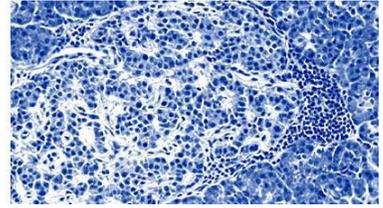




nPOD

Network for Pancreatic Organ
Donors with Diabetes



BETA CELL DEVELOPMENT, DIFFERENTIATION, REGULATION

O1: Loss of Hypusine Biosynthesis in the Developing β -cell Results in Diabetes Onset in Young Mice

AUTHORS

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PURPOSE

Diabetes is a chronic disease characterized by the destruction or dysfunction of the insulin-producing pancreatic β -cells. The development of novel cellular therapeutics to prevent or treat diabetes requires a comprehensive understanding of pathways regulating β cell fate. To date, many studies have focused on the transcriptional cascades that instruct β cell development and function; however, the role of mRNA translation in the formation of functional β cells is not well known. Eukaryotic initiation factor 5A (eIF5A) functions during mRNA translation to promote the elongation of a largely undefined subset of transcripts. To engage its function in translation, a specific lysine residue in eIF5A must be post-translationally modified (“hypusinated”) by the enzyme deoxyhypusine synthase (DHPS), which generates the active or hypusinated form of eIF5A (eIF5A^{Hyp}). Interestingly, treatment with N1-Guanyl-1,7-diaminoheptane (GC7), which inhibits DHPS and hypusine biosynthesis, has been shown to improve the diabetic phenotype in a mouse model of type 1 diabetes.

The purpose of this study is to evaluate the role that hypusine biosynthesis plays during β -cell development.

METHODS

We created and analyzed a mouse model harboring a β -cell-specific deletion of *Dhps* (hereafter referred to as *Dhps*-KO^{beta}). Recombination was confirmed to be specific and robust with the use of a Cre-dependent reporter (*R26R^{Tomato}*). For all analyses, *Dhps*-KO^{beta} animals were compared with littermate controls (*Dhps^{fl/fl}* and *Dhps^{+/+};Ins1-Cre⁺*). Fed blood glucose and body weight measurements as well as histological analysis was performed on all groups at 1 week of age. In a different cohort of mice, we measured fed blood glucose and body weight in animals from 3 to 6 weeks of age. Results from these longitudinal measurements suggested that glucose tolerance tests (GTTs) were required in animals at 4 weeks of age. Pancreas tissue was obtained at 4 and 6 weeks of age for histological analysis.

SUMMARY OF RESULTS

Compared with controls, *Dhps*-KO^{beta} mice at the 1-week of age showed no change in body weight and fed blood glucose. Similarly, the *Dhps*-KO^{beta} animals demonstrated normal body weight and fed blood glucose levels at weaning (3 weeks) and 4 weeks of age. In contrast, *Dhps*-KO^{beta} animals displayed hyperglycemia at 5 weeks of age, which was further increased at 6 weeks of age. GTTs performed at 4 weeks of age showed *Dhps*-KO^{beta} mice to be glucose tolerant. Preliminary data suggest that the complement of insulin-expressing β -cells is similar between the control and *Dhps*-KO^{beta} mice at 1 and 4 weeks of age; however, fewer beta cells were observed at 6 weeks of age.

CONCLUSIONS

Deletion of *Dhps* in the developing β -cells leads to the rapid onset of hyperglycemia and diabetes in mice between 4 and 5 weeks of age. These data suggest that hypusine biosynthesis and subsequent eIF5A-dependent mRNA translation may be critical as β -cells mature. As our mutant mouse displays a reproducible onset of diabetes, ongoing studies are utilizing this model to understand how β -cells progress from a prediabetic to a diabetic state.

O2: RNAseq of carbonic anhydrase II (CAII)-negative human major pancreatic ductal cells confirms progenitor-like phenotype

AUTHORS

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PURPOSE

We have previously described a population of cells with progenitor-like characteristics within the major pancreatic ducts (MPDs) of the human pancreas. These cells are characterized by the expression of PDX1, its surrogate surface marker P2RY1, and the BMP receptor 1A (BMPR1A), also known as activin-like kinase 3 (ALK3). However, they do not express carbonic anhydrase II (CAII), a ductal differentiation marker. Active BMP signaling induces their proliferation, and the withdrawal of ALK3 agonists is permissive for their multipotent differentiation. PDX1⁺/ALK3⁺/CAII⁻ cells are also present in non-human primates, suggesting evolutionary conservation. Here we set out to further investigate the phenotype of these cells by RNAseq.

