Network for Pancreatic Organ donors with Diabetes

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CATEGORICAL LISTING

Beta Cell Development, Differentiation & Regeneration

Development of a new strategy for the accurate assessment of beta-cell mass and function in health and disease

Proteome-wide and matrisome-specific alterations during human pancreas development and maturation

Endocrine cell function during early postnatal development of the human pancreas

Multi-omics study uncovers the influence of Senescence on Beta Cell Functionality

Evidence for extensive elimination of cells during postnatal pancreas growth

Reappearance of C-peptide during the third trimester in type 1 diabetes pregnancy: pancreatic regeneration or fetal hyperinsulinism?

Beta Cell Physiology and Dysfunction

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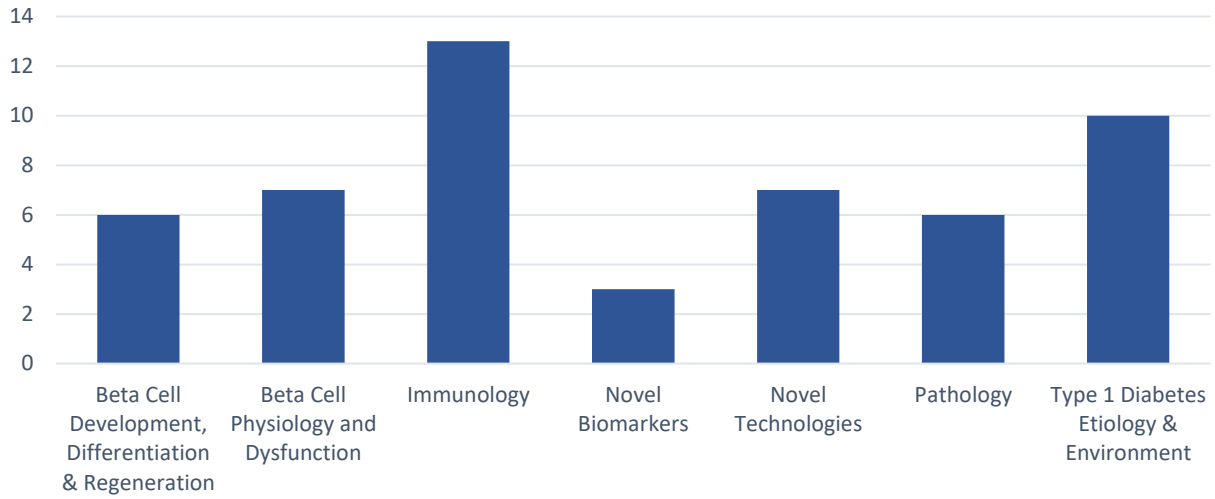

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Abstract #1

Endocrine cell function during early postnatal development of the human pancreas

Authors

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PURPOSE

Studying human pancreatic beta cells and islets is essential if we are to understand the pathogenesis of type 1 diabetes. Although much has been learned about human islet biology over the last decade, there are major gaps in our understanding of human pancreatic islet development. While anatomical studies provide a first glance that the islet is dramatically rearranged during the first years of life, there is no information about how islets become fully responsive to glycemic fluctuations and how they start regulating glucose homeostasis. The working hypothesis is that beta cells become fully responsive to glucose once the islets attain their adult cell arrangement.

METHODS

Most of what we know about the postnatal development of the pancreatic islet is derived from anatomical studies in mice or from isolated human islets. Our work is focused on the functional maturation of the human islet. Given the complex structural rearrangements that occur during the first decade of life, it is clear that the function of maturing islets can only be studied while embedded in their natural environment. Thus, we are using viable juvenile human tissue slices obtained from human donors aged 0 to 10 years old. Slices are produced and distributed by the nPOD Organ Processing and Pathology Core (OPPC) at the University of Florida. We combine functional recordings in living human pancreas tissue slices with anatomical studies of islet cytoarchitecture and measurements of hormone secretion.

SUMMARY OF RESULTS

Hormone release from juvenile human slices demonstrated significant changes during maturation. Insulin secretion patterns from slices of newborn donors showed no glucose responsiveness, but became progressively responsive with increasing age. Glucagon secretion on the other hand seems to be sensitive to a drop in glucose concentrations earlier in life. Anatomically, human juvenile islets undergo major changes in composition and cytoarchitecture during this phase. We are currently examining dynamic Ca2+ responses to specific stimuli to determine changes in endocrine cell function and cell communication within the islet

CONCLUSIONS

Our preliminary data show that juvenile human islets differ significantly from adult islets, both functionally and anatomically. These findings point at the first decade of life as a period of islet functional maturation that is likely orchestrated by multiple inputs including nutrient nature and supply, intrinsic and systemic factors, which collectively tune islet responses to changes in glycemia.

Abstract #2

Multi-omics study uncovers the influence of Senescence on Beta Cell Functionality

Authors

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PURPOSE

Cellular senescence, a stable cell cycle arrest, is associated with various pathological conditions, including cancer and aging. It has also been suggested that senescence contributes to diabetes. The senescence program is activated by the tumor suppressive p53-p21 pathway, and the p16Ink4a-Rb pathway, often working in concert. It can be triggered by DNA damage, various stresses, and other physiologic cues. In addition, the senescence-associated secretory phenotype (SASP) is activated by inflammatory regulators, including NFkB, and promoted by damage. p16, encoded by the CDKN2A gene, is upregulated in pancreatic beta cells during aging, raising the question of whether these cells undergo senescence, and if so, how does this affect their phenotype and function. We recently found that p16 expression and cellular senescence influence pancreatic beta-cell functionality, boosting glucose-stimulated-insulin-secretion (GSIS). A negative role of beta-cell senescence in diabetes was also reported, mediated by pro-inflammatory effects of the SASP. However, the roles of beta-cell senescence in aging-associated glucose metabolism and diabetes are currently poorly characterized. We aimed to uncover the changes in chromatin organization and gene regulatory circuits in senescent beta cells and how these influence cell function and phenotype.

METHODS

Senescence induction was carried out in EndoC-bH3 human pancreatic cell line by tamoxifen-inducible Cre mediated excision of SV40-LT and hTERT for three weeks. We conducted transcriptomic profiling of senescent and control EndoC-bH3 cells, and employed multiplexed-ChIP-Seq using antibodies against histone marks for active promoters (H3K4me3 and H3K27ac), enhancers (H3K4me1 and H3K27ac), and repressive marks (H3K9me3 and H3K27me3).

SUMMARY OF RESULTS

Transcriptomic analysis indicated the activation of functional genes parallel to the silencing of cell cycle and proliferation genes. Senescence did not involve SASP activation. Analysis of chromatin modifications supports the transcriptomic data in an additional layer. We systematically mapped active promoters and enhancers in the senescent and non-senescent EndoC-bH3 cells based on active chromatin marks H3K4me3, H3K27ac, and H3K4me1 as well as the repressive marks H3K9me3, H3K27me3. Interestingly, beta-cell functionality genes were mostly activated through increased H3K27ac on enhancers involved in insulin secretion, pancreas development, and GSIS. Many of these enhancers overlap with known islet enhancer hubs, which interact with multiple functional promoters. In contrast, cell cycle gene silencing was achieved mostly by depleting H3K27ac from these genes' promoters.

CONCLUSIONS

Senescence induction in EndoC-bH3, boosting cell functionality and maturation, is associated with hyperacetylation of enhancers linked to beta-cell functionality, and is also associated with hypoacetylation of cell cycle gene promoters.

Abstract #3

Evidence for extensive elimination of cells during postnatal pancreas growth

Authors

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PURPOSE

It is typically assumed that organ growth is an efficient process in which cell proliferation leads to increased cell number and tissue mass, with a minor participation of programmed cell death (particularly during morphogenetic stages, such as digit formation). However, there is little evidence regarding the extent of cell death during development of most organs, largely because of the difficulty in quantifying dying cells that are rapidly cleared. We designed experiments to assess if cell proliferation can fully account for the observed increased in pancreas size during postnatal life of mice and humans.

METHODS

Organ growth is a function of the change in cell size and cell number, as follows:
Δ pancreas weight = Δcell size x Δcell number + extracellular volume =
=Δcell volume x Δtime x (cell proliferation rate – cell death rate) + extracellular volume.
Thus, a measurement of organ weight, cell size and proliferation rate should allow inference of cell death, assuming that extracellular matrix volume is negligible.

SUMMARY OF RESULTS

Using accurate measurements of cell size and organ mass in the pancreas of newborn and adult mammals we have estimated the number of exocrine pancreatic cells during postnatal growth. Pancreatic cell number is mice increases 2.5 fold from birth to adult life, accompanied by a major component of cell growth (hypertrophy). Pancreatic cell number in human increases ~3 fold during the first year of life, in accordance with the increase in organ mass.

Strikingly, multiple independent approaches for assessing cell proliferation predict much more extensive increase in acinar cell numbers than observed. For example, immunostaining for markers of proliferating cells (Ki67, PCNA, Phospho histone H3) in humans between birth and 1 year of age predicts cell number at one year that is orders of magnitude larger than observed. Immunostaining

analysis of the mouse pancreas gives similar results. Analysis of heavy isotope labeling data reveals label dilution that also predicts massive expansion of the pancreas, beyond the actual growth observed. Histologic analysis does reveal evidence for cell death in the newborn mouse and human pancreas that exceeds what is observed in adults.

CONCLUSIONS

Integrating measurements of pancreas size, acinar cell size and acinar cell proliferation rate in mice and humans suggests extensive elimination of cells during postnatal pancreas development. We speculate that cell elimination reflects a selection process for the fittest cells. Strict selection in humans may lead to slow growth and delayed aging, while loose selection in small rodents may lead to faster growth at the expense of more rapid aging.

Abstract #4

Reappearance of C-peptide during the third trimester in type 1 diabetes pregnancy: pancreatic regeneration or fetal hyperinsulinism?

Authors

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PURPOSE

There is controversy about whether endogenous insulin secretion increases in late gestation in type 1 diabetes pregnancy. We aimed to assess longitudinal patterns of maternal C-peptide concentration and to investigate the mechanism for the changes seen during pregnancy.

METHODS

C-peptide concentration was measured on serum samples at 12, 24 and 34 weeks from 127 participants in the continuous glucose monitoring in type 1 diabetes pregnancy trial (CONCEPTT). Maternal serum C-peptide and cord blood C-peptide were measured using a highly sensitive direct and solid-phase competitive electrochemiluminescence immunoassay respectively

SUMMARY OF RESULTS

Three discrete patterns of maternal C-peptide trajectory were identified: Pattern 1 undetectable throughout pregnancy, n=74 (58%, maternal C-peptide <3 pmol/l); Pattern 2 detectable at baseline, n=22 (17%); Pattern 3 undetectable C-peptide at 12 and 24 weeks which became detectable at 34 weeks, n=31 (24%; maternal C-peptide 4-26 pmol/l at 34 weeks). The baseline characteristics and third trimester glucose profiles of women with pattern 1 and pattern 3 C-peptide trajectories were similar. The offspring of women with pattern 3 C-peptide trajectories had markedly increased rates of neonatal hypoglycemia (42% vs 14%; p=0.001), large-for-gestational-age (90% vs 60%; p=0.002) and neonatal intensive care admission (45% vs 23%; p=0.023), with elevated cord blood C-peptide (geometric mean 1319 vs 718 pmol/l; p=0.007) compared to offspring of women in pattern 1.

CONCLUSIONS

Increased C-peptide concentration at 34 weeks is associated with fetal hyperinsulinism, suggesting fetal to maternal transfer. We found no evidence for improved maternal beta cell function. First appearance of C peptide in late pregnancy could be used to identify pregnancies at highest risk of neonatal complications

Abstract #5

Proteome-wide and matrisome-specific alterations during human pancreas development and maturation

Authors

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PURPOSE

The purpose of this study is to characterize the overall proteome changes at distinct time points throughout human development, with a specific focus on proteins associated with the extracellular matrix (ECM) in the whole pancreas, as well as in the islet and acinar compartments.

METHODS

Human pancreata were collected from donors in four age groups: fetal (18-20 weeks gestation, N=6), juvenile (5-16 years old, N=5), young adult (21-29 years old, N=6) and older adult (50-61 years old, N=6). For each donor, whole pancreas tissue was lysed and prepared for liquid chromatography tandem mass spectrometry analysis, and pieces were fixed and stored for immunohistochemistry. Multiplexed quantitative mass spectrometry was performed on all samples. Specific ECM proteins of interest were identified through the comparative mass spec study and used for immunofluorescent staining to quantify relative expression of those proteins in the islet and acinar regions.

SUMMARY OF RESULTS

We identified 3,523 proteins in all four developmental groups, of which 1,570 had significantly changed expression levels in at least one of the four developmental groups by ANOVA analysis. We identified 185 ECM proteins, of which 117 were quantified throughout all four age groups. Of these, 84 proteins (72%) had significant changes among the groups, revealing that the matrisome of the pancreas is dynamically remodeled throughout development. A closer look at structural ECM proteins (in the collagen, proteoglycan, and glycoprotein families) revealed trends in the expression patterns of many proteins among the acinar and islet regions, of which some proteins were primarily expressed only in fetal tissue, and others were enriched in islets only in adult donors. Furthermore, although the total protein lysates of younger adult and older adult donors had very little significantly changed ECM proteins by total abundance, several ECM proteins were enriched in the older adult compared to

younger adult islets. We have developed a searchable database for the exploration of the entire set of proteins quantified in this study.

CONCLUSIONS

The matrisome of the human pancreas changes dynamically throughout development, with 72% of identified ECM proteins having significant changes among the four age groups. Furthermore, the ECM environment of the acinar and islet regions of the pancreas also change over developmental time, including in older adulthood.

Abstract #6

RNA and Proteins in Exosomes from Human β Cells May Reflect Early β Cell Stress in Type 1 Diabetes

Authors

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PURPOSE

Type 1 diabetes (T1D) is characterized by immune-mediated destruction of pancreatic β -cells. Immunomodulatory interventions initiated at Stage 3 disease onset have shown limited efficacy in inducing disease remission. As such, there is increasing emphasis on efforts to test disease-modifying therapies in Stage 1 and 2 disease. Whereas it has been proposed that proteins and RNA species released by β cells can be monitored as biomarkers of stress in early stages, little remains known about the mechanisms by which these species are e released into the circulation as biomarkers. In this study we sought to identify the mechanisms by which RNAs and proteins might be channeled into exosomes in response to cellular stress in β cells and islets.

METHODS

Min6, INS-832/13, human EndoC βH1 cell line and human pancreatic islets were treated in vitro with or without pro-inflammatory cytokines (IL-1β and IFN-γ) to mimic stress conditions seen in T1D. Exosomes were isolated using either a precipitation method or by serial ultracentrifugation, and vesicle derived mRNA levels were measured by RT-PCR and proteins were identified by mass spectrometry. Untreated and cytokine treated human islets were subjected to poly-ribosome profiling (PRP) to assess the cytokine mediated global mRNA translation initiation block. Digital PCR was used to measure the circulating levels of insulin mRNA from T1D animal models and human subjects with T1D. Single molecular FISH was performed to identify the co-localization of INS mRNA with stress granule markers.

SUMMARY OF RESULTS

The treatment of mouse rodent β-cell lines, human EndoC- βH1 β cells, and human islets with pro-inflammatory cytokines or with arsenite (a chemical inducer of cell stress) resulted in a block in mRNA translation initiation and decreased protein synthesis as assessed by polyribosomal profiling and puromycin incorporation, respectively. Human islets treated with cytokines or arsenite also exhibited increased formation of stress granules, P-bodies, and sec-bodies, as observed by immunofluorescence and immunoblotting. Moreover, studies using single molecular FISH further confirmed that human β

cells treated with cytokines and arsinite have increased colocalization of INS mRNA with G3BP1 (a marker of stress granules). EndoC- βH1 human β cells transfected with fluorescently-labeled G3BP1 and CD63 (a marker of exosomes) and treated with cytokines or arsenite exhibited co-localization of stress granules with exosomes, suggesting that stress granule contents are secreted extracellularly. Preliminary proteomics data from isolated exosomes from untreated and cytokine treated human islets revealed the presence of preproinsulin, preproglucagon, HLA class I, DPP4, proinflammatory proteins such as HSP70, HSP90, and 14-3-3b, components of sec-bodies (SEC23A, SEC31A, COP9) and mitochondrial proteins, suggesting that the contents of exosomes reflect the underlying activation of inflammation in the cell. Human preproinsulin (PPI) mRNA was recoverable almost exclusively in the exosomal fraction isolated from supernatants of stressed human islet cultures, also suggesting that translationally inhibited mRNAs are shuttled into exosomes. Pharmacological inhibition of exosome release decreased the PPI mRNA into the culture media, further suggesting that PPI mRNA is selectively released from stressed β cells via exosomes. Digital PCR analysis demonstrated that circulating mouse PPI mRNA was detectable in circulation of several diabetic mouse models prior to the onset of diabetes. Likewise, we observed significantly higher levels of PPI mRNA in circulation of new-onset T1D subjects when compared to age-matched healthy controls.

CONCLUSIONS

Our data suggest that inflammatory stress in β cells leads to the sequestration of translationally inhibited mRNAs into stress granules which are subsequently shuttled into the endosomal pathway leading to exosomal formation. Moreover, these exosomes carry with them intracellular protein species that reflect both a state of cellular stress and the cell from which the exosome originated. We conclude that exosomes contain a dynamically alterable pool of RNAs and proteins that may reflect cellular states of stress that could correlate with stage of disease in T1D.

Abstract #7

Pancreatic slices give new insights into stimulus secretion coupling

Authors

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PURPOSE

The pancreatic slice method is emerging as an excellent tool to understand the structure of islets. We know that beta cells within the slices are polarised and each cell makes a discrete point of contact with the islet capillaries. How this might affect stimulus-secretion coupling is unclear. Here, we have focused on functional assessment of beta cells within slices in terms of glucose-induced insulin secretion and calcium responses.

METHODS

Pancreatic slices and isolated islets were prepared from mice as previously described. They were cultured overnight and imaged using multiphoton microscopy. Calcium measures were made in preparations from transgenic Ins1Cre knock-in mice crossed with loxP GCaMP6s mice. Exocytosis was measured using SRB as an indirect reporter of granule fusion. Immunostaining and insulin secretion were performed as described previously.

SUMMARY OF RESULTS

We used diazoxide to discriminate between glucose-induced triggering and amplification and compare pancreatic slices with isolated islets. Our data show that in slices, triggering is enhanced and amplification is reduced compared with isolated islets. We used calcium responses to assess triggering. At 2.8 mM glucose calcium spikes were observed in slices but not in isolated islets. Each spike originated at the capillary interface and then spread as a wave across the beta cell. Increased glucose concentrations led to phasic calcium responses that were induced with shorter latencies and more rapid kinetics in slices compared with isolated islets. Furthermore, we observed coordination of responses in both slices and isolated islets indicating that gap junctional communication was preserved in both preparations.

Studies of the distribution of glucose-induced granule fusion showed a very tight targeting to the capillaries in slices that was more diffuse in isolated islets. Consistent with this, immunostaining showed that presynaptic scaffold proteins, like liprin, were strongly enriched at the capillary interface of beta cells in slices but were widely spread across the cell membrane in isolated islets.

