups and baseline (V1) (transformed from log-fold change). The DESeq2 package provides methods for estimating and testing differential expression using negative-binomial generalized linear models. Empirical Bayes methods are used to estimate dispersion and log2(fold change) with data-driven prior distributions. No pre-filtering is applied to the data prior to analysis. The DESeq2 model corrects for library size using the median ratio method from Anders and Huber (2010). Dispersions are estimated with the Cox Reid-adjusted profile likelihood method developed by McCarthy et al. (2012). Log2 fold changes are estimated via Tikhonov/ridge regularization with a zero-centered normal prior distribution with variance calculated using the observed distribution of maximum likelihood coefficients. DESeq2 performs independent filtering on probes prior to applying the false discovery rate p-value adjustment in order to increase power. This will cause some probes to have no p-value.

Summary of Results

The analysis of differential expression involves a Chi Square for repeated measures for all 3 follow-up samples followed by assessment of differential expression for pairs of time points for those miRNAs that were significantly different in the analysis of repeated measures after correction for multiple comparisons. This analysis revealed that 338 miRNAs showed significant changes, and for 214 these changes remained significant after correction for multiple comparisons.

For these 338 miRNAs, the software then assessed differential expression between the EV-positive samples and the first and last samples, separately.

Comparing the initial sample to the EV-positive sample, our analysis identified 101 miRNAs that were differentially expressed; 63 miRNAs were significantly downregulated (Mean log2 FC ± SD: -2.1 ± 0.6) and 38 were upregulated (Mean log2 FC ± SD: 1.76 ± 0.3); mean log2 FC ranges were between -4.23 and 2.42. However, none of these miRNAs remained significant after correction for multiple comparisons.

Comparing the first sample to the last sample, 6-months post-EV infection, our analysis revealed 149 differentially expressed miRNAs; there were 31 downregulated miRNAs (Mean log2 FC ± SD: -1.85 ± 0.3) and 118 upregulated miRNAs (Mean log2 FC ± SD: 3.54 ± 2.2); the mean log2 FC ranged between -2.54 and 12.0). We found that 35/148 miRNAs remained significant after adjustment for multiple comparisons.

We then evaluated whether any of these miRNAs overlapped with 148 miRNAs that were previously reported to be associated with T1D and/or insulin secretion. Of 148 miRNAs previously reported, 45 were found to have significant changes between timepoints but only one remained significant after corrections for multiple comparisons: miR-339-3p, which has been reported to have roles in T1D risk and insulin release, exhibit elevated counts, by a factor of 10, more than six months after infection.

Conclusions

Results from this pilot study suggest that EV infections may lead to changes in levels of circulating miRNAs, which were observed both acutely, coincidental with the detection of EV RNA, and after 6 months when EV RNA was no longer detected, which could potentially suggest an impact of viral infection beyond the acute phase. This observation may also be related to the possibility that EV infections

may become chronic. This pilot study provides support for future investigations involving a larger cohort, including subjects from the DIPP study who developed islet autoimmunity and/or T1D, and the study of follow-up samples from later time-points to establish stability of change (>1 year post infection).

Funding acknowledgement. NIAID 1R01AI123258.

Preferred Presentation Format

No Preference

Research Category

Type 1 Diabetes Etiology & Environment

30: Laurin Herbsthofer (CBmed GmbH Center for Biomarker Medicine)

Abstract Title

IsletViewer: An interactive web-tool for visualization and analysis of pancreatic islets

Authors

Laurin Herbsthofer, Barbara Ehall, Kaddour Bounab, Joakim Franz, Barbara Prietl, Thoms R. Pieber

Purpose

In type 1 diabetes, pancreata contain islets of Langerhans in all shapes and sizes. Together with variance introduced by clinical features, this heterogeneity makes hypothesis-driven analysis of large image data sets difficult and favors exploratory approaches. In previous work, we established an automated islet detection and staging algorithm based on fluorescent multiplex immunohistochemistry that classifies images of murine pancreatic islets into either healthy or one of four stages of beta cell destruction. Using this method, we detected and staged over 1,600 islets in a cohort of NOD mice. However, without a suited software solution being available, the sheer number of images complicated the exploratory analysis of this big data problem, making it difficult to generate new insights.

Methods

As a solution, we developed IsletViewer, an interactive, web-based tool that enables scientists to effortlessly browse our data set and study islet properties in a collaborative fashion. To this end, IsletViewer enables users to:
(1) view clinical data of available samples, create overview plots, and filter data based on clinical features (e.g., blood glucose levels, donor age, sample ID, etc.),
(2) browse a large collection of islet images and study extracted islet features (e.g., islet size and phenotype composition) of selected samples,
(3) manually change assigned islet stages in case of misclassification, and
(4) plot graphs and statistical results to compare groups of islets for exploratory analysis.
IsletViewer was created in python using the streamlit package, making it easy to add new features and incorporate new data sets.

Summary of Results

We actively used IsletViewer for several months in a multi-center research project and continually updated it with new features based on feedback from scientists. The function to manually change islet stages ensured that all images were correctly labelled before any statistical analysis was performed. Additionally, we used IsletViewer for statistical analysis and plotting of results to identify correlations between changes in islet stage frequencies and different clinical features, helping us generate new hypotheses.

All plots in IsletViewer are interactive to enable the identification of outliers and interesting phenomena, making it easy to trace them back to the sample donor and individual islet to further study their properties. The installation-free app can be accessed on any device with an internet browser and by multiple users simultaneously.

Conclusions

After several months of sustained use, we found IsletViewer to be a valuable tool for exploratory analysis of a large and growing data base of murine pancreatic islet images. Its main strength is the simple and fast integration of new features based on feedback from scientists. We plan to make IsletViewer available to the scientific community to enable other researchers to upload and analyze their data and suggest new software features. Specifically, the adaptation of IsletViewer to human samples is planned for the near future. We envision that this platform will become a central hub for data integration from multiple labs to foster research collaboration and generate new insights into type 1 diabetes. An online demo of IsletViewer is available upon request.

Preferred Presentation Format

Oral Presentation

Research Category

Novel Technologies

31: Eddie James (Benaroya Research Institute)

Abstract Title

Recognition of a splice variant neo-epitope by CD4+ T cells in subjects with type 1 diabetes

Authors

Perrin Guyer, David Arribas-Layton, Cate Speake, Carla Greenbaum, Decio Enzirik, Sally Kent, Roberto Mallone, Eddie James

Purpose

A recent discovery effort investigated tissue specific mRNA splice variants and other novel secretory granule antigens within human islets, demonstrating that unique peptide sequences from these proteins are present within HLA-class I peptidome of human β cells and documenting their recognition by CD8+ T Cells from peripheral blood and human islets. Our goal was to investigate the relevance of CD4+ T cell recognition of epitopes derived from these target antigens.

Methods

In this study, we applied a systematic epitope discovery process to identify novel CD4+ T cell epitopes derived from mRNA splice variants and novel secretory granule antigens. We first predicted potential epitopes spanning unique junctions of mRNA splice variants and within conventional secretory granule antigens contained in the data set. Peptides with DRB1*04:01 motifs were screened for in vitro binding and used to generate HLA class II tetramers. The corresponding tetramers were used to assess peptide immunogenicity, isolate T cell clones, and label and detect CD4+ T cells specific for these putative epitopes in peripheral blood. We further investigated the relevance of these epitopes by examining their characteristics in subjects with established T1D and by investigating their recognition by islet derived T cell lines.

Summary of Results

We observed detectable populations of T cells that recognize three novel epitopes in the peripheral blood of subjects with T1D at frequencies that were similar to an immunodominant proinsulin epitope. T cells that recognized these epitopes were present in peripheral blood at higher frequencies in subjects with T1D than in controls and predominantly exhibited a Th1-like surface phenotype. Among the three novel epitopes, responses to a peptide derived from the CCNI-008 splice variant tended to be the most frequent. T cells with this specificity also exhibited a more differentiated memory phenotype. Furthermore, T cells that respond to these epitopes were present among islet infiltrating T cells.

Conclusions

These results reveal novel epitopes that are recognized by CD4+ T cells in human T1D. This further establishes alternative splicing as a mechanism that contributes to the loss of tolerance in T1D.

Preferred Presentation Format

No Preference

Research Category

Immunology

32: Jamie Felton (Indiana University)

Abstract Title

Type I interferon-driven changes in B cell metabolism in type 1 diabetes

Authors

Jamie Felton, Hollu Conway, Mark Kaplan, Carmela Evans-Molina

Purpose

Type 1 diabetes (T1D) results in immune-mediated destruction of insulin-producing beta cells in pancreatic islets. Islet autoantibodies are the earliest predictor of T1D and a prerequisite for nearly all immune intervention trials to date. However, the presence of islet autoantibodies indicate that critical tolerance thresholds have already been breached and an immune response has already been initiated, limiting the efficacy of immunomodulatory interventions initiated at this time. Understanding the events that precede islet autoimmunity has the potential to uncover earlier, more efficacious targets for immune intervention. Two changes have been identified in the blood of individuals at risk for T1D, prior to the appearance of autoantibodies: an increase in expression of type I interferon (IFN)-inducible genes, followed by the disappearance of anergic, or tolerant, islet-reactive B cell subsets, presumably due to their successful activation and migration to the pancreatic lymph nodes and islet. Changes in B cell metabolism have been implicated in loss of B cell tolerance in other autoimmune diseases. Specifically, restrictions in energy metabolism have been shown to restrain pathologic, autoreactive B cells in the periphery. However, whether type I IFN-induced inflammation is required for these metabolic changes to permit B cell escape from anergy in T1D is not known. We sought to determine how type I interferons influence B cell metabolism prior to seroconversion.

Methods

To test the hypothesis that type I IFNs alter B cell metabolic pathways to promote autoimmunity, we used flow cytometric detection of fluorescence to assess glucose uptake, mitochondrial mass, and mitochondrial polarity throughout disease progression in lymphocytes in pancreatic lymph nodes (PLNs) and islets in non-obese diabetic (NOD) mice. To determine whether type I interferon-induced changes were specific to autoreactive B cells, we used extracellular flux analysis to assess oxygen consumption rates, and flow cytometry to

identify changes in markers of B cell development and activation in NOD and non-autoimmune prone C57BL/6J mice, with and without treatment with IFN-alpha.

Summary of Results

Relative glucose uptake was increased in islet-infiltrating B cells compared to T cell subsets in young NOD mice (<8 weeks). Glucose uptake in islet-infiltrating B cells was higher than glucose uptake in B cells in pancreatic lymph nodes early in disease development, but similar as disease progressed. Patterns of mitochondrial function in PLNs and islets were similar for T and B cell subsets throughout disease progression. Compared to non-autoimmune prone B cells, NOD B cells demonstrated increased oxidative phosphorylation in response to treatment with interferon alpha. Of note, baseline oxidative phosphorylation was decreased in NOD compared to C57BL/6J mice. B cell developmental subsets in NOD mice were skewed toward the marginal zone compartment and activation marker CD69 was increased in both NOD and C57BL/6J B cells but increased to a greater extent in NOD B cells, indicating that NOD B cells are more sensitive to treatment with IFN-alpha.

Conclusions

These findings suggest that type I IFNs alter B cell metabolism in NOD mice. It is possible that autoreactive B cells exist in a state of anergy in NOD mice, indicated by reduced oxidative phosphorylation, and that this anergy is reversed upon treatment with IFN-alpha, given the increase in maximal respiratory capacity and upregulation of the activation marker CD69. Future studies are needed to determine whether type I IFN-driven inflammation drives similar changes in human B cells, explore mechanisms of alteration in B cell metabolism, and to determine whether these changes drive T1D development. Understanding mechanisms of tolerance loss will facilitate detection of key molecular targets for earlier intervention in individuals at risk for T1D, in order to improve the efficacy of immunomodulation.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

33: Charanya Muralidharan and Olha Melnyk (Indiana University)

Abstract Title

Induction of islet autophagy in response to IFN-alpha

Authors

Charanya Muralidharan, Olha Melnyk, Michelle Martinez, Justin Crowder, Amelia Linnemann

Purpose

Type 1 diabetes (T1D) is a multifactorial disease involving genetic and environmental factors. One of the factors implicated in disease pathogenesis is early life viral infection. A typical immune response to viral infection includes the production of type 1 interferons (IFN), such as IFN-alpha, which can induce stress in the pancreatic β-cells. Intrinsic cellular stress response mechanisms exist, including autophagy, a process that serves to degrade and recycle cellular components to promote homeostasis, including in response to infection. We recently discovered that autophagy is impaired in the residual β-cells of human organ donors with T1D as well as in islets of the diabetic non-obese diabetic (NOD) mouse model of autoimmune diabetes. Additionally, we observed a significant accumulation of autophagosomes and defective lysosomes in the β-cells of autoantibody-positive donors, suggesting that autophagy is perturbed prior to diabetes onset. Thus, we hypothesize that an ineffective autophagic response to elevated circulating IFN-alpha may play a role in T1D

pathogenesis. There is currently no literature linking IFN-alpha to autophagy in the β-cell, so we set out to determine if β-cell autophagy is modulated by acute IFN-alpha exposure in vitro and in vivo.

Methods

INS1 832/13 cells, human islets obtained from IIDP or the University of Alberta, and wildtype C57Bl/6J mice were used to assess autophagy induction upon IFN-alpha exposure. All samples were evaluated for stimulation of autophagy after treatment with IFN-alpha, in the presence/absence of chloroquine-diphosphate to inhibit autophagic flux.

For in vitro experiments, INS1 832/13 cells or human islets were split into four groups with an approximately equal number of cells or islets. Two groups were pre-treated with 100uM chloroquine-diphosphate for 3 hours followed by vehicle or 2000 U/mL IFN-alpha for 1 hour. Two other groups were treated with either vehicle or 2000 U/mL IFN-alpha for 1 hour without pre-treatment. Total protein was assessed for the IFN-alpha second messenger, pSTAT2, and the autophagosome marker LC3.

For in vivo imaging experiments, 7-week old wild type C57Bl/6J mice were intraperitoneally injected with approximately 3.44*1012 genomic copies of a custom β-cell-selective fluorescent autophagy biosensor (AAV8-INS-mCherry-EGFP-LC3B). Three weeks later, animals were anesthetized and the pancreas was externalized for live imaging. Islets were identified based on GFP+mCherry fluorescence, then imaged using LEICA SP8 DIVE two-photon microscope fitted with a 40x/1.1NA water objective. Baseline images were collected for 2 min, followed by 30 min of imaging (every 2 min) after IP injection of saline (100μL) or 100mg/kg Chloroquine. Following this, 1.8*105 IU of mouse IFN-α was retro-orbitally injected, and images were acquired every 2 min for 20 min. Raw images were 3D-drift corrected using a registration plugin for drift correction in ImageJ. The processed images were then background subtracted, and mCherry puncta count and Pearson correlation of mCherry: EGFP was calculated using CellProfiler/ ImageJ.

Summary of Results

We observe a significant stimulation of autophagy, as measured by changes in the LC3-II/LC3-I ratio, in response to IFN-alpha in INS1 cells (p=0.01), and a more modest stimulation in human islets in vitro. Our in vivo data corroborates these findings, where we observe a rapid and robust stimulation of endogenous pancreatic β-cell autophagic flux within minutes of IV injection of recombinant mouse IFN-alpha.

Conclusions

We report stimulation of pancreatic β-cell autophagy in response to IFN-alpha exposure both in vitro and in vivo. This suggests autophagy as a compensatory mechanism to mitigate the cellular stress caused by IFN-alpha. In the context of our prior observations suggesting defective autophagy in the prediabetic stage, these data support our hypothesis that loss of autophagic response to IFN-alpha-induced cellular stress could promote β-cell dysfunction and death in T1D pathogenesis. Further studies will be required to thoroughly test our hypothesis and determine both the mechanism and timing of the autophagic decline in T1D.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

34: Geming Lu (Icahn School of Medicine at Mount Sinai)

Abstract Title

Dextran Sulfate Ameliorates Type 1 Diabetes by Enhancing Suppressive Myeloid Cells via HGF/cMET Signaling

Authors

Geming Lu, Jiamin Zhang, Randy Kang, Yansui Li, Carolina Rosselot, Kara Beliard, Adolfo Garcia-Ocana

Purpose

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

Methods

1. Eight-week-old NOD/Ltj (NOD) mice were intraperitoneally injected with different doses of DS. Non-fasting blood glucose was measured by a portable glucometer and hyperglycemia was considered when blood glucose was > 250 mg/dl for two consecutive days. At week 22, we collected plasma to measure insulin and HGF, analyzed immune cells phenotypes in PBMC, spleen, pancreatic lymph nodes, and bone marrow, and harvested pancreases for histology staining. 2. Analyzed cMET expression on beta cells, myeloid cells, and T cells with flow cytometry, fluerecency staining, and RNA seq, 3. Non-diabetic NOD mice were treated with DS or saline or cMET inhibitor (PH665752) for 4 weeks and lymphocytes from spleen and pancreatic lymph nodes were analyzed by flow cytometry to profile T cells and DC/Macrophage compartments by checking IFNγ-producing CD4+cells and Foxp3+ regulatory T cells and MHC II/CD86/CD80/PD-1L-expressing myeloid cells. 4. in vitro study showed DS enhanced HGF-cMET signaling in shifting the myeloid cells into suppressive MDSC, which recapitulated suppressive phenotype in vivo.

Summary of Results

Previous evidence indicate DS can stabilize and increase the half-life of hepatocyte growth factor (HGF) in vitro and in vivo. In our studies, we found that pre-diabetic NOD mice treated daily with DS, i.p., for 10 weeks displayed elevated circulating levels of HGF, which strongly correlated with the absence of diabetic onset in these NOD mice. Based on this, we analyzed cMET (hepatocyte growth factor receptor) expression in both islets and immune cells. cMET mRNA expression was significantly increased in cytokine-treated human islets; immunofluorescence staining showed that cMET protein expression was increased in beta cells of immune cell infiltrated islets in NOD mice compared with non-infiltrated islets; and, flow cytometry analysis revealed that cMET was highly enriched in the myeloid cells and comparatively low in the lymphoid cells. Taken together, these studies indicate that HGF/cMET signaling system is upregulated by DS in beta cells and myeloid cells in the autoimmune environment of NOD mice. Indeed, treatment of NOD mice with DS plus the cMET inhibitor, PH665752, blocked DS-induced amelioration of diabetes, eliminated DS-induced increased beta cell survival and lower islet insulitis, and led to increased activated T cells. We next found that treatment with DS combined with HGF in vitro significantly reduced the production of inflammatory cytokines (IL12, IL1b and IL6) in the LPS/IFNg treated bone marrow-derived myeloid cells. Surprisingly, DS+HGF significantly enhanced the polarization of the myeloid-derived suppressive cells (MDSCs) driven by GM-CSF and IL6. Most importantly, DS+HGF treated MDSCs markedly blocked proliferation of aCD3/aCD28-treated T cells. Finally, the transfer of HGF+DS-treated MDSCs significantly delayed T1D onset in NOD-SCID mice adaptively transferred with splenocytes from diabetic NOD mice.