METHODS

MPDs typically harbor both PDX1⁺/ALK3⁺/CAII⁻ and PDX1⁺/ALK3⁺/CAII⁺ cells, often in an interspersed pattern. Using for the first time in pancreatic tissue the Method for Analyzing RNA following Intracellular Sorting (MARIS), here we report on the RNAseq signature of both populations.

SUMMARY OF RESULTS

Our studies confirm that PDX1⁺/ALK3⁺/CAII⁺ cells are archetypal mature ductal cells, with high expression of all the expected markers (SOX9, KRT19, FOXA2, ONECUT1, HNF1B, etc.). In contrast, the expression of most ductal genes is downregulated in the PDX1⁺/ALK3⁺/CAII⁻ population. Gene Ontology (GO) analysis also indicates that CAII⁻ cells exhibit differential upregulation of numerous progenitor cell-related pathways. Interestingly, we also observed a significant upregulation of cellular stress response pathways in CAII⁻ vs. CAII⁺ cells without concomitant upregulation of senescence or cell death programs, which rules out the possibility that CAII⁻ cells are apoptotic or otherwise impaired. Stress has been widely linked to de-differentiation of cells in several organ systems, including the pancreas. This observation opens the door to the alternative notion that PDX1⁺/ALK3⁺/CAII⁻ may be regular ductal cells that have de-differentiated and

acquired progenitor-like multipotency in response to a yet-to-determine stimulus. Ongoing single-cell RNAseq analyses are expected to shed additional light on the biology and origin of these cells.

CONCLUSIONS

Our analyses confirm the progenitor-like phenotype of a specific subpopulation of major ductal cells in the human pancreas. Their responsiveness to ALK3 agonists could be the basis of novel *in situ* regenerative therapies.

O3:

Dual DYRK1A-GLP1R Modulation Synergistically and Dramatically Increases Human Beta Cell Proliferation and Numbers in vivo and in vitro In Normal Islet Donors and in Type 2 Diabetes.

AUTHORS

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**please let the Program Committee members/reviewers know that the first author is a graduate student, so it might be appropriate to include this I an a session for next-gen researchers.

PURPOSE

There is a compelling need in T1D and T2D to identify drugs that can induce human beta cells to proliferate and to expand beta cell numbers, while retaining differentiated secretory function. Drugs that inhibit the kinase, DYRK1A, are able to induce low rates of human beta cell proliferation, but greater rates would be preferable. Further, there is need to deliver mitogenic drugs for diabetes in a way that specifically targets human beta cells. With these goals in mind, we explored the combination of DYRK1A inhibitors (harmine, INDY, leucettine, 5-IT, GNF4877) with a broad range of GLP1 receptor (GLP1R) agonists, several of which are in widespread clinical use (GLP1, exenatide, liraglutide, lixisenatide, semaglutide).

METHODS

Human islets from 81 normal donors and 11 T2D donors were studied using Ki67- and BrdU- co-staining with insulin, under control conditions, in response to the panel of DYRK1A inhibitors, in response to the panel of GLP1R agonists, and assessed for Ki67/BrdU insulin co-immunolabeling, as well as FACS-quantification of human beta cells. Beta cells derived from human ES cells were also studied for increases in beta cell numbers. Intracellular calcium and cAMP signaling pathways were also assessed. Assessment of beta cell differentiation and function was assessed through markers of differentiation (qPCR and immunocytochemistry) and GSIS.

SUMMARY OF RESULTS

GLP1R agonists, as expected, had no effect on human beta cell proliferation. Harmine and other DYRK1A inhibitors (INDY, leucettine, 5-IT, GNF4877) induced Ki67 and BrdU incorporation in 2-3% of beta cells. Remarkably, the combination of ANY DYRK1A inhibitor with ANY GLP1R agonist led to dramatic further increases in human beta cell Ki67 labeling, averaging 3-6%, and reaching 15-20% in some islet preparations. This occurred with the combination of DYRK1A inhibitors with liraglutide, exenatide, lixisenatide, and semaglutide, drugs currently in widespread clinical use. True synergy was observed, both at high doses of GLP1R agonists and DYRK1A inhibitors, and also with low doses of each drug that have no effect on their own. Notably, the increase in beta cell proliferation *markers* was accompanied by a readily measurable 30-50% increase in beta cell *numbers* in both human islets as well as in hESC-derived beta cells. The mechanism underlying the synergistic effects requires both cAMP-PKA-EPAC2 as well as DYRK1A signaling. Concerned that proliferation might lead to beta cell de-differentiation, we explored markers of differentiation (NKX6.1, MAFA, PDX1, INS, PCSK1, SLC2A2, GLPR, etc) by qPCR and immunohistochemistry and GSIS. Rather than reducing differentiation markers, these actually increased, implying enhanced beta cell differentiation. Finally, the synergistic increase in proliferation was observed not only in islets from normal donors, but also in islets from donors with T2D.