CONCLUSIONS

We conclude that the pancreatic slices preserve beta cell structure and this impacts on cell function. Overall insulin secretion is different in slices, compared with isolated islets, and this is a reflection of intrinsic differences within beta cells including different calcium responses and local control of granule fusion.

Abstract #8

A distinct beta cell type of human islets contains proinsulin but does not express proprotein convertase 1/3, the hormone involved in the conversion of the proinsulin to insulin.

Authors

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PURPOSE

The insulin precursor proinsulin is processed into insulin and c-peptide in secretory vesicles by the enzymes proprotein convertases (PC)1/3, PC2 and carboxypeptidase E. Previous observations indicated that human beta cells show significant variation in the expression level of PC1/3. The current study sought to determine whether the heterogeneity in enzyme expression is correlated with levels of proinsulin and mature insulin.

METHODS

The co-expression of proinsulin, PC1/3 and insulin was examined by high resolution confocal microscopy in tissue sections of pancreas from non-diabetic donors (n=5) and of donors with Type 2 diabetes (n=4) provided by nPOD.

SUMMARY OF RESULTS

Results- This analysis identified a human beta cell type that expressed proinsulin but lacked PC1/3 (ProIN+PC1/3-). This beta cell type is absent in rodent islets and is scarce in islets from early postnatal donors. A second cell type discovered expressed both ProIN and variable levels of PC1/3 (ProIN+PC1/3+). Islets of adults contain nearly identical percentages of ProIN+PC1/3- and ProIN+PC1/3+ cells. A third, and less abundant, beta cell type identified was less abundant, lacked proinsulin but expressed the convertase (ProIN-PC1/3+). Pancreas from T2D donors contained islets with normal phenotype next to islets with decreased PC1/3 levels and with ProIN expression dispersed in the cytoplasm.

CONCLUSIONS

This analysis suggests the three beta cell types represent sequential changes in functional beta cell phenotypes with processing of proinsulin in the ER in ProIN+PC1/3- cells, then in secretory granules in ProIN+PC1/3+ cells and replenishing the content of the prohormone in the ProIN-PC1/3+ cell type followed by its shuttle to the RER. Moreover, that preservation of the three cell phenotypes and their sequential inter-conversion is linked to normal beta cell function.

Establishing whether or not the expression these functional markers is dysregulated in synchrony during the development of diabetes will provide in insight into the identity of factors involved in the progress of the disease.

Abstract #9

Evidence of increased telolysosomes presence in alpha and beta cells of autoantibody positive human organ donors

Authors

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PURPOSE

Autophagic degradation is a dynamic recycling process that contributes to cellular homeostasis and mitigation of cellular stress. While there are multiple forms of -phagic degradation processes, a common endpoint is the final degradation step in the lumen of the acidic lysosomes. Post-mitotic cells, including beta cells, can accumulate tertiary lysosomes called telolysosomes which contain oxidized proteins and lipids that are undigestible. We recently found that autophagy is impaired in the beta cells of individuals with T1D and also in islets of diabetic NOD mice. We also observed a significant increase in the number of telolysosomes in the beta cells of autoantibody positive individuals. We have now extended our analyses to the alpha cells of autoantibody positive organ donors.

METHODS

We used the electron microscopic images of nPOD pancreas tissue sections, available from www.nanotomy.org/OA/nPOD. Telolysosomes in the alpha cells were identified and quantified in tissue sections from nondiabetic and autoantibody positive organ donors.

SUMMARY OF RESULTS

By implementing immunofluorescence analysis and electron microscopy, we previously observed an increase in the number of autophagosomes ($p=0.0458$) and telolysosomes ($p=0.0024$) in the beta cells of autoantibody positive organ donors when compared to nondiabetic organ donors. Currently, by taking advantage of electron microscopic images from the nanotomy repository, we also report a significant increase in telolysosomes ($p=0.03$) in the alpha cells of autoantibody positive individuals when compared to alpha cells of nondiabetic organ donors.

CONCLUSIONS

We now report our observation of an increased number of telolysosomes in the alpha cells of autoantibody positive individuals, suggesting an accumulation of lysosomes with highly oxidized proteins and lipids in both alpha and beta cells of autoantibody positive individuals. This observation in conjunction with our previous observation of increased autophagosomes in beta cells of autoantibody positive donors suggest a possible defect in the lysosomes in the islet cells during disease pathogenesis, prior to the development of hyperglycemia. Further studies will be required to confirm these observations and address when lysosomes become defective during the pathogenesis of type 1 diabetes.

Abstract #10

MAFA S64F Missense Mutation Causes Sex-Dependent Islet Beta Cell Aging and Senescence

Authors

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PURPOSE

A naturally occurring, missense mutation of the pancreatic islet-enriched transcription factor MAFA (termed S64F MAFA) was identified in patients characterized by familial diabetes or insulinomatosis, with men more prone to diabetes. This variant causes the normally unstable MAFA protein to be unusually stable. We have generated a heterozygous germline mouse model expressing this variant (S64F MafA Het) which mimics the sex-dependent phenotypes in humans: Het males have impaired glucose tolerance while Het females have hypoglycemia. Here we performed functional and molecular assays to better explore the pathophysiology of this variant in mouse and human beta cells.

METHODS

We performed bulk RNA-sequencing (RNA-seq) on male and female Het mouse islets and their wild type counterparts to identify globally affected pathways in an unbiased manner. These pathways were confirmed by functional analysis, qPCR and immunostaining on primary tissues. Affected pathways of interest were then interrogated in the human EndoC-bH2 beta cell line after lentiviral transfection of S64F MAFA.

SUMMARY OF RESULTS

Both sex-dependent and sex-independent pathways were found in S64F MafA Het islets by bulk RNA-seq. Calcium regulatory pathways were commonly affected in male and female islets, and functional analyses demonstrated disturbed calcium signaling after high glucose stimulation. Interestingly, these results suggested unique beta cell subpopulations within each group, particularly in females, which comprised both non-responsive and hyper-responsive beta cell subpopulations.

Our analysis focused on S64F MafA Het males because increased MafA protein stability was found to

precede glucose intolerance in males with upregulation of genes involved in aging/senescence by bulk RNA-seq. We confirmed increased beta cell expression of senescence markers gH2AX, 53BP1, p21, and senescence-associated b-galactosidase (SA-b-gal) staining, which were not seen in female Het islets. These results implicate accelerated aging/senescence in islet beta cell dysfunction in Het males. We also find increased cellular senescence in S64F MAFA expressing EndoC-bH2 cells compared to those expressing WT MAFA.
As senescent cells are highly active and produce a SASP (senescence-associated secretory phenotype) signature for paracrine induction of senescence in a cell non-autonomous fashion, media from WT MAFA or S64F MAFA-expressing human beta cells were prepared and applied to untransduced EndoC-bH2 cells. Only the conditioned media from S64F MAFA-expressing cells increased expression of SA-b-gal in these naïve cells and induced a human senescence and SASP signature with upregulation of P21, BCL2, ICAM3, IGFBP2, and IGFBP4.

CONCLUSIONS

S64F MAFA was recently identified as a de facto MODY gene, with men predominantly presenting with diabetes and women with hypoglycemia. We generated a mouse model harboring this mutation which produces similar sex-dependent phenotypes. Glucose intolerance in male Het mice were associated with accelerated cellular aging and senescence, which was also identified in human EndoC-bH2 cells expressing S64F MAFA. Importantly, two independent studies have shown that senescent beta cells in established mouse models of T1D (NOD mice) and T2D (insulin resistant on high fat diet) causes beta cell dysfunction. However, senescent beta cells in these models were quite rare (<10%) compared to rates upwards 60% in human T2D beta cells. We find pervasive senescence in beta cells expressing S64F MAFA, which mimics the human T2D condition. Future studies will include analyzing the molecular subtypes of the beta cell populations produced in male and female S64F MAFA Het mice as well as extend experimentation to human islets using pseudoislet technology to evaluate the broader significance of these processes in aging beta cells and diabetes.

Abstract #11

Lipid droplets protect human islet β cell from lipotoxic-induced stress and senescence

Authors

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PURPOSE

Transplanted human islet β cells, and not mouse, were first observed to accumulate lipid droplets (LDs) in high fat diet fed immunodeficient NSG mice. Significantly, we have now established that LD accumulation in the intact human pancreas is age dependent, as few LDs are found in juvenile islet β cells in relation to adults. LDs were also enriched in type 1 and type 2 diabetes (i.e., T1D and T2D) islet cells, suggesting an association between LD accumulation and islet dysfunction. In contrast, LD accumulation was nearly undetectable in the adult rodent pancreas, even in hyperglycemic and hyperlipidemic models or aged mice. Here we sought to obtain mechanistic insight into how LDs influence human β cell function.

METHODS

Perilipin 2 (PLIN2), a key structural protein of LDs in human islets, was targeted to manipulate LD levels by lentivirus-based shRNA knockdown (P2KD) or over-expression (P2OE) in the EndoCβH2-Cre, a functional representative human β cell line. Functional and unbiased molecular characterization was performed on free fatty acids (FFA) treated and untreated cells.

SUMMARY OF RESULTS

As expected, P2KD reduced LD accumulation upon exposure of EndoCβH2-Cre cells to FFA, while levels were increased in P2OE. In addition, glucose stimulated insulin secretion was blunted in P2KD cells and improved after P2OE treatment, suggesting that LD levels regulate human β cell activity. An unbiased transcriptomic analysis of P2KD cells revealed that limiting LD formation induced effectors of endoplasmic reticulum stress followed by induction of senescence, compromised β cell function and identity gene expression. In contrast, essentially none of these signatures of β cell dysfunction were induced upon elevating LD levels in P2OE cells.

CONCLUSIONS

Our working model is that LDs are important to adult human islet β cells under both normal and pathological settings. We further propose that differences in LD formation/breakdown contributes to diabetes susceptibility by impacting the ability of β cells to defend against lipotoxicity.

Abstract #12

Dimethyl fumarate antagonizes islet autoimmunity in NOD mice

Authors

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PURPOSE

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

METHODS

NOD mice with recent diabetes onset or 10-week old mice received DMF (100 mg/Kg) or vehicle daily by oral gavage for 12-weeks. Mice were followed for additional 12-weeks from the end of treatment and monitored for diabetes relapse or diabetes development, respectively. Diabetes reversal was defined as the absence of glycosuria and a return to normal blood glucose concentrations. Mice in this group were implanted with a subcutaneous insulin pellet lasting 3-4 weeks to mimic studies in humans. Peripheral blood, spleen, lymph nodes (LN), pancreatic LN and pancreatic islets were analyzed by flow cytometry. Histology studies were also performed. For the prevention group, the absence of glycosuria and hyperglycemia defined as two consecutive blood glucose measurements exceeding 250 mg/dL indicated the lack of diabetes development. Immunophenotyping from various tissues was performed by flow cytometry. In this group we also measured the glucose uptake by adding 100µM 2-NBDG analog (Life Technologies) during the last 2 hours of splenocytes stimulated with anti-CD3 for 5 hours in vitro. In addition, we assessed the expression of the early activation maker CD69 by flow cytometry and cytokines production after 4h of in vitro stimulation with Leukocyte Activation Cocktail (BD Biosciences).

SUMMARY OF RESULTS

In mice with recent diabetes onset, 69.2% of DMF-treated mice (n= 13) achieved durable diabetes remission and continued to remain diabetes-free for the 12-weeks follow-up after the secession of treatment, while all vehicle-treated mice did not achieve durable remission (n=9) ($p<0.0001$). DMF-treated mice contained CD4+Foxp3+ T-cells in the islets that express CD25 and Foxp3 at higher levels compared to vehicle-treated mice (MFI, 1602 vs 474 and 2016 vs 1479 respectively for CD25 and Foxp3) and a robust population of cKit+ Natural Killer (NK) cells within islets. Pancreas from 2 mice with diabetes reversal and euglycemic at the end of treatment had less significant insulitis (defined as >25% islet infiltration) compared to 4 age-matched euglycemic mice. No major changes were

observed in the peripheral blood, spleen, LN, and pancreatic LN. In the ongoing prevention study, none of the DMF-treated mice (n=27) developed diabetes after 7 weeks of treatment compared to 44% of control mice that develop diabetes (n=26) (p=0.0015). We also observed that DMF treatment in vitro (100 μM) of stimulated splenocytes from 4 weeks treated mice significantly inhibited glucose uptake in CD4 T cells by 41.1% in Vehicle-treated (P<0.0001, One-Way ANOVA) and by 55.8% in DMF-treated mice (P=0.0036). Moreover, in vitro DMF treatment significantly inhibited the upregulation of CD69 in CD4 T-cells by 32.5% in Vehicle-treated mice (P=0.0179 One Way ANOVA) and by 69.7% in the DMF-treated mice (P= 0.0001). Direct comparison between DMF and Vehicle treated mice showed significantly more inhibition of CD69 upregulation (P=0.0021, Two-Way ANOVA). A similar trend was observed in CD8 T-cells. In both the diabetes reversal and prevention studies we observed less production of IL-2, IFNγ, and TNFα after stimulation in the presence of DMF, while there was no effect on the production of inhibitory cytokines, IL-4 and IL-10.

CONCLUSIONS

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

Abstract #13

Revisiting the role of gamma delta T cells in type 1 diabetes

Authors

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PURPOSE

Type 1 diabetes (T1D) is caused by immune mediated destruction of insulin-producing pancreatic β-cells and Islet-specific CD4+ and CD8+ T cells are the main drivers of antibody formation and β-cell destruction. Indeed, conventional CD8+ and CD4+ T cells αβ T cells are well represented among human islet infiltrates. However, immune cell infiltrates of pancreatic islets also include B cells, antigen presenting cells, and innate-like T cells such as NK cells and γδ T cells. Due to their presence in islets and pancreatic lymph nodes, past studies in both rodents and man have addressed possible roles for γδ T cells in T1D, but the conclusions reached have been inconsistent, suggesting either pathogenic function (e.g. direct recognition of β-cells or production of inflammatory cytokines) or a protective role (e.g. production of inflammatory cytokines). Our recent observations that γδ T cells are prominent among pancreatic islet infiltrates of some pancreatic organ donors prompted us to revisit the role of gamma delta T cells in T1D.

METHODS

To address this question, we investigated the presence of γδ T cells within T cell lines grown from the islets of donors without diabetes, GADA+ donors with no clinical diagnosis of diabetes, and donors with T1D. Lines were isolated from handpicked islets using established procedures. These isolated islets were received from the Network of Pancreatic Organ Donors with Diabetes, Vanderbilt University (Alvin Powers), or the University of Pennsylvania's Human Pancreatic Analysis Program. Focusing on T cell lines that included a CD4-CD8- population, we surface stained for CD3, Vδ1, Vδ2 and αβ TCR to unambiguously identify γδ T cells. To assess function, we intracellularly stained these islet derived T cell lines for IFNγ and TNFα. To further probe the significance of γδ T cells, we measured their total percentage and Vδ1 to Vδ2 ratio in subjects with T1D and healthy controls and assessed their cytotoxic potential by measuring levels of CD16.

SUMMARY OF RESULTS

We observed that γδ T cells were present within multiple T cell lines grown from the islets of donors without diabetes, donors with islet-specific autoantibodies but no clinical diagnosis of diabetes, and donors with T1D. Notably γδ T cells were present in multiple cell lines and that γδ T cells from a donor with T1D produced TNFα, suggesting inflammatory function in that instance. In peripheral blood, we observed that subjects with T1D exhibited a significant elevation in their Vδ1 / Vδ2 ratio.

Furthermore, γδ T cells had higher levels of CD16 expression in subjects with T1D than in controls, indicating increased cytotoxic potential in those donors.

CONCLUSIONS

Based on these observations, we conclude that γδ T cells infiltrate the islets not only in subjects with T1D, but also in subjects with no diagnosis of T1D. These infiltrating γδ T cells appear to include Vδ1 (primarily thought to be pathogenic) and Vδ2 (primarily thought to be protective) and show evidence of effector function (TNFα) and cytotoxic potential (based on CD16 expression). Furthermore, γδ T cells become imbalanced in peripheral blood after the onset of T1D, pointing to a dysregulation of these cells in disease. Consequently, γδ T cells are worthy of further investigation to elucidate their potential role in the etiology of T1D.

Abstract #14

A Novel PD-1:MAdCAM Bifunctional Antibody for the Treatment of T1D

Authors

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PURPOSE

Assess efficacy of a PD-1:MAdCAM bifunctional antibody at modulating immune responses in NOD mice

METHODS

Using a phage display library human/mouse/cyno cross-reactive antibodies, specific for PD-1 or MAdCAM, were isolated. PD-1 antibodies were screened for their ability to antagonize or agonize the PD-1 pathway. A single triple-species cross-reactive clone that was specifically agonistic with no evidence of antagonism was identified and incorporated into the final bifunctional antibody. Similarly, a triple species cross-reactive non-blocking anti-MAdCAM antibody was identified using a multi-tiered screening approach. These two antibodies were combined to generate an IgG-scFv fusion with the anti-PD1 moiety comprising the IgG component and the anti-MAdCAM moiety comprising the scFv moiety . We term this antibody PD1A07T.

NOD mice at various ages (10-16 weeks) were treated IV or SC with PD1A07T or vehicle alone. At multiple timepoints post-treatment tissues (MLN, PLN, Pancreas) were harvested to assess for in vivo localization of test article by probing with anti-human IgG antibody specific for the FC portion of the bifunctional antibody. At multiple timepoints post-treatment cells were isolated from lymph nodes or spleen by mechanical dissociation or from the pancreas by intraductal injection of collagenase IV solution. Isolated cells were stained with a cocktail of antibodies to assess expression of the following markers; CD3, CD4, CD8, Live Dead, CD44, PD-1, Tim3, and IGRP-tet. For efficacy studies 6week-old mice were treated once with a 500ug bolus of anti-PD-L1 antibody (10F.9G2) and 250ug every two days after. Test article was administered at days 0 and day 7 via subcutaneous injection.

SUMMARY OF RESULTS

Starting at 13 weeks of age bifunctional antibody could be detected concurrently with MAdCAM expressing structures in the mesenteric lymph node, pancreatic lymph node, and pancreas after a single subcutaneous injection. Treatment with PD1A07T was able to specifically induce Tim3 on IGRP-tet+ cells which peaked at 4 days post-treatment and was undetectable after 7 days. Additionally, PD1A07 treatment led to a significant reduction of PD-1 on bulk CD8 T cells as well as on IGRP-tet+ cells. In an accelerated PD-L1 blockade mediated model of NOD hyperglycemia simultaneous administration of PD1A07T with PD-L1 blocking antibody resulted in significantly delayed induction of hyperglycemia compared to blockade alone.

CONCLUSIONS

PD1A07T was able to modulate antigen-specific and bulk CD8 T cells in pre-hyperglycemic NOD mice and was able to delay PD-L1 blockade accelerated insulitis/hyperglycemia in NOD mice. Whether the modulations observed in CD8 T cells are causally or coincidentally related to the observed delay in PD-L1 accelerated disease remains an open question for further inquiry. Single-cell RNAseq experiments are planned to identify unique PD biomarkers associated with PD1A07T treatment, and their relationship to delayed hyperglycemia. We hypothesize that the results from these pre-clinical in vivo models are the first step towards generating a novel PD-1 agonist therapy for the treatment of T1D.