Conclusions

Collectively, these results indicate that DS reduces the onset of T1D in NOD mice via HGF/cMET signaling in myeloid cells, leading to increased number and suppressive function of MDSCs to reduce T cell activation and inflammation. Combination treatment of HGF and DS can potentially be of great value for treating T1D.

Preferred Presentation Format

Poster Presentation

Research Category

35: Jorge Santini-Gonzalez (University of Florida)

Abstract Title

Stem cell derived beta-like cells engineered to present tolerogenic factors in an autoimmune diabetes model

Authors

Jorge Santini-Gonzalez, Roberto Castro-Gutierrez, Matthew Becker, 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 or combined with a biomaterial delivery strategy 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 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. We reasoned that sBC grafts should not present immediate immune rejection due to HLA mismatch but may experience eventual rejection due recognition of other xenoantigens in this fully immune competent model.

NOD-cMHCI-/--A2 mice underwent transplantation of 1,000 sBC under the kidney capsule and were separated in two groups, iP sBC (N=15) and iP-BKO sBC (N=13), each with +/- DOX. The viability of the sBC transplants was longitudinally monitored by IVIS bioluminescence imaging at days 1, 3, 7, and 14 after surgery. After 14 days, the pancreas and kidney were retrieved for histology. Cryosections were stained with H&E and immunostained for insulin, PD-L1 and CD45 or CD3, then imaged via confocal microscopy. Blood serum was also collected the day the tissue was collected and evaluated for human C-peptide with an ultrasensitive human C-peptide ELISA assay.

Summary of Results

A two-way ANOVA of the total photon flux detected longitudinally by IVIS showed a statistical difference (p-value <0.01) for female mice transplanted with A2 sBCs on standard or doxycycline feed at day 3. Although luciferase signal dropped below detection limits by day 14 for most mice, immunostaining showed surviving insulin positive cells, PD-L1 expression and infiltration of CD3+ and CD45+ cells in the iP + DOX sBC graft. Although sBC transplants were insufficient to restore euglycemia in severely diabetic mice, ultrasensitive human C-peptide ELISA detected low levels of circulating human C-peptide in several mice.

Conclusions

This study focused on transplantation of stem cell-derived beta-like cells in a mouse model with spontaneous diabetes and matching human MHC class I immunity. Our data suggest PD-L1 expression by the engineered sBC is likely to improve graft survival, but the

immune environment in this model was so aggressive that many cells did not have a chance to engraft. Nonetheless, 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. These results suggest that primary and adaptive immune responses (we did not use rapamycin) compromised the sBC graft at early time points but that significant numbers of PD-L1 expressing cells managed to survive in this harsh immunological environment. 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.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

36: Virginia M Stone (Karolinska Institutet)

Abstract Title

A Coxsackie B virus prevents virus-accelerated diabetes in NOD mice.

Authors

Virginia M. Stone, Marta Butrym, Minna M. Hankaniemi, Amir-Babak Sioofy-Khojine, Vesa P. Hytonen, Malin Flodstrom-Tullberg

Purpose

Coxsackie B viruses (CVBs) have been associated with type 1 diabetes, however whether they cause the disease remains to be confirmed. An attractive strategy to determine their involvement in type 1 diabetes involves vaccinating genetically at-risk individuals with a CVB vaccine and the subsequent monitoring of disease incidence. We have developed and pre-clinically tested CVB vaccines and demonstrated that they are safe and immunogenic in murine models for type 1 diabetes and in non-human primates. A similar vaccine made for human use has been developed and it recently entered clinical trials. We have previously shown that this type of vaccine prevents acute CVB infections in NOD mice and virus-induced diabetes in the SOCS-1-tg mouse model for virus-mediated beta-cell destruction. Here, we present our studies which aimed to establish if a CVB vaccine can protect against virus-accelerated diabetes onset in the NOD mouse.

Methods

CVB1 vaccine was made by the formalin inactivation of infectious CVB1 virus. Female NOD mice (aged 6-7 weeks old) were left untreated, mock vaccinated (vaccine buffer) or vaccinated on three occasions (days 0, 21 and 35) with 1.8 ug doses of CVB1 vaccine. Serum was collected throughout the study to measure virus neutralising antibody titres by standard plaque reduction assay. One week after the final mock- or CVB1-vaccine dose animals were infected with CVB1 virus by i.p. injection. Diabetes incidence was monitored up to 30 weeks of age through the weekly measurement of blood glucose levels. At diabetes onset/the terminal time point, the pancreas was collected for histological analysis.

Summary of Results

We first confirmed previous results showing that CVBs can accelerate diabetes onset in pre-diabetic female NOD mice. Diabetes developed significantly earlier in mice infected with CVB1 compared to their untreated counterparts. Next, young female NOD mice were left untreated, mock-vaccinated or vaccinated with CVB1 vaccine and the latter two groups were also infected with CVB1 one week after the final vaccination. Protective CVB1 neutralising antibodies were detected in CVB1 vaccinated mice at the time of infection. CVB1-infection accelerated the onset of diabetes in control animals, however vaccination protected against this, and the diabetes incidence curve of vaccinated animals mirrored that of the untreated group. The mean age at diabetes onset was significantly lower in control infected mice compared to untreated and vaccinated groups. Histological assessment of the pancreas revealed that the vaccine also prevented virus-mediated destruction of the exocrine tissue.

Conclusions

In this study we show for the first time that a CVB vaccine can prevent virus-accelerated diabetes in the NOD mouse model. Combined with our previous studies showing that CVB vaccines prevent the direct infection of the beta-cell, this pre-clinical proof-of-concept work provides compelling evidence for the use of the newly developed human CVB vaccine in determining the involvement of CVBs in type 1 diabetes. If proven to be causal, vaccine strategies could be employed to prevent virus-mediated diabetes.

Preferred Presentation Format

No Preference

Research Category：Type 1 Diabetes Etiology & Environment

37: Peter Duinkerken (University of Medical Center Groningen)

Abstract Title

A zebrafish model to address if pancreatic exocrine malfunction triggers beta cell stress

Authors

Peter Duinkerken, Noura Faraj, Anouk Wolters, Tessa Vergroesen, Elizabeth Carroll, Ben Giepmans

Purpose

Abnormalities in the exocrine pancreas might precede T1D as has become evident in several nPOD studies. In our lab, we focused on large-scale electron microscopy (EM; aka 'nanotomy'). Dysfunction of the exocrine pancreas might induce beta cell stress, which may ultimately evoke the immune attack. While exocrine/endocrine cell interactions are found in nPOD donors, the human tissue does not allow an in vivo cause/consequence relationship assessment. To dynamically asses whether exocrine damage can indeed affect the functional integrity of beta cells, we create a zebrafish model that allows modulation of exocrine cells and read out of beta cells in living Islets of Langerhans in situ.

Methods

Zebrafish are modified to allow (1) conditional ablation of exocrine cells and (2) functional readout of beta cell dynamics. To retain spatio-temporal control and allow the assessment of multiple ablation strategies, ablation will be in both an enzyme dependent and photosensitizer dependent manner. Beta cell function is assessed in vivo with light sheet microscopy using fluorescent probes designed to monitor calcium dynamics, ER stress, mitochondrial stress, neoantigen production and glucose response. RNAseq will be performed and compared to expression patterns known to be involved in T1D. Ultrastructural (nanotomy) analysis at endpoints will be conducted and compared to abnormalities found in the nPOD large-scale EM repository.

Summary of Results

The five days old larval zebrafish pancreas, including Islets, show high similarity to adult human pancreas at the ultrastructural level. Moreover, imaging of calcium dynamics indicates the presence of functional, as also demonstrated by others. Ablation of exocrine cells through an NTR- and SuperNova-dependent manner look promising to induce exocrine cell stress.

Conclusions

Abnormalities related to the exocrine pancreas mapped in the large-scale EM nPOD repository and other studies have driven us to assess whether the zebrafish could be usable in investigating exocrine-endocrine interactions dynamically in situ. Pancreatic development in zebrafish is similar to humans and its small size and transparency in the early larval stages allows functional assessment of beta cells using light sheet microscopy. These features pave the way for more in depth analysis of exocrine-endocrine interactions potentially preceding T1D.

Preferred Presentation Format

Poster Presentation

Research Category

Beta Cell Physiology and Dysfunction

38: Martin Haupt-Jorgensen (Bartholin Institute)

Abstract Title

Reduced levels of phosphatidylcholine species in the colonic mucus layer of NOD mice

Authors

Mia Mønsted, Kristina Pedersen, Mesut Bilgin, Marek Kuzma, Petra Tomasova, Blank Sediva, David P Funda, Dennis Sandris Nielsen, Josue Leonardo Castro Mjia, Flemming Pociot, Simranjeet Kaur, Krsten Buschard, Laurits J Holm, Martin Haupt-Jorgensen

Purpose

Several factors implicate the intestine in the development of type 1 diabetes (T1D). We hypothesize that the level and composition of phosphatidylcholine (PC) species in the mucus layer contributes to the compromised intestinal barrier function observed in T1D, which was analyzed here by an omics approach.

Methods

Non-obese diabetic (NOD) mice were compared to C57BL/6 mice at ages 4, 8 and 13 weeks. Mucus samples from jejunum and colon (4 and 13 weeks) were examined for lipid composition through quantitative shotgun lipidomics. The plasma metabolome (4, 8 and 13 weeks) was determined by NMR and LC-MS. Total RNA from jejunal enterocytes (4 weeks) were hybridized to the GeneChipTM Mouse Gene 2.0 ST array to get a complete mRNA expression profile.

Summary of Results

The lipidomics analysis showed that several PC species were reduced in the colonic mucus layer of NOD mice compared to C57BL/6 mice at 4 and 13 weeks of age, which was also the case in serum as measured by metabolomics. This included decreased levels of the PC species PC(36:4) in NOD mice versus C57BL/6 mice; a lipid species that has consistently shown to be decreased in human T1D. Our GeneChip analysis of jejunal enterocytes revealed decreased mRNA levels of the cholesterol and phospholipid transporter ABCA1 in NOD mice versus C57BL/6 mice at 4 weeks.

Conclusions

The results suggest that there is an aberrant transport of phospholipids from the intestinal lumen, resulting in decreased levels of PC in the blood, which again reduces the amount of PC that is transported into the colonic mucus layer. This could potentially contribute to the compromised intestinal barrier that is seen in T1D.

Preferred Presentation Format

Oral Presentation

Research Category

Type 1 Diabetes Etiology & Environment

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

Abstract Title

Elevated islet prohormone ratios as indicators of insulin dependency in islet transplant recipients

Authors

Yi-Chun Chen, Agnieszka Klimek-Abercrombie, Kathryn Potter, Lindsay Pallo, Galina Soukhatcheva, Dai Lei, Melena Bellin, C. Bruce Verchere

Purpose

Pancreatic islet transplantation has therapeutic potential in T1D and is also an established therapy for patients with chronic pancreatitis; however, long-term transplant outcomes are modest. Identifying indicators and predictors of graft function could aid in the improvement of transplant outcomes and glycemic control.

Methods

We analyzed plasma beta-cell (pro)hormones including proinsulin, C-peptide, amidated islet amyloid polypeptide (IAPP), and proIAPP1-48 levels in a retrospective cohort of autologous total pancreatic islet transplant patients ($n = 28$), as well as in a mouse model of optimal versus sub-optimal human islet transplantation. We also performed histological analysis of islet grafts retrieved from mice to evaluate islet prohormone processing machinery in situ.

Summary of Results

Proinsulin-to-C-peptide (PI/C) and proIAPP-to-total IAPP (proIAPP/IAPP) ratios measured at 3 months post-autologous islet transplant were significantly higher in patients who remained insulin dependent at 1 year follow-up (PI/C: 8.83 ± 1.03 vs. 5.76 ± 0.93, $p < 0.05$; proIAPP/IAPP: 0.62 ± 0.10 vs. 0.30 ± 0.02, $p < 0.05$). In a mouse model of sub-optimal human islet transplantation, we found that mice that later became hyperglycemic displayed significantly higher PI/C ratios than mice that remained normoglycemic (1.08 ± 0.10 vs. 0.25 ± 0.41, $n = 8$ and 41, respectively; $p < 0.05$). Histological analysis of islet grafts retrieved from mice showed reduced insulin- and proinsulin-positive area, but elevated glucagon-positive area in grafts that experienced greater secretory demand. Increased prohormone convertase 1/3 (PC1/3) immunoreactivity was detected in glucagon-positive cells, and glucagon-like peptide 1 (GLP-1) immunoreactive area was elevated in grafts from mice that displayed hyperglycemia or elevated plasma PI/C ratios, demonstrating intra-islet incretin production in metabolically challenged human islet grafts.

Conclusions

In failing islet transplants, incomplete beta cell prohormone processing may be an early indicator of graft dysfunction and future insulin dependency. Alpha cell prohormone processing is also likely altered, leading to intra-islet GLP-1 production.

Preferred Presentation Format

Oral Presentation

Research Category

Novel Biomarkers

40: Nagesha Guthalu Kondegowda (Arthur Riggs Diabetes and Metabolism Institute)

Abstract Title

Humoral Factors and Circulating Extracellular Vesicles in Type 1 Diabetes Induce Beta Cell Cytotoxicity.

Authors

Nagesha Guthalu Kondegowda, Joanna Filipowska, Nancy Leon-Rivera, Rollie Hampton, Rosemary Li, Selassie Ogyaadu, Clive Wasserfall, Mark Atkinson, Helna Reijonen, Yuan Yate-Ching, Navneet Dogra, Daniel Roeth, Markus Kalkum, Carol Levy, Susmita Sahoo, Rupangi C. Vasavada

Purpose

The goal of these studies is to examine the role of humoral factors and circulating extracellular vesicles (cEVs) in the pathogenesis of Type 1 diabetes (T1D). T1D is an autoimmune disease in which beta cell loss and dysfunction play a vital role in its pathogenesis. EVs, small membrane bound structures, are secreted into the circulation by almost every cell type. Due to their distinct cargo, EVs act as molecular messengers and inter-organ communicators, and play an important role in both normal and pathophysiological conditions. EV cargo content is based on the environment of the tissue it is being secreted from and can alter the phenotype of recipient cells. Humoral factors in the serum from T1D subjects can be cytotoxic to rodent beta cell lines and islets. Also, serum EVs of long term T1D patients carry distinct miRNA cargo and cause defect in β-cell secretion. Therefore, we hypothesized that cEVs from T1D subjects, at different stages of the disease, are detrimental to islet health, through their differential RNA and protein cargo, with the potential to serve as disease biomarkers.

Methods

Serum and plasma samples were obtained from early (1-5 years since diagnosis) and late (>10 years since diagnosis) stage T1D patients, from autoantibody positive (Aab+) donors, and age, sex and ethnicity matched non-diabetic healthy donors (HD). cEVs and EV-depleted fractions prepared from T1D and HD plasma using ultracentrifugation and exoquick methods, were characterized by Dynamic light scattering (DLS), Nanoparticle Tracking Analysis, Western blotting and transmission electron microscopy. PKH26 labeled cEVs were used for uptake studies in human islet cells. Cytotoxicity assays were performed on rat insulinoma cell line (INS1), mouse and human islet cells cultured for 24h in media in which fetal calf serum (FCS) was substituted for human serum (10%v/v), or in islet media treated with cEVs, from the donors indicated above. Cleaved caspase 3 staining, TUNEL with insulin and glucagon co-staining, were used to assess cell death. Mouse serum from T1D NOD female mice at different stages of the disease was examined similarly. Seahorse analysis was used to assess the effects on mitochondrial function in human islets. RNAseq and proteomic analyses of cEV cargo and the functional contribution of candidate miRNAs towards β-cell cytotoxicity was assessed.

Summary of Results

Our initial findings show that there were no obvious differences in physical characteristics, cell surface markers, size and number, in cEVs from T1D versus HD plasma. cEVs from T1D and HD donors tagged with PKH26 dye showed uptake in INS1 cells and human

beta cells. Serum and plasma from T1D donors (both early and late-stage) (n=5-9) significantly increased cell death in INS1 cells, and in human beta cells, comparable to cell death levels induced by pro-inflammatory cytokines, suggesting that humoral cytotoxicity may persist with disease progression. The same findings hold true when serum from early and late stage of the disease from female NOD mice was used. Our initial data indicate that serum from Aab+ subjects (n=8) induce cytotoxicity in human beta cells, similar to that induced by serum from T1D subjects, suggesting that the humoral beta cell cytotoxicity occurs early, before disease onset. Seahorse analysis suggests that T1D serum-treatment reduces the mitochondrial respiratory spare capacity of human islets compared to HD serum (n=4). We then tested our hypothesis that the humoral cytotoxicity against beta cells seen in the serum and plasma of T1D patients is mediated by cEVs. Indeed, cEVs from T1D donors, but not HD (n=8/group), significantly induced human beta cell death, and this was not observed with the EV-depleted fraction. Relevant to disease pathology, T1D cEV-induced cytotoxic effect was specific to human beta cells but did not induce cell death in human alpha cells in the same islet prep. To investigate the cargo and molecular mechanisms, we performed a pilot RNASeq and proteomic analysis on cEVs (n=5) from T1D and HD subjects. We identified differential miRNA, lncRNA, mRNA and protein cargo in cEVs from T1D vs HD subjects. A functional analysis of candidate miRNAs in T1D cEVs showed specific miRNAs are detrimental to beta cells.

Conclusions

Serum, plasma, and plasma-derived cEVs from human T1D subjects induce human beta cell but not alpha cell death in vitro. Humoral cytotoxicity on beta cells may initiate early in pre-disease at-risk Aab+ individuals, and likely persist in late-stage disease. The differential RNA and protein cargo in T1D cEVs likely mediates the detrimental effects on human beta cells. Our findings suggest that cEVs, at various stages of the disease, play a role in the pathogenesis of T1D as related to the beta cell. Our ongoing studies are investigating the effects of the cEVs on the immune system, their molecular mechanisms of action, as well as the differential RNA and protein cargo in cEVs from different stages of the disease, with the potential to develop new biomarkers and therapeutics for T1D.