CONCLUSIONS

Combination of ANY DYRK1A inhibitor with ANY one of a widely used class of diabetes drugs, the GLP1R agonist class, converts the GLP1R class from drugs that are unable to activate human beta cell proliferation, into drugs that induce therapeutically relevant rates of human beta cell proliferation. These rates into authentic increases in beta cell numbers, while enhancing beta cell differentiation. Importantly, GLP1R agonists provide a degree of beta cell specificity to proliferation not previously achieved. Remarkably, these beneficial effects on proliferation and differentiation occur not only in beta cells from normal donors, but also in islets from donors with T2D.

O4:
**Single-Cell Analysis Of Human Pancreatic α And β Cell
Maturation Provides Insights Into Molecular Mechanisms
Underlying Type 2 Diabetes**

AUTHORS

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PURPOSE

Multiple mechanisms including β -cell death or dedifferentiation and α -cell dysregulation have been suggested to explain reduced β cell mass and islet dysfunction in type 2 diabetes (T2D). Recently, an alternative pathway has been invoked that involves β -cell de-differentiation or even complete loss of β -cell identity – but not cell death – to explain the reduced functional β -cell mass in T2D. However, only limited evidence is available supporting the notion of transient or permanent dedifferentiation of human β -cells during the pathogenesis of diabetes and fundamental questions remain as to the precise nature of these cellular states, their prevalence, level of plasticity and functional outcome.

METHODS

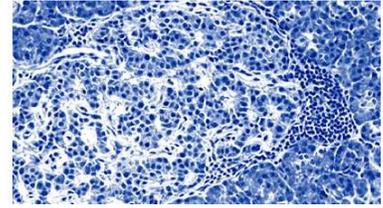
We exploit high-depth single cell RNAseq analysis of human pancreatic β - and α -cells during ontogeny and in T2D to detail the molecular programs driving the identity loss and dysfunction of diabetic β - and α -cells.

SUMMARY OF RESULTS

Our analysis shows that human α - and β - cells undergo distinct maturation processes. Remarkably, a fraction of T2D β -cells closely resemble β -cells from the neonatal organ donor, strongly suggesting either a restoration of an immature state or neogenesis of new β cells. Further, T2D α -cells express high levels of G6PC2, which likely contributes to glucagon hypersecretion.

CONCLUSIONS

We propose de-maturation, without a loss of lineage identity, of β -cells as an alternative path to islet dysfunction. Furthermore, we describe a possible molecular mechanism in α cells, which may contribute to the pathologic hyperglycagonemia that occurs in diabetics.



(LB-14)

Combining gene editing and human stem cells to model diabetes due to HNF1A deficiency

AUTHORS

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PURPOSE

MODY3 is a form of Maturity Onset Diabetes of the Young that accounts for 60% of cases of MODY and is caused by mutations of the *HNF1 α* gene, leading to impaired β -cell mass and reduced insulin production. HNF1 α is a transcription factor known to play an important role in β -cell development, proliferation, viability and glucose sensing/insulin release. It is likely that subtler derangements of the function of these genes and their pathways account for some proportion of susceptibility to more prevalent forms of diabetes. By generating insulin-producing pancreatic β -cells from MODY3-patient's iPSCs and *HNF1 α* mutant hESCs, we can characterize the cellular and molecular defects due to HNF1 α deficiency and thus get a better understanding of the mechanisms underlying β -cell development, function and survival.

METHODS

To this purpose, by using genome-editing CRISPR/Cas9 technology, I have generated mutation-corrected and homozygous mutant cell lines for *HNF1 α* on the same genetic background. Gene manipulation of *HNF1 α* by CRISPR/Cas9 technology was also performed in a GFP-reporter hESCs cell line that harbors a GFP gene knock-in in one INS locus to enable

isolation of viable INS-GFP+ cells *in vitro* and *in vivo*. I will generate glucose-responsive pancreatic β -cells from these isogenic genotypes and characterize the cellular and molecular phenotypes caused by *HNF1a* deficiency using the following assays: 1) measurement of insulin processing, storage and secretion; 2) measurement of cell proliferation and apoptosis by BrdU/Ki67 and TUNNEL staining; 3) global transcriptional analysis by global and single-cell RNAseq after sorting for INS-GFP+ cells; 4) transplantation of mutant and “corrected” β -cells into mice to assess insulin secretion *in vivo*.