Abstract #15

Senescent phenotype of islet-infiltrating CD8+ T cells from donors with type 1 diabetes

Authors

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PURPOSE

Type 1 diabetes (T1D) is thought to be caused by islet-specific T cell destruction of insulin-producing pancreatic β-cells. However, comprehensive phenotyping to better understand the composition and function(s) of islet-infiltrating T cells is needed. To this end, upon receipt of isolated islets, we have hand-picked islets from the isolates and immediately enumerated and phenotyped islet-infiltrating T cells.

METHODS

Islets isolated from donors without diabetes were supplied by Prodo Labs and the Integrated Islet Distribution Program (IIDP) We received tissues and isolated islets from the Network of Pancreatic Organ Donors with Diabetes (nPOD), the Human Pancreas Analysis Program (HPAP, University of Pennsylvania) and from Vanderbilt University (Alvin Powers). We received freshly isolated islets with autologous pancreatic draining lymph nodes (pLN) and/or spleen from donors without a clinical diagnosis of T1D, but who were GADA+ upon demise (T1D-GADA+, HPAP) and donors with 0.42-31 years of T1D duration (nPOD and Vanderbilt University). Upon receipt, spleen and pLN were processed into single cells suspensions. Islets were hand-picked, recombinant trypsin-dispersed, stained for viability and markers for T cells subsets, memory, activation, tissue residence, and cellular senescence and analyzed by flow cytometry. Islet isolation material from one donor, nPOD 6414 (23 year old male, 0.42 years of T1D duration) was also assayed for similar markers by CyTOF.

SUMMARY OF RESULTS

The frequency of CD4+ and CD8+ T cells derived from hand-picked islets was not statistically different among the T1D-GADA+ donors (n=4), donors with new-onset to moderate-term T1D (0.42-8 years duration, n=14), and donors with long-term T1D (10-31 years duration, n=15), but each was statistically more frequent when compared to those from control donor islets. For the donors with T1D and T1D-GADA+ donors, both CD4+ and CD8+ T cells derived from hand-picked islets demonstrated a combination of effector (TEM) and central memory (TCM) phenotypes with CD8+ cells from one T1D-GADA+ donor being 60% effector (TEFF) phenotype. From hand-picked islets of 4 donors with T1D (2, 6, 8, and 8 years duration), a subpopulation of CD8+ T cells (12-50%) were CD57hi/+PD-1+; this was also seen from 0.27% of pLN CD4+ T cells from one donor with 2 years

duration of T1D. This phenotype was not seen in the islet-derived CD4+ T cells, in CD4+ and CD8+ T cells from any spleen or other pLN and not in CD4+ or CD8+ T cells from the islets/spleen/pLN from 4 donors with longer-term T1D (12, 15, 22, and 31 years duration). The phenotype of both CD8+CD103+ (tissue resident) and CD8+CD103- T cells from the islet isolation material from nPOD 6414 showed increased expression of CD57 (CyTOF).

CONCLUSIONS

A subpopulation of islet-infiltrating CD8+ T cells, from donors with new-onset to moderate-term T1D exhibited markers for 1) possible senescence, repeated antigen exposure, and/or cytotoxic potential (CD57) and 2) potential suppression of function by any islet cell, including β cells, expressing a ligand for PD-1, PD-1L [PMID: 29844327, 30269996] and/or PDL-2. These data have implications for the role of CD8+ T cells in progression of T1D pathogenesis, at the site of β-cell damage, and can provide targets for therapeutic interventions in T1D.

Abstract #16

Quantitative analysis of pancreatic T cell infiltration in type 1 diabetes: a numbers game

Authors

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PURPOSE

Islet infiltration is considered a hallmark of type 1 diabetes (T1D). Several definitions of insulitis have been established over the years based on multiple immune cell populations, which has made data difficult to standardize. Current consensus defines insulitis as a minimum of three islets infiltrated by ≥ 15 CD45+ cells or by ≥ 6 CD3+ cells. While these are useful references, none of these definitions accurately consider the size of the islets. Thus, we aimed to quantitatively analyze the extension and density of T cell infiltration in the pancreas of non-diabetic (ND), autoantibody positive (AAb+) and T1D donors.

METHODS

T cell infiltration was quantified in all the islets from 11 ND (n=4797), 2 single and 5 double AAb+ (n=1526) and 10 T1D donors (n=2235, 0-2 years of disease duration). Frozen sections were stained for insulin, glucagon, CD3 and CD8 by immunofluorescence. CD3+ and CD8+ cells were identified as areas of staining above background and optimized threshold values for intensity and cell size by using QuPath (University of Edinburgh). All images were manually checked to identify insulin containing islets (ICIs) or insulin deficient islets (IDIs), and corrected for any possible errors.

SUMMARY OF RESULTS

The proportion of infiltrated islets (≥ 1 CD3+ cells inside or immediately adjacent to the islet) was 17.2% in ND, 28.7% in AAb+ and 40.7% in T1D donors. When the threshold for insulitis was applied (≥ 6 CD3+), 0.4% in ND, 3.6% in AAb+ and 8.2% of the islets in T1D donors were accounted for. Similar values were found for CD8+ cells using 1 or 6 cells as threshold. Interestingly, the proportion of islets with at least 1 CD4+ cell was significantly higher in both, AAb+ (16.1%) and T1D donors (21.7%) compared to ND donors (7%). As expected, in T1D donors, ICIs were preferentially infiltrated independently of the T cell population.

Then, CD3+ density was calculated for all the islets. It was significantly higher in T1D (74.8 CD3+ cells/mm2) compared to AAb+ (34.2 CD3+ cells/mm2) and ND (16.1 CD3+ cells/mm2) donors. When the analysis was restricted to islets that contained ≥ 1 CD3+ cells, cell density remained significantly higher in T1D donors. However, when the threshold for insulitis was used, mean cell density per se was not statistically different between the groups, regardless of the cell population. Next, cell density was compared between ICIs and IDIs from the same individuals within the T1D group. When all the islets were analyzed (without any threshold), there was a tendency to increased T cell density in ICIs compared to IDIs. When the threshold of ≥ 1 CD3+ cells was applied, T cell density was comparable between both islet types, whereas in insulitic islets, it tended to be higher in IDIs than in ICIs regardless of the T cell population. Lastly, we aimed to define a threshold value of islet T cell density

able to distinguish ND from T1D donors. The optimal threshold was found to be 33.5 CD3+ cells/mm2 (average 93% specificity and 87% sensitivity) when a minimum of 40 islets per donor were analyzed, and was validated in an external image dataset obtained from nPOD.

CONCLUSIONS

Using novel image analysis tools, we show that a high proportion of infiltrated islets and high islet T cell density are defining features in T1D. Our data suggest that it is the proportion of infiltrated islets rather than the number of infiltrating cells what dramatically changes during the course of T1D. This indicates that a “mild wave “of infiltrating cells slowly extends in the pancreas as disease progresses, whereas large accumulations of cells are rare. Furthermore, our data shows that, in T1D donors, while the proportion of infiltrated ICIs is significantly high, T cell density values do not statistically differ between ICIs and IDIs. This indicates that the number of cells that infiltrate an islet (in relation to the islet size) is similar in the presence or absence of insulin, but a larger proportion of ICIs are actually infiltrated at any given time. Lastly, we believe that the use of cell density values, which account for magnitude of infiltration and size of the islets, rather than absolute cell numbers per islet should be implemented. Therefore, we proposed a new threshold to define insulitis based on T cell density. Further analysis and establishment of a T cell density range to complement the current definition of insulitis will help to fulfill the need of a better understanding of the role of T cells in the course of T1D.

Abstract #17

Glycolysis Inhibition delays the onset of Type 1 Diabetes by inducing CD4+ T cell Exhaustion

Authors

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Purpose

Invasion of pancreatic islets by immune cells is a hallmark of Type 1 Diabetes (T1D), where the innate and adaptive immune systems work cooperatively to mediate β cell damage. This attack is largely orchestrated by self-reactive CD4+ T cells, which are fundamental drivers of disease pathology. Importantly, distinct metabolic programs dictate T cell differentiation and function over the course of the T cell lifecycle. Notably, CD4+ T cells undergo metabolic reprogramming to the less efficient aerobic glycolysis during activation to support clonal expansion and effector cytokine secretion. This is true too for diabetogenic CD4+ T cells, who upregulate the glycolysis pathway upon encounter with β cell antigen. Targeting the glycolysis pathway as a means to maintain tolerance has been investigated in a number of autoimmune disease models, including Lupus, Multiple Sclerosis, and Rheumatoid Arthritis, but remains a largely understudied therapeutic approach to control self- reactivity against islet β cells T1D. Based on these previous studies, we hypothesized that administration of the anti-glycolytic PFK15 would inhibit the activation, proliferation, and effector capabilities 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.

SUMMARY OF RESULTS

Our data demonstrate that 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 56% 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. Further, immunofluorescence staining of pancreatic tissue sections revealed a reduced T cell infiltrate of treated animals with increased and sustained expression of PD-1 within islets compared

to controls, indicating possible 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 is irreversible with checkpoint blockade.

CONCLUSIONS

These findings support that inhibition of glycolysis 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.

Abstract #18

Changes in HLA class I expression in pancreatic beta cell line EndoC-βH1 exposed to poly I:C and interferons

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PURPOSE

HLA class I (HLA-I) molecules play a crucial role in cell-mediated immunity by presenting peptide antigens to CD8+ T-cells. A pathological hallmark of Type 1 diabetes (T1D) is the hyperexpression of HLA-I in pancreatic islets with residual insulin producing beta cells. In addition to expression on the cell surface, HLA-I can also be found in a soluble form (sHLA-I) in the plasma. Intriguingly, sHLA-I is significantly elevated in the serum of patients with T1D and some viral infections. We have investigated the impact of interferons and poly I:C, a viral dsRNA mimetic, on sHLA-I expression in human islets and on surface and soluble HLA-I in the human pancreatic beta cell line EndoC-βH1.

METHODS

EndoC-βH1 and human islets were treated with IFNα, IFNγ or poly I:C and expression levels of total and surface HLA-I were measured in cells. Soluble HLA-I was measured in culture media of cells and islets.

SUMMARY OF RESULTS

Following exposure to IFNα and IFNγ: total and surface HLA-I levels were significantly upregulated in EndoC-βH1, release of sHLA-I was significantly upregulated in culture media of EndoC-βH1 and islets exposed to poly I:C, IFNα or IFNγ.

CONCLUSIONS

In this study we show that pancreatic beta cells respond to IFNα and IFNγ by extensively upregulating total and surface HLA-I expression. In addition, our data provide strong evidence for the first time that sHLA-I release from pancreatic islets and beta cells is significantly upregulated following exposure to interferons and poly I:C, further strengthening the association between sHLA-I elevation and loss of beta cells.

Abstract #19

Preproinsulin-reactive CD4 T cells in the islets of type 1 diabetes organ donors

Authors

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PURPOSE

Proinsulin is an abundant protein that is selectively expressed by pancreatic beta cells and has been a focus for developing antigen-specific immunotherapies for type 1 diabetes (T1D). In this study, we sought to comprehensively evaluate pancreatic islet infiltrating CD4 T cell reactivity to preproinsulin from organ donors having T1D.

METHODS

We identified T cell receptor (TCR) sequences expressed by CD4 T cells in the islets of six recent-onset T1D organ donors and expressed frequent TCR clonotypes on T-hybridoma cells to analyze their antigen specificity. TCR transductants were tested for the response to 99 truncated preproinsulin peptide pools in the presence of autologous EBV (Epstein-Barr Virus)-transformed B cells. We further determined HLA class I molecules presenting peptides to TCR clonotypes responding to preproinsulin. We also compared frequencies of preproinsulin-reactive CD4 and CD8 T cells in the islets of individual donors.

SUMMARY OF RESULTS

Among 187 TCR clonotypes studied, 14 TCRs responded to preproinsulin peptides. Epitopes were found across all of proinsulin (insulin B-chain, C-peptide, and A-chain) including four hot spot regions containing peptides commonly targeted by TCR clonotypes derived from multiple donors. Only one of the four hot spots, located in insulin A-chain, was overlapped with that for islet CD8 T cells. The 14 TCR clonotypes recognized peptides presented by various HLA class II molecules, but there was a trend for dominant restriction with HLA-DQ especially T1D risk alleles DQ8, DQ2, and DQ8-trans. In terms of frequency of preproinsulin-reactive CD4 T cells in the islets, four of the six T1D donors had at least one proinsulin-reactive CD4 TCR clonotype, and these TCRs were expressed by 10-25% of islet CD4 T cells in each donor. Donors having high frequency of preproinsulin-reactive CD4 T cells did not necessarily have many preproinsulin-reactive CD8 T cells in the islets.

CONCLUSIONS

Pancreata of T1D organ donors contain proinsulin-reactive CD4 T cells, but frequencies of proinsulin-reactive T cells varied by individual donors. The characteristics of the tri-molecular complex including proinsulin peptide, HLA-DQ molecule, and TCR derived from CD4 T cells in islets, provides an essential basis for developing antigen-specific biomarkers as well as immunotherapies.

Abstract #20

Use of a Computational Biology Workflow to Identify Altered Pre-mRNA Splicing Events in Subjects with New-Onset T1D

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PURPOSE

Pre-mRNA splicing events enhance genetic diversity in mammalian systems by increasing the repertoire and number of protein isoforms. Alternative splicing within the pancreatic β cell has been proposed as one potential pathway that may unmask novel immunogenic protein epitopes and thus initiate/exacerbate autoimmunity. Here, we employed a computational strategy to catalog and prioritize significant splicing events in human circulation of subjects with new-onset T1D and to relate these events to those appearing in the human islets treated with pro-inflammatory cytokines.

METHODS

RNA sequencing of alternatively spliced transcripts in whole blood was performed in 12 new-onset T1D subjects (age: 12 ± 4 ; sex: 8 M/4 F; BMI: 19.15 ± 5.07) and 12 age- and sex-matched healthy controls (age: 12 ± 4; Gender: 8 M/4 F; BMI: 19.52 ± 2.52).Total RNA was extracted and subjected to 2 x 75 bp paired-end RNA sequencing at a 180 million sequencing depth using an Illumina HiSeq4000. Sequenced libraries were mapped to the human genome (GENCODE GRCh37) using a STAR RNA-seq aligner. We applied replicate multivariate analysis of transcript splicing (rMATS) to identify splicing events induced by cytokine treatment. Events with more than half of each comparison group replicate having a sum of inclusion junction counts and skipping junction counts per sample greater than 10 were retained. To be considered significantly changed, a cut-off of 10% on ΔPSI and 5% FDR were used. Differential expression analysis was performed using edgeR. Human islets from 10 cadaveric donors (6 male; 4 female; average age 33 ± 11 yrs; average BMI 28.89 ±4.68 kg/m2) were treated with or without pro-inflammatory cytokines (IL-1β and IFN-γ) for 24 hrs. RNA sequencing of human islets was analyzed using the same computational workflow at a 50 million sequencing depth.

SUMMARY OF RESULTS

RNA sequencing was performed at a depth of approximately 180M reads per sample in the whole blood of subjects with new-onset (≤48 h after diagnosis) T1D. Our data revealed 662 differentially expressed, alternatively spliced transcripts in subjects with T1D compared to age- and sex-matched controls without disease. The majority (44.3%) of splicing events involved a skipped exon; 23.6% of events were categorized as a mutually exclusive exon; 11.3% involved a 3’ alternative splice site; 6.8% involved a 5’ alternative splice site, and 14% of events resulted from a retained intron. Among this set of transcripts, gene ontology analysis revealed enrichment for biological pathways related to DNA recombination, positive regulation of oxidoreductase activity, methylation and lipid homeostasis. In human islets, 970 splicing events in 753 unique mRNAs were identified following cytokine treatment. Notably, 19 of the alternatively spliced transcripts in whole blood overlapped with those seen in human islets exposed to cytokines, suggesting a potential signature in the circulation that reflects the inflammatory stress observed in islets.

CONCLUSIONS

Taken together, this analysis illustrates the utility of transcriptomic datasets to identify and prioritize alternative mRNA splicing events in human blood that correlate to those seen in human islets under stress conditions mimicking T1D. Future studies will test the functional impact of these splicing events on circulation cell types and immunogenicity and test key events as potential biomarkers of T1D risk and heterogeneity.

Abstract #21

Establishment of pro-islet amyloid polypeptide as a biomarker in T1D

Authors

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PURPOSE

Synthesis and secretion of beta cell peptide hormones closely reflect beta cell health, and failure in hormone biosynthesis and processing may result in disproportional secretion of prohormones in diabetes. Indeed, the plasma ratio of proinsulin-to-C-peptide is elevated in type 1 diabetes (T1D) and pre-T1D subjects. Parallel to insulin, islet amyloid polypeptide (IAPP) is first synthesized in beta cells as a 67-amino acid precursor molecule, proIAPP1-67. Through sequential endopeptidase cleavage and amidation, proIAPP is processed into an intermediate form (proIAPP1-48), and eventually becomes mature IAPP (IAPP1-37). Utilizing a sensitive ELISA developed in-house, we previously showed that the ratio of proIAPP1-48 to total IAPP is elevated in plasma from T1D subjects and islet transplant recipients. We aimed to confirm this finding in an additional T1D cohort, to validate the performance of our proIAPP1-48 and IAPP1-37 ELISAs through the JDRF Core for Assay Validation (CAV), and to analyze the relationship between proinsulin and proIAPP levels in T1D plasma and islets from T1D organ donors.

METHODS

To evaluate the reproducibility of our in-house developed ELISAs, we measured proIAPP1-48 and IAPP1-37 levels in blinded plasma samples from T1D subjects from JDRF CAV, and calculated the percent coefficient of variation (%CV) of triplicate aliquots. To establish the proIAPP1-48-to-total IAPP ratio as a biomarker in T1D, we measured proIAPP1-48 and IAPP1-37 levels in plasma from the

Indiana University cohort of new-onset T1D and age-matched subjects, and performed regression analysis on the proinsulin:C-peptide and proIAPP1-48:IAPP ratios. We also examined the expression patterns of proIAPP and proinsulin by performing immunostaining in pancreas sections from T1D and BMI- and age- matched donors.

SUMMARY OF RESULTS

Both proIAPP1-48 and IAPP1-37 ELISAs displayed good reproducibility (%CV = 16.28 and 12.61, respectively) in intra-assay tests, whereas the proIAPP1-48:IAPP ratio displayed excellent reproducibility (%CV = 3.73). The proIAPP1-48:IAPP ratio was significantly elevated in plasma from new-onset T1D subjects (0.37±0.02 vs. 0.22±0.02; $p < 0.0001$) in the Indiana University cohort, and ratios of proIAPP1-48:IAPP and proinsulin:C-peptide showed a significant linear correlation ($r = 0.445$; $p < 0.05$). Immunostaining of nPOD tissue indicated that proinsulin and proIAPP are co-expressed in beta cells of pancreatic sections from T1D donors.

CONCLUSIONS

We validated our ELISA for proIAPP1-48 and IAPP1-37 in human plasma and confirmed the proIAPP1-48:IAPP ratio as a biomarker of beta cell dysfunction in T1D. The co-localization of proIAPP and proinsulin in T1D beta cells, and correlation of proinsulin:C-peptide and proIAPP1-48:IAPP ratios in T1D plasma, suggest that the same residual beta cells in T1D likely secrete both proinsulin and proIAPP and are responsible for persistent prohormone secretion in T1D.