Preferred Presentation Format

Oral Presentation

Research Category

Type 1 Diabetes Etiology & Environment

41: Barbara Ehall (Medical University of Graz)

Abstract Title

Re-evaluating diabetes diagnosis in NOD mice shows striking similarity to the human disease

Authors

Barbara Ehall, Laurin Herbsthofer, Kaddour Bounab, Joakim Franz, Ceren Karacay, Clemens Harer, Petra Kotzbeck, Barbara Prietl, Thomas R. Pieber

Purpose

Investigating T1D progression inevitably involves investigating the pancreas and its islets of Langerhans. A very useful model currently in use is the NOD mouse as it exhibits a very similar pathogenesis as in human T1D. To better understand diabetes progression in NOD mice, we previously established a new staging approach based on islet health characterized by fluorescent multiplexed immunohistochemistry (fm-IHC). Additionally, we automated the procedure of detecting and staging islets by using a specialized fm-IHC compression method that enables rule-based detection and staging of islets. In-depth characterization of murine T1D pathogenesis will also improve transferability of future results to the human disease.

Methods

Methods:
Formalin fixed paraffin embedded (FFPE) pancreatic tissue slides of NOD mice were stained with a multiplexed, fluorescent panel that simultaneously targets markers for insulin, glucagon, CD45, CD8 and CD4 in addition to nuclear staining with DAPI. Pancreatic tissue sections of female, NOD mice aged 5, 10, 20, 30, 40 (n=4 each) and 35 (n=9) weeks with last blood glucose (BG) measurements below 200 mg/dl as well as diabetic NOD mice with BG above 350 mg/dl and healthy control C57BL6 mice were used. We stained five FFPE sections per mouse approx. 80 µm apart. We applied Cell2Grid, a novel fm-IHC image compression algorithm, followed by cell-based rules for islet detection and staging, enabling quantitative high-throughput analysis as well as compiling all data in a digital database ("IsletViewer").

Summary of Results

Our staging classifies islets by presence and absence of stained markers (insulin and immune cell markers). We verified stage 0, the "healthy islet", with C57BL/6 mice and stage 4, the pseudo-atrophic, insulin deficient islet, with diabetic NOD mice with BG above 350 mg/dl.
We then characterized the non-diabetic NOD cohort regarding islet stages and correlated it to the average BG over the last two weeks before sacrification (last 4 measurements), thus reducing the impact of BG fluctuations. Islets exhibiting insulin production (stages 0 to 2) are found predominantly in NOD mice with BG values under 140 mg/dl. At an average BG of >140 mg/dl islets are already mostly insulin deficient (stages 3 and 4) and the total amount of islets also decreases.

Conclusions

Evaluating the overall health and functionality of pancreatic islets requires an islet staging that reflects beta cell destruction by the typical autoimmune attack. Our results indicate that this process is already in its final phase at a much lower average BG than expected. This means that classifying NOD mice with a BG <200 mg/dl as non- diabetic can lead to huge variability regarding the actual endocrine state in those animals. Moreover, comparing the threshold of 140 mg/dl (average BG) to the human disease classification of >125 mg/dl (fasting BG) implies that the NOD mouse model is more comparable than previously thought.

Preferred Presentation Format

Oral Presentation

Research Category

Pathology

42: Heikki Hyoty (Tampere University)

Abstract Title

Enterovirus vaccine induces good antibody response in children with increased risk to develop type 1 diabetes

Authors

Amirbabak Sioofy-Khojine, Jussi Lehtonen, Noora Nurminen, Jutta Laiho, Johanna Lempanen, Jormal Ilonen, Jorma Toppari, Riitta Veijola, Mikael Knip, Heikki Hyoty

Purpose

Enterovirus infections have been linked to the initiation of the beta-cell damaging process that leads to type 1 diabetes (T1D). The first vaccine targeting T1D-associated enteroviruses, group B coxsackieviruses, is currently studied in a phase 1 human trial for its safety and immunogenicity. In case this trial shows favorable results, this vaccine can be considered as a feasible candidate for T1D prevention trials. Some studies have implicated that T1D patients may generate lower responses to virus vaccines that healthy control subjects.

Therefore, it would be important to know whether children who carry T1D susceptibility genes and/or who develop islet autoimmunity respond in a proper way to enterovirus vaccines. The purpose of this study was to evaluate the response to the licensed enterovirus vaccine, the poliovirus vaccine, is such children.

Methods

Study subjects comprised children who have been followed from birth in the Type 1 Diabetes Prediction and Prevention (DIPP) study. Antibody response to enterovirus vaccine (formalin-inactivated poliovirus vaccine) was analysed in two different case-control sets that were nested in the DIPP study cohort: 1) The HLA case-control set included samples taken at the age of 18 months from children (N=110) who carried HLA-DQ genotypes that are associated with increased risk of T1D and non-risk children (N=65) who did not carry such HLA-DQ genotypes and were matched for the time of birth, sex and the city of residency. All these children were autoantibody negative and did not develop T1D. 2) Islet autoimmunity case-control set included similar samples from children (N=111) who developed multiple biochemical islet autoantibodies and control children who were pairwise matched for the time of birth, sex and the city of residency, all having HLA-DQ genotypes that confer increased risk for T1D. All children had received three poliovirus immunizations at the age of 3, 5 and 12 months. Neutralizing antibodies against poliovirus 1 Sabin strain were analyzsed using plaque reduction assay (end-point titration).

Summary of Results

In the HLA case-control set neutralizing poliovirus antibody titers did not differ between children who carried either T1D risk or non-risk HLA-DQ genotypes [OR=1.03, (95%CI 0.9-1.2); p=0.642]. In the islet autoimmunity case-control set poliovirus antibody levels were comparable between the case and control children [OR=1, (95%CI 0.85-1.18), p=1.00]. There were no differences in poliovirus antibody levels between the case and control groups in either of these case-control sets when boys and girls were analyzed separately. No difference was seen in poliovirus antibodies when the islet autoimmunity case-control set was stratified by the nature of the first appearing autoantibody (IAA or GADA), or by the age of autoantibody appearance (before or after the age of 18 month).

Conclusions

The response to poliovirus vaccine was similar in children with increased genetic risk of T1D and in children who developed islet autoimmunity when compared to control children. The results suggest that enterovirus vaccine induces good protective immunity in children who are at risk of developing T1D. This finding support the idea of testing the efficacy of other enterovirus vaccines in the prevention of T1D.

Preferred Presentation Format

No Preference

Research Category

Type 1 Diabetes Etiology & Environment

43: Sami Oikarinen (Tampere University)

Abstract Title

Most of the enteroviruses detected in pancreatic samples belong to enterovirus B species

Authors

Jutta E. Laiho, Hanna Honkanen, Jussi Lehtonen, Leena Puustinen, Riitta Veijola, Kalle Kurppa, Johanna Lempainen, Mikael Knip, Jorma Toppari, Lars Krogvold, Rick Lloyd, Knut Dahl-Jørgensen, Heikki Hyöty

Purpose

In type 1 diabetes (T1D) pancreatic beta-cells are damaged, leading to deficient insulin synthesis and hyperglycemia. Enterovirus infections have been linked to T1D in many studies. The association has been seen in prospective series that have screened enteroviruses from stool and serum samples, but also in pancreas samples in e.g. the nPOD and DiViD studies. Interestingly, only part of the 120 different enterovirus genotypes have shown association with T1D, most notably enterovirus B species, including coxsackie B viruses (CBV).

Enteroviruses must pass mucosal surface and other anatomical and immunological barriers to be able to spread into the blood and infect internal organs such as pancreas. In this study, the genotype distribution of enteroviruses detected in the pancreas was compared to that in blood and stools to find out whether the viruses which can reach the pancreas show any characteristic genotype profile. The hypothesis is that the viruses causing infection in the pancreatic islets have properties which make them invasive and tropic to these sites.

Methods

Enterovirus RNA was screened from pancreas (nPOD, DiViD) stool (DIPP) and serum (DIPP) samples using enterovirus specific RT-PCR method targeting a part of the 5'UTR region. Further, all positive samples were genotyped by sequencing part of the viral protein (VP1) coding region and part of the 5'UTR using both traditional Sanger sequencing and amplicon sequencing with the Illumina platform. The sequences were blasted against GenBank sequence database to genotype the detected viruses

Summary of Results

The genotype distribution differed significantly between the tested sample types. Especially, enterovirus B species were more frequent in serum samples (34,3%) compared to the stool samples (24,7%) based on the VP1 coding region. Similarly, analysis of 5'UTR region from the pancreas samples suggested that the majority of the detected enteroviruses belonged to enterovirus B species (88,9%), including CBV's. Interestingly, several different enterovirus strains coexisted in some pancreas samples.

Conclusions

Various enterovirus genotypes were found in the stool samples, but more invasive infections were caused only by a limited number of enterovirus genotypes. Especially, in pancreas samples the majority of the detected enteroviruses belonged to the enterovirus B species, including CBVs. The results support the earlier findings that enterovirus B group may be a risk virus for the development of T1D. This could link to its property to cause invasive viremic infections and tropism to the pancreatic islets.

Preferred Presentation Format

Poster Presentation

Research Category

Type 1 Diabetes Etiology & Environment

44: Ji-Ming Feng (Louisiana State University)

Abstract Title

Detection of Human Papillomavirus Immunoreactivity in Pancreas Tissues with Type-1 Diabetes

Authors

Ji-Ming Feng

Purpose

Type-1 diabetes (T1D) is insulin-dependent diabetes mellitus (IDDM) caused by multiple environmental factors associated with a strong genetic predisposition. Virus infections is one of such environmental factors. Several viruses have been reported as possible triggers in human type-1 diabetes. HPV is one the most common sexually transmitted infections worldwide, affecting 50-70% of sexually active individual. A very high percentage of cervical and anal cancers is caused by HPV infections. Vaccines against HPV have been proven to effectively protect against these HPV-associated cancers, and it has been introduced for female adolescents at age of 9-14 years old. While proven to be effective, some reports raised a concern on a possible link of this vaccine with pancreatis and T1D. This project is to determine the presence of human papillomavirus (HPV) in pancreas tissue with T1D.

Methods

Paraffin sections of human pancreatic tissue from nPOD were stained by immunohistochemistry with human papillomavirus monoclonal antibody (specific to the major virus capsid protein L1) in conjunction with other islet cell markers (insulin and glucagon antibodies).

Summary of Results

HPV L1 immunostaining were performed in pancreas tissues from 8 nondiabetic controls, 10 T1D patients. We have found that HPV L1 immunoreactivity is present in pancreas tissues from two patients (#6235 and #6045) with T1D, but absent in nondiabetic controls. The two HPV L1 positive T1D pancreas tissues show different immunostaining pattern: 1) in #6235, the HPV L1 immunostaining is mainly present in exocrine acinar cells, absent in ductal cells and endocrine islet cells; 2) in #6045, the HPV L1 immunostaining is mainly present in ductal cells and endocrine islet cells, absent in exocrine acinar cells.

Conclusions

To our knowledge, this is the first report to show the presence of HPV L1 immunoreactivity in T1D pancreas tissues, which raised the question on whether the HPV virus plays a role in some cases of T1D. It may also provide some clue to explain the link between HPV vaccine and T1D.

Preferred Presentation Format

No Preference

Research Category

Type 1 Diabetes Etiology & Environment

45: Ahmad Alsahaf (University Medical Center Groningen)

Name of abstract presenter(s):

Anouk Wolters

Abstract Title

FAIR data management of the nPOD Nanotomy Datasets

Authors

Ahmad Alsahaf, Anouk Wolters, Pascal de Boer, J. Paul van Schayck, Dieuwke Roelofs-Prins, Aneas Hodselmans, Morris Swertz, Ben Giepmans

Purpose

In 2020, an open-access dataset that contains large-scale electron microscopy (EM; aka 'nanotomy') images of nPOD pancreas tissue was created and made publicly available [1]. Analysis of the dataset led to the discovery of novel anomalies in type 1 diabetes samples [2].

Moreover, the open access status of the dataset (hosted on nanotomy.org) led to its reuse in several other studies. Due to the large size of the images in the dataset, and the richness and diversity of the metadata, further efforts were needed to increase the dataset's access and reusability, especially for automated learning approaches, and to make it compatible with FAIR data principles [3].

Methods

1) A Python-based software application was created for converting the image data from proprietary file formats of microscope vendors to a standardized microscopy file format (OME-TIFF).

2) Rich metadata (experimental, technical, and patient) were linked to the dataset using a MOLGENIS4 data model for microscopy.

3) Data sets directly refer to the newly created metadata by nPOD on NCBI [7].

Summary of Results

The dataset was shared on the public repositories Image Data Resource [5] and BioImage Archive [6], making it available to a wider group of researchers worldwide.

Conclusions

The implementation of standardized data stewardship practices such as FAIR will increase the visibility and reusability of the nPOD EM dataset, and that will consequently increase its potential for scientific discovery.

References:
[1] www.nanotomy.org/OA/nPOD
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[3] Wilkinson, Mark D., et al. "The FAIR Guiding Principles for scientific data management and stewardship." Scientific data 3.1 (2016): 1-9.
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[5] https://doi.org/10.17867/10000168
[6] https://www.ebi.ac.uk/biostudies/studies/S-BIAD217
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Preferred Presentation Format

Poster Presentation

Research Category

Type 1 Diabetes Etiology & Environment

46: Christina Martins-Cargill (University of Pittsburgh)

Abstract Title

Therapeutic Targeting of the Glycolysis Enzyme PFKFB3 Terminally Exhausts Diabetogenic CD4+ T cells

Authors

Leann New, Erin O'Connor, Dana Previte, Kasey Cargill, Isabelle Tse, Sunder Sims-Lucas, Jon Piganelli

Purpose

Autoreactive CD4+ T cells are critical and required for Type 1 Diabetes (T1D) pathogenesis, and the importance of metabolic programs in mediating proper T cell function have gained better appreciation in recent years. It is now well- established that during activation, effector T cells undergo metabolic reprogramming to the less efficient aerobic glycolysis to support clonal expansion and effector function. Targeting the glycolysis pathway to control aberrant T cell responses has been investigated in several autoimmune disease models, including Systemic Lupus Erythematosus, Multiple Sclerosis, and Rheumatoid Arthritis, but remains a largely understudied therapeutic approach to control self- reactivity against islet β cells T1D. Moreover, current therapies targeting the glycolysis pathway using the prototypical inhibitor 2-DG (a non- metabolizable glucose analog) have failed to promote a durable phenotype, with cessation of treatment associated with re-emergent disease. Based on these previous studies, we hypothesized that administration of the small molecule PFK15, an inhibitor of the rate limiting glycolysis enzyme PFKFB3, would inhibit aberrant activation of autoreactive CD4+ T cells, thereby delaying the onset of T1D in relevant animal models of disease.

Methods

To determine the ability of PFK15 to inhibit CD4+ T cell responses to diabetes relevant antigens in vitro, we used the NOD.BDC.2.5.TCR-Tg mouse (BDC2.5), which recognize a β cell protein and can transfer diabetes. Splenocytes from these mice were stimulated in vitro for 24- 72 hours with their cognate peptide +/- PFK15. Supernatants were collected for lactate and effector cytokine production, and T cells were stained for activation markers and analyzed by flow cytometry. To evaluate the ability of PFK15 to prevent T1D in vivo, isolated CD4+ T cells from BDC2.5 animals were activated and expanded ex vivo, and transferred into NOD.scid recipients. This is a robust model that induces diabetes in approximately 7-14 days. A cohort of animals received 25 mg/kg PFK15 soluble drug i.p. or vehicle control every other day for 2 weeks. Animals were monitored biweekly for onset of hyperglycemia, and body weights were measured to assess toxicity of the drug. Pancreata, spleens, and peripheral blood were harvested at sacrifice for histological analyses, immunofluorescence staining, and downstream flow cytometric analyses, respectively. To determine durability of the exhaustion phenotype induced, we performed reversibility studies using the BDC2.5 T cell clone in vitro, and a T cell transfer model in vivo where a subset of T cells or recipient animals received immune checkpoint blockade (αPD-1 and/or αLAG-3).

Summary of Results

PFK15 treatment interrupted metabolic reprogramming to glycolysis upon activation with β cell antigen, and reduced T cell responses to diabetes relevant antigens in vitro. In in vivo studies, PFK15 treatment delayed the onset of T1D, with 57% of animals remaining diabetes free for the duration of the experimental study. Protection correlated with increased expression of inhibitory receptors PD-1 and LAG-3 on CD4+ T cells in the peripheral blood and spleens of treated animals, while immunofluorescence staining of pancreatic tissue sections revealed a reduced T cell infiltrate, which demonstrated an increased and sustained expression of PD-1 within islets, indicating T cell exhaustion. Mechanistic in vitro studies treating BDC2.5 T cell clones with PFK15 during 2- week restimulation cultures confirmed that PFK15 treatment of diabetogenic CD4+ T cell clones induces functional and metabolic T cell exhaustion, a phenotype that was irreversible via restimulation, IL-2 supplementation, or checkpoint blockade in vitro and in vivo. These findings were specific to targeting of PFKFB3, as use of 2-DG failed to recapitulate the exhaustion phenotypes associated with PFK15 treatment.

Conclusions

While the development of T cell exhaustion is detrimental in cancer and chronic infection, the opposite is true in autoimmunity, where inducing a hyporesponsive phenotype can mediate protection of self- tissues from autoimmune destruction. Our data and recent manuscript demonstrates that targeting of the glycolysis enzyme PFKFB3 with the small molecule inhibitor PFK15 delays the onset of

T1D in a T cell transfer model by inducing CD4+ T cells to become terminally exhausted. T cell exhaustion is thought to primarily occur because of chronic antigen exposure; however, our work demonstrates an ability to terminally exhaust T cells through inhibition of the rate limiting glycolytic enzyme PFKFB3, independently of persistent antigenic stimulation. These findings support that PFKFB3 inhibition drives T cell exhaustion, similarly to that observed when nutrients are limiting in the tumor microenvironment, and that metabolic modulation may serve as a novel therapeutic target to control aberrant T cell responses in T1D.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

47: Marcus Lundberg (Uppsala University)

Abstract Title

Alpha cells in laser-capture microdissected islets of typ 1 diabetes donors with a long duration of disease

Authors

Louise Granlund, Anders Hedin, Olle Korsgren, Marcus Lundberg, Oskar Skog

Purpose

In donors with a long duration of type 1 diabetes (T1D), alpha cells constitute the largest fraction of pancreatic islet cells. However, T1D patients have an impaired glucagon response, and dissociated alpha cells obtained from isolated islets of T1D donors have been shown to be dysfunctional. The purpose of this study was to characterize the transcriptome of laser-capture microdissected (LCM) islets in T1D donors with a long duration of diabetes to examine especially alpha cells that have not been exposed to enzymatic digestion.

Methods

Pancreatic biopsies obtained through the Nordic Network For Clinical Islet Transplantation, from 7 T1D donors with a long duration of disease, and 8 matched non-diabetic donors, were examined. Consecutive sections from flash-frozen biopsies were either stained for insulin, glucagon and somatostatin using immunofluorescence protocols, or utilized for LCM. RNA of LCM-extracted islets was sequenced using AmpliSeq.

Summary of Results

All T1D islets were histologically devoid of insulin. The median islet cell proportion in subjects with T1D was 76% alpha cells, 0 % beta cells and 24% delta cells (with a similar estimated cell type proportions in extracted islet tissue using Multi-subject Single Cell deconvolution). Islets from T1D and non-diabetic donors clustered separately on a principal component analysis (PCA). To more specifically address whether the alpha cell population in the present T1D islets had been altered, previously reported data from sorted alpha cells were used to define 526 highly expressed genes in alpha cells, as well as 40 alpha-cell specific genes. The rank of the 526 genes in the reported single-cell sorted alpha-cell data set were compared to the rank in the present extracted T1D islet data set. The alpha-cell specific genes were compared between the present T1D and non-diabetic islets. Preliminary comparisons indicate several differences, which are currently further analyzed.

Conclusions

We present the transcriptome profile of LCM-extracted islets obtained from long-standing T1D donors. T1D islets have an altered profile, and bioinformatical studies are ongoing to further answer which alterations can be attributed to changes specific to the alpha cells present. Furthermore, optimization of single-cell LCM is ongoing to enable extraction of single cells to further increase the precision of the analysis.

Preferred Presentation Format

Poster Presentation

Research Category

Pathology

48: Janet Wezlau (University of Colorado Anschutz)

Abstract Title

A B-chain Hybrid Insulin Peptide is a potent antigen for an insulin-reactive T cell clone

Authors

Janet Wezlau, James DiLision, Rocky Baker, Kathryn Haskins

Purpose

Insulin is considered the predominant and initiating antigen in both Type 1 Diabetes (T1D) in humans and autoimmune diabetes in the NOD mouse. The insulin B-chain sequence, B:9-23, has been a primary focus of research and there is strong evidence that insulin-reactive T cells play a key role in pathogenesis of disease. Our lab previously determined that hybrid insulin peptides (HIPs) are antigens for autoreactive CD4 T cells in both the NOD mouse and in human T1D patients. HIPs are formed through a unique mode of post-translational modification whereby insulin C-peptide fragments are fused to sequences from other β-cell granule proteins. Because the responses of insulin-reactive T cell clones to insulin B:9-23 as a peptide antigen are relatively weak, we hypothesized that the ligands for these T cells are HIPs comprised of sequences from B:9-23 (vs insulin C-peptide) linked to other β-cell peptides. The resulting non-genomically encoded neoantigens may provide an explanation for the antigencity of the insulin B:9-23 peptide.

Methods

We constructed combinatorial B-chain HIP libraries, representing over 300 potential B-chain HIPs, and tested pools of these HIPs for ability to stimulate a panel of B:9-23-reactive T cell clones. Synthetic B-chain HIP candidates, deduced from positive pools, were used to validate reactivity of B-chain HIP-reactive T cell clones and to detect T cell responses to B-chain HIPs in splenocytes from diabetic NOD mice. MHC class II tetramers loaded with the novel B-chain HIPs were used to detect B-chain HIP-specific T cells in the islet infiltrate of NOD mice.

Summary of Results

We constructed combinatorial B-chain HIP libraries, representing over 300 potential B-chain HIPs, and tested pools of these HIPs for ability to stimulate a panel of B:9-23-reactive T cell clones. Synthetic B-chain HIP candidates, deduced from positive pools, were used to validate reactivity of B-chain HIP-reactive T cell clones and to detect T cell responses to B-chain HIPs in splenocytes from diabetic NOD mice. MHC class II tetramers loaded with the novel B-chain HIPs were used to detect B-chain HIP-specific T cells in the islet infiltrate of NOD mice.

Conclusions

Similar to C-peptide-containing HIPs that are potent antigens for T cells in T1D and NOD mice, insulin B:9-23 sequences can also form HIPs with other secretory granule peptides that stimulate diabetogenic T cells in autoimmune diabetes. B-chain HIPs may provide an explanation for T cell reactivity to native B:9-23 epitopes and provide unique targets for antigen-specific immunotherapies.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

49: Leeana Peters (University of Florida)

Abstract Title

Interrogation of pancreatic lymph node and pancreas derived T cell phenotypes though integration of single cell transcriptomic and epigenomic data

Authors

Leeana Peters, Maigan Brusko, Mollie Huber, nPOD Immunology Core

Purpose

Single cell technologies provide potential for defining tissue specific regulatory and diabetogenic cell subsets at high resolution. In particular, multimodal analysis of both transcriptomic and epigenetic data permits assessment of the molecular mechanisms underlying T cell phenotypes, including the influence of noncoding risk variants. We chose to apply these technologies, namely single cell RNAseq and ATACseq, to cells derived from nPOD donor PLN and T cells expanded from pancreatic slices. The capacity to integrate these multiple modalities has the potential to aid in informing translational efforts in developing optimized pathway targets and cellular therapies for T1D.

Methods

Transcriptomic analysis was performed using whole homogenized PLN from control and T1D donors or T cells expanded from pancreatic slices using the 10x genomics 5'v1.1 kit. Epigenetic analysis was performed using CD8+ T cells sorted from PLN of control, autoantibody positive and control donors using 10x genomics Single Cell ATAC v1.1 kit. Transcriptomic and immune receptor repertoire data were analyzed and visualized using the R packages Seurat and ScRepertoire, and epigenetic data was analyzed using Signac and ArchR.

Summary of Results

Unsupervised clustering of transcriptomic data using Seurat identified 21 clusters, 4 of which are T cell clusters that are enriched in T1D PLN. These include a cluster of KLRB1+ T cells expressing costimulatory marker ICOS, a cluster reflecting a potentially TH17 skewed subset (STAT3, IL6ST, TNFAIP3), and a cluster of CD8 T cells which express molecules indicative of an effector phenotype (CCL5, NKG7, CCL4, GZMK, KLRD1). Paired TCRab sequence analysis of expanded slice T cells identified clusters of T1D antigen reactive CD8+ T cells with a similar phenotype. Accessibility analysis from CD8+ T cells also identifies an upregulation in IFNG and enrichment in RUNX1 and LEF1 TF binding motifs in AAB+ relative to control.

Conclusions

Analysis of single cell transcriptomes of immune cells derived from PLN and pancreatic slices revealed enrichment of clusters with potentially cytotoxic and proinflammatory profiles, including effector CD8 T cells. Enrichment of motifs for transcription factors which could drive this phenotype were evident in CD8+ T cell epigenetic data. We intend to substantiate and further these observations with more subjects and the addition of functional studies, including gene editing, as well as add additional analysis investigating the impact of risk variants on regulatory network function.

Preferred Presentation Format

No Preference

Research Category

Immunology

50: Abdel Hamad (Johns Hopkins)

Abstract Title

Updates on dual expressers: discovery of new functional pathogenic autoantibody-T cell diabetogenic axis

Authors

Rizwan Ahmed

Purpose

In 2019, we published a Cell paper describing a novel lymphocyte that coexpresses TCR and BCR called dual expressers (DEs). A comment during 12th nPOD meeting by Dr. Atkinson and publication of "matter arising" study raised doubts among some members of the community in the varsity of our original findings. However, during the last two years since our original publication we have made striking progress in extending our findings. We hope that we will be given the opportunity to update the nPOD community in the progress we have made and its implications for the field.

Methods

T cell activation and proliferation assays; dual staining with DQ8 tetramers complexed with x-autoantigen and insulin-mimotope; repertoire analsys and immunosequencing; public data search using http://clonesearch.jdrfnpod.org/; iReceptor Gateway: https://gateway.ireceptor.org/login; Tcrdb: http://bioinfo.life.hust.edu.cn/TCRdb/#/search; immunoprecipitation and western blotting; molecular dynamic simulations.

Summary of Results

Using x-mAb as a prototype we identified thousands of stereotypic autoantibodies bearing the invariant x-motif. Synthetic peptides from CDR3 of these autoantibodies including PLNs of nPOD subjects are highly immunogenic especially in DR3/DR3 heterozygous subjects. Using x-mAb to identify interacting T cells, followed by sorting and repertoire analysis, we identified a large family of public TCRs with characteristically invariant CDR3 sequences. We found more than 30 thousands of the same published three clonotypes using public database, including from T1D. However, these TCRs are highly enriched among DEs.
Immunoprecipitation and molecular simulations show that x-mAb interacts directly with TCRs.
We will also show update on published data

Conclusions

Our results identify a new pathogenic axis between BCR and TCR with invariant CDR3 that appears to play important role in the diabetogenic process.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

51: Denise Drotar (University of Florida)

Abstract Title

Functional assessment of regional endocrine and exocrine pancreas heterogeneity

Authors

Denise Drotar, Ana Karen Mojica-Avila, Josephine Brown, Helmut Hiller, Mollie Huber, Edward Phelps, Irina Kusmartseva, Mark Atkinson, Stephan Speier

Purpose

In type 1 diabetes (T1D), infiltrating immune cells cause pancreatic beta cell destruction in a patchy, lobular pattern. Beyond beta cell loss, recent efforts have demonstrated that both endocrine and exocrine compartments of the pancreas are affected in T1D. While histological evidence of regional heterogeneity within the human pancreas is extensive, the functional significance of these differences remains unknown. Thus, we aim to coregister regional differences in secretory function from endocrine (insulin, glucagon) and exocrine (amylase, lipase, trypsinogen) pancreatic tissue together with morphology; all in organ donors with and without T1D using the novel pancreas tissue slice platform. Our hypothesis is that interregional differences in endocrine and exocrine cell physiology drive heterogeneity of insulitis and beta cell destruction in the human T1D pancreas.

Methods

Pancreata from non-diabetic (ND) and T1D organ donors were processed in line with the Network for Pancreatic Organ donors with Diabetes (nPOD) Organ Processing and Pathology Core (OPPC) standard operating procedures. Tissue slices of 120µm thickness were freshly generated from the pancreatic head (PH), body (PB) and tail (PT) regions, and viability was evaluated by immunofluorescent staining. Islet and acinar cell secretory function was assessed from PH, PB, and PT slices obtained from seven ND and two T1D organ donors in response to glucose and other stimuli. Secreted hormones and enzymes were measured using commercially available ELISA and radioimmunoassay kits. After functional assessment, slices were fixed and shipped to the Paul Langerhans Institute Dresden for 3D morphometrical analysis. Fixed slices were stained for endocrine and immune cell markers (insulin, glucagon and CD3), and whole slice imaging was performed on a Zeiss LSM 780 NLO equipped with an automated stage. Image processing and analysis were accomplished semi-automatically using Fiji and MorphoLibJ.

Summary of Results

Here, we compare dynamic insulin and glucagon secretion patterns as well as pancreatic enzyme release using live pancreas tissue slices obtained from the PH, PB, and PT regions of eight ND organ donors (age range 4-33 years). Our data, while preliminary, suggests insulin and glucagon secretory capacity in Here, we compare dynamic insulin and glucagon secretion patterns as well as pancreatic enzyme release using live pancreas tissue slices obtained from the PH, PB, and PT regions of eight ND organ donors (age range 4-33 years). Our data, while preliminary, suggests that insulin and glucagon secretory capacity in response to changes in glucose concentrations (1mM to 5.5mM and 1mM to 11.1mM) is similar in slices obtained from the PH, PB and PT of ND individuals; this, despite known differences in

islet cell composition across the three regions. While pancreatic exocrine enzyme release showed heterogeneity between the donors we have examined, amylase and lipase secretion capacity in response to carbachol appears to be comparable across the three regions.
We also investigated slices from two T1D pancreata, one with new onset disease (25 years of age; GADA+, ZnT8A+) and a second with 7 month diabetes duration (20 years of age; GADA+, ZnT8A+, mIAA+ and IA-2A+). In both cases, insulin secretion capacity was dramatically diminished in response to a glucose challenge when compared to ND donors. Interestingly, in the new onset disease case, insulin response to 11.1mM challenge was higher in PH as compared to PT, while insulin secretion could not be detected from PB. Glucagon release was measured from the same slices at the same time and showed an increase at 11.1mM glucose in all regions. Stimulated amylase and lipase secretion was within the ND range in slices from both T1D donors. Experiments are in progress to further evaluate the functional relationship between the endocrine and exocrine compartments in pancreas slices from T1D donors and those at increased risk for this disease (autoantibody positive). Furthermore, ongoing 3D morphometry analysis of the perifused tissue slices will allow us to identify the distinct roles of endocrine cell mass and function.

Conclusions

This work provides proof that islet and acinar cell function can be simultaneously studied across the human pancreas of organ donors using the pancreas tissue slice platform, to identify potential regional heterogeneity of cell function. 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.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

52: Mollie Huber (University of Florida)

Abstract Title

Investigating β cell functionality during T1D pathogenesis using the live pancreas tissue slice model

Authors

Mollie Huber, Marjan Slak Rupnik, Denise Drotar, Helmut Hiller, Maria Beery, Irina Kusmartseva,Maigan Brusko, Alberto Pugliese, Mark Atkinson, Clayton Matthews, Edward Phelps

Purpose

Type 1 diabetes (T1D) results from the immune-mediated destruction of the insulin-producing β cells of the pancreas. Here we explore the hypothesis that islet autoimmunity and T cell infiltration trigger dysfunctional β cell glucose responses during early stages of T1D progression and contribute to dysglycemia prior to β cell loss using human pancreas slices in conjunction with Ca2+ imaging and insulin secretion assays.

Methods

Live human pancreas tissue slices were prepared from organ donors without diabetes or autoantibodies (ND, n=9), donors positive for one or more autoantibodies without a diagnosis of T1D (AAb+, n=6), and donors with short duration T1D within 4 years of diagnosis (T1D, n=4). Imaging studies were performed on 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. Insulin secretion was measured in parallel using a Biorep perifusion system. Analysis of Ca2+ images utilized a data analysis pipeline involving segmentation of regions of interest, extraction of Ca2+ traces, and signal processing to detect features of individual Ca2+ events. This analysis determines the glucose responsiveness of islets from the level of individual cells as well as for the entire islet.

Summary of Results

For each nPOD slice case we imaged many CD3 and ENTPD3 stained islets (approx.15-50) across multiple slices to assess the general degree of CD3+ T cell infiltration and to search for insulitic islets with remaining ENTPD3+ β cells to measure glucose and KCl responsiveness. We also sought to identify non-infiltrated islets with normal morphology from within the same donor to serve as an internal control. No islets measured from ND or AAb+ donors had substantial numbers of infiltrating CD3+ T cells. For donors with T1D, most ENTPD3+ islets had some degree T cells infiltration with several islets meeting the standard to be categorized as insulitic with ≥15 CD45+ (in this case CD3+) immune cells in contact with the islet. Between two and six successful Ca2+ imaging experiments were acquired for each slice donor. Islets in slices from ND and AAb+ donors exhibited strong Ca2+ and insulin secretory responses to high glucose and KCl, indicating that the β cells were alive and functional. In contrast, islet function in T1D cases was heterogeneous, with some islets remaining responsive to both glucose and KCl while other islets exhibited dysfunction indicated by a lack of glucose response yet confirmed to be viable by responding to KCl. One short duration T1D case (#6551) was particularly informative where insulitic islets exhibited functional heterogeneity. We recorded multiple islets from case #6551 with heavy insulitis (>50 CD3+ T cells) that had no Ca2+ response to high glucose or a diminished response, yet other insulitic islets maintained good glucose responsiveness despite the presence of many T cells. Together, these data demonstrate the existence of large numbers of remaining β cells in T1D cases that fail to respond appropriately to glucose. This dysfunction is linked to the presence of CD3+ cells within dysfunctional islets during early stages of T1D.

Conclusions

The live pancreas tissue slice model allows for endogenous immune cell activity to be observed simultaneously with β cell function. Our data suggest that human β cells tend to lose glucose responsiveness when large numbers of T cells infiltrate the islet, although this dysfunction was not universally observed for all infiltrated islets. This heterogeneity in β cell dysfunction indicates that while immune cell infiltration is likely important, other factors in the β cell such as changes in glucose metabolism, microenvironmental breakdown, and mitochondrial stress also play a role. Our next challenge is to determine the mechanistic factors that differentiate functional from dysfunctional β cells within insulitic islets. To better control the stages of insulitis we are exploring introduction of engineered human T cells into slices to create “investigator initiated insulitis.” Pancreas slices from mouse models of T1D will also provide valuable insight. In summary, we have demonstrated dysfunctional glucose responses in β cells undergoing an endogenous autoimmune insult during progression of T1D in humans. These results contribute to a better understanding of human T1D etiopathology and perhaps broaden the opportunity for therapeutic intervention in new-onset T1D patients.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

53: James DiLisio (University of Colorado)

Abstract Title

Induction of antigen-specific tolerance with hybrid insulin peptides suppresses autoreactive T cells in islet graft transplant recipients

Authors

James E. DiLisio

Purpose

Autoreactive T cells are thought to drive β-cell destruction in both the NOD mouse and human autoimmune diabetes. Our panel of diabetogenic CD4 T cell clones was derived from diabetic NOD mice and the prototype BDC-2.5 T cell clone was determined to be highly responsive to a hybrid insulin peptide (HIP) termed the 2.5HIP. In the NOD mouse, CD4 T cells recognizing the 2.5HIP are abundant in the pancreas and T cells from the BDC-2.5 TCR transgenic (Tg) mouse readily transfer disease in NOD.scid recipients. Previous work in our lab demonstrated that the 2.5HIP, coupled to tolerogenic poly(lactide-co-glycolide) (PLG)-nanoparticles (NPs), delays diabetes in the BDC-2.5 disease transfer model. Currently, we are investigating the tolerogenic potential of 2.5HIP NPs to prolong islet graft function in diabetic NOD mice. The aim of this work is to determine the mechanisms by which antigen-specific tolerance is induced by 2.5HIP NPs and whether there is linked suppression whereby tolerance extends to T cells of other antigen specificities.