Abstract #22

Cell2grid Compression of Fluorescent Multiplexed Immunohistochemistry Images Enables Structural Analysis of Pancreatic Islets and Novel Biomarker Discovery

Authors

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PURPOSE

Fluorescent multiplexed immunohistochemistry (fm-IHC) images provide detailed insights into the structure and change of pancreatic islets during pathogenesis of type 1 diabetes. However, these high-resolution images rely on hundreds of pixels to represent each individual cell, resulting in low information density whenever overall cell phenotypes are deemed more relevant than intra-cellular marker variance. As a result, whole-slide fm-IHC image files are big (>12 GB/cm² of tissue at 0.5 µm/px resolution), making large-scale data sharing within the research community challenging. In addition, there are typically only few images available which is challenging for state-of-the-art machine learning methods. We aim to solve these problems for a large data set of murine pancreas samples by using a specialized fm-IHC compression method that enables rule-based creation of synthetic image data.

METHODS

We applied cell2grid (Herbsthofer et al. 2020), a novel fm-IHC image compression algorithm, to compress images of pancreas tissue slides from NOD mice and healthy non-NOD controls. Based on these compressed images, we defined cell-based rules for the robust and automated identification of pancreatic islets and their immune cell environment in healthy mice and in different stages of beta cell destruction. These rules utilize the multiplex staining of cellular markers to define a set of cell phenotypes and their spatial distribution in the islets. Furthermore, we used this set of rules to

develop a procedural algorithm to create synthetic fm-IHC images of pancreatic islets for pre-training convolutional neural networks.

SUMMARY OF RESULTS

We successfully used cell2grid to compress a large data set of whole-slide fm-IHC images of pancreas tissue slides stained for insulin, glucagon, CD4, CD8, CD45 and DAPI, achieving a compression ratio of > 100 (5 µm target grid) in 2.2 s/mm² tissue on a single CPU core. Cell-based rules allowed the robust identification of pancreatic islets in various stages of beta cell destruction using phenotype characterization and cell counts in the islet core (mostly alpha and beta cells) and in the local islet immune environment (mostly helper and cytotoxic T cells). We validated our findings with manual annotations and used the cell-based rules to generate synthetic fm-IHC images modelled after our empirical data. We created 1000 synthetic images of islets in under 4 min on a single CPU core.

CONCLUSIONS

Our results show that cell2grid is a viable image compression method to simplify storage, sharing and analysis of large fm-IHC data sets of murine pancreas samples. We are currently focusing on the use of synthetic image data to pre-train convolutional neural networks for automated image analysis to facilitate the extraction of novel, interpretable biomarkers. After validation with additional murine samples, we aim to study the structure of human pancreatic islets.

Abstract #23

Confident identification of hybrid insulin peptides as autoantigens in T1D

Authors

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PURPOSE

Within the pancreatic beta cell, insulin fragments are sometimes post-translationally fused with other peptides to form hybrid insulin peptides (HIPs). HIPs are key targets of autoimmunity in the non-obese diabetic (NOD) mouse model of type 1 diabetes (T1D), and mounting evidence suggests they are important autoantigens in human T1D. In order to characterize the role of the immune response to HIPs in T1D pathogenesis, robust approaches for identifying HIPs in primary islets are needed. Tandem mass spectrometry is the best available technology for this task. In tandem mass spectrometry, peptides in complex biological samples are fragmented and the masses of the resultant fragments are measured to generate fragmentation spectra. To identify a peptide, an attempt is made to match its corresponding fragmentation spectrum to predicted spectra for peptides in a database. Fragmentation spectra are difficult to predict and are often ambiguous (i.e., one observed spectrum can be matched to multiple peptides in a database). When identifying HIPs, these and other problems result in a high risk of false identifications. The gold standard for validating individual peptide matches is to compare the observed spectrum to the spectrum for a synthetic version of the proposed peptide sequence. However, this comparison is highly subjective, leaving many matches open for dispute. We therefore sought to develop a more rigorous, objective approach for validating mass spectrometry-based HIP identifications.

METHODS

We developed a novel approach in which internal standard peptides are used to determine the degree of similarity that can be expected between two fragmentation spectra for the same peptide. Using this information as a reference, the spectrum for a biological peptide in question and the spectrum for a synthetic version of the proposed sequence match are objectively compared to determine if the match is valid. We generated a freely-available computer program that automates and standardizes the data analysis portion of this workflow. The accuracy of this approach was evaluated by testing its ability to distinguish between pairs of highly similar but distinct peptides. Finally, the approach was used to evaluate the validity of putative HIP identifications.

SUMMARY OF RESULTS

The average accuracy of the approach across five replicate benchmarking experiments was 93%, with no false positives observed. Using the approach, we confirmed with high confidence the presence of multiple HIPs in both mouse and human primary islets.

CONCLUSIONS

Multiple lines of evidence indicate that HIPs are important autoantigens in T1D. In order to further characterize the immune response to HIPs, methods are needed for confidently identifying HIPs in islets. However, current methodologies fail to provide the required rigor. We therefore developed a novel approach that enables confident identification of HIPs in islets, providing a foundation for future efforts to study their role in T1D.

Abstract #24

Whole exome sequencing and analysis of 207 nPOD cases

Authors

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PURPOSE

Type 1 diabetes (T1D) is an autoimmune disease caused by the immune-mediated destruction of pancreatic beta cells. Some people initially diagnosed with T1D are later found to have a protein coding variant in a beta cell gene that reduces pancreatic insulin production, or an immune cell gene that causes the autoimmune attack on the insulin-producing beta cell. We generated a genetic dataset using cases selected from the Network of Pancreatic Organ Donors (nPOD) that can be used to screen for coding variants in known or suspected diabetes causing genes, and that can serve as a sequence dataset for a control population of T1D individuals.

METHODS

Whole exome sequencing (WES) was performed on genomic DNA from 207 nPOD cases, including 147 cases clinically diagnosed with T1D. WES data was processed to generate a list of coding variants for each nPOD case. Coding variants were annotated with a variety of useful metrics, such as the Combined Annotation Dependent Depletion (CADD) score for predicting protein deleteriousness, the American College of Medical Genetics (ACMG) classification for predicting disease pathogenicity, and the Genome Aggregation Database (gnomAD) allele frequency for predicting how common a variant may be in the human population.

SUMMARY OF RESULTS

Our current analysis suggests that several T1D cases in the nPOD collection may harbor coding variants in a beta cell or immune cell genes that underlies their diabetes. These candidates will require additional studies to determine their pathogenicity.

CONCLUSIONS

These data will provide a valuable resource to nPOD investigators and the broader T1D research community. The study of protein-coding variants in the T1D population may provide insight into disease mechanisms and enable screening methods for monogenic diabetes.

Abstract #25

Islet composition beyond insulitis: Re-evaluating grading of beta cell destruction via fluorescent multiplexed immunohistochemistry (fm-IHC)

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PURPOSE

Currently, pancreatic islet health and insulitis progression are routinely determined by hematoxylin and eosin (H&E) staining. This has proven useful to determine the degree of inflammation in insulitis. Here we demonstrate that without a more specific staining, H&E fails to distinguish later stages of beta cell destruction. We propose a new staging of islet health based on images stained with fluorescent multiplexed immunohistochemistry (fm-IHC).

METHODS

Formalin fixed paraffin embedded (FFPE) pancreatic tissue slides of non-obese diabetic (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. We used the Opal staining workflow that is based on primary specific antibodies to stain distinct cellular markers and a detection system based on an HRP-conjugated secondary antibody with a specific detection substrate. Imaging was performed using a PerkinElmer Vectra3 platform, the inForm software (Akoya Biosciences) and the Halo™ Image Analysis software (Indica Labs) for whole slide image analysis. Pancreatic tissue sections of 9 female, 35-week-old, non-diabetic NOD mice with blood glucose below 200 mg/dl and healthy C57BL6 mice were used. We stained a minimum of 5 FFPE slides/mouse, with sections approx. 20 µm apart from each other. Adjacent sections were also H&E stained to directly compare both staining methods.

SUMMARY OF RESULTS

We were able to identify 5 different stages of beta cells destruction in pancreatic islets of NOD tissue samples. We found that conventional H&E insulitis staging was insufficient to evaluate islet health. By using fm-IHC we achieved a more comprehensive staging system where we classified islets by presence/absence of stained markers and allocated them to 5 stages. Stage 1 has healthy islets, stage 2 is characterized by immune cells adjacent but not yet surrounding the islet, stage 3 has islets encircled by immune cells with progressing disintegration of classical islet structure, stage 4 is characterized by (pseudo-) atrophic islets without insulin producing beta cells while immune cells are still abundant, and stage 5 comprises (pseudo-) atrophic islets without immune cells and beta cells. Stages 1 to 3 were similar to conventional H&E insulitis grades. However, while stages 4 and 5 are known to exist (Campbell-Thompson et al. 2013, Atkinson et al. 2020), they are not considered in H&E insulitis grades even though the absence of insulin is highly relevant in evaluating islet health.

CONCLUSIONS

Studies that solely use H&E staining do not distinguish islets in very advanced stages of beta cell destruction, which we consider important in evaluating the overall health and functionality of the pancreas. Our proposed staging approach could also be applied to more complex staining methods such as imaging mass cytometry (Wang et al. 2019) or combinations such as conventional H&E with the addition of insulin staining. Compared with classical IHC staining, fm-IHC has the advantage to identify more than one marker simultaneously, hence displaying the amount of autoimmune destruction and residual insulin production, and to minimize inter-observer variability by using automated systems and digital analysis. Our findings suggest that this staging based on fm-IHC can also be used to gain a deeper insight into human beta cell destruction in type 1 diabetes.
In future work we aim to automate islet detection and staging, thus reducing subjective bias in staging and also reducing workload.

Abstract #26

Alterations in the Molecular Pathways Involved in the Expression of Hyaluronan in Human Pancreatic Islets

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PURPOSE

Hyaluronic acid (HA) has been found to accumulate in the extracellular matrix (ECM) of insulitic islets. Low molecular weight HA (LMW-HA) is associated with activation of inflammatory pathways and recruitment/activation of T-cells involved in the progression of Type 1 diabetes (T1D). This study used a previously described dataset of transcriptomes from 260 individual islets to evaluate the changes in transcription of genes from these pathways in normal vs. insulitic islets.

METHODS

Analysis of prior data from 260 laser-captured human pancreatic islet samples from nPOD organ donors. Samples were obtained from 3 disease states: Control, AB+, and T1D. Tissue staining produced four types of islets: Ins+CD3-, Ins+CD3+, Ins-CD3+, and Ins-CD3-. RNA transcriptomes were collected and data analyzed by unpaired, uneven t-tests. Literature review and data mining with WebGestalt into geneontology and KEGG pathways were performed to identify specific genes of interest – which were then analyzed for expression differences in Ins+CD3- vs. Ins+CD3+ islets (normal vs insulitic).

SUMMARY OF RESULTS

Hyaluronan Lyase 2, a gene responsible for cleaving high molecular weight hyaluronic acid (HMW-HA) to the proinflammatory low molecular weight form, was increased in islets from AB+ donors compared to both controls and T1D donor islets. Expression levels above 600 units were seen in 13/77 islets from AB+ donors (17%) but not in islets from control (0/73) or T1D donors (0/110). LMW-HA binding with receptors such as CD44 and TLR4 on T cells may cause cascade events which promotes leukocyte recruitment and infiltration. Multiple genes involved in pathways activated by LMW-HA were found to be differentially expressed in islets with insulitis vs those without. Those genes are associated with CD44 cascade signaling, leukocyte recruitment, apoptosis, ECM degradation, angiogenesis, as well as β cell function and differentiation.

CONCLUSIONS

Our data show an increased expression of LMV-HA in 17% of islets from AB+ donors but not in islets from controls and T1D donors. Genes from pathways that are downstream activated by LMV-HA were increased in insulitic (Ins+CD3+) islets compared to normal (Ins+CD3-) islets. This suggests an important role for HYAL2 in the early stages of islet pathology.

Abstract #27

The Vbeta13 T cell receptor monoclonal antibody reduces hyaluronan and CD68+, CD3+ and CD8+ cell infiltrations to delay diabetes in congenic BB DRLyp/Lyp rats

Authors

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PURPOSE

The depleting Vβ13a T cell receptor monoclonal antibody (mAb) 17D5 prevents both induced and spontaneous autoimmune diabetes in BB rats. We administered the 17D5 mAb in congenic DRLyp/Lyp rats, all of which spontaneously develop diabetes, to examine the CD3-positive (CD3+) and CD8+ T cell and CD68+ monocyte infiltration and whether modulation of islet mononuclear cell infiltration affects hyaluronan (HA) deposition in islets.

METHODS

Starting at 40 days of age, BBM DRLyp/Lyp rats were injected once weekly with either saline, His42 Vβ16 mAb (directed against TCR Vβ16), or 17D5 mAb and monitored for hyperglycemia. Treatment continued until the rats developed diabetes. It was predetermined that DRLyp/Lyp rats that remained diabetes-free at 95 days of age would be killed at about 100 days of age. The rats were weighed and blood glucose was measured daily beginning at 40 days of age. Pancreata were collected and processed for immunohistochemistry to determine the presence and severity of insulitis, to quantify the islet-infiltrating CD3+, CD8+, and CD68+ cells, and to measure the insulin-positive and HA-stained areas.

SUMMARY OF RESULTS

Diabetes occurred in 100% (n = 5/5) of saline-treated rats (median age, 66 days; range 55-73), and in 100% (n = 6/6) of His42-treated rats (median age, 69 days; range 59-69). Diabetes occurred in fewer (n = 8/11, 73%) 17D5-treated rats at a later age (median 76 days, range 60-92). Three (27%) of the 17D5-treated rats were killed at 101-103 days of age without diabetes (17D5 no-diabetes rats). Survival analysis demonstrated that 17D5 mAb delayed diabetes onset. Saline- and His42-treated rats had severely distorted islets with substantial loss of insulin-positive cells. These rats exhibited prominent HA staining, with the intra-islet HA+ accumulations occupying 36 ± 12% of islet area, and severe (grade 4) insulitis with abundant infiltration by CD68+, CD3+ and CD8+ cells. The 17D5 mAb-treated rats with delayed diabetes onset exhibited less severe insulitis (predominantly grade 3).

In contrast, the 17D5 no-diabetes rats had mostly normal islets, with insulin+ cells representing 76 ± 3% of islet cells. In these rats, the islet HA deposits were significantly smaller than in the diabetic rats; the intra-islet HA+ areas accounted for 8 ± 1% of islet area. Also, islet-associated CD68+ and CD3+ cells occurred less frequently (on average in 60% and 3% of the islets, respectively) than in the diabetes rats (present in >95% of the islets). No CD8+ cells were detected in islets in all 17D5 no-diabetes rats.

CONCLUSIONS

Diabetes occurred in 100% (n = 5/5) of saline-treated rats (median age, 66 days; range 55-73), and in 100% (n = 6/6) of His42-treated rats (median age, 69 days; range 59-69). Diabetes occurred in fewer (n = 8/11, 73%) 17D5-treated rats at a later age (median 76 days, range 60-92). Three (27%) of the 17D5-treated rats were killed at 101-103 days of age without diabetes (17D5 no-diabetes rats). Survival analysis demonstrated that 17D5 mAb delayed diabetes onset. Saline- and His42-treated rats had severely distorted islets with substantial loss of insulin-positive cells. These rats exhibited prominent HA staining, with the intra-islet HA+ accumulations occupying 36 ± 12% of islet area, and severe (grade 4) insulitis with abundant infiltration by CD68+, CD3+ and CD8+ cells. The 17D5 mAb-treated rats with delayed diabetes onset exhibited less severe insulitis (predominantly grade 3).

In contrast, the 17D5 no-diabetes rats had mostly normal islets, with insulin+ cells representing 76 ± 3% of islet cells. In these rats, the islet HA deposits were significantly smaller than in the diabetic rats; the intra-islet HA+ areas accounted for 8 ± 1% of islet area. Also, islet-associated CD68+ and CD3+ cells occurred less frequently (on average in 60% and 3% of the islets, respectively) than in the diabetes rats (present in >95% of the islets). No CD8+ cells were detected in islets in all 17D5 no-diabetes rats.

Abstract #28

HLA class II can be expressed in the islets of type 1 diabetic patients

Authors

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PURPOSE

Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing β cells are damaged by the immune system. However, the immunological mechanisms that lead to T1D have remained incompletely understood. It has been suggested that HLA class II (HLA-II) can play a role in T1D pathogenesis, and certain mutations in genes encoding for HLA-II molecules are associated with different risks of developing T1D. HLA-II is known to be widely expressed by antigen-presenting cells (APCs), and macrophages were found to be the most predominant APC islet infiltrate in T1D donors. Additionally, it has been suggested that HLA-II can be upregulated in β-cells exposed to proinflammatory cytokines in vitro. However, its expression in the islet β-cells of T1D patients has been historically controversial and its specific role in T1D is still undefined. The aim of this project is to characterize and quantify the expression of HLA class II in human pancreatic tissue sections obtained from the network of pancreatic organ donors (nPOD), to investigate the cellular sources of HLA-II in the pancreas and to analyze the induction of HLA-II expression in isolated human islets (Integrated Islet Distribution Program, IIDP) and in human pancreatic organoids (InSphero).

METHODS

Antibodies for HLA class II (DP, DQ, DR), HLA-I (A, B, C), CD31, CD68, and Insulin were independently optimized in Formalin Fixed Paraffin Embedded (FFPE) sections of human tonsils. Multicolor immunofluorescent imaging panels were optimized in FFPE sections of tonsils, pancreas, and in isolated human islets. Human pancreatic FFPE nPOD sections from six non-diabetic, five auto-antibody positive (Aab+), and five T1D donors were stained with HLA-II, CD68, and Insulin. Isolated human islets and human islet organoids were cultured for four days with proinflammatory cytokines (IFN-γ, TNF-α, IL-1β) of varying concentrations. After culture with cytokines, islet function was measured by glucose-stimulated insulin secretion (GSIS), and islets were subsequently stained with HLA-II, HLA-I, and Insulin. Images of whole tissue sections were obtained using Zeiss AxioScan Z1 slide scanner and were analyzed using Qupath. High resolution images of randomly selected regions of interest from the tissue sections and of the stained isolated human islets and islet organoids were acquired with the Zeiss LSM780 confocal microscopy system. Image analysis was performed on confocal images with Zen Blue and ImageJ.

SUMMARY OF RESULTS

First, we observed that of non-islet cells, CD68+ was a more important source of HLA-II than CD31. After that, we stained 16 nPOD cases with HLA-II, CD68 and Insulin. We analyzed the totality of the tissue sections retrieving data from 7416 islets (4034 islets from non-diabetic, 2951 from Aab+, and 431 islets from T1D cases). We observed a statistically significant higher percentage of HLA-II-positive

area in the insulin-containing islets (ICIs) of T1D cases (24.31% of islet area) compared to non-diabetic (3.82%), Aab+ (2.31%), and T1D insulin-deficient islets (IDIs) (0.67%). Within T1D ICIs, many of the β-cells were found to express HLA-II, since 45.9% of the total Insulin signal colocalized with HLA-II. In the non-diabetic and Aab+ cases, most of the Insulin signal of the β-cells did not colocalized with HLA-II (5.9% and 4.1%, respectively). In the peri-islet region, T1D ICIs also exhibited a higher percentage of positive area for HLA class II (18.9%) when compared with the other groups (expressing 2.6-4.8%). Significantly higher CD68+ macrophage infiltration was observed within and around ICIs in T1D cases (5.06% of islet area and 6.55% of peri-islet area) when compared with the other cases, and 17.09% of the HLA-II signal within T1D ICIs colocalized with CD68. Interestingly, we also observed a higher expression of HLA-II and CD68 in the exocrine tissue of T1D patients when compared with the non-diabetic and Aab+ cases. Then we tested if pro-inflammatory cytokines were able to induce HLA-II expression in isolated human islets (IIDP) and in islet organoids (InSphero). Upon treatment of the islets with inflammatory cytokine stressors (combinations of IFN-γ, TNF-α +/- IL-1β) we observed an impairment in islet function assessed by GSIS, and the induction of HLA-II and HLA-I expression (which was not observed in control islets cultured with media). Different concentrations of TNF-α by itself did not impair the islet function or induce HLA class II expression. However, different concentrations of IFN-γ by itself produced altered GSIS responses and an increased expression of HLA-II and HLA-I, although this increase was not statistically significant.