Methods

Tolerance induction and islet transplant of diabetic NOD mice: Diabetic NOD mice were treated i.v. with the 2.5HIP or HEL (as an irrelevant control peptide) NPs on days -7, +1, and +7 relative to islet transplant. At day 0, the mice received a transplant of 500 syngeneic NOD.scid islet equivalents under the kidney capsule, reverting to normoglycemia with two days. Mice were then monitored for recurrence of disease.
T cell phenotyping: Islet grafts and spleens of recipient NOD mice were dissociated and the cell suspensions were stained with various tetramers and antibodies, with or without mitogen stimulation, to determine the phenotype and functionality of T cells after tolerance induction.
Single cell RNA sequencing (ScRNASeq) of graft-infiltrating T cells: Cell suspensions from islet grafts were stained with tetramers and antibodies labeled with both fluorochromes and oligo barcodes. Live CD4 and CD8 T cells were sorted by FACS, followed by single cell sequencing via the 10X Genomics single cell platform.

Summary of Results

Post-transplant normoglycemia was maintained approximately four times as long in NOD mice treated with the 2.5HIP NPs compared to HEL NP-treated or untreated controls. Graft-infiltrating 2.5HIP tetramer+ CD4 T cells exhibited an anergic phenotype with reduced effector function after 2.5HIP NP treatment compared to either control. Of note, CD4 and CD8 T cells specific for other known islet autoantigens also exhibited reduced effector function after 2.5HIP NP treatment.

Conclusions

We have demonstrated that antigen-specific tolerance induction to a hybrid insulin peptide T cell epitope prolongs islet graft function in NOD mice. Treatment with the 2.5HIP NPs not only induced tolerance in graft-infiltrating 2.5HIP tet+ CD4 T cells, but also in other islet-specific T cells. Targeting HIP-reactive T cells through antigen-specific tolerance induction could inform future type 1 diabetes immunotherapies.

Preferred Presentation Format

No Preference

Research Category

Immunology

54: Abhishek Kulkarni (University of Chicago)

Abstract Title

12-Lipoxygenase Activates the Integrated Stress Response in Pancreatic Islets, Suppresses PD-L1 Production, and Promotes the Development of T1D

Authors

Abhishek Kulkarni, Sarah A. Tersey, Fei Huang, Annie R. Pineros, Farooq Syed, Hongyu Gao, Kara Orr, Yunlong Liu, Maureen Gannon, Marcia McDuffie, Jerry L. Nadler, Margaret A. Morris, Raghavendra G. Mirmira

Purpose

In recent years, type 1 diabetes (T1D) has become viewed as a disease initiated and propagated by islet β cells. 12-lipoxygenase (12-LOX), an enzyme in arachidonic acid metabolism, is expressed in β cells and macrophages, and prior studies have shown that its deletion globally can protect against T1D in the NOD mouse model. In this study, we hypothesized that 12-LOX within the β cell may be an initiator of autoimmunity in T1D through activation of the integrated stress response (ISR).

Methods

We interrogated NOD mice with islet-specific deletion of the gene encoding 12-LOX for activation of the ISR and diabetes outcome, and leveraged tissues from the nPOD repository for interrogation of the ISR in human T1D. In addition, we utilized an inhibitor of the human 12-LOX enzyme (ML355) in studies of human islets and in studies using NOD mice containing targeted replacement of the mouse enzyme with the human enzyme ("humanized mice").

Summary of Results

We backcrossed both mice with floxed alleles of the gene encoding 12-LOX (Alox15) and mice with the PdxPB-CreERT transgene onto the NOD background at greater than 99.7% of genomic loci. Tamoxifen-induced deletion of islet Alox15 at 6 weeks of age led to preservation of β-cell mass, suppression of insulitis, and near-complete protection against autoimmune diabetes. Single cell RNA-sequencing and mass cytometry analyses revealed that the loss of islet Alox15 led to an increase in a population of β cells expressing Cd274, encoding the immune checkpoint protein PD-L1 [5], and promoted the reprogramming of the immune response. The reprogramming included the expansion of anti-inflammatory macrophages and regulatory T cells and suppression of cytotoxic T cells. The increase in PD-L1 protein was coincident with the suppression of the integrated stress response (ISR) in these mouse islets, as evidenced by reductions in stress granules and levels of phospho-eIF2α. Administration of a PD-L1 blocking antibody led to recovery of the diabetes phenotype in islet Alox15 knockout mice. In humans, increases in islet 12-LOX and the ISR (evidenced by formation of stress granules) was observed in pancreas tissue sections from AAb+ donors. In human islets, inhibition of either 12-LOX using ML355 or the ISR using ISRIB resulted in the upregulation of PD-L1 levels on both β cells and in their liberated exosomes. To assess if inhibition of 12-LOX using the human-specific 12-LOX inhibitor modifies T1D progression, we generated mice in which the gene encoding mouse 12-LOX (Alox15) was replaced by the gene encoding human 12-LOX (ALOX12). These "humanized" mice were subsequently backcrossed onto the NOD background, then treated in the prediabetic phase with the 12-LOX inhibitor ML355. Male and female humanized mice developed T1D at the expected frequency, but those receiving ML-355 showed significant delay of T1D.

Conclusions

Our results support several key, previously unappreciated findings: (a) that cell-autonomous inflammatory signaling via 12-LOX in the β-cell dictates a dialog that propagates innate and adaptive immunity, likely via the suppression of the immune checkpoint PD-L1, (b) 12-LOX activity is linked to the ISR in T1D, and that the ISR may be increasing the susceptibility of β cells to autoimmune attack, and (c) targeting of the 12-LOX pathway and the ISR provides an opportunity for the potential modification of T1D disease progression.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

55: Feyza Engin (University of Wisconsin-Madison)

Abstract Title

Genetic targeting the beta cell UPR in type 1 diabetes

Authors

Hugo Lee, Gulcan Semra, Yash Sonthalia, Feyza Engin

Purpose

The purpose of our abstract is to summarize the ongoing research in our laboratory focusing on identification of role of the beta cell ER stress and the UPR in T1D initiation and progression.

Methods

Single cell RNAseq, immunophenityping, genetic mouse knockout, immunofluorescence and imaging, RNA and protein biochemistry.

Summary of Results

Type 1 diabetes (T1D) results from immune-mediated destruction of β-cells. Aberrant β-cell unfolded protein response (UPR) has recently been linked to T1D, however molecular mechanisms by which UPR affect T1D progression remain elusive. Here, we show that deletion of a UPR sensor, IRE1 in beta cells of NOD mice (IRE1β-/-) prior to insulitis leads to a transient dedifferentiation, reduced autoantigen levels and differential expression of genes involved in antigen presentation. Reduced CD8 T cells and impaired diabetogenic activity of T cells in these mice contribute to the protection from T1D development. We also show that deletion of ATF6 in β-cells of NOD mice (Atf6β-/-) prior to islet inflammation results in markedly reduced diabetes incidence. Atf6β-/- mice presented substantially reduced insulitis, β-cell apoptosis, and preserved insulin secretion. We will compare the phenotype and the molecular mechanisms of diabetes protection in these genetic models of the UPR and discuss the role of ER stress in T1D.

Conclusions

Investigating the specific functions of the each UPR sensor in β-cells during different stages of T1D in preclinical models will not only advance our understanding of the specificity of cellular stress responses, but will also be critically important for developing novel non-immune-based therapeutic strategies for prevention of this disease.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

56: Jason Spaeth (Indiana University School Medicine)

Abstract Title

The Pdx1-recruited Chd4 Helicase Controls Genes Critical for β-cell Function

Authors

Rebecca Davidson, Nolan Casey, Sukrati Kanojia, Jason Spaeth

Purpose

Diabetes is associated with loss of transcription factors (TFs) from a subset of failing β-cells. Among these TFs is Pdx1, which drives expression of numerous genes involved in maintaining β-cell function and identity. Pdx1 transcriptional activity is modulated by transcriptional coregulators and has recently been shown to interact with the Chd4 ATPase subunit of the Nucleosome Remodeling and Deacetylase complex. Herein, we aim to evaluate the role of Chd4 in controlling Pdx1 and β-cell function, and test the hypothesis that interactions between Pdx1 and Chd4 in the β-cell are reduced in settings of type 2 diabetes.

Methods

Conditional, β-cell specific Chd4 knockout mice were generated using the Tamoxifen-inducible Mouse insulin promoter-Cre driver line (termed Chd4Δβ). Tamoxifen administration induced the removal of Chd4 in β-cells and metabolic phenotyping was performed (IPGTT, Plasma measurements). To evaluate the mechanisms of β-cell dysfunction, islets isolated from control and Chd4Δβ mutant mice were subjected to: 1. Islet perifusion 2. Tdtomato+ flow sorting followed by RNA-Seq and 3. Transmission electron imaging. The role the diabetogenic milieu has on Pdx1:Chd4 interactions was evaluated by performing proximity ligation assays for Pdx1 and Chd4 in pancreatic tissues from high-fat fed mice and from non-diabetic and type 2 diabetic human donor tissues.

Summary of Results

4 weeks following tamoxifen administration, to remove Chd4 from islet β-cells, we found Chd4Δβ mice are glucose intolerant, with ad libitum fed and fasting hyperglycemia. Moreover, plasma insulin was reduced in Chd4Δβ mice at both fasting and 2 minutes post glucose injection, suggesting a defect in the 1st peak of insulin secretion. This was confirmed by perifusion analyses, where Chd4Δβ islets displayed defects in insulin secretion under high glucose and depolarizing (KCl) conditions. Based on this, we speculated that Chd4Δβ mutants had deficiencies in mature insulin granule formation, although expression of Ins1/2 and Insulin detection by immunofluorescence is unchanged. To this end, Transmission Electron Microscopy revealed that Chd4Δβ β-cells have an increase in immature:mature granule formation. RNA-sequencing was performed on flow-sorted β-cells from control and Chd4Δβ islets, which uncovered over 400 downregulated (i.e. MafA) and 1000 upregulated (Mycl, HK2) gene targets. Current investigation links the differential gene expression from Chd4Δβ β-cells to markers of β-cell dysfunction and loss of cellular identity.

To determine how diabetogenic stressors influence Pdx1:Chd4 interactions in vivo, we utilized a diet-induced obesity model of T2D. C57BL/6J male mice were placed on a high fat diet (HFD) regimen that contained 60 kCal% from fat to mimic the induction of obesity and metabolic stress in humans. 8 week old mice placed on HFD for 4 weeks displayed glucose intolerance and had reduced expression of β-cell functional genes (i.e. MafA), similar to Chd4Δβ β-cells. To determine whether Pdx1:Chd4 interactions were altered, we performed PLAs with Pdx1 and Chd4 antibodies, which revealed a significant reduction in the number of Pdx1:Chd4 interactions in β-cells in 4W HFD fed mice. We translated these findings by performing PLA in human donor tissue sections from nPOD, where we discovered that PDX1:CHD4 interactions in T2D donor β-cells are significantly reduced in comparison to an age/sex/BMI matched non-diabetic donors.

Conclusions

Collectively, out data establishes Chd4 controls genes critical to maintain β-cell health. We establish that in vivo metabolic stress alters Pdx1:Chd4 association and that type 2 diabetes donor tissues have significantly reduced Pdx1:Chd4 interactions.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

57: Nicolai Doliba (University of Pennsylvania)

Abstract Title

Changes in alpha cell function and gene expression precede the insulin secretory deficit during development of T1D

Authors

Nicolai Doliba, Andrea Rozo, Jeffrey Roman, Wei Qin, Daniel Traum, Long Gao, Jinping Liu, Elisabetta Manduchi

Purpose

Multiple islet autoantibodies (AAb) predict type 1 diabetes (T1D) and hyperglycemia within 10 years. By contrast, T1D develops in just ~15% of single AAb+ (generally against glutamic acid decarboxylase, GADA+) individuals; hence the single GADA+ state may represent an early stage of T1D amenable to interventions.

Methods

Here, we functionally, histologically, and molecularly phenotype human islets from non-diabetic, GADA+ and T1D donors. Insulin and glucagon secretion rates were measured by perifusion of isolated islets. The proportions of endocrine cells in donor pancreata were determined by flow CyTOF of single islet cell suspensions stained simultaneously with a panel of 36 antibodies. Transcriptome data of alpha and beta cells was collected by single cell RNA sequencing (scRNAseq), and data were analyzed by gene set enrichment analysis.

Summary of Results

Similar to the few remaining beta cells in T1D islets, GADA+ donor islets demonstrated a preserved insulin secretory response to glucose and to cAMP elevation by IBMX. By contrast, alpha cell glucagon secretion was dysregulated in both T1D and GADA+ islets with impaired glucose suppression of glucagon secretion and a pronounced augmentation of IBMX-stimulated glucagon secretion Islet endocrine cell composition, morphology, and immune cell infiltration were unremarkable in GADA+ donors; however, scRNAseq of GADA+ alpha cells revealed distinct abnormalities in glycolysis and oxidative phosphorylation pathways and a marked downregulation of PKIB, providing a molecular basis for the loss of glucose suppression and the increased effect of IBMX observed in GADA+ donor islets.

Conclusions

The striking observation of a distinct early defect in alpha cell function that precedes beta cell loss in T1D suggests that not only overt disease, but also the progression to T1D itself, is bihormonal in nature.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Physiology and Dysfunction

58: Suzanne Shapira (University of Pennsylvania)

Presenter of Abstract

Klaus Kaestner

Abstract Title

Spotlight on the Human Pancreas Analysis Program (HPAP) and Data Repository (PANC-DB)

Authors

Nilanjana Samantha, Suzanne Shpaira, Diane Saunders, Marcela Brissova, Anna Gloyn, Kyle Gaulton, Golnaz Vahedi, Ben Voight, Seung Kim, Robert Farayabi, Mark Atkinson, Ali Naji, Alvin Powers, Klaus Kaestner.

Purpose

The Human Pancreas Analysis Program (HPAP) consortium was established in 2016 as part of the Human Islet Research Network to study the human pancreas with the full spectrum of physiological, imaging, genetic, and genomic technologies available to date.The consortium performs deep phenotyping of the human pancreas, with the goal of developing an improved understanding of the cellular and molecular events that lead to beta cell loss in type 1 diabetes (T1D) and beta cell insufficiency in type 2 diabetes (T2D). To date, the program has collected over 100 human pancreatic samples. Importantly, all generated data is deposited in an open-access database, PANC-DB (https://hpap.pmacs.upenn.edu/), available to the entire research community before publication. Currently, the database includes 24 T1D cases, 22 T2D cases and 58 controls for both studies, providing users with over 4.9 terabytes of data.

Methods

The database provides users access to donor demographics, clinical information, traditional tissue histology, imaging mass cytometry (IMC), CyTOF, perifusion, calcium imaging, oxygen consumption, bulk and single cell RNA-seq and ATAC-seq, as well as characterization of immune cells from the spleen, peripheral blood and pancreatic lymph nodes for T1D samples. For T2D cases, CODEX imaging, genotyping and Patch-seq studies are also performed. The database allows users to download all data files and standard operating protocols produced by the HPAP effort. Downloads are navigated using either a simple-to-use folder structure that allows for multiple-donor data selection or a new and fast FTP download feature allowing mass download directly from the server. Additionally, a bulk download feature organized by experimental categories and specific filters is also available for users. Thus far, HPAP data have contributed to over 15 peer-reviewed articles.

Summary of Results

Recently, the HPAP database has successfully linked its imaging (Histology and IMC) and genomic data to two external databases, namely Pancreatlas and the Common Metabolic Diseases Genome Atlas. These platforms provide more advanced viewing and download features, as well as capabilities with protein filters and more. Additionally, the program has implemented RRIDs for every donor sample to improve tracking publications using HPAP biosamples. We are in the process of hosting genotypic risk score data for our T2D samples with link to dbGaP where the data will be hosted.

Conclusions

Altogether, HPAP and PANC-DB will continue to perform innovative studies and host multiple types of data to assist investigators with their research related to T1D and T2D.

Preferred Presentation Format

Poster Presentation

Research Category

Beta Cell Physiology and Dysfunction

59: Klaus Kaestner (University of Pennsylvania)

Abstract Title

An improved PancDB platform for easy analysis of multi-omics single-cell analysis of human pancreatic islets from the HPAP consortium

Authors

Robert Farayabi, Maria Fasolino, Gregory Schwartz, Abhijeet Patil, Aanchal Mongia, Nilanjana Samantha, Donbu Hu, Michael Stauffer, Colin McGovern, Jonathan Schug, Suzanne Shapira, Golnaz Vahedi, Klaus Kaestner.

Purpose

The Human Pancreas Analysis Program (HPAP) generates high volume scRNA-seq and scATAC-seq data from pancreatic islets from deceased organ donors that are non-diabetic, non-diabetic but positive for islet autoantibodies, T1D and T2D. These multimodal analysis are enabling novel discoveries about disease pathogenesis. Curated datasets are made available to the research community at large via PancDB (https://hpap.pmacs.upenn.edu), now in an easily searchable form by implementing cellxgene platform.

Methods

Pancreatic islets from more than 60 deceased organ donors were subjected to scRNA-seq and scATAC-seq as well as protein-based assay such as flow CyTOF and imaging mass cytometry. Data were integrated using multimodal analysis. The open source platform 'CellxGene' developed by the Chan Zuckerberg Initiative was implemented on PancDB for easy user-driven analyses.

Summary of Results

In the first phase, pancreatic islets from 24 organ donors were subjected to scRNA-seq, flow CyTOF and imaging mass cytometry, resulting in data from >80,000 cells by cells using single-cell transcriptomics, >7,000,000 cells using cytometry by time-of-flight, and >1,000,000 cells using in situ imaging mass cytometry. Among the main findings, we discovered that AAb+ donors exhibit many of the same transcriptome changes in endocrine and even exocrine cell populations than those with T1D, despite retaining normoglycemia. In addition, we found that in single GADA+ individuals, anti-GAD titers correlate with transcriptomic changes in a subpopulation of beta cells. Perhaps most remarkable is our observation that ductal cells from T1D – but not AAb+ - organ donors express high levels of MHC Class II proteins and have activated interferon pathways, findings confirmed by imaging mass cytometry. These MHC Class II high ductal cells are surrounded by CD4+ T cells and dendritic cells, and display a transcriptome reminiscent of tolerogenic dendritic cells, suggesting an unappreciated role of these exocrine cells in modulating T cell activity in long-term T1D. We have now expanded our single cell omics data to more than 60 organ donors. Importantly, in order to enable easy access and further in-depth analysis of these valuable data by the diabetes research community, we have now implemented a facile CellxGene platform on the HPAP website, PancDB. Diabetes researchers can visualize gene expression at the level of an entire dataset or a particular subset of cells to help identify cell types, which can in turn help define stages and/or subtypes of diabetes. CellxGene also allows researchers to keep track of and annotate cell types as they define them in the HPAP dataset, and enables plotting one gene's expression level against another's to compare how these different genes are expressed across a dataset.