CONCLUSIONS

HLA-II can be expressed by pancreatic β-cells in T1D patients. We observed a higher expression of HLA-II in the ICIs and the peri-islet regions of T1D donors when compared with non-diabetic and Aab+ donors. Aab+ cases did not exhibit higher levels of HLA-II than the controls in any pancreatic region. HLA-II was homogeneously expressed in the exocrine and endocrine pancreatic tissue of both non-diabetic and Aab+ donors. T1D cases also presented a higher expression of HLA-II in the exocrine pancreas when compared with the other groups, however, it was substantially higher in the islet and peri-islet regions. In general, a higher expression of CD68+ was present in the islet, peri-islet, and exocrine regions of the T1D cases. HLA-II expressing CD68+ macrophages are frequently found in and around T1D ICIs, and often in close contact with surrounding β-cells. Further studies may investigate the functional state of infiltrating macrophages in T1D cases, and investigate potential macrophage-islet cross-reaction in vitro. HLA-II expression is inducible in the β-cells of isolated human islets and human islet organoids in vitro by treating them with a combination of pro-inflammatory cytokines. Our findings support a potential pathogenic role for HLA-II in T1D.

Abstract #29

Characterization of the anti-viral immune response against Coxsackievirus in type 1 diabetes

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PURPOSE

Despite accumulating epidemiological and histopathological evidence for an association between Coxsackievirus B (CVB) and type 1 diabetes (T1D), a causal link is missing. Very little is known about the T-cell response mounted against CVB infection and the epitopes targeted. Moreover, the upcoming T1D prevention trials using CVB vaccination require the exploration of possible cross-reactivities with islet antigens that may precipitate disease. We identified the HLA Class I (HLA-I) viral peptidome of CVB-infected beta cells and aimed to characterize the CD8+ T-cell response against these peptides.

METHODS

HLA-I-bound peptides presented by CVB-infected human beta-cell lines were identified by mass spectrometry and those restricted for and confirmed to bind to HLA-A2 or -A3 were retained. We optimized the analysis of combinatorial HLA-I multimer assays and selected the immunodominant peptides recognized by circulating CD8+ T cells from CVB-seropositive healthy donors and in nPOD splenocytes. CD8+ T-cell clones were generated, and we evaluated the frequency and phenotype of CD8+ T cells recognizing the immunodominant CVB peptides thus identified in T1D and healthy children.

SUMMARY OF RESULTS

Infected beta cells presented only few selected CVB peptides, and only a fraction of them was recognized by circulating CD8+ T cells from healthy seropositive donors. Moreover, only another sub-fraction of these epitopes was targeted by CD8+ T cells with an effector/memory phenotype. Although cross-reactivity with some islet antigens was documented, this was limited to subdominant CVB epitopes. Two major immunodominant epitopes were identified in both the blood and spleen. Ten clones generated from one donors against one of these immunodominant peptides were cytotoxic and carried the same TCR. The beta chain of this TCR was identical to that of 3.3% CD8+ T cells sorted from the pancreatic lymph nodes of a CVB+ HLA-I-matched T1D nPOD donor. The frequency and phenotype of circulating CD8+ T cells recognizing CVB epitopes in T1D and healthy children will be presented.

CONCLUSIONS

CVB infection seems to induce a limited CD8+ T-cell memory response in terms of antigen coverage, which may favor the repeated or chronic infections reported to be associated with islet autoimmunity. We will discuss whether this poor memory response is preferentially found in T1D children. The epitopes identified can be used to evaluate the efficacy of CVB vaccines. Moreover, their mapping to different CVB regions can distinguish the vaccinal response from that triggered by natural infection.

Abstract #30

Virus-mediated dysbiosis alters immune populations to promote type 1 diabetes onset

Authors

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PURPOSE

In combination with genetic determinants, susceptibility to autoimmune diseases such as Type 1 Diabetes (T1D) is established by various environmental factors including infection, microbial dysbiosis, antibiotic use, and vitamin D deficiency. Viruses such as coxsackievirus B (CVB) have been identified as important cofactors associated with diabetes development and pathogenesis. We examined how infection with CVB4 may be an instigating factor which alters the microbiome and this microbial change may be sufficient to skew immune populations and promote autoimmunity in a genetically-susceptible mouse model.

METHODS

We used a non-obese diabetic (NOD) mouse model to determine how infection with CVB4 can modulate host microbiome to predispose for type 1 diabetes development. Fecal samples were taken in the 21 days following infection for 16S rRNA sequencing to understand how infection impacts microbial communities. To determine if these changes in the microbiome are sufficient to promote T1D onset, naïve NOD mice were treated with a course of broad-spectrum antibiotics before performing fecal microbiome transfers (FMTs) and monitoring for diabetes incidence. Various assays, histology, and flow cytometry were used to determine specific changes in humoral responses to microbial antigens, gut physiology, and immune profiles following infection with CVB4 which may be contributing to T1D pathogenesis.

SUMMARY OF RESULTS

Infection with CVB4 accelerates onset of diabetes and results in intestinal dysbiosis which resembles that of a spontaneously diabetic mouse. This is characterized by a loss of microbial diversity and changes in microbe composition particularly within Actinobacteria, Verrucomicrobia, and Firmicutes phylum. Furthermore, introducing this “diabetogenic” infection-induced microbiome into recipient mice through the use of FMTs can accelerate diabetes onset and alter immune profiles in the gut and pancreas. Infection results in changes within intestinal physiology including breakdowns of physical barriers between luminal microbes and gut-resident immune cells. This includes increased gut permeability, loss of mucus layers, and change in tight junction protein expression. Ultimately this loss of intestinal integrity results in bacterial translocation and modifications to regulatory and inflammatory T cell populations.

CONCLUSIONS

Our data highlights the role of environmental factors in T1D and the potential for significant cross-talk between CVB infection, the microbiome, and gut-resident immune cells to contribute to diabetes pathogenesis and susceptibility.

Abstract #31

Genetic and environmental factors regulate the type 1 diabetes gene CTSH by differential DNA methylation

Authors

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PURPOSE

CTSH is a type 1 diabetes (T1D) susceptibility gene that can be regulated by environmental influences. Pro-inflammatory cytokine has been shown to attenuate CTSH expression in human islets. Using Mendelian randomization analyses, we previously found that T1D genetic risk at the CTSH locus could be mediated by DNA methylation. We hypothesized that gene and pro-inflammatory cytokines modulate CTSH transcription and translation by regulating its DNA methylation levels and that these changes potentiate the destruction of pancreatic beta-cells in T1D.

METHODS

Human islets were treated with a pro-inflammatory cytokine cocktail containing IL-1β , TNF-α, and IFN-γ. Gene expression, DNA methylation level, promoter activity, TET hydroxylase activity, and genotyeps were evaluated.

SUMMARY OF RESULTS

In purified human islets, CTSH down-regulation by IL-1β + TNF-α + IFN-γ was coupled with DNA hypermethylation in an open chromatin region in intron 1. Using luciferase assay, methylation of three cytosine-phosphate-guanine (CpG) residues in intron 1 were shown to reduce promoter activity. We found that cytokine induced intron 1 hypermethylation is caused by lowered Tet1/3 activities. Interestingly, the T1D risk variants correlated with reduced methylation variability, whereas the protective variants correlated with increased methylation variability thus were more sensitive to environmental influence.

CONCLUSIONS

In summary, DNA methylation in CTSH intron 1 is a key mediator of T1D risk. Inflammatory cytokines and the genetic risk may contribute to T1D development through different mechanistic pathways at the CTSH locus.

Abstract #32

Circulating insulin C-peptide levels mirror pancreatic beta-cell loss across all ranges of age and disease duration of type 1 diabetes

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PURPOSE

C-peptide (CP) levels decline in type 1 diabetes (T1D), although many patients have low but detectable levels of CP for many years after diagnosis. Currently there is little understanding of how the levels of circulating CP relate to pancreatic beta cell retention and if this varies with age at diagnosis. In this study we used a large clinical T1D cohort and unique T1D pancreas biobanks to independently assess the CP levels in the blood and beta cell loss within the pancreas, in subjects diagnosed at increasing ages and with differing diabetes durations.

METHODS

We studied 4,079 serum CP from the UK Genetic Resource Investigating Diabetes (UK GRID) cohort, diagnosed at <16 years as well as 235 pancreas samples recovered from people with T1D (diagnosed <18 years) from the network for Pancreatic Organ donors with Diabetes (nPOD) biobank and the Exeter Archival Diabetes Biobank (EADB). Cases were stratified by age at diagnosis (<7, 7-12, ≥13 years) and grouped by diabetes duration (<1, 1-5, 5-10, ≥10 years). We report the proportion of individuals from the UK GRID cohort with detectable CP (>9 pmol/L) and the proportion of donors from the two independent pancreatic biobanks having residual insulin containing islets (ICIs), within each age group over increasing diabetes durations.

SUMMARY OF RESULTS

Detectable CP levels persisted in some individuals in all age groups over time, although this was least common in those diagnosed <7 years (detectable CP: <7 group: 372/1666 (22%), 7-12 group: 981/1887 (52%), ≥13 group 363/526 (69%)). A similar pattern was observed in the proportion of individuals with residual ICIs in the pancreas, (residual ICIs: <7 group: 33/87 (38%), 7-12 group: 51/89

(57%), ≥13 group: 42/62 (68%). In all groups, the number of individuals with detectable CP levels declined beyond the first year after diagnosis, but was most marked in those diagnosed at younger ages (detectable CP <1 year post diagnosis: <7 group: 18/20 (90%), 7-12 group: 107/110 (97%), ≥13 group 58/61 (95%) vs. detectable CP 1-5 years post diagnosis: <7 group: 172/522 (33%), 7-12 group: 604/995 (61%), ≥13 group: 225/289 (78%). This was mirrored by an equally rapid decline in individuals with residual ICIs in the pancreas (residual ICIs <1 year post diagnosis: <7 group: 24/26 (92%), 7-12 group: 32/33 (97%), ≥13 group: 22/25 (88%) vs. residual ICIs 1-5 years post diagnosis: <7 group: 1/12 (8.3%), 7-12 group: 8/13 (62%), ≥13 group: 7/8 (88%). Only 4.3% (21/489) of children diagnosed < 7y had detectable C-peptide 10y post diagnosis and a similar proportion (3/34 (8.8%)) retained cells with immunoreactive insulin positivity at this time.

CONCLUSIONS

Circulating C-peptide levels reflect residual beta-cell mass in patients with T1D at all ages and duration of disease, indicating progressive beta-cell loss is the main contributory factor to the decline in endogenous insulin secretion.

Abstract #33

Analysis of long-read whole genome sequence data from T1D pancreatic tissues for pathogenic HERV-W-env sequences

Authors

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PURPOSE

HERV-W (Human Endogenous Retroviruses, W subgroup) has recently been associated with T1D. Particularly, the envelope protein of HERV-W, named HERV-W-Env, was detected in pancreas from T1D patients and was shown to display pro-inflammatory properties and direct toxicity toward pancreatic beta cells [1]. T1D patients were also shown to have antibodies targeting HERV-W peptides [2]. However, a genomic copy or genomic variants encoding the HERV-W-Env pathogenic protein has not already been identified. We aim to understand a major unanswered question about HERV/HERV-W-env copies in the T1D genomes by identifying non-referenced HERV / HERV-W-env insertions generated through retrotransposition or reinfection or non-homologous recombination. This will help us to identify T1D genetic risk factors or contributors among mobile genetic elements of the human genome, with an emphasis on HERV and HERV-W-env sequences.

METHODS

We strive to identify HERV-W-env copies in the T1D genomes using long-read whole genome sequencing (WGS) approach in parallel with an inverse PCR and sequencing approach. With regard to the long-read WGS data, we use a two-prong approach to identify the non-reference insertions of HERV-W-env in those samples. A reference assembly-based approach, in which reads from each sample are aligned to the reference human genome (hg38) using tools like Pbmm2 or NGMLR. Then structural variants (SVs) are called using tools such as PBSVs or Sniffles. We then analyze the non-reference insertions identified by those tools to detect the presence of HERVW-env sequences. Use of multiple tools would increase the sensitivity of SV detection. Secondly, we are using a reference assembly free approach, and generates de novo assembly for each sample using a tool called Canu. This would help us to circumvent any biases induced by reference assembly. The de novo assembled sequences and the unassembled sequences from each sample are further examined for the presence of HERV-W-env insertions using homology-based searches (RepeatMasker). Those homologous regions are further evaluated to differentiate any new copies from the known HERV-W-env copies present in the reference genome. In addition to long-read WGS approach, we are also using an inverse PCR, cloning and sequencing approach to identify novel/unknown insertion sites of HERV-W-env in the above described samples. This will help us in identifying somatic insertions that could be of very low frequency but unable to pick up with sequencing approach.

SUMMARY OF RESULTS

Using ‘Single Molecule Real-Time’ (SMRT) sequencing technology from PacBio, we generated whole genome sequences (WGS) for four T1D pancreas samples, one control sample and one positive control cell line. Each sample is sequenced > 60X coverage, and have ~500Gb data, and have reads up to ~160K bp long. The N50 read length metric, the read length at which 50% of the bases are in reads are > 30K bp. We use reference assembly-based and de novo assembly-based approaches for identifying pathogenic HERV-W-env in the samples. Utilizing the reference assembly, the insertions are identified in every sample using the Pbmm2 (aligner) and PbSV (SV caller) and are investigated for

its homology to HERVW-env sequences. We have not identified any non-reference insertions containing an intact HERVW- env. As part of the sanity check, we successfully identified known non-reference insertions of another HERV family (HERVK) in those whole genome sequences. Since use of multiple tools would be necessary to enhance the sensitivity of SV calls, we are repeating the similar analysis using another aligner and SVcaller (e.g NGMLR and Sniffles).

As part of de novo assembly approach, we have generated assembles of three samples and rest three are underway. Even though, this approach is very computationally intensive and time consuming, (with 80 CPUs and 754 GB memory, more than a month would be required to finish an assembly), this would help us in overcoming the some of the limitations of a reference assembly-based approach. A de novo assembly enables the identification the structural variants and repeats associated with a genome far more perfectly. We identified regions homologous to HERV-W-env in the de novo assembled sequences and checked if they differ from the known copies in the reference assembly. So far, we have not identified any non-reference copy of HERV-W-env in the de novo assemblies and in unassembled the sequences associated with them. We will be analyzing the rest of the samples as described, as the assemblies are generated.

The complimentary inverse PCR, cloning and sequencing approach to identify HERV-W-env insertions has been tested and validated in the cell line sample tested. This approach picked up many known insertion sites of HERV-W-env along with flanking sequence in the sample tested. The validated approach will be used for T1D tissue samples to identify non-reference HERV-W-env insertions.

CONCLUSIONS

We have generated at high coverage long read data set using SMRT technology for four T1D samples and for two control samples. We have aligned these long reads to the human reference assembly (GRCh38) and called structural variants. We have also generated de novo assemblies for three samples so far. We have not identified a non-reference intact HERVW-env copy yet. However, these are valuable data that will be available to the community for those who are interested in genetic/ genomic analysis. The inverse PCR approach that we validated also can be used for identifying similar non-reference insertions.

Abstract #34

DR15-DQ6 remains dominantly protective against type 1 diabetes throughout the first five decades of life

Authors

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PURPOSE

Among white European children developing type 1 diabetes, the otherwise common HLA haplotype DR15-DQ6 is rare, and highly protective. Adult onset type 1 diabetes is now known to represent more overall cases than childhood onset, but it is unknown if DR15-DQ6 is protective in older onset type 1 diabetes. We sought to quantify DR15-DQ6 protection against type 1 diabetes as onset age increased.

METHODS

In two independent cohorts we assessed the proportion of type 1 diabetes cases presenting through the first 50 years of life with DR15-DQ6, compared to population controls. In ADDRESS2 (n=1458) clinician diagnosed type 1 diabetes was confirmed by ≥1 positive islet specific autoantibody. In UK Biobank (n=2502), we estimated type 1 diabetes incidence rates relative to baseline HLA risk for each HLA group using Poisson regression. Analyses were restricted to White Europeans and was performed in three age groups by type 1 diabetes onset: 0-18 years, 19-30 years and 31-50 years.

SUMMARY OF RESULTS

DR15-DQ6 was protective against type 1 diabetes through age 50 (odds ratio [OR] <1 for each age group, all p<0.001). An attenuation of protection was observed with age >30 years [p=0.003] in ADDRESS2: ages 5-18 OR 0.16 (95% Confidence interval 0.08-0.31); ages 19-30 OR 0.10 (0.04-0.23); and ages 31-50 OR 0.37 (0.21-0.68). DR15-DQ6 also remained highly protective at all ages in UK Biobank. Without DR15-DQ6, the presence of either major type 1 diabetes high risk haplotype (DR3-DQ2 or DR4-DQ8) was associated with increased risk of type 1 diabetes.

CONCLUSIONS

Protection against type 1 diabetes afforded by DR15-DQ6 attenuates with older age at onset, yet still affords significant dominant protection across the first five decades of life.

Abstract #35

The dominant presence of Preproinsulin-specific CD8 T cells in the human pancreas

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PURPOSE

Type 1 diabetes (T1D) is a chronic autoimmune disease where CD8 T cells infiltrate the islets and are sequestered in the exocrine pancreas in high numbers. CD8 T cells reactive against islet and viral antigens can be detected in the blood of patients with T1D, and similar frequencies have been reported in healthy individuals. However, little is known about their numbers in the target organ. Therefore, we quantified and compared the frequencies of beta-cell and virus-specific CD8 T cells in the exocrine pancreas and islets at different stages of diabetes progression.

METHODS

We performed in situ immunofluorescence staining using different HLA-A2-restricted tetramers (Preproinsulin, PPI15-24; glucose-6-phosphatase catalytic subunit-related protein, IGRP265-273; cytomegalovirus, CMV495-503; Epstein-Barr Virus, EBV426-434; Influenza A virus, Flu58-66; enterovirus Coxsackievirus B3, EV1587-1596) combined with CD8 on frozen human pancreas section from donors with T1D, autoantibody-positive, and autoantibody-negative healthy controls. In summary, we analyzed tissue samples from 22 donors obtained through the Network for Pancreatic Donors with Diabetes (nPOD), 5 donors from the Human Pancreas Analysis Program (HPAP), and tissue samples from 4 recent-onset individuals collected by the Diabetes Virus Detection (DiViD) Study. Sections were scanned using a Zeiss Axio.Scan Z1 slide scanner (Zeiss). Afterward, the same tissue sample was treated with hydrogen peroxide to inactivate the fluorophores and re-stained for

insulin and glucagon. The analysis of the entire tissue section was performed manually using the ZEN 2.5 lite software (Zeiss).

SUMMARY OF RESULTS

Among the 22 donors (6 non-diabetic, 5 aab+, 11 T1D; nPOD), PPI-specific CD8 T cells were present in the exocrine pancreas of non-diabetic donors, but their numbers increased significantly close to and within the islets during disease progression. In donors with T1D, a mean of 40% of CD8 T cells specific for PPI were detected in insulin-containing islets compared to 20% in insulin-deficient islets.

The mean frequency of IGRP-specific CD8 T cells was significantly lower (less than 8%) compared with PPI-specific CD8 T cells in 2 donors with autoantibodies and 2 T1D donors (HPAP).
In donors with recent-onset T1D (DiViD), the percentage of CD8 T cells reactive against cytomegalovirus that infiltrated the pancreas was relatively high in 3 out of 4 individuals (around 5%) but was lower than the frequency of PPI-specific CD8 T cells (9.5%-12.8%). Overall, the frequency of CD8 T cells reactive against Epstein-Barr Virus, Influenza A virus, and the enterovirus Coxsackievirus B3 was relatively low (less than 5%) for all the DiViD samples compared to PPI-specific CD8 T cells.

CONCLUSIONS

In contrast to their previously documented presence in the blood of healthy human donors in relatively low numbers, we found that PPI-reactive CD8 T cells are abundantly present in the exocrine portion of the pancreas in non-diabetic and T1D donors. However, they preferentially infiltrate the remaining insulin-containing islets in T1D donors, suggesting a possible role in disease pathogenesis.

The fact that frequencies against IGRP were lower than against PPI additionally points to PPI\'s importance as a prominent antigen in T1D. Our data suggest that the frequencies of antigen-specific T cells are higher in the pancreas than previously reported in the peripheral blood of patients with T1D, thus highlighting the importance of studying T cell reactivities in the pancreas. Our findings contribute to the current limited existing knowledge on T cell specificity in the pancreas and indicate that therapeutic approaches directed towards PPI could have a high clinical impact.

Abstract #36

Large-scale electron microscopy database for human type 1 diabetes

Authors

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PURPOSE

The underlying mechanism(s) initiating beta cell destruction resulting in type 1 diabetes (T1D) are still poorly understood. T1D etiology demands full knowledge of cellular composition and microenvironment of the islets of Langerhans. Electron microscopy (EM) allows to study ultrastructure, but typically only reveals high resolution of limited subcellular areas.

METHODS

We routinely perform large-scale EM for unbiased analysis of complete islet cross-sections at nanometer-resolution, which we call ‘nanotomy’ for nano-anatomy and thereby transform biobanked material from the Network for Pancreatic Organ donors with Diabetes (nPOD) into an open access EM database1. The repository currently contains over 50 datasets from 45 donors (asymptomatic autoantibody-positive (n=13), T1D (n=16), and control (n=16)). Analysis of these gigabytes grey-scaled data was aided by label-free elemental fingerprinting of secretory granule content.

SUMMARY OF RESULTS

Our analysis of the database revealed significant increased presence of specific mast cell subtypes in both autoantibody-positive ($p = 0.02$) and T1D ($p = 0.005$) compared to control donors. Moreover, we found that endocrine cells co-appearing with exocrine granules were present in a greater extent among autoantibody-positive (23%) and T1D donors (38%) compared to control donors (13%). Furthermore, these ‘intermediate’ cells in both autoantibody-positive and T1D donors display a stressed morphology.

CONCLUSIONS

The first datamining showed innate immune cell alterations as well as aberrant exocrine and endocrine interactions that fit with the growing notion that T1D is a pancreas-wide disease. We are currently addressing a possible cause-consequence relationship between exocrine alterations and T1D

pathology in a model for dynamic in vivo imaging experiments. In conclusion, we present the largest repository for human EM data. The information-dense character of these zoomable EM maps is excellent for data reuse and can now be accessed by researchers worldwide to address their own questions on T1D pathology at the nanometer scale.

1. de Boer, P. et al. Large-scale electron microscopy database for human type 1 diabetes. Nat. Commun. 11, 2475-020-16287-5 (2020)

Abstract #37

Multiomics single-cell analysis of human pancreatic islets reveals novel cellular states in health and type 1 diabetes

Authors

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PURPOSE

Type 1 diabetes (T1D) is an autoimmune disease of only partially defined etiology in which immune cells destroy insulin-producing beta cells. Using single-cell transcriptomics and an advanced analytical strategy to assess pancreatic islets of T1D, autoantibody-positive, and non-diabetic organ donors, we identified both canonical cell types and rare insulin-expressing cells with a hybrid mixture of endocrine and exocrine gene signatures within all donors. We further found elevated expression of MHC Class II pathway genes in exocrine ductal cells of T1D donors, which we confirmed through CyTOF, in situ imaging mass cytometry, and immunofluorescence analysis. Taken together, our multimodal analyses identify novel cell types and processes that may contribute to T1D immunopathogenesis and provide new cellular and molecular insights into human pancreas function.

METHODS

Type 1 diabetes (T1D) is an autoimmune disease in which immune cells attack and destroy insulin-secreting pancreatic beta cells. This complex disease affects millions of individuals, incurring billions of dollars in medical costs in the United States alone. The initial pathogenic events that trigger autoimmunity have remained elusive due to the inability to biopsy the pancreas and the fact that clinical diagnosis is only made once massive beta cell destruction has occurred. Therefore, a better understanding of the earliest molecular events in T1D pathogenesis remains critical for biomarker identification and disease prevention. Here, we generated the single-cell transcriptomic maps in pancreatic islets of human organ donors. This work is unprecedented for three major reasons:

1. The Human Pancreas Analysis Program at the University of Pennsylvania has collected high quality pancreatic tissues in three well-characterized disease categories: those clinically diagnosed with T1D, those normoglycemic but with circulating autoantibodies (AAB) towards pancreatic islet proteins (prediabetics), and age-matched non-diabetic controls. We have generated high-resolution single-cell maps of gene expression in pancreatic tissues of these donor groups where AAB+ donors represent the earliest molecular events in T1D pathogenesis. We employed additional data modalities such as multiplexed In situ Imaging Mass Cytometry (IMC) across the same donors to corroborate our single-cell RNA-seq results.
2. We developed a novel analytical tool 'TooManyCells', employing hierarchical spectral clustering and Newman-Girvan modularity for cell partitioning. TooManyCells, which became online on March 2nd, 2020 (https://www.nature.com/articles/s41592-020-0748-5) has an exceptional ability to visualize cellular relationships, enabling us to detect rare cell populations and their relationships to canonical cell fates in the pancreas.
3. We developed a data portal and provided easy access to the entire single-cell RNA-seq data generated in this study, enabling users to examine the expression of a gene of interest across clinically

well-characterized donors corresponding to annotated cell fates (http://faryabi05.med.upenn.edu:8050/).

SUMMARY OF RESULTS

Specific unexpected findings arising from our study are:

1. Endocrine and non-endocrine cells from AAB+ donors are transcriptionally similar to T1D donors.
2. Single cell profiling enables the identification of ductal-endocrine hybrid cells, which predominantly arise in AAB+ and T1D donors.
3. T1D ductal cells are indicative of an inflammatory environment and unexpectedly express MHC Class II proteins.
4. Immune cells infiltrating pancreatic islets segregate based on inflammation and disease state.

CONCLUSIONS

Specific unexpected findings arising from our study are:

1. Endocrine and non-endocrine cells from AAB+ donors are transcriptionally similar to T1D donors.
2. Single cell profiling enables the identification of ductal-endocrine hybrid cells, which predominantly arise in AAB+ and T1D donors.
3. T1D ductal cells are indicative of an inflammatory environment and unexpectedly express MHC Class II proteins.
4. Immune cells infiltrating pancreatic islets segregate based on inflammation and disease state.

Abstract #38

Off-Therapy Effects of Golimumab in Type 1 Diabetes: Week 104 Follow-up Results of the T1GER Study

Authors

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PURPOSE

Type 1 diabetes (T1D) is an autoimmune disease characterized by progressive loss of pancreatic β cells. Golimumab (GLM) is a human IgG1κ monoclonal antibody specific for tumor necrosis factor α. The T1GER study (ClinicalTrials.gov identifier, NCT02846545) assessed the on- and off-therapy effects of GLM in children and young adults with newly diagnosed stage 3 T1D. This abstract focuses on the off-therapy data.

METHODS

GLM was administered for 52 weeks, followed by a 52-week off-therapy period. 84 participants (6-21 years of age) were enrolled (GLM, n = 56; PBO, n = 28); of these, 74 participants (GLM, n = 49; PBO, n = 25) entered the off-therapy period. The primary endpoint was C-peptide area under the curve (AUC) at Week 52 after a 4-hour mixed-meal tolerance test. Insulin use, HbA1c, hypoglycemia rates were also assessed. A 52-week off-therapy period was planned to continue to monitor these outcomes and safety.

SUMMARY OF RESULTS

At Week 52 of treatment, there was significant preservation of C-peptide and lower insulin use in those receiving GLM. During the off-therapy period, there was a decline in C-peptide AUC in both the GLM and PBO groups. However, C-peptide AUC continued to be higher in the GLM group at Weeks 78 and 104 ($p < 0.05$) without any difference in insulin dose or HbA1c. Biochemically confirmed hypoglycemic events were reduced in the GLM group during the off-therapy period. Off-therapy and during the study as a whole, there were no new safety signals in either group. During the entirety of the study (including the 52-week on-therapy and 52-week off-therapy periods), there was a delay in disease progression, defined by percentage of C-peptide loss, in the GLM group by 10.9 months.

CONCLUSIONS

In children and young adults newly diagnosed T1D, in addition to clinical and metabolic benefits during 52 weeks of treatment with GLM, C-peptide AUC persists higher in the GLM treated group compared to placebo suggesting that GLM may lead to continued benefits that may extend at least an additional 52 weeks post-therapy in some patients.

Abstract #39

A multivalent Coxsackievirus vaccine does not accelerate the onset of diabetes in NOD mice

Authors

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PURPOSE

An extensive concerted effort has been made by the scientific community to elucidate the exact cause(s) of Type 1 diabetes (T1D), however despite this, the disease aetiology continues to remain elusive. In combination with a genetic component, a number of different environmental factors having been implicated in T1D, including infections with common enteroviruses and more specifically the Coxsackievirus Bs (CVBs). Despite a significant body of research investigating CVBs in the context of T1D, whether these infections are causal is yet to be confirmed. Vaccination strategies targeting genetically at-risk populations provide a viable option to clarify the involvement of CVBs. As such, we have created a multivalent CVB1-6 vaccine and undertaken proof-of-concept studies that have demonstrated the protective capacity of this vaccine against virus induced diabetes in an experimental mouse model. We have also shown that the vaccine is highly immunogenic in a non-human primate model. As an extension to these studies, here we addressed in the commonly used NOD mouse model for T1D, whether the vaccine had an impact on immune cell infiltration in the pancreatic islets of Langerhans and/or diabetes development.

METHODS

CVB1-6 serotypes were inactivated by formalin treatment and mixed to create the multivalent vaccine. Female NOD mice aged between 4.5 - 7.5 weeks old were vaccinated with either CVB1-6 vaccine or buffer on two or three occasions with two to three-week intervals between vaccinations. A third group of similarly aged female mice were left untreated. Serum was collected prior to each vaccination and at the end of the study. Weight and blood glucose levels were monitored weekly. Mice were either followed to 11 - 13.6 weeks of age when they were sacrificed, and the pancreas was collected for histological analysis of islet immune cell infiltration (insulitis) or up to 30 weeks of age to monitor the incidence of diabetes. Serum CVB-specific neutralising antibody titres were assessed by standard plaque reduction assay.

SUMMARY OF RESULTS

The multivalent CVB1-6 vaccine was highly immunogenic and induced neutralising antibodies to all 6 CVB serotypes. The vaccine was well tolerated and had no adverse effects on weight and blood glucose levels. Insulitis scores in pre-diabetic mice were comparable between the buffer treated and

CVB1-6 vaccinated groups and no statistical differences were seen. Furthermore, no differences were detected in either the average age at diabetes onset or in the cumulative diabetes incidence curves when comparing the buffer-treated, CVB1-6 vaccinated and untreated groups.

CONCLUSIONS

Our CVB1-6 vaccine had an excellent safety profile in NOD mice and was highly immunogenic. Moreover, the vaccine did not alter the degree of infiltrating immune cells surrounding and within the islets in prediabetic animals, nor the onset of diabetes in this model for T1D. These proof-of-concept studies provide data to support the use of an equivalent vaccine in human trials to establish whether CVBs are involved in T1D. If their involvement is confirmed such a vaccine could provide a viable preventative treatment for the disease.

Abstract #40

Development of a new strategy for the accurate assessment of beta-cell mass and function in health and disease

Authors

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PURPOSE

In both Type 1 and Type 2 diabetes (T1D and T2D), hyperglycemia results from insufficient insulin secretion, caused by a combination of reduced beta-cell mass and beta cell dysfunction. However, the extent of these defects varies between patients and contributes differently to the etiology of the diseases. Insulin immunostaining is central for the measurement of beta-cell mass; similarly, immunodetection of pancreatic islet insulin content serves as a standard parameter for the normalization of secreted insulin in the well-established glucose-stimulated insulin secretion (GSIS) assay for the ex-vivo study of beta-cell function. Recent studies have reported that metabolic stress, such as prolonged hyperglycemia might cause beta-cell degranulation (loss of insulin due to over secretion), loss of beta-cell molecular identity or complete dedifferentiation: such phenomena might result in insulin loss and the subsequent underestimation of beta-cell mass and flawed assessment of beta cell function. Thus, there is a pressing need to develop an accurate method for the unbiased measurement of beta cells in islets and pancreatic sections, independent of insulin staining.

METHODS

To quantify beta cells in islets, we have developed an experimental platform based on cell type-specific DNA methylation signatures to measure beta cell DNA. Specifically, we quantify beta-cell DNA based on several loci in the human genome, each one containing five to eight CpG sites that are unmethylated in beta cells while almost fully methylated in other cell types present in the pancreas (alpha, delta, acinar, duct, endothelial and blood cells). We are using Next Generation Sequencing (NGS), to sequence amplicons of bisulfite converted human islet DNA and identify beta cell DNA. The specificity and accuracy of the assay were carefully tested.

SUMMARY OF RESULTS

We calibrated this procedure to assess the fraction of all pancreatic (and non- pancreatic) cell-types in human islets and pancreatic histologic sections. This strategy will help us understand how islet composition affects insulin secretion ex-vivo. Moreover, it will allow us to examine cellular heterogeneity within the human pancreas, including the presence and identity of immune cell types, using histological sections of healthy and diabetic donors.

In addition, We are using this assay to measure beta cell DNA per insulin-stained area on pancreatic

sections from healthy and diabetic donors to assess whether as reported (for mice) in several studies, metabolically stressed beta cells survive but lose their identity.

CONCLUSIONS

Taken together, Our study based on the development of a reliable method for the assessment of beta-cell (and additional cell type) DNA in histological sections and pancreatic islets, will greatly benefit analysis of beta-cell phenotype (both identity and function) and allow for a more precise investigation of the mechanisms underlying beta-cell failure in diabetes.

Abstract #41

Altered in-situ expression of proinsulin-insulin in pancreatic islets reflects metabolic and molecular defects in Type 2 Diabetic and Glucose Intolerant living donors

Authors

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PURPOSE

Our previous results have shown that in pancreatic islets of impaired glucose tolerant (IGT) and type 2 diabetic (T2D) patients, PI intracellular localization was altered. Moreover, we demonstrated that colocalization coefficient insulin-proinsulin (INS-PI) as well as PI levels and PI/INS ratio gradually increased from NGT to IGT and T2D pancreatic islets and were related to the loss of glucose tolerance and impaired β-cell function. However, the molecular mechanisms driving such alterations are not fully characterized neither their occurrence were correlated with patients' metabolic profile. To fill in this gap, we investigated the correlation between altered PI expression and phenotypic/functional changes of β-cells during metabolic stress in extensively clinical characterized IGT and T2D individuals, compared to NGT.

METHODS

We analyzed microdissected pancreatic islets collected from pancreas biopsies during partial pancreatectomy from n=4 NGT, n=7 IGT and n=4 T2D patients. We evaluated the expression of ER stress genes and β cell mature phenotype-related genes. Given the high heterogeneity among pancreatic islets we also performed a single islets analysis in n=88 islets from n=3 NGT, n=3 IGT and n=3 T2D patients.

SUMMARY OF RESULTS

We observed that PDIA1, GRP78 and XBP1, genes involved in unfolded protein response (UPR) were significantly upregulated in pancreatic islets of IGT and T2D patients vs NGT ($p<0.05$) and were positively correlated with in-situ PI/INS ratio ($r=0.6$; $p=0.01$) and colocalization ($r=0.5$, $p<0.05$), with in-vivo measurements of glucose intolerance ($r=0.6$-0.8; $p<0.001$) and β cell functional reduction ($r=-0.6$; $p=0.04$).

Single islets phenotyping approach revealed a progressively increased heterogeneity from NGT to IGT and T2D patients. Of note, in-situ PI/INS ratio and colocalization were positively correlated with the expression of UPR genes ($r=0.3$; $p<0.01$) and negatively with those associated to β cell identity ($r=-0.2$; $p<0.01$).

CONCLUSIONS

Our data demonstrated that altered PI processing in β-cell reflected metabolic and molecular defects in IGT and T2D patients. Further, single islet phenotyping analysis revealed a high heterogeneity among pancreatic islets in terms of ER stress and β-cell differentiation profile during metabolic alterations.

Abstract #42

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

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PURPOSE

IL-17 is a pro-inflammatory cytokine, important in shaping host immune responses against pathogens. IL-17 is also attributed to cause tissue damage and chronic inflammation in autoimmune diseases such as psoriasis, rheumatoid arthritis, and multiple sclerosis. An increase in IL-17 secreting CD4+ T cells and Th17 cells were observed in pancreatic lymph nodes and peripheral blood of subjects with type 1 diabetes (T1D). However, it is not known whether IL-17 is expressed in human pancreata in healthy conditions and during T1D or T2D pathogenesis. Last year we reported increased IL-17 expression in islets of donors with Type 1 and Type 2 diabetes with alpha and beta cells being the major cellular source. Since then, we have stained more cases from every diagnosis group and evaluated infiltrating CD45+ lymphocytes contribution to the observed IL-17 expression in T1D and T2D donors.

METHODS

IL-17, CD45, Insulin, and Glucagon antibodies were individually optimized in FFPE human tonsil and pancreas sections. 21 Human pancreatic FFPE tissues sections from non-diabetic (n=5), autoantibody positive (n=5), T1D (n=6) and T2D (n=5) donors were stained for IL-17, Insulin, and Glucagon. Consecutive sections from selected T2D (n=1), T1D (n=2), and non-diabetic (n=1) cases were stained for IL-17, CD45, and Insulin. Whole tissue sections were imaged using Zeiss AxioScan Z1 slide scanner. High resolution images of islets were acquired using LSM780 Confocal Microscopy system. Quantitative image analysis was performed through Zen Blue and ImageJ software to find percentage of positive area and colocalization data for each marker within islet areas.