Conclusions

Multiomics single cell analyses of human pancreatic islets from diabetes-relevant donors help to elucidate the molecular pathology of the disease. The upgraded PancDB website enables further analysis of these large and unique datasets by scientists worldwide.

Preferred Presentation Format

Oral Presentation

Research Category

Novel Technologies

60: Michelle Lee (University of Pennsylvania)

Abstract Title

Single nucleus-resolved chromatin accessibility profiles of pancreatic islet cells

Authors

Michelle Lee, Jonathan Schug, Elisabetta Manduchi, Eric Waite, Erin Duffy, Danielle Jaffe, Ali Naji, Mark Atkinson, Alvin Powers, Klaus Kaestner.

Purpose

Deep molecular profiling of pancreatic islet cells via single-cell RNA-sequencing (scRNA-seq) has revealed cell type specific transcriptome profiles. However, scRNA-seq suffers from 'dropout', or missing signals, for genes expressed at low levels, such as those encoding transcription factors or signaling molecules. In addition, there is significant of cross-contamination of ambient RNA from lysed cells in each cell, complicating the detection of rare and/or transitional cell types or states. Therefore, additional molecular profiles are required to strengthen our understanding of cell type identity and reveal potential subpopulations of cells. Single nucleus Assay for Transposase-Accessible Chromatin sequencing (snATAC-seq) reveals regulatory element sequence and key transcription factor motifs that function in a cell type specific manner, complementing gene expression. Therefore, we employed snATAC-seq to advance our understanding of cell type identity for key human pancreatic cell types.

Methods

We performed snATAC-seq on pancreatic islets obtained from 33 deceased organ donors that were non-diabetic, auto-antibody positive but normoglycemic, type 1 diabetic, or type 2 diabetic, and performed computational analyses.

Summary of Results

By unsupervised clustering, we found that chromatin accessibility profiles alone can identify the major pancreatic cell types, including five endocrine cell populations. Furthermore, the accessible chromatin regions of the various cell types showed enrichment for specific transcription factor binding motifs. Moreover, we observed a population of cells displaying a transitional phenotype related to but distinct from alpha cells. Cells of this population showed accessibility at the glucagon promoter, the defining marker of alpha cells, but also displayed multiple open chromatin regions enriched for the binding motifs of developmental transcription factors, raising the possibility that even adult human islets contain a subset of transitional and possibly progenitor type cells.

Conclusions

We demonstrate that chromatin accessibility captures important characteristics of cell type identity in the human pancreas. Moreover, snATAC-seq discovered a subpopulation of cells that show a progenitor-like phenotype. Thus, snATAC-seq reveal novel information and putative transitional cell types that complement gene expression analysis by scRNA-seq. Future integration of these data promises to further our understanding of cell type identity and function of human pancreatic cells.

Preferred Presentation Format

Poster Presentation

Research Category

Pathology

61: Daniela Fignani (University of Siena)

Abstract Title

Long and short isoforms of SARS-CoV-2 receptor Angiotensin I-Converting Enzyme type 2 (ACE2) are increased in pancreatic islets of type 2 diabetic patients

Authors

Daniela Fignani, Giada Licata, Noemi Brusco, Elena Aiello, Giuseppina E. Grieco, Caterina Formichi, Laura Nigi, Lorella Marselli, Piero Marchetti, Guido Sebastiani, Francesco Dotta.

Purpose

SARS-CoV-2 can infect human pancreatic islets through β cell-specific expression of ACE2 receptor and other accessory proteins which are determinant for virus entry. We and others have previously shown that β cells do express the novel short-ACE2 (s-ACE2) isoform alongside with S-protein binding long ACE2 (l-ACE2).
It is reported that type 2 diabetes (T2D) is a major comorbidity of COVID-19, and T2D patients are predisposed to poor prognosis, worse outcome and exacerbated altered glycometabolic control after SARS-CoV-2 infection. These consequences may be due to several reasons including poor baseline glucose control, pre-existing immune dysfunction, proinflammatory status or other comorbidities, such as cardiovascular disease. Moreover, considering the expression of ACE2 in pancreatic islets, we should take into account the hypothesis of a partial insulin deficiency due to direct damage and/or dysfunction of β cells during SARS-CoV-2 infection in T2D patients.
Here, we aimed at elucidating the in-situ expression pattern of ACE2 in T2D respect to non-diabetic donors.

Methods

In order to evaluate the staining pattern of ACE2 in T2D pancreatic tissues, we take advantage from the large biorepository of T2D donor tissues from University of Pisa. We analyzed multiple FFPE sections from n=20 adult non-diabetic subjects (CTR) (F=9 M=11; age mean±S.D.: 70.6 ±7 y; BMI: 26.2 Kg/m2±4.1 Kg/m2) and from n=20 type 2 diabetic patients (T2D) (F=6 M=14; age mean±S.D.: 71.7±7.6 y; BMI: 27.1 Kg/m2±2.7 Kg/m2). Whole pancreata were obtained and processed following standardized procedures at the University of Pisa. The sections were subjected to triple immunofluorescence staining using monoclonal mouse anti-human ACE2 (R&D-MAB933; detecting s-ACE2), polyclonal rabbit anti-human ACE2 (Abcam-ab15348; detecting both ACE2 isoforms) and with polyclonal Guinea Pig anti-human Insulin (Dako). Stained sections were analyzed using confocal microscopy; colocalization rate, intensity values and 3D deconvolution analyses were performed using LasAF software. Additionally, previous bulk RNA sequencing datasets of T2D and CTR islets were interrogated.

Summary of Results

The analysis of n=1082 islets across all donors confirmed the expression of s-ACE2 and l-ACE2 in pancreatic β cells, both in T2Ds and CTRs. A high rate of heterogeneity among islets was observed, as previously shown. We confirmed the prevalence of s-ACE2 respect to l-ACE2 in β cells (mean colocalization rate in all donors s-ACE2: 10.8%; l-ACE2: 8.2%). Furthermore, we observed a significant increase of colocalization rate (%) of s-ACE2 [MAB933]/INS in T2D compared to CTR (T2D mean±S.D.: 12.5 ± 7.6% and CTR 9.23 ± 7.0%, p<0.001) and of l-ACE2 [ab15348]/INS in T2D compared to CTR (T2D mean±S.D.: 11.45 ± 13.7% and CTR 5.3% ± 5.3%; p<0.001, n=1082 islets). Moreover, the normalized signal intensity analysis (s-ACE2 and l-ACE2 mean pixel intensity/islet area) confirmed the upregulation of both ACE2 isoforms in T2D patients respect to CTR patients (mean normalized grey scale values of s-

ACE2 in T2D=52.5±34.6 and in CTR=37.1±28.1, $p<0.001$; grey scale values of l-ACE2 in T2D=53.2±63.5 and in CTR=27.3±22, $p<0.001$; n=1082 islets). The analysis of bulk RNA sequencing of collagenase isolated T2D islets vs CTRs confirmed the upregulation of ACE2 mRNA in T2D vs age-matched controls.

Conclusions

We observed the upregulation of s-ACE2 and l-ACE2 in pancreatic islets of T2D donors. High ACE2 expression in T2D islets might increase their susceptibility to SARS-CoV-2 infection during COVID-19 disease in T2D patients, thus exacerbating glycometabolic demise due to a potential direct damage or dysfunction of β cells.

Preferred Presentation Format

No Preference

Research Category

Beta Cell Physiology and Dysfunction

62: Mia Smith (Barbara Davis Center for Diabetes)

Abstract Title

Activation and trafficking of insulin-binding B cells to the pLN in nPOD donors

Authors

Zachary Stensland, Hali Broncucia, Adam Magera, Peter Gottlieb, Mia Smith

Purpose

In healthy individuals autoreactive B cells that escape central tolerance mechanisms are normally silenced by anergy, a type of B cell tolerance wherein autoreactive cells occupy peripheral lymphoid organs but are antigen unresponsive. Previously we have found that young new-onset T1D subjects exhibit a loss of anergic insulin-binding B cells (IBCs) in their peripheral blood. Given that an increase in B cells in both the blood and pancreas is associated with the aggressive form of T1D that is seen in younger patients, we hypothesize that loss of anergic IBCs in the peripheral blood reflects their activation and trafficking to tissues rich in autoantigen (e.g. pancreas and pancreatic lymph node (pLN)) where they participate in disease pathogenesis.

Methods

Since antigen-specific B cells occur in very low frequency in the periphery, we developed a magnetic nanoparticle-based scheme to enrich for IBCs from blood and tissue. Aided by enrichment, we explored the frequency of various IBC and non-IBC subsets and their activation status in the peripheral blood of T1D subjects and spleen and pLN of nPOD donors using both mass cytometry and spectral flow cytometry.

Summary of Results

Both manual gating and unsupervised clustering algorithms identified ‘anergic’ B cells in the peripheral blood of T1D donors are highly activated (e.g. increased expression of CD86, CD11c, and CXCR3), particularly in insulin-binding B cells compared to non-insulin-binding B cells. Analysis of the spleen and pLN of nPOD donors revealed similar findings with these B cell subsets further enriched in these tissues, especially the pLN. Trajectory and phenotypic analysis suggests these activated ‘anergic’ B cells could be precursors to extrafollicular antibody secreting cells, as well as act as potent antigen-presenting cells to diabetogenic T cells.

Conclusions

Our results suggest that autoreactive B cells that are normally silenced by anergy in healthy individuals, become activated in T1D subjects and traffic to the pLN, where they likely participate in disease pathogenesis through antibody secretion and/or antigen presentation to T cells.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

63: Doris Stoffers (University of Pennsylvania)

Abstract Title

Alpha cell dysfunction in islets from non-diabetic GADA+ individuals

Authors

Nicolai Doliba, Andrea Rozo, Jeffrey Roman, Wei Qin, Daniel Traum, Long Gao, Jinping Liu, El;isabetta Manduchi, Chengyang Liu, Maria Golson, Golnaz Vahedo, Ali Naji, Franz Matschinsky, Mark Atkinson, Alvin Powers, Marcela Brissova, Klaus Kaestner, Doris Stoffers

Purpose

Multiple islet autoantibodies (AAb) predict type 1 diabetes (T1D) and hyperglycemia within 10 years. By contrast, T1D develops in just ~15% of single AAb+ (generally against glutamic acid decarboxylase, GADA+) individuals; hence the single GADA+ state may represent an early stage of T1D. Here, we functionally, histologically, and molecularly phenotype human islets from non-diabetic, GADA+ and T1D donors.

Methods

GADA+ autoantibody-positive organ donors were identified by the nPOD team working with the NIDDK-funded Human Pancreas Analysis Program (HPAP; ; https://hpap.pmacs.upenn.edu). The pancreas was processed and islets isolated by HPAP. We characterized the physiology and metabolic state of 39 human islet preparations isolated from 23 control (20 from HPAP, 3 from Penn's Human Islet Resource Center; age 27+/-10 years, BMI 27+/-7 kg/m2, HbA1c 5.3+/-0.6 [mean+/-SD]), 10 GADA+ (age 24+/-5 years, BMI 27+/- 4 kg/m2, HbA1c 5.4+/-0.2) and 6 T1D-donors (age 20+/-8 years, BMI 19+/-5 kg/m2, HbA1c 10.1+/-0.6). HPAP employs two complementary and validated islet perifusion protocols to assess insulin and glucagon secretion (at Penn and at Vanderbilt). scRNASeq analysis was performed using the Single Cell 3' Reagent Kit and scRNASeq data were preprocessed using Seurat. Flow CyTOF experiments were performed with 36 metal-conjugated antibodies and analyzed using the Cytobank implement. Imaging mass cytometry was performed to assess hormone expression and immune cell markers. Confocal imaging was performed on human pancreatic sections stained for pCREB. 500 endocrine cells per donor were manually counted and pCREB and glucagon expression was evaluated.

Summary of Results

Utilizing donor tissues and cells from HPAP, we examined both insulin and glucagon secretion from non-diabetic, GADA+, and T1D islets. GADA+ donor islets undergoing the Penn perifusion protocol demonstrated a normal insulin secretory response. By contrast, alpha cell glucagon secretion was dysregulated in both T1D and GADA+ islets with impaired glucose suppression of glucagon secretion. Further, potentiation of glucagon secretion by IBMX, a phosphodiesterase inhibitor that maximally increases intracellular cAMP

concentrations, was ~50% greater in islets from GADA+ donors compared to controls. There was no difference in KCl-stimulated glucagon secretion. Analysis of the same islet preparations with the Vanderbilt protocol confirmed decreased suppression of glucagon secretion by glucose, increased response to low glucose plus epinephrine and a trend toward increased response to IBMX, with no change in the KCl effect.

To evaluate if differences in islet composition might explain this observation, we determined the proportions of endocrine cells in donor pancreata by flow CyTOF of single islet cell suspensions which revealed no statistically significant change in the alpha cell fraction in GADA+ islets. Furthermore, we analyzed data from imaging mass cytometry (IMC) performed on a subset of these donor islet preparations and again found no statistically significant difference in endocrine cell populations between the two groups. To assess whether immunological infiltrates in or near islets are already present at the single GADA+ stage, we performed detailed image analysis of immune cell infiltrates and the distance relationships between pancreatic islets and CD4+ and CD8+ T cells, proliferating (activated) and non-proliferating macrophages, B cells, and Treg cells, but found no significant differences.

Single cell RNA sequencing (scRNASeq) of GADA+ alpha cells revealed distinct differences in the glycolysis and oxidative phosphorylation pathways, and a marked downregulation of PKIB, providing a molecular basis for the loss of glucose suppression and the increased effect of IBMX observed in GADA+ donor islets. To seek further evidence for altered cAMP signaling in alpha cells of GADA+ individuals, we performed immunofluorescent staining of human pancreatic sections using anti-phospho CREB (P-CREB) antisera and found that percent of alpha cells expressing nuclear P-CREB was significantly greater in GADA+ islets ($p<0.05$).

Conclusions

The observation of distinct early defects in alpha cell function that precede beta cell loss in T1D suggests that not only overt T1D, but also the progression to T1D itself, is bihormonal in nature.

Preferred Presentation Format

No Preference

Research Category

Type 1 Diabetes Etiology & Environment

64: Hyo Jeong Yong (Florida State University)

Abstract Title

Gene Signatures of NEUROGENIN3+ Endocrine Progenitor Cells in the Human Pancreas

Authors

Hyo Jeong Yong, Gengqiang Xie, Chengyang Liu, Wei Wang, Ali Naji, Jerome Irianto, Yue Wang.

Purpose

NEUROGENIN3+ (NEUROG3+) cells are considered to be pancreatic endocrine progenitors. Our current knowledge on the molecular program of NEUROG3+ cells in humans is largely extrapolated from studies in mice. We hypothesized that single-cell RNA-seq enables in-depth exploration of the rare NEUROG3+ cells directly in humans.

Methods

To explore gene signatures of rare NEUROG3+ cells, we aligned four large single-cell RNA-seq datasets from postnatal human pancreas and performed differential expression and transcriptional network analysis. The results from single-cell RNA-seq were confirmed with

immunostaining of pancreatic tissue sections. To examine whether NEUROG3+ cells in the postnatal human pancreas shared characteristics with NEUROG3+ cells during development, we compared gene signatures of NEUROG3+ cells with qPCR data from human fetal pancreatic cells and NEUROG3+ cells derived in vitro, respectively.

Summary of Results

Our integrated data revealed 10 NEUROG3+ epithelial cells from a total of 11,174 pancreatic cells. Human NEUROG3+ cells clustered with mature pancreatic cells and epsilon cells displayed the highest frequency of NEUROG3 positivity. We confirmed the co-expression of NEUROG3 with endocrine markers and the high percentage of NEUROG3+ cells among epsilon cells at the protein level. We further revealed that novel transcription factors may act jointly with NEUROG3. We also outline the similarities of these postnatal NEUROG3+ cells with NEUROG3+ cells in the fetal pancreas.

Conclusions

Utilizing existing single-cell RNA-seq datasets, we confirmed the presence of NEUROG3+ in the postnatal human pancreas and revealed the gene signatures of NEUROG3+ cells. Our study demonstrated that postnatal NEUROG3+ cells are likely resident endocrine progenitors that can be further functionally and bioinformatics explored to gain insights on diabetes therapies.

Preferred Presentation Format

No Preference

Research Category

Beta Cell Development, Differentiation & Regeneration

65: Lea Bogensperger (Graz University of Technology, Austria)

Abstract Title

A fiducial-less reconstruction of electron tomograms to visualize the autoimmune attack within NOD mice

Authors

Lea Bogensperger, Erich Kobler, Dominique Pernitsch, Petra Kotzbeck, Barbara Ehall, Thomas R. Pieber, Thomas Pock, Dagmar Kolb

Purpose

Visualizing the ultrastructure of the endocrine pancreas can be very indicative of cellular interactions in type 1 diabetes research. This can be achieved with electron tomography, which allows to reconstruct 3D samples from a tilt series of 2D projection images. However, the measured 2D projection images inherently contain unwanted translatory shifts that have to be corrected for, which is traditionally done using fiducial markers. These can then be used to align the projection images prior to reconstruction.
We have developed a joint alignment and reconstruction algorithm to solve for both the unknown reconstruction and the unintentional shift without the need of fiducial markers, thereby decreasing labour and material costs. Our algorithm enables 3D reconstructions of pancreatic samples of healthy C57BL/6J and non-obese diabetic (NOD) mice with the aim of visualizing the autoimmune attack in type 1 diabetes on beta cells within the islets of Langerhans in the pancreas.

Methods

To obtain reliable 3D reconstructions, it is crucial to align the tilt series such that all 2D projections are devoid of any remaining translatory shifts. Iteratively solving in an alternating manner for the disruptive shift within the projection images and the reconstruction

volume yields an updating scheme where estimates for both unknown variables continuously improve and benefit each other.
The algorithm is first empirically demonstrated on a synthetic phantom with artificially induced shifts within the projection images. Electron microscopy, especially electron tomography is used to generate tomograms and scanning transmission electron microscopy (STEM) additionally provides us with enlarged overviews on the islets. The algorithm is then applied to reconstruct tomograms of healthy C57BL/6J and prediabetic NOD mice and allows for a direct comparison of pancreatic samples with blood glucose values of 119 and 123 mg/dl, respectively.