SUMMARY OF RESULTS

IL-17 signal found in islets of non-diabetic and auto-antibody positive cases, had a weak, sparse pattern accounting for an average of 2.4% and 4.6% of islet areas, respectively. In donors with T1D, insulin containing islets (ICIs) had a stronger expression of IL-17, accounting to an average of 7.8% of total islet area. Insulin-deficient islets (IDIs) from T1D donors had a lower IL-17 expression, similar to the level seen in islets from non-diabetic cases. IL-17 expression was highest in islets from T2D donors, accounting for an average of 14.9% of islet area. Much of the IL-17 production was accounted by either beta or alpha cells, while in many cases beta cells were the major source. In ICIs of T1D

cases, 27.8% of the IL-17 expression was contributed by beta cells, while 16.9% was contributed by alpha cells. In the islets of T2D cases, 37.4% of the IL-17 expression was contributed by beta cells, while 33.5% was contributed by alpha cells. The proportion of beta cells expressing IL-17 was significantly higher in the T2D cases (31.6%) and T1D ICIs (16.5%) compared to the cases from other diagnosis groups. The proportion of alpha cells expressing IL-17 was significantly higher in the T2D cases (22.3%) compared to the other groups. Subsequent staining of consecutive sections with CD45, IL-17, and Insulin showed CD45 accounted for a very small percentage of the total islet area (between 0.19 to 1.1%) and almost none of the IL-17 expression was attributable to CD45+ infiltrating lymphocytes. CD45 only contributed 0.23% of the IL-17 expression in the T1D cases and 0.025% in the T2D case.

CONCLUSIONS

According to literature, expression of IL-17 is usually restricted to Th17, gamma delta T cells and some cell types of the innate immune system. Our finding that IL-17 can be expressed in islet cells of T1D or T2D is quite intriguing and could be a result of beta cell dysfunction induced by either metabolic or immune stress. Further mechanistic studies in human islets or human islet organoid models may be helpful to determine under which conditions IL-17 expression can be induced, at least in vitro, in human islets.

Abstract #43

Identification of Enteroviruses in nPOD cases by Mass Spectrometry

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PURPOSE

To utilize proteomic and liquid chromatography-mass spectrometry technologies to identify and characterize enterovirus proteins/peptides in disease stratified nPOD tissue samples.

METHODS

Proteins from different types of pancreas tissue samples from nPOD were isolated and processed for liquid chromatography–mass spectrometry analysis. Different instrument platforms with complementary scanning methods were used to generate peptide mass spectra. Mass spectrometry data were processed and used in protein database searches for identification, qualitative and quantitative comparative analyses of viral protein expression.

SUMMARY OF RESULTS

In these studies, we comprehensively analyzed pancreata from 82 disease stratified nPOD donors. We identified enterovirus peptides from different EV serotypes in 28 donors, including 10 T1D donors. The identified serotypes include those that were associated with T1D in previous clinical studies.

CONCLUSIONS

Our data demonstrate the presence of enterovirus proteins in the pancreas, including samples from donors with T1D.

Abstract #44

Glutamate Decarboxylase and Islet Antigen-2 autoantibody titres at diagnosis in people with Type 1 Diabetes showed bimodality and an age of diagnosis association

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PURPOSE

Islet autoantibodies (GADA, IA2A and ZnT8A) are commonly used in the diagnosis of type 1 diabetes (T1D). However, often just the presence of an islet autoantibody is considered useful, rather than its level (titre). The role of autoantibody titre at diagnosis of T1D is not known. This study aimed to assess the distribution of islet autoantibody titres and their association with genetic, clinical and presentation characteristics at diagnosis in people with T1D.

METHODS

We studied 1536 white European clinically diagnosed T1D patients from the UK-wide ADDRESS-2 study (median age at diagnosis 20.8 y, [range 4.4-75.3 y]). Autoantibody positivity and titre of GADA, IA-2A and ZnT8A were determined using gold-standard radiobinding assays at the University of Bristol at a median of 10.1 weeks diabetes duration. We assessed the distribution of titre and its association with type 1 diabetes genetic risk score, HLA genotypes (DR3, DR4), presentation and clinical characteristics.

SUMMARY OF RESULTS

GADA and IA-2A, but not ZnT8A, titres exhibited a bimodal distribution at diagnosis in those positive for that autoantibody. We used the trough between the two modes to divide bimodal distribution into low and high titre groups for GADA and IA-2A. The mean titre of the low and high GADA titre groups were 180.3 DK U/ml (±118.4, n=711) and 760.9 DK U/ml (±229.3, n=557), respectively. Whereas they were 41.2 DK U/ml (±37.4, n=285) and 301.0 DK U/ml (±88.3, n=749) for IA-2A.

GADA and IA-2A titres was significantly associated with age of diagnosis but in the opposite direction. Those in the high titre GADA group were diagnosed later compared to the low GADA titre group (median 27.1 y [IQR 17.5-38.8 y] vs 19.4 y [12.9-29.7 y])($p<0.0001$). Whereas the people in the high titre IA-2A group were diagnosed earlier compared to the low IA-2A titre group (median 17.0 y [IQR 11.9-25.2 y] vs 22.4 y [13.1-33.5 y]) ($p<0.0001$). There were no other significant differences between high and low titre categories of GADA and IA-2A for presentation and clinical characteristics.
People with HLA DR3/X (OR=1.49 [1.16-1.91]) and DR3/3 (OR=1.57 [1.10-2.23]) relative to DRX/X were more likely to have high titre GADA compared to low titre GADA. Whereas, those with HLA DR4/X (OR=2.04 [1.52-2.73]) relative to DRX/X were more likely to have high titre IA-2A compared to low titre IA2A. However, there was no difference in overall T1DGRS between the low and high titre groups for GADA and IA2A.

CONCLUSIONS
Autoantibody titres for GADA and IA-2A exhibit a bimodal distribution at diagnosis of T1D. This bimodality of autoantibody titre appears to have no effect on presentation characteristics. The bimodality is strongly associated with age of diagnosis and DR3 and DR4 genotypes but not T1DGRS, suggesting that low titre islet positivity should not deter from a diagnosis of T1D.

Abstract #45

Development of an islet "micropunch" to isolate and characterize single islets from donors with and without T1D through the nPOD pancreatic slice program

Authors

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PURPOSE

Human islets are known to be very heterogeneous, even within a single donor's pancreas. Not only does the size and cellular constituency (α-, δ-, γ-, and β-cell proportion) vary from islet to islet within any individual human pancreas, but also in donors with T1D, some islets may be infiltrated by inflammatory cells, while other islets in the same donor pancreas may appear to be uninflamed. Since islet inflammation may influence islet function, endocrine cell numbers, and individual islet cell gene expression, we have sought to develop assays that assess those variables from a single human donor. The successful development of such techniques will for the first time provide data on questions like:

1. How does an individual islet's cell constituents influence that islet's insulin and glucagon secretion in response to physiological stimuli?
2. Do β-cells (or α-cells) from islets with infiltrating T cells display unique gene expression patterns relative to islets from the same donor without demonstrable insulitis?
3. Are the functional responses of inflamed islets demonstrably different from those of uninflamed islets of similar size and cellular makeup?

Data addressing these critical knowledge gaps may shed new light on the basic pathophysiological processes underlying T1D, and therefore identify potential therapeutic targets

METHODS

We have developed a custom micropunch tool that allows us to sample individual islets and surrounding tissue from viable pancreas slices, using biopsy needles of 350, 560, or 860 µm bore to accommodate islets of various sizes. The punched islets from donors with or without T1D are recovered in a well of an HTS Transwell® plate. After overnight culture to recover from isolation shock, the transwell is acclimated in basal glucose Krebs-Ringer Bicarbonate + HEPES (KRBH) solution for 1 hour. The transwell insert is then serially passed into custom-printed low volume reservoir plates containing basal glucose KRBH, high glucose KRBH, low glucose KRBH + epinephrine, and KCl. Incubation time in each buffer is 15 minutes. The insert is gently centrifuged after each step to minimize carry over and maximize recovery of solutions. These culture supernatants are then assayed for insulin and glucagon content using commercially available ELISA kits. The same islets studied for their functional responses are then dissociated into single cells, and the islet's cellular constituents and each individual cell's transcriptome is determined using techniques well-established in our laboratory.

SUMMARY OF RESULTS

We have developed techniques and will present data showing:

1. A micropunch tool with which we can isolate single, viable islets and peri-islet T cells from pancreas slices
2. Static assays for determining the function of individual islets, i.e. insulin and glucagon secretion in low and high glucose concentrations
3. The cellular constituents (i.e. endocrine cell subsets and presence or absence of peri-islet or infiltrating T cells) of single islets.
4. The transcriptome of single cells from individual islets worked out for traditionally isolated islets and now being adapted for micropunch isolated islets (in process).

We are now further developing these techniques to allow them all be applied to a single islet, revealing that islet's secretory functionality, cellular constituents, and each cell's gene expression profile.

CONCLUSIONS

Many of the remaining fundamental knowledge gaps in our understanding of T1D pathophysiology relate to the patchy "vitiligo-like" nature of the disease, e.g. why are some islets inflamed while others are not, and what impact do infiltrating or peri-islet T cells have on an islet's function, cellular constituents, and individual cell's gene expression. The successful completion of the techniques we're developing can provide a platform for gaining insight into such issues.

Abstract #46

Isolation and proteome profiling of plasma-derived extracellular vesicles from the non-obese diabetic (NOD) mouse

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PURPOSE

The mechanism(s) through which pancreatic β-cells are destroyed in type 1 diabetes (T1D) remain to be fully understood but may comprise of more than one distinct pathophysiological mechanism (leading to so-called disease endotypes). Discovery of one or several endotype-specific biomarkers during the pre-diabetic period may open up the potential of personalized disease interventions. In the last decade, much attention has been paid to small vesicles (30-500 nm), so-called extracellular vesicles (EVs), which are excreted from cells under normal and pathological conditions. Due to their presence in all types of body fluids and the fact that they contain cargo reflecting their parental cell, EVs may harbor biological markers which reflect disease conditions, including the pre-diabetic stage(s) of T1D. Here, we describe the enrichment and characterization of EVs from plasma collected from non-obese diabetic (NOD) mice, a model for T1D. We also performed proteomic profiling of EVs isolated from pre-diabetic NOD mice.

METHODS

Plasma samples from 8-10 week old prediabetic NOD mice were collected for EV enrichment by size exclusion-chromatography (SEC) and membrane affinity purification (MA) based methods. For EV characterization, nanoparticle trafficking analysis (NTA), protein concentration measurements and transmission electron microscopy (TEM) were performed. Before LC-MS/MS analysis, the efficacy of an affinity-based depletion column in decreasing the presence of highly abundant plasma proteins from both whole plasma and EV samples was evaluated. Databases including Vesiclepledia (http://microvesicles.org/) and Exocarta (http://exocarta.org/) were used to identify typical EV markers in proteomic data sets.

SUMMARY OF RESULTS

SEC enriched samples contained a higher number of particles exhibiting the “characteristic” EV cup-shape morphology than those isolated by MA. In contrast, the MA-based enrichment yielded a particle pool with a higher protein content than SEC, and the particles were also atypical in mean/mode size. When protein profiles were compared, MA-purified EV samples had a higher presence of proteins that are abundant in plasma than EVs enriched by SEC. The column-based

depletion successfully removed albumin, transferrin and IgGs from both whole plasma and plasma-purified EVs, but the number of proteins identified by MS was only increased in whole plasma samples and not in EVs. An optimized LC-MS/MS based analysis of EVs enriched by SEC yielded a total of 680 proteins and confirmed the presence of some of the most characteristic EV markers, such as CD9, CD81 and ALIX.

CONCLUSIONS

SEC can be successfully employed to enrich EVs from NOD mouse plasma. The method that has been established could be used to identify new and endotype specific biomarkers in stage 1 and 2 T1D, which can serve to provide insight into disease endotypes and may be used for patient stratification in intervention studies.

Abstract #47

Hormone detection in formaldehyde-fixed paraffin-embedded pancreata using chemical analyte decrosslinking-based mass spectrometry imaging

Authors

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PURPOSE

To enable the use of biobanked formalin-fixed tissues samples with mass spectrometry imaging, the tissue must be decrosslinked; the goal is an efficient approach of decrosslinking of peptide hormones to characterize their spatiochemical distributions. This decrosslinking approach allows mass spectrometry imaging analyses of large sets of well-preserved paraffin-embedded formaldehyde-fixed human clinical specimens available at the Network for Pancreatic Organ Donors with Diabetes (nPOD).

METHODS

To develop method, we prepared seven model peptides with Ac-VEL-X-VLL amino acid sequence and used them in our in vitro analysis. The formaldehyde-reactive amino acids marked X are arginine, cysteine, glutamine, histidine, lysine, tryptophan and tyrosine. Model peptides were treated with 4% formaldehyde in phosphate buffer to produce a crosslinked form of the peptide exhibiting methylol or Schiff base moieties at the nucleophilic sites. For detection of peptidyl hormones in formaldehyde-fixed paraffin-embedded (FFPE) tissues, fresh rat pancreata were dissected after quick transcardial blood replacement using ice-cold physiological saline. The tissues were immersed in same formaldehyde solution used in in vitro experiments and kept at 4°C for 48 h. After the fixation, specimens were washed twice with PBS buffer and kept at -80°C freezer until analysis. Another set of samples were prepared from human FFPE pancreatic tissues collected and tissue banked by nPOD. University of Florida Institutional Review Board approved, and informed consent was obtained from each donor's legal representative (IRB201600029). In both cases, thin tissue slices were cut and mounted onto the ITO glass slides.

To enable a detection of model peptides or peptide hormones residing in the rat and human tissue slides, specimens were treated using chemical decrosslinking solution containing 1 M hydroxylamine hydrochloride in acidic tris(hydroxymethyl)aminomethane (TRIS) buffer at pH 3. The treatment of the samples containing model peptides was performed inside of tubes at 90°C for 30 min (in vitro). Human pancreas samples were deparaffinized using xylene, and series of ethanol solutions containing 100%, 95% and 70% ethanol. Rat and human tissues deposited onto ITO glass slides were treated inside of a Coplin jar at 60°C for 2 h. To remove remaining salts and lipids, specimens were thoroughly washed 3 times with ice-cold ethanol. Before analysis using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS), 50% MeOH plus 0.1% trifluoroacetic acid solution containing 50 mg/mL in) 2,5-dihydroxybenzoic acid (DHB) MALDI matrix was sprayed onto the slides to enable the detection of peptidyl hormones in chemically-decrosslinked pancreata.

SUMMARY OF RESULTS

Localization of the hormones in the tissue was successfully determined using MSI in the slices of formaldehyde-fixed rat pancreata processed with the analyte decrosslinking approach. In contrast no corresponding signals are detected in the control non treated tissue slices. Chemical decrosslinking by hydroxylamine hydrochloride in acidic tris(hydroxymethyl)aminomethane solution exhibited no obvious detrimental effects on observed distributions of peptidyl hormone. High-spatial resolution MSI demonstrated the characteristic for the pancreatic islets beta cell (represented by insulin, m/z 5800.9) localization in islet's center, while alpha (represented by glucagon, m/z 3481.5) and gamma cell (represented by pancreatic polypeptide, m/z 4397.2) are surrounding the core. In contrast, another approach involving sample's heat treatment in common for immunohistochemistry antigen retrieval 10 mM TRIS (pH 9), TRIS-EDTA (pH 8) or citrate (pH 6) buffers could not restore the detectability of the hormones. Buffer containing only formic acid at pH 2 which is lower than pH 3 of the decrosslinking solution also did not work for analyte retrieval. These results demonstrate that presence of hydroxylamine hydrochloride, alpha effect nucleophile, is essential for restoring the intact forms of peptides.

To understand mechanisms of analyte retrieval, we used seven model peptides having one formaldehyde-reactive amino acid residue in the middle of sequences. Their formaldehyde-derivatized structure were evaluated for the recovery of the intact forms by chemical decrosslinking. Presence of cysteine, lysine and arginine which have nucleophilic moieties lead to the restoration of intact model peptides as well endogenous peptides in formaldehyde-fixed pancreata. The quantitative surface analysis of 13C-formaldehyde-treated pancreata further proved formaldehyde could be successfully removed by chemical decrosslinking of biological tissues. At last, the developed approach allowed the detection of pancreatic hormones localized in the islet of Langerhans of human FFPE pancreas biobanked for several years by the nPOD. Our results demonstrated that localization of observed insulin-containing spots have a positive correlation with amylin distribution and are not correlated with glucagon, perhaps as insulin and glucagon are released from different cells in the islet. This approach facilitates larger scale investigation of the relationships between peptidyl hormones and type one diabetes progression using biobanked by nPOD FFPE tissues.

CONCLUSIONS

Developed new chemical analyte decrosslinking approach allows quantitative characterization of peptidyl hormones in FFPE pancreata using mass spectrometry imaging approach. We expect this approach will be applied to a range of multi-platform measurements and will involve different types of mass spectrometry technologies including mass spectrometry imaging. Specifically, the approach is capable of permitting of quantitative comparison of peptidyl hormone contents in the FFPE T1D-affected pancreata and other organs.

Support: American Diabetes Association Visionary Award #1-18-VSN-19

Abstract #48

Cellular distribution of Golli protein in human pancreatic islets

Authors

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PURPOSE

This project is to determine the cellular distribution of Golli protein in human pancreatic islets. Golli (gene expressed in oligodendrocyte lineage) is an alternatively spliced product of myelin basic protein (MBP) gene featured by the presence of a unique Golli domain which contains a 133-amino-acid sequence fused in the N-terminal of classic MBP. The Golli peptide shares 79% homology between the human and the mouse. Our works in mouse have identified that Golli protein serves as an important regulator of Ca2+ release –activated Ca2+ (CRAC) channel in T lymphocytes and voltage-dependent calcium channel (VDCC) in oligodendrocytes and neurons. This proposal start from our original findings in mice that: Golli protein is expressed in β cells of mouse Langerhans islet and plays a critical role in insulin secretion. We propose here to investigate the cellular distribution of Golli protein in human pancreas (both normal and type-1 and -2 diabetic) tissues, and we aim to build up a direct correlation of our findings of Golli in rodents to type-1 and type-2 diabetes in human.

METHODS

Paraffin sections of human pancreatic tissue from nPOD were stained by immunohistochemistry with Golli antibody (specific to human Golli peptide) in conjunction with other islet cell markers (insulin, glucagon, IAPP).

SUMMARY OF RESULTS

With the generous support by the Helmsley Charitable Trust George S. Eisenbarth nPOD Award for Team Science, we have finished a pilot experiment of Golli immunostaining in pancreas tissue from 5 nondiabetic controls (#6015, 6017, 6030, 6034 and 6254), 5 T1D patients (#5000, 6035, 6045, 6046 and 6299), and 1 T2D patient (#6249) with amyloid. Interestingly, we have found that 1) Golli immunostaining is found in all type of islet endocrine cells from nondiabetic human pancreas; 2) Dramatic (greatly) reduced Golli immunostaining in Ins+/GCG+ islets of T1D patients (#5000 and #6046, these two patients still have insulin positive islets, as early-staged T1D), reduced level of Golli immunostaining in all islet endocrine cells, especially evident in β cells; 3) In late-staged T1D patients whose islets contain only α cells, Golli immunostaining reduced slightly (some islets in #6035) or no change (#6045 and 6299); 4) Moderate Golli immunostaining is found in islet amyloid plaque of T2D patient (#6249).

CONCLUSIONS

Golli protein is specifically expressed in islet endocrine cells (both alpha and beta cells) in healthy human islets. Their expression level in beta cells is dramatically reduced in early staged type-1 diabetic patients when impaired insulin secretion occurs, suggesting a role of Golli protein in regulation of insulin secretion during type-1 diabetes. The presence of Golli immunostaining in amyloid of T2D islets may indicate a role of Golli protein in islet amyloidosis during typ-2 diabetes.

Abstract #49

Postnatal Development of Islet Macrophages

Authors

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PURPOSE

The purpose of this study is to understand the physiological profile of the pancreatic islet resident macrophage at different stages during human postnatal development. The predominant leukocyte in the islet is the resident macrophage. This cell gauges the activation status of beta cells by detecting beta cell-derived ATP and adjusts its functions to contribute to islet tissue homeostasis. In the juvenile human pancreas, decreased levels of the ectonucleotidase NTPDase3 in beta cells may lead to increased levels of extracellular ATP in the islet. A large increase in ATP is an inflammatory signal and may lead to dysregulated immune responses that lead towards the beta cell-directed autoimmunity which emerges in early life. In the present study, we examine the functional phenotype of macrophages in pancreata from non-diabetic and type 1 diabetic (T1D) donors ranging from 3 months to 20 years old.

METHODS

Living human pancreas slices were obtained from nPOD and incubated in HEPES solution (125 mmol/L NaCl, 5.9 mmol/L KCl, 2.56 mmol/L CaCl2, 1 mmol/L MgCl2, 25 mmol/L HEPES, 0.1% BSA, pH 7.4, and 10 mg/mL aprotinin). To visualize macrophages in situ, we used fluorescence conjugated antibodies for CD45 (1:50) and CD14 (1:50). To measure Ca2+ responses to stimuli, slices were incubated in the Ca2+ indicator dye Fluo-4 as well. Glucose was added to the buffered solution to give a basal glucose concentration of 3 mmol/L. All stimuli were bath applied. Confocal images were acquired in a Z-stack of 9-10 images every 3-5s using a Leica SP8 confocal laser-scanning microscope.

SUMMARY OF RESULTS

In pancreata from young donors (3 months – 8 years), we observed that islet macrophages had a higher baseline activity and larger increases in intracellular Ca2+ in response to exogenous ATP. In contrast, macrophages from older individuals (12 – 20 years) had less activity at baseline and smaller responses to exogenous ATP.

CONCLUSIONS

The data from our study show that in young T1D and non-diabetic donors, macrophages are more active at baseline and respond strongly to ATP, as compared to macrophages from older donors. This may be due to increased availability of ATP signals in the juvenile pancreas. These results show that the islet resident macrophage undergoes functional changes throughout postnatal development. Moreover, they represent initial data that show the importance of measuring the functional maturation of immune cells and its relation to the development of T1D.

Abstract #50

Single cell transcriptomic and epigenetic analysis of pancreatic lymph node tissues in T1D

Authors

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PURPOSE

Despite the progress made in defining cell types that are thought to contribute to type 1 diabetes (T1D), there remains a paucity of information as to the phenotype and function of these subsets in disease relevant tissue. The advent of single cell technology and bioinformatics now allow for more comprehensive analysis of immune cell subsets, including the integration of transcriptomic and epigenetic data, which is of particular importance due to the number of risk variants that fall in noncoding regions. We therefore chose to perform single cell RNA sequencing and ATAC-seq using cells derived from nPOD donor pancreatic lymph nodes (pLN). The potential for defining diabetogenic subsets at higher resolution, as well as interrogating the role of risk variants in the promotion of these subsets, has important implications for the development of rational therapies for T1D.

METHODS

Transcriptomic analysis was performed using whole homogenized PLN from control (n=2) and T1D (n=2) donors. Samples were stained with oligonucleotide-barcoded antibodies and dextramer reagents and run using the 10x genomics platform. Epigenetic analysis was performed using CD8+ T cells sorted from PLN of control (n=1), autoantibody positive (n=1) and control (n=1) donors using 10x genomics single cell ATAC-seq assay. Data were analyzed and visualized using the R packages Seurat and Signac.

SUMMARY OF RESULTS

We were able to recover data from 23,000 immune cells after quality control. 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). Unsupervised clustering of ATAC-seq data from CD8+ T cells also identifies clusters with accessibility at genes involved in effector function such as NKG7, as well as TH1 associated transcription factor STAT4.

CONCLUSIONS

Single cell technologies permit an in-depth and unbiased view of cell subsets which may contribute to T1D pathogenesis. Analysis of single cell transcriptomes of immune cells derived from PLN revealed enrichment of clusters with potentially cytotoxic and proinflammatory profiles, including effector CD8 T cells. Utilizing epigenetic data from CD8 T cells, we were also able to identify clusters defined by accessibility of genes responsible for effector function. We intend to substantiate and further these observations with more subjects and the addition of functional studies, including gene editing, to determine key markers and pathways which may influence the development of these phenotypes and contribute to disease pathogenesis.

Abstract #51

Experimental SARS-CoV-2 infection of the human pancreas

Authors

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PURPOSE

The relationship between coronavirus-disease 2019 (COVID-19) and diabetes has been proposed to exhibit a “bidirectional” dynamic: pre-existing diabetes may exacerbate the COVID-19 course, and infection with SARS-CoV-2, the etiological agent of COVID-19, may precipitate diabetes. However, while the role of T1D/T2D as significant co-morbidities for COVID-19 is by now firmly established, the epidemiological evidence for SARS-CoV-2 infection as a diabetes trigger is much less compelling. Consistent with the latter contention, we and others have recently reported that expression of the viral receptor ACE2 in human pancreatic tissue sections is largely restricted to ductal and microvascular structures rather than endocrine cells (PMID 33207244 & 33207245). Here, we have employed living pancreatic cells and tissues to interrogate the principal feasibility of SARS-CoV-2 to target and infect beta and other pancreas cell subsets.

METHODS

Tissues: primary human islets and dispersed islet cells; human pluripotent stem cell-derived ACE2-deficient beta-like cells; human pancreatic tissue slices. Basic experimental design: in vitro infection of pancreatic cells and tissues with SARS-CoV-2 (WA1/202 “Seattle strain” / BEI Resources) at low multiplicities of infection (≤0.025). Experimental readouts: quantification and delineation of virus-infected cell subsets by flow cytometry, mass cytometry, scRNAseq, and confocal microscopy; determination of infectious virus titers and soluble markers of inflammation in tissue culture supernatant (TCS; Olink Proximity Extension Assay platform); functional beta cell responses (dynamic glucose-stimulated insulin secretion / GSIS).

SUMMARY OF RESULTS

Our results indicate that ACE2 is expressed by small subpopulations of live dispersed islet cells including alpha, beta and other cells; is reduced in response to escalating glucose concentrations; and, interestingly, is broadly found on dying and dead cells emphasizing the importance for live/dead cell distinction in these analyses. The major target of in vitro SARS-CoV-2 infection are CD34+ endothelial

cells that lack CD31 expression as well as ~5% of beta cells (~1-10% range). Beta cell infection is not associated with a reduction of insulin expression and dynamic GSIS experiments are currently pending. Other endocrine cell subsets (alpha, gamma, delta) as well as acinar, ductal and CD31+ endothelial cells exhibit a lesser degree of infection; in addition, macrophage subsets present in the cell preparation also demonstrate viral antigen expression. Importantly, antibody-mediated ACE2-blockade readily prevents infection of beta, alpha and other cell subsets, and we have generated ACE2-deficient beta-like cells to further define a presumably irreducible role for ACE2 in productive SARS-CoV-2 infection of beta and other pancreatic cells. Lastly, scRNAseq analyses are ongoing; infected and control pancreatic tissue slices have been generated to visualize the in situ extent and distribution of viral antigen; and TCS of infected and control islets has been collected for Olink analyses.

CONCLUSIONS

Human beta and other pancreatic cell subsets can be readily infected in vitro with SARS-CoV-2 in an ACE2-dependent manner. However, the extent of infection is limited, and the molecular, phenotypic and functional consequences are expected to have only a minor impact on overall beta cell activity. Together with the restricted ACE2 expression in pancreatic histology specimens, it would therefore appear unlikely that direct targeting of beta cells by SARS-CoV-2 contributes to the enhanced development of diabetes.

Abstract #52

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

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PURPOSE

A goal of type 1 diabetes (T1D) research is to understand the complex islet microenvironment to determine the mechanisms of autoimmune-mediated beta cell dysfunction and loss. Here, we investigate the use of live pancreas tissue slices as a genuine and native three-dimensional microenvironment in the study of the islet-immune cell interface within the context of type 1 diabetes. In this system, endogenous tissue-resident or pathological immune cells can be studied in slices to identify translatable differences between donors at varying levels of T1D risk and development. Additionally, islet functionality at various disease stages can be assessed through Ca2+ imaging and dynamic insulin secretion assays. This approach allows for observations of islet health and function under insulitic conditions and can provide insights into disease mechanisms and progression.

METHODS

Live human pancreas tissue slices were prepared from donors without diabetes or diabetes-relevant autoantibodies (ND, n=9), autoantibody-positive donors without a diagnosis of T1D (AAb+, n=5), and donors with short duration of 4 years or fewer T1D (T1D+, n=3) through methods co-developed by the nPOD/OPPC at the University of Florida and the Paul Langerhans Institute Dresden. Slices were imaged by time-lapse confocal microscopy on a Leica SP8 confocal equipped with an automated stage and incubation system. Live human pancreas tissue slices were stained with anti-CD3-APC and HLA-multimers to identify and track endogenous T cells within the tissue enabling the determination of in situ T cell activity around and within islets. Reflected light was used to locate islets due to their granularity from insulin content. An anti-ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3) antibody was used to identify islets with beta cells. Beta cell functionality was assessed through changes in intracellular Ca2+ concentrations through the use of Fluo-4 Ca2+ indicator dye. Overall, the viability and state of islets within the slices were determined through SYTOX Blue staining for viability, anti-ENTPD3 staining for beta cell identification, Ca2+ imaging for functionality, and staining with anti-CD3 and HLA multimers for T cell identification.

In addition to human pancreas tissue slices, live mouse pancreas tissue slices were prepared from NOD-Rag1-/- and NOD-Rag1-/--AI4α/β TCR transgenic (NOD.AI4) mice. Endogenous T cells were stained with anti-CD3-APC and anti-CD8-APC and tracked in the tissue. Oregon Green 488 BAPTA-1 Ca2+ indicator dye was used to stain the mouse slices in order to record changes in intracellular Ca2+ concentrations. Perifusion experiments were also performed on the slices and insulin secretion traces will be obtained from hormone measurements using ELISAs.

SUMMARY OF RESULTS

Slices generated from ND donors had functional beta cells and few T cells in the immediate vicinity of most islets. An nPOD donor representative of this trend is case 6516 (ND). Islets from case 6516 exhibit strong Ca2+ responses following either 16.7 mM glucose or 30 mM KCl stimulations. Following perifusion, slices in this case produced insulin peaks following high glucose and KCl stimulations. Similarly, in AAb+ tissues, islets had strong Ca2+ responses to 16.7 mM glucose and KCl, as was noted in a GAD AAb+ case 6530. Again, insulin peaks during perifusion were recorded following high glucose and KCl stimulations. In contrast, islet functionality decreased in T1D+ slice cases. For example, T1D+ case 6533 had some T cell infiltrated islets with ENTPD3+ cells that were unresponsive to high glucose but maintained KCl responses. Within this same case, an ENTPD3+ islet without infiltrating T cells was observed that retained both high glucose and KCl responses. Insulin traces following perifusion from this case were abnormal with a small peak in low glucose and a strong peak following KCl stimulation, indicating dysfunctional glucose responsiveness, but residual insulin secretory capacity. This suggests a possible role for infiltrating T cells in beta cell dysfunction prior to beta cell killing.

Additionally, T cell populations were identified more specifically through co-staining with antibodies and HLA multimers. Slice studies using these multimers identified IGRP and GAD reactive T cells in or near islets from slices of recent onset human T1D organ donors. When endogenous CD3+ cells were examined in live pancreas slices from a GAD AAb+ donor with T1D case 6469, one islet was found with >18 CD3+ T cells forming an apparent focal insulitis. To our knowledge, this is the first image of live, endogenous human immune cells attacking live insulin-producing beta cells from an autoantibody-positive organ donor with T1D in situ. Single cell RNAseq was applied to slices from T1D+ case 6472 and autoreactive T cell receptors specific to GAD were identified. Confirmatory studies using mouse pancreas tissue studies support the finding from human slices that the presence of T cells in the islet coincides with poor islet glucose sensitivity. Islets in slices from NOD.AI4 experiencing heavy insulitis exhibited no Ca2+ responses to high glucose. These same islets also were found to have lost all

reflectivity. Conversely, islets from T1D-prone NOD.AI4 mice with few to no T cells as well as from non-diabetic NOD-Rag1-/- mice continued to have Ca2+ responses to both high glucose and KCl.

CONCLUSIONS

Endogenous immune cell activity can be observed within live human and mouse pancreas tissue slices. Features such as in situ islets and endogenous immune cells remain intact. T cell behaviors and interactions in and around the islet can be recorded. The impacts of T cell activity on beta cells can be determined through functional studies such as Ca2+ recordings and perifusion experiments. Through the application of the slice method and the techniques discussed above, we have observed impaired beta cell function in response to glucose stimulation in the presence of T cells. Though this observation will need to be supported by future experiments, these studies will help to give a clear idea of what is occurring within the pancreas at different disease stages and the functional impacts on beta cells that these events cause. These studies will be critical in understanding if T cells cause beta cell dysfunction during T1D progression or only beta cell loss. This technology has strong potential as a fully human platform for understanding the etiopathology of T1D and testing interventional therapies that act in the local islet immune microenvironment. These initial successes are guiding our development of pancreas slices for further detailed three-dimensional studies of islet—immune cell interactions relevant to human T1D.

Abstract #53

x-mAb produced by dual expresser lymphocytes identifies a universal autoreactive T cell receptor

Authors

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PURPOSE

Despite significant advances in understanding pathogenesis of T1D, our knowledge about autoreactive T cells and mechanisms underlying their activation remains incomplete. Key questions include whether there are public TCRs and their roles in driving the autoimmune processes. Also unclear is how islet autoantigens with characteristically weak stimulatory ability drive pathogenic T cells to potently attack and destroy insulin-producing pancreatic beta cells. *We hypothesize that dual expressers (DEs), a recently discovered hybrid adaptive immune cell that coexpresses TCR and BCR, plays a key role in the diabetogenic process by production of a unique diabetogenic germline-encoded antibody (x-mAb) that cross-activates pancreatic islet-reactive T cells*. In T1D patients, DEs are predominated by a public BCR clonotype (x-mAb) that is autoreactive as demonstrated by the ability to stimulate autologous T cells, including insulin-reactive T cells. The purpose of this study is to phenotype x-mAb-reactive T cells, analyze their TCR repertoires and relevance to the disease process.

METHODS

We used EBV-transformed DE cell that produce x-mAb, affinity chromatography, immunoprecipitation, Western blot, flow cytometry, high-throughput sequencing and public database search using new comprehensive site http://bioinfo.life.hust.edu.cn/TCRdb/# and http://clonesearch.jdrfnpod.org/.

SUMMARY OF RESULTS

Using x-mAb produced by an EBV-transformed DE clone, we identified a distinct subpopulation of autologous T cells that are recognized by x-mAb in T1D patients. Immunoprecipitation and western blot analyses showed that the x-mAb interacts with T cells directly through the TCR. X-mAb-reactive T cells (hereafter called IgM+) are antigen-experienced and included CD4 and CD8 T cells. High-throughput analysis of TCR repertoires of IgM+ T cells (n= 7 subjects) revealed oligoclonal TCR repertoires that are enriched for few public TCR clones. Most striking, public database search of the new comprehensive site http://bioinfo.life.hust.edu.cn/TCRdb/#/search show that key enriched IgM+ clones are widely present in T1D and other autoimmune diseases, cancers, and cancer patients treated with checkpoint inhibitors (some develop overt T1D). We identified one particular IgM+ clone that we defined as a “universal public TCR”. This designation is based on detection of this clone 3173 times in published repertories and 99 times in repertoires of 99 nPOD subjects (mainly in PLNs. Strikingly, these clones, including the public clone, are enriched several orders of magnitude in

frequency among IgM+ T cells as compared to autologous IgM- T cells and T1D as well as published results in other different diseases.

CONCLUSIONS

We conclude that x-mAb identifies widely used public TCR across species and diseases. We propose a two-hit model for natural progression of TID whereby islet-reactive T cells are first primed by islet specific autoantigens and then potently stimulated by x-mAb upon activation of DEs by exogenous antigen to drive disease pathogenesis. If proved correct, this could lead to the development of new specific immunotherapies that spare useful T cells.

Abstract #54

The (sugar) Science

Authors

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PURPOSE

Despite the(sugar)science is a dynamic, collaborative scientific community that transcends institutional and geographic boundaries to bring together researchers, information, and funding resources to advance the treatment and cure for Type 1 Diabetes (T1D).

METHODS

We spoke to T1D focused scientists from around the world and asked what they would like to see in a digital collaborative platform. We gained input from early career scientists, graduate students, post-docs, and thought leaders in the field. Feedback from clinical researchers and academics was also considered. Our team designed a digital platform to reflect the specific needs of these scientists, and we launched www.thesugarscience.org on July 1, 2020.

SUMMARY OF RESULTS

In the last month, our platform has gained 1.7K global visitors who are eager to participate in our growing ecosystem. Our team has produced over 60 podcasts, 80 woven topics blogs, and several video webinars. Metrics show our website and socials are growing at a robust rate. the(sugar)science currently has 45.5K tweet impressions in the past 7 days alone. the(sugar)science is the first and only scientific social and collaborative network. We provide a space for scientists to connect and discover creative synergies across T1D disciplines in a secure, private “ethical oasis”. We are committed to never sharing our user’s personal data.

In addition to our social network, we publish curated newsletters, resource links, and podcasts. We conduct informative and original events, including:

- "Ask the Expert" Digital Cafes - Live opportunities for early-career scientists to listen to and interact with thought leaders in the T1D research field
- "Off the Record" Digital Salons - Private, interdisciplinary conversations for scientists to discuss topics pertaining to T1D in a secure, unrecorded, digital venue
- Pitch Competitions - Bi-annual events to showcase young researchers who pitch research ideas, win cash prizes, and meet esteemed members in the field of T1D science

The(sugar)science has continued to receive positive feedback and endorsements from across the scientific community, which further confirms this platform is filling an unmet need.

CONCLUSIONS

The(sugar)science is creating a novel scientific ecosystem to foster connection and collaboration in the T1D research community. The pandemic has served to demonstrate that global collaboration can accelerate research and drive clinical outcomes. As the market for Type 2 diabetes grows exponentially, it is critical to continue to recruit, connect, and leverage the best and brightest minds to study Type 1 diabetes and to continue to momentum in research for this 24/7/365 chronic disease. One hundred years after the discovery of insulin, the time is now to create a new paradigm for discovery of a cure for autoimmune diabetes. the(sugar)science is providing such a paradigm.