Summary of Results

Quantitative verification of the algorithm on synthetic data entitles the presentation of qualitative reconstruction results for healthy C57BL/6J and NOD samples. Thus, a baseline of a healthy C57BL/6J beta cell containing organelles and typical structures found within healthy looking phenotypes enables a comparison with the NOD reconstruction. Within the NOD beta cell, the presence of a cytotoxic immune cell can be clearly observed, which can also be located within the STEM overview.
Moreover, taking a closer look at different optical slices reveals close interactions between the invading immune cell and the insulin secretory granules that are found within beta cells, which are of particular interest. Using a more profound 3D visualization technique further strongly suggests that the process of internalization of a granule into the immune cell's cytoplasm has been captured. This could represent a key step in immune cells recognizing and furthermore attacking beta cells.

Conclusions

Our novel joint alignment and reconstruction algorithm proves to be valuable when integrated into our workflow as it decreases labour and material costs by omitting the preprocessing step of labeling the sample with fiducial markers. It thus facilitates further research targeted directly towards investigating the immune system's attacks in different stages of type 1 diabetes in pancreata of NOD mice by enabling convenient and high-quality reconstructions. This will provide more insight into the dynamic immune cell attack on beta cells including the role and fate of the insulin secretory granules.

Preferred Presentation Format

Oral Presentation

Research Category

Novel Technologies

66: Doris Stoffers (University of Pennsylvania)

Presenter of Abstract

Nicolai Doliba

Abstract Title

Ethnic Differences in Pancreatic Alpha Cell Function in Health and T2D

Authors

Nicolai Doliba, Jeffery Roman, Andrea Rozo, Wei Qin, Chengyang Liu, Ali Naji, Franz Matschinsky, Mark Atkinson, Alvin Powers, Marcela Brissova, Klaus Kaestner, Doris Stoffers

Purpose

Type 2 diabetes (T2D) occurs at a greater frequency, has an earlier age of onset and is associated with poorer glycemic control in African American and Hispanic populations. The reasons for these disparities are multifactorial, involving environmental and cultural determinants. However, these factors do not fully account for differences in T2D frequency and therapeutic outcomes, suggesting that biological differences in insulin production, secretion, or action are important contributors. We tested the hypothesis that a critical biologic determinant of T2D disparities is based on ethnicity-related differences in pancreatic islet function.

Methods

Utilizing donor tissues from the NIDDK-funded Human Pancreas Analysis Program (HPAP; https://hpap.pmacs.upenn.edu), we examined both insulin and glucagon secretion from non-diabetic and diabetic Caucasian, African American and Hispanic donor islets. Demographic information is listed below:

Caucasian Controls: n=21, Age 38.8 ± 9.1 yrs, BMI 29.0 ± 6.7, HbA1c 5.5 ± 0.6
African American Controls: n=5, Age 36.8 ± 17.7 yrs, BMI 33.8 ± 3.8, HbA1c 5.4 ± 0.5
Hispanic Controls: n=4, 35.0 ± 13.5 yrs, BMI 30.5 ± 7.0, HbA1c 5.5 ± 0.7

Caucasian T2D: n=7, 45.0 ± 10.2 yrs. BMI 32.6 ± 5.9. HbA1c 7.7 ± 2.2
African American T2D: n=7, 45.4 ± 8.7 yrs, BMI 33.7 ± 8.9, HbA1c 7.8 ± 2.1
Hispanic T2D: n=3, 52.7 ± 3.1 yrs, BMI 34.1 ± 5.5, 7.1 ± 0.5

HPAP employs two complementary and validated islet perifusion protocols to assess insulin and glucagon secretion (at Penn and at Vanderbilt). The Penn perifusion protocol, for which results are described here, involves pre-perifusion with substrate-free medium followed by a physiological amino acid mixture (total concentration 4 mM) to stimulate glucagon secretion, followed by first low and then high glucose (3 and 16.7 mM) to inhibit glucagon secretion and to stimulate insulin secretion. During the high glucose step, IBMX (0.1 mM) is added to maximally increase intracellular cAMP levels and stimulate secretion of both hormones. Finally, a brief washout period with substrate-free medium is used to remove all secretagogues, followed by addition of 30 mM KCl to depolarize the islet cells and quantify the readily releasable pool of secretory granules. Analysis using the Vanderbilt protocol is currently in progress. Insulin and glucagon concentration in perifusates and islet extracts was measured by radioimmunoassay or ELISA. Statistical comparisons were drawn by repeated measures ANOVA.

Summary of Results

Comparing non-diabetic islets, we observed overlapping insulin secretion profiles among all ethnicities; however, glucagon secretion was distinctly greater in both non-diabetic African American (+111%, p = 0.006) and Hispanic (+188%, p = 0.001) islets compared to non-diabetic Caucasian islets, under all interventions. Further, comparison of insulin and glucagon secretion profiles between non-diabetic and T2D donors also showed distinct differences according to ethnicity. In T2D, all ethnic groups displayed markedly reduced stimulation of insulin secretion by high glucose and potentiation by IBMX; however, the reduction in insulin secretion was significantly greater in African American T2D donors (high glucose -76%, p = 0.001; IBMX -76%, p = 0.002), with a trend to greater reduction in Hispanic T2D donors (high glucose -60%, p = 0.232; IBMX -50%, p = 0.142) compared to Caucasian T2D donors (high glucose -47%, p = 0.144; IBMX -50%, p = 0.052). More strikingly, glucagon secretion in T2D donors was markedly different based on ethnicity. Whereas Caucasian T2D islets exhibited a similar baseline of glucagon secretion compared to Caucasian controls, there was a marked reduction of glucagon secretion overall in African American T2D donors (-70%, p = 0.012) and a similar trend observed in Hispanic T2D donors (-45%, p = 0.312) compared to their corresponding controls. The largest detected difference was in IBMX-potentiation of glucagon secretion, which is higher in Caucasian T2D donors (+103%, p = 0.163) but significantly decreased in African American T2D donors (-76%, p = 0.015) with no significant change in Hispanic T2D donors (-10%, p = 0.433). Preliminary analysis of hormone content in a subset of the donor islet preparations revealed no differences according to ethnicity. Our current efforts are directed at increasing the sample size, comparing these findings with results from a complementary perifusion protocol, and integrating the physiological observations with calcium imaging, mitochondrial respiration, islet composition and architecture, and advanced molecular profiling.

Conclusions

We propose that ethnicity-related differences in non-diabetic islet function may contribute to the enhanced risk of T2D. Ethnicity-specific defects in T2D islet function should be further explored to inform therapeutic efforts seeking to combat diabetes in these high-risk populations.

Preferred Presentation Format

No Preference

Research Category

Beta Cell Physiology and Dysfunction

67: Yuval Dor (The Hebrew University of Jerusalem)

Abstract Title

Lessons on human beta cell biology from comprehensive methylome analysis

Authors

Yuval Dor, Ayelet Peretz, Judith Magenheim, Netanel Loyfer, Agnes Klochendler, Benjamin Glaser, Tommy Kaplan.

Purpose

The methylome of cells encodes and memorizes the combined influence of pre-existing genetics, cell differentiation and maintenance programs, and environmental exposures. Previous studies have described extensive plasticity of the beta cell methylome during healthy development and in diabetes. However such studies have typically relied on material from whole islets, where different preparations may have different cellular compositions. In addition, analyses have often examined the methylation status of individual CpG sites, while the actual functional unit of DNA methylation, controlling chromatin organization and protein binding, is a block of multiple adjacent CpGs that are either all methylated or all unmethylated. The purpose of this study was to characterize the methylome of human beta cells as compared to all other major human cell types.

Methods

We have generated a comprehensive cell type-specific human methylome atlas, based on whole genome bisulfite sequencing of individual cell types sorted from dissociated surgical material. The atlas contains the methylomes of 210 samples from 86 cell types, including triplicates of samples from alpha, beta and delta cells from non-diabetic donors; duplicates of alpha, beta and delta cells from donors with type 2 diabetes; and triplicates of samples from acinar cells and duct cells.

Summary of Results

Comparative analysis of the atlas provides interesting insights, including:
1. When examined through the lens of methylation blocks, the methylomes of beta cells from different donors are nearly identical. Out of 3 million methylation blocks in the genome, only 1000 (0.03%) vary in their methylation patterns between beta cells of healthy individuals. This degree of variation is seen also when comparing material from healthy donors and people with type 2 diabetes.
2. The methylome of beta cells is most similar to that of alpha and delta cells, followed by similarity to acinar cells, duct cells and hepatocytes. Strikingly, the methylomes of beta cells and neurons are highly dissimilar, despite the phenotypic similarity. Thus, methylomes reflect the lineage history of beta cells more than their current phenotype.
3. There are hundreds of loci unmethylated uniquely in beta cells. These are typically enhancers that control expression of beta cell genes.
4. Loci that are methylated uniquely in beta cells are typically binding sites to CTCF, suggesting that these are regulators of beta cell-specific chromatin looping.

Conclusions

The methylome of beta cells, examined in the context of the methylomes of other cell types, opens a window into beta cell biology including regulatory circuits, interpretation of GWAS hits and more.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Development, Differentiation & Regeneration

68: Gregory Golden (University of Pennsylvania)

Abstract Title

Immunological Atlas of Pancreatic Lymph Nodes in Type 1 Diabetes

Authors

Gregory Golden

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 secondary lymphoid organs, 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 20 cell populations.

Summary of Results

Although relative percentages of immune cell populations did not strongly differ between disease states, immune cell sub-populations had altered activation and differentiation markers between ND and T1D within the PLNs. Importantly, various T cell populations within PLNs had significantly altered surface levels of CD27, CD127, CD69, and HLA-DR. Further, B cells and NK cells showed similar affects.

Conclusions

This initial characterization of the PLNs in ND, AAb+, and T1D and other lymphoid tissue gives an in-depth glimpse into the autoimmune response during T1D and T1D development.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

69: Alexandra Title (InSphero AG)

Abstract Title

Establishing a platform for the robust evaluation of β-cell function and proliferation and function in human islet microtissues using 3D high-content imaging

Authors

Alexandra Title, Maria Karsai, Ozlem Yavas Grining, Chantal Rufer, Sayro Jawurek, Felix Forschler, Joan Mir, Thomas Klein, Burcak Yesildag

Purpose

Restoration of β-cell mass through the induction of proliferation represents an attractive therapeutic approach for the treatment of T1D. To date, several β-cell proliferative compounds have been identified in studies that employ cell lines and animal models, however, effects of very few have successfully been translated to the primary human β-cell. When cultured in vitro, intact or dispersed primary islets suffer from deteriorating viability and function, posing a significant challenge for their experimental use. Here, we describe a novel method for the assessment of compound effects on β-cell proliferation using reaggregated primary human islets, or islet microtissues (MTs), which display homogeneous size, tissue architecture and cellular composition as well as robust and stable functionality and viability in culture. The highly standardized islet MT size provides more uniform compound penetration, simplifies evaluation of changes in islet size and β-cell fraction. In addition, robust and uniform functionality of islet microtissues enables parallel assessment of functional endpoints (e.g., glucose-stimulated insulin secretion) in a one-islet-per-well format, while improved viability in culture (> 28 days) allows assessment of long-term compound effects and wash-out studies. As a proof-of-concept study, we utilized this platform to evaluate the dose-dependent short- and long-term effects of harmine on β-cell proliferation and function.

Methods

We established a platform combining 3D confocal imaging and automated image analysis, as well as functional endpoints, using human islet MTs. In brief, native human islets are dispersed and reaggregated to generate MTs that are uniform in size and composition. Following compound treatment and EdU incorporation, MTs are stained and confocal-imaged for DAPI (nuclear marker), NKX6.1 (β-cell marker), and EdU (proliferation marker), allowing the automated analysis of total cell count, β-cell count and fraction, proliferating β- and non-β-cell counts, and MT volume. In parallel, functional analyses including glucose-stimulated insulin secretion, assessment of total insulin and ATP contents, and Caspase 3/7 activity are carried out to obtain a comprehensive overview of the functional and proliferative effects of the compounds being evaluated.

Summary of Results

Evaluation of harmine in a range of concentrations (0 to 10 µM) utilizing our platform identified a dose-dependent increase in β- and non-β-cell proliferation, as well as a mild increase in β-cell count and fraction, over the course of 4 days of harmine treatment. Interestingly, long-term treatment for 15 days led to a general reduction in its proliferative effect as well as different dose-dependent trends, suggesting that excessive or continuous harmine dosing may impede its effects on proliferation. Furthermore, mean intensity of NKX6.1 expression was correlated with harmine dose, suggesting additional positive effects of harmine on β-cell identity. Functionally, harmine led to increased basal and stimulated insulin secretion, with maximal fold-stimulation achieved at moderate doses of harmine. Total insulin content was slightly reduced at highest harmine doses, correlating with increased chronic secretion. ATP content and caspase activity were not significantly altered.

Conclusions

Using our newly established high-throughput compatible β-cell proliferation platform, we confirmed a potent role for harmine in inducing islet cell proliferation and stimulating insulin secretion, with effects highly dependent on treatment dose and duration. This platform thus provides the opportunity to evaluate a variety of proliferation-related parameters, such as total islet cell count and MT volume, as well as proliferative rates, in a representative 3D human islet model, and to correlate this descriptive data with functional endpoints. Such a platform will likely be instrumental in the discovery of novel therapeutic agents to restore β-cell mass in T1D patients.

Preferred Presentation Format

Oral Presentation

Research Category

Beta Cell Development, Differentiation & Regeneration

70: Alberto Pugliese (University of Miami)

Presenter of Abstract

Melinee D'silva

Abstract Title

Characterization of pancreas transplant pathology in recipients with recurrent type 1 diabetes (T1DR). The nPOD Transplantation Group.

Authors

Peter Arvan, Marika Bogdani, George W. Burke, Francesco Dotta, Melinee D'silva, Ivan Gerling, Jaresley Guillen, Leena Haataja, Mark Huising, Heikki Hyoty, Eva Korpos, Jake A. Kushner, Irina Kusmartseva, Carol J. Lam, Pia Leete, Yaima Lightfoot, Clayton Matthews, Armando Mendez, Noel Morgan, Jerry Nadler, Laura Nigi, Ron Piran, Alberto Pugliese, Sarah J. Richardson, Isaac V. Snowhite, Francesco Vendrame.

Purpose

Simultaneous pancreas-kidney (SPK) transplantation is indicated for patients with type 1 diabetes (T1D) who develop end-stage renal disease. SPK transplantation reverses diabetes and restores kidney function in most recipients, for several years, under the cover of chronic immunosuppression that prevents rejection. However, about 5% of SPK recipients, in our cohort at the University of Miami, have developed T1D recurrence (T1DR) requiring insulin therapy, several years after successful transplantation and typically in the absence of significant rejection. In a previous report, we described the clinical course of T1DR in three recipients and pancreas transplant pathology demonstrated basic features of islet autoimmunity (insulitis, beta cell loss). Here, we describe 3 additional patients with T1DR, and comprehensively describe pancreas transplant pathology by including the collaborative assessment of multiple changes that have been linked to the development of the disease in the native pancreas of T1D patients near the time of diagnosis. We contrast results with pancreas transplant pathology findings in an SPK recipient with T1DR in the presence of rejection and in an SPK recipient who passed as an organ donor with a functioning graft.

Methods

The three SPK recipients with T1DR (cases 3678, 3626, 3681, all males) all had developed multiple T1D-associated autoantibodies (against GAD65, IA-2, or ZnT8) developing 2-9 year after transplantation, with elevations in HBA1c followed by symptoms of T1D developing 2-8 years after autoantibody conversion. Pancreas transplant biopsies were obtained following a clinical diagnosis of T1DR requiring insulin therapy, and these three patients also underwent a mixed meal tolerance test (MMTT) to evaluate residual insulin

secretion. One SPK recipient, case 3717, was a female recipient whom 9 years after transplant developed moderate to severe rejection of both pancreas and kidney transplants, as well as hyperglycemia. Finally, case 6403 was an SPK recipient who passed away as an organ donor with a functioning graft. We examined pancreas transplant tissue (formalin-fixed, paraffin-embedded and frozen). Serial sections were distributed to multiple investigators who performed a variety of assessments, by immunohistochemistry and immunofluorescence. For some studies, RNA from laser-capture dissected islets were used to conduct gene expression analysis.
We examined pancreas pathology for signs of rejection and autoimmunity, and specifically evaluated: 1) islet inflammation, including insulitis, the phenotype of islet-infiltrating cells (CD3, CD20, CD4, CD8, CD45RO, CXCR3), hyper-expression of HLA class I molecules, and expression of MICA, CXCL10, and 12-lipoxygenase; 2) the severity of beta cell loss, presence of glucagon-positive endocrine cells, the expression of urocortin (a marker of mature beta cells), beta cell replication (by assessing Ki67 expression in insulin-positive or synaptophysin-positive islet cells), ATP synthase and glucose kinase; 3) Alterations of extracellular matrix (ECM) components, including assessment of peri-islet basement membranes (BM), hyaluronan (HA) and hyaladherins; 4) The presence of the enteroviral protein VP-1 and assessment of the enterovirus CAR receptor, together with the retrospective assessment of serial serum samples to measure neutralizing antibodies to multiple enterovirus serotypes (CBV1-6).

Summary of Results

Main observations for the three SPK recipients with T1DR include the presence of insulitis and beta cell loss, demonstrating islet autoimmunity, affecting significant proportions of islets, in the absence of significant rejection (grade I and II for cell-mediated rejection, stages 1 and 2 for chronic allograft rejection). Moreover, the following was observed:
1) The insulitis lesions were characterized by a CD20 low phenotype, with CD8 T cells representing the dominant infiltrating cell type; significant proportions of infiltrating T cells expressed the memory marker CD45RO; CXCR3-positive infiltrating T cells were also observed. There was additional evidence of inflammation exemplified by hyperexpression of HLA class I molecules in islet cells, detection of MICA and CXCL10 in approximately 50% and 30% of the islets; gene expression analysis of laser dissected islets showed increased levels of CXCL9 and CXCL10 transcripts, which are ligands for CXCR3. There was increased expression by beta cells of the proinflammatory 12-lipoxygenase enzyme.
2) The severity of beta cell loss varied among patients, and across pancreas blocks, with 8-30% of the islets scored as insulin deficient. However, for all 3 patients with T1DR, the MMTT revealed a compromised insulin response; there were decreased proportions of insulin-positive cells and increased proportions of glucagon-positive cells within the islets, and increased proportions of insulin/glucagon double-positive cells. Urocortin-3 is a marker of mature beta cells, and the frequency of urocortin-3 bright beta cells was much reduced in patients 3681, 3626, and 3717, while it was not reduced in case 3678 compared to the control donor 6403. There was no evidence of beta cell replication by assessing Ki67 expression in insulin-positive or synaptophysin-positive islet cells. We also found that key proteins involved in glucose-stimulated insulin secretion (ATP synthase, glucose kinase) had reduced expression in residual beta cells of cases 3681 and 3678, suggesting beta cell dysfunction. Consistently, canonical gene pathways there were active in laser captured islet cells included mitochondrial dysfunction and oxidative phosphorylation, in addition to those for antigen presentation, allograft rejection signaling, and autoimmune thyroid disease signaling.
3) We observed multiple alterations of ECM components, including loss of peri-islet BM at the site of leukocyte penetration, which was associated with the expression of the proteolytic enzyme cathepsin S, suggesting that cathepsin S produced by CD45+ islet-infiltrating leukocytes might contribute to the degradation of peri-islet BM. No changes were observed at the endothelial BM of blood vessels at the site of leukocyte infiltration, however Meca79+ vessels were detected in close vicinity to immune cell accumulation suggesting that the extravasation of autoreactive immune cell from the circulation might occur through the high endothelial venules. We observed altered hyaluronan (HA) and hyaladherin patterns with an accumulation of HA along the peri- and intra-islet microvessels and infiltrating immune cells and co-localization of Hyaladherins co-localize with HA in the enlarged HA-rich regions.
4) The enteroviral protein VP-1 was detected in insulin-containing islets, particularly those with increased HLA class I expression; beta cells were found to express the enterovirus CAR receptor. The retrospective assessment of serial serum samples collected on follow-up revealed positivity for neutralizing antibodies to multiple enterovirus serotypes (CBV1-6) and interestingly spikes in titers were observed preceding or coinciding with the elevation of autoantibody titers.
In contrast, the recipient with clinical rejection (case 3717) demonstrated pancreas pathology findings consistent with moderate to severe chronic rejection, extensive inflammatory cell infiltrates throughout the pancreas affecting both the exocrine tissue and islets, with a dramatic loss of exocrine tissue and islet hypertrophy. While autoimmune infiltration of the islets is possible, it could not be clearly ascertained although this recipient had also developed autoantibodies on follow-up. The pancreas from an SPK recipient who passed as an organ donor with a normally functioning pancreas graft did not exhibit the features observed in the patients with recurrent T1D and had no significant rejection.

Conclusions

Most of the same features that characterize pancreas pathology in T1D are observed in the transplanted pancreas of recipients with T1DR, which were detected in the absence of significant rejection. Moreover, the demonstration of impaired stimulated insulin secretion

during a MMTT near the time of pancreas transplant biopsy support the existence of beta cell dysfunction contributing to insulin-dependence in the face of partial beta cell loss. To our knowledge, this is the most comprehensive characterization of T1DR in pancreas transplantation, made possible by the collaborative study of these patients performed by the nPOD-Transplantation group.

Preferred Presentation Format

Poster Presentation

Research Category

Pathology

71: Alberto Pugliese (University of Miami)

Presenter of Abstract

Helena Reijonen

Abstract Title

Altered frequencies of TEMRA and CXCR3-positive cells among autoreactive CD4 T cells associated with recurrent islet autoimmunity in recipients of simultaneous pancreas-kidney transplants

Authors

Helena Reijonen, Isaac Snowhite, Francesco Vendrame, George W. Burke III, Alberto Pugliese

Purpose

Patients with autoimmune, type 1 diabetes (T1D) and end stage renal disease may become recipients of simultaneous pancreas-kidney (SPK) transplants to restore insulin secretion and kidney function. We previously reported that some SPK recipients may develop T1D recurrence (T1DR) on follow-up despite immunosuppression that prevents rejection; in some patients T1DR was confirmed by the demonstration of insulitis and beta cell loss in a pancreas transplant biopsy. Overall, we have observed T1DR in about 5% of SPK recipients on extended follow-up. We previously reported that seroconversion for multiple autoantibodies is a risk factor for T1DR. The aim of this study was to determine whether autoreactive T cells in the circulation of SPK recipients are associated with T1DR and define key phenotypic features of these cells.

Methods

We studied 7 SPK recipients who had developed T1DR and 16 with normal glucose tolerance (NGT), of whom 5 were classified as autoantibody converters at risk for future T1DR (NGT-C) and 11 were autoantibody negative or had stable autoantibody levels from prior to the transplant (stable, NGT-S). We evaluated autoreactive T cells in peripheral blood using a pool of HLA class II tetramers loaded with T1D-associated peptides from multiple autoantigens (GAD65, proinsulin and ZnT8), and a viral antigen (flu) was used as a control. Besides antigen specificity, T cells were analyzed for lineage and phenotype by flow cytometry staining CD4, CD45RA, CD45RO, PD1, CXCR3, CCR7, CCR6 and CCR4. We examined naïve (CD45RA+, CCR7+), effector memory (EM, CD45RO+, CCR7-), central memory (CM, CD45RO+, CCR7+), total memory (EM+CM) and terminally differentially memory (TEMRA, CD45RA+, CCR7-). We measured these phenotypes in the total CD4 T cell compartment and in tetramer positive CD4 T cells. We assessed number of tetramer+ cells/1x106 CD4 T cells and as % of the various subset among the tetramer+ CD4 T cells; Value ranks among patient groups were compared using the non-parametric Mann-Whitney test; two-tailed p values are reported. For some analysis, we used ROC (Receiving Operating Curves).

Summary of Results

T1DR patients had significantly higher numbers of autoreactive CD4 T cells compared to NGT-S patients, and this was observed for naïve, total memory and effector memory compartments. In contrast, T1DR patients had decreased numbers of autoreactive CD4 T cells among TEMRAs compared to NGT-S recipients. For NGT-C patients, numbers of autoreactive CD4 T cells were similar to those of NGT-S patients, except these numbers were higher among naïve T cells.
We then examined the frequency of various subsets among autoreactive CD4 T cells, specifically naive, total memory, effector memory and TEMRA. In T1DR patients there were increased proportions of naïve and total memory autoreactive CD4 T cells compared to NGT-S patients. T1DR patients had very low frequencies of TEMRA autoreactive CD4 T cells compared to NGT-S patients (% mean 1.143 + SEM 0.6335 vs 46.82 + 8.781, respectively, $p<0.0026$); NGT-C patients also had lower frequency TEMRAs among the autoreactive CD4 T cell (% mean 14.60 + SEM 10.35) compared to NGT-S patients ($p= 0.0279$). The T1DR and NGT-C patients combined had much lower frequencies of TEMRA autoreactive CD4 T cells compared to NGT-S patients ($p<0.00001$). Using ROC, the proportions of TEMRA autoreactive CD4 T cells distinguished T1DR and NGT-C from NGT-S patients (AUC= 0.94, $p=0.0003$, 90% sensitivity, 92% specificity). We did not observe significant differences in the frequencies of naïve and memory subsets among the three patient groups, except that TEMRA CD4 T cells were reduced in T1DR compared to NGT-S patients (% mean 3.286 + SEM 1.358 vs 6.250 + 1.023, respectively, $p<0.04$).
The frequency autoreactive CD4 T cells expressing PD1, CCR6, or CCR4 did not differ among the three patient groups. However, the frequency of CXCR3-positive CD4 autoreactive T cells was significantly higher in T1DR and NGT-C patients compared to NGT-S patients. The frequency of CXCR3-positive autoreactive CD4 T cells among T1DR and NGT-C patients combined was % mean 25.25 + SEM 6.361 vs % mean 4.455 + SEM 3.431 among NGT-S patients ($p= 0.0063$). ROC analysis showed that the proportions of CXCR3-positive autoreactive CD4 T cells distinguished T1DR and NGT-C from NGT-S patients (AUC= 0.80, $p=0.01$, 82% sensitivity, 75% specificity).

Conclusions

Autoreactive CD4 T cells were increased in T1DR compared to NGT-S patients. We show that this applied to both naïve and total memory compartments. NGT-C patients had higher frequencies then NGT-S patients only in the naive compartment. Remarkably, the autoreactive CD4 T cells had very low frequency in the TEMRA compartment of T1DR and NGT-C patients, and this was a distinguishing feature that may be exploited as a biomarker of T1DR. Our results also demonstrate an association of CXCR3-positive autoreactive CD4 T cells with T1DR and autoantibody conversion. The expression of CXCR3 by circulating autoreactive CD4 T cells help identify a stage in which autoreactive T cells may migrate to the pancreas and infiltrate the islets. While our findings are from a relatively small number of patients, given the low frequency of T1DR among SPK recipients, they support CXCR3 as a potential therapeutic target to antagonize recurrent islet autoimmunity.

Preferred Presentation Format

No Preference

Research Category

Immunology

72: George Burke (University of Miami)

Abstract Title

Increase in C-peptide and proinsulin in kidney-pancreas transplant recipient with type 1 diabetes recurrence (T1DR) after one year of treatment with dulaglutide (a GLP-1 agonist) and Risankizumab (an anti-IL-23 monoclonal antibody).

Authors

Francesco Vendrame, Helena Reijonen, Aberto Pugliese, George Burke

Purpose

A 54-year-old Caucasian female was diagnosed with T1D at the age of 14. She later developed T1D-related nephropathy which progressed to end stage renal disease. At the age of 40 she underwent a kidney-pancreas transplant. The transplant normalized kidney function and restored euglycemia, and the patient became insulin-independent. However, she developed recurrent autoimmunity 4.3 years following transplantation, as evidenced by appearance of autoantibodies to GAD65, IA2, and ZNT8, and she also carried that high-risk HLA DR3 and DR4 (Vendrame, F. AJT 2016 June 16(1):235-245). At 10.4 years after transplantation, she became hyperglycemic (HbA1c between 6.5 and 7.5%) and returned to insulin therapy, despite fasting C-peptide levels of 3.0 ng/ml.

Methods

She began using a continuous glucose monitor and was started on a beta cell protective regimen of metformin and dulaglutide, a GLP-1 agonist, and was also treated with insulin degludec. Additional immunosuppression, to complement the beta cell-protective regimen was added in an attempt to treat T1D Recurrence (T1DR).

Summary of Results

In November 2018 she was treated with low-dose thymoglobulin therapy which had been shown to be beneficial in newly diagnosed T1D patients (Haller MJ, et al. Diabetes Care 2018;41(9):1917-1925). However, her glycemic control did not improve, and in fact deteriorated, peak MMTT C-peptide fell from 5.04 ng/ml to 1.43 ng/ml. She was then treated with Risankizumab, a monoclonal antibody against IL-23, which is used to treat psoriasis. This therapy was started in January 2020 and continued for a year. She underwent a Mixed Meal Tolerance Test (MMTT) before and after finishing the one-year course of Risankizumab. Comparison of the two MMTTs demonstrated: 1) C-peptide AUC increased from 77,135 to 83,664, suggesting improvement; and 2) proinsulin AUC increased from 696.8 to 1532.
Current HbA1c is 6.1% with fasting C-peptide 0.86 ng/ml, continuing on insulin degludec 24 U/day

Conclusions

In summary, this patient has received combinational therapy including 1) complementary beta-cell-protective agents (metformin and dulaglutide) and 2) Risankizumab resulting in improved C-peptide and proinsulin levels. This is consistent with a recent study demonstrating preserved beta cell function in a population of T1D patients receiving liraglutide, a GLP-1 analog and an anti-IL-21 monoclonal antibody (von Herrath M at al. Lancet Diabetes Endocrinol 2021; 9:212-24). Our patient continues on a second year of this regimen, with the possibility that the improved levels of C-peptide and proinsulin will translate to improved beta cell function, although the increase in proinsulin may reflect ongoing beta cell stress.

Preferred Presentation Format

Poster Presentation

Research Category

Immunology

73: Christoph Nowak (Diamyd Medical)

Presenter of Abstract

Johnny Ludvigsson

Abstract Title

Intralymphatic GAD-alum (Diamyd®) improves glycemic control as measured by continuous glucose monitoring in Type 1 diabetes patients carrying HLA DR3-DQ2

Authors

Ulf Hannelius, Cristoph Nowak, Johnny Ludvigsson

Purpose

Residual beta cell function in Type 1 diabetes (T1D) is associated with lower risk of complications. Autoantigen therapy with GAD-alum (Diamyd®) given in three intralymphatic injections with oral Vitamin D has shown promising results in persons with T1D carrying the HLA DR3-DQ2 haplotype in the phase IIb trial DIAGNODE-2 (Ludvigsson et al. Diab Care 2021, PMID:34021020). We aimed to further explore the efficacy of intralymphatic GAD-alum compared to placebo on blood glucose recorded by continuous glucose monitoring (CGM).

Methods

DIAGNODE-2 (NCT03345004) was a multicenter, randomized, placebo-controlled, double-blind trial of 109 recent-onset T1D patients aged 12–24 years with GAD65 antibodies and fasting C-peptide >0.12 nmol/L, which randomized patients to either three intralymphatic injections of 4 µg GAD-alum and oral vitamin D, or placebo. Here, we report results for exploratory endpoints assessed by 14-day CGM at Months 0, 6 and 15. Treatment arms were compared by mixed-effects models for repeated measures adjusting for baseline values.

Summary of Results

We included 98 patients with CGM recordings of sufficient quality (27 Diamyd-treated and 15 placebo-treated DR3-DQ2-positive patients) with a median (mean) recording length of 14 (13) days. In HLA DR3-DQ2-positive patients, % time in range (3.9-10 mmol/L; 70-180 mg/dL) declined less between baseline and Month 15 in Diamyd-treated compared to placebo-treated patients (-5.1% and -16.7%, respectively, P = .0075), with reduced time (P = .0036) and number of excursions (P = .0072) above 13.9 mmol/L (250 mg/dL), and significant benefits on the glucose management indicator (P = .0025). No differences were detected for hypoglycemia.

Conclusions

Intralymphatic GAD-alum (Diamyd®) improves glycemic control in recently diagnosed T1D patients carrying HLA DR3-DQ2.

Preferred Presentation Format

Oral Presentation

Research Category

Immunology

74: Alberto Pugliese (University of Miami)

Presenter of Abstract

Isaac Snowhite

Abstract Title

miRNA responses during stimulated insulin secretion in pancreas slices from organ donors and living subjects.

Authors

Isaac Snowhite, Irina Kusmartseva, Helmut Hiller, Maria Beery, Jay Sosenko, Shari Messinger, Ricardo Pastori, Roberto Mallone, Alberto Pugliese

Purpose

During the last two years nPOD has launched the pancreas slice program to support investigations of live tissue in its entirety, so that not only one can examine islet cells but also investigate infiltrating immune cells and the exocrine cell compartment. Thus, islets are examined in the natural tissue environment. Pancreas slices are subjected to in vitro glucose stimulated insulin secretion (GSIS) assays. As part of these core studies we measured miRNA levels during GSIS with financial support from the Helmsley Charitable Trust George Eisenbarth nPOD Award for Team Science. While progress is being made in the identification of circulating miRNA biomarkers in T1D, the source of the circulating miRNAs is unknown. The aim of this pilot analysis was to determine changes in miRNA levels occurring during GSIS in pancreas slices for organ donors with and without T1D, and compare those with changes in miRNA levels obtained in patients with type 1 diabetes (T1D) during a mixed meal tolerance test (MMTT), to determine whether miRNA changes during the MMTT reflect changes occurring in pancreas slices during GSIS, which could help determine the tissue of origin and relevance to insulin secretion of miRNAs detected in the circulation.

Methods

miRNA levels were assessed in perifusate samples from GSIS assays performed on pancreas slice specimens and from serum samples obtained during the course of a MMTT; miRNA levels were measured using the HTG Molecular Diagnostics EdgeSeq miRNA assay, which combines a quantitative nuclease protection assay (qNPA) with a Next Generation Sequencing (NGS) to enable semi-quantitative analysis of targeted genes in a single assay, reporting levels for 2,083 miRNAs. We assessed levels form GSIS perifusate samples from 20 organ donors without diabetes (13 males, 7 females, age range 4.6-27.1 years) and 5 T1D donors in whom residual insulin secretion was still detectable during GSIS (3 males, 2 females, age range 2.8-29.8 years of age, T1D duration 0, 0, 1, 1.5, and 2.5 years, respectively). We also measured miRNAs in serum samples from the baseline MMTT obtained from 4 T1D patients within 3 months from diagnosis (3 males, 1 female, age range 21.1-32.2 years, age of onset 21, 21.1, 22.3, and 32 years). All patients had a peak stimulated C-peptide > 0.2 pmol/l during the MMTT.

Summary of Results

We used the HTG Reveal 4.0 software (based on the R package) to calculate differential expression among the time points of the GSIS and MMTT assays in terms of miRNA levels. We determined that changes in miRNAs were most apparent between the 3mM and 16 mM glucose time points of the GSIS in slices, corresponding to the first peak in insulin release, and between the -10 and 30 minute samples of the MMTT. We chose a log2 FC> +1.3 (corresponding to FC> +2.4 on the linear scale) as a conservative cutoff, given the limited number of samples examined and the large number of miRNAs examined. We first compared results from the GSIS of the 20 donors without diabetes to the MMTT data from the 4 T1D patients. We found that 34/2,083 (1.7%) of these miRNAs showed Log2 FCs> +1.3 in the same direction in both MMTT and GSIS studies, with 25 and 9 miRNAs being upregulated or down-regulated, respectively. Among these miRNAs were miR-21-5p, miR-375-3p, miR-324-3p, miR-4302, all of which were previously linked to T1D in studies of circulating miRNAs. We then repeated the same analysis for the MMTT data from the 4 T1D patients and the GSIS data from 5 T1D organ donors with residual insulin secretion. We identified 109/2,083 (5.2%) with Log2 FCs> +1.3 in the same direction in both MMTT and GSIS studies, with 81 and 28 miRNAs being upregulated or down-regulated, respectively. Among these miRNAs we observed again miR-21-5p and miR-375-3p.

Conclusions

Ongoing efforts have identified circulating miRNAs that improve prediction of future T1D and miRNAs that are associated with T1D or with C-peptide decline, but their cellular/tissue origin is unknown. While these data are limited by small sample size, the lack of data from MMTT in subjects without diabetes, and differences in the GSIS and MMTT stimulations, the results of this pilot investigation support that it is possible to 1) measure miRNA changes during stimulated insulin secretion in vitro and in vivo, and 2) observe parallel

changes in miRNA levels during stimulated insulin secretion that could assist in establishing the relevance of circulating miRNAs to pancreas physiopathology. Future studies will be needed to identify critical miRNA responses and their alterations during the progression of T1D.

Preferred Presentation Format

Poster Presentation

Research Category

Novel Biomarkers