SUMMARY OF RESULTS

Preliminary results show that *HNF1a* mutant β -cells display increased insulin content and reduced basal insulin secretion *in vitro*. RNAseq analysis indicates that genes involved in β -cell function and development are major players in this phenotype. Mutant β -cells have an impaired insulin secretion *in vivo* and fail to restore normoglycemia in a STZ-induced diabetes mouse model.

CONCLUSIONS

These studies should advance treatment of this relatively rare disorder (<5% of non-immune diabetes), but also provide insights relevant to more prevalent forms of diabetes. Such cells might ultimately be used for transplantation therapies.

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<input type="checkbox"/> Core Lab	<input type="checkbox"/> Type 1 Diabetes Etiology & Environment
<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):

BETA CELL PHYSIOLOGY, DYSFUNCTION

O5:

SNAP-23 depletion in pancreatic β -cells paradoxically promotes insulin secretion, improving glucose homeostasis in diabetes

AUTHORS

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PURPOSE

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins synaptosomal protein of 25 kD (SNAP25), syntaxin and synaptobrevin assemble into a SNARE complex to mediate membrane fusion including exocytosis (i.e. insulin secretion). SNAP23 is the ubiquitous SNAP25 isoform in non-neuronal cells that mediates secretion similar to SNAP25 in neurotransmitter release. However, some secretory cells such as pancreatic islet beta-cells contain abundance of both SNAP25 and SNAP23, where SNAP23 is believed to play a redundant role to SNAP25. Here, we show that SNAP23 actually plays an inhibitory role to SNAP25, by binding the exocytotic partners that SNAP25 bind to, and thus SNAP23 depletion facilitated SNAP25-mediated insulin secretion. In type 1- and type 2 diabetes wherein beta-cell is reduced in mass and secretory efficiency, respectively, blocking SNAP23 or reducing SNAP23 levels in beta-cells could enhance insulin secretion to improve glucose homeostasis.

METHODS

SNAP23 flox mice were injected with AAV8-RIP-Cre to delete beta-cell SNAP23. Glucose homeostasis determined by IPGTT and ITT were performed; islets then isolated for islet perfusion glucose-stimulated insulin secretion (GSIS) assay and single beta cell TIRF microscopy to assess insulin granule exocytosis. SNAP23 were depleted in normal and T2D human islet beta-cells by an shRNA virus to assess insulin secretion, and examined by electrophysiology to assess calcium sensitivity of exocytosis and calcium channel activity. Immunoprecipitation of INS cells assessed the exocytotic proteins pulled down by SNAP25 and SNAP23. Superresolution TIRF microscopy assessed whether SNAP23 deletion or overexpression would influence SNAP25 interaction with the voltage-gated calcium channel (Cav1.2) and insulin granule approach to the Cav1.2. Finally, the SNAP-23 shRNA virus was injected retrograde into T2D Goto-Kakizaki rats (wherein islet defect rather than insulin

sensitivity is the cause of the T2D) pancreatic duct to infect the islets *in vivo* and see if this can affect insulin secretion and improve glucose homeostasis over a period of several months.

SUMMARY OF RESULTS

SNAP23 depletion in mouse beta-cells *in vivo* and human beta-cells (from normal and T2D patients) *in vitro*, surprisingly greatly increased first- and second-phase GSIS corresponding to increases in exocytosis of predocked and newcomer insulin granules we've previously showed to be mediated by distinct SNARE complexes. This increased secretion by SNAP23 depletion of human beta cells was not due to changes in calcium sensitivity of exocytosis or on Cav1.2 channel activity. SNAP25 and SNAP23 seems equal in forming SNARE complexes with syntaxins and synaptobrevins and could block each other in this action. However, we found SNAP25 binds different Caves better than SNAP23, and is also able to better bind priming (Munc13, RIM2) and calcium sensor (synaptotagmins-1 and -7) proteins; and the levels of these large SNAP25 exocytotic complexes increased with stimulation. Mechanistically, SNAP23 depletion promoted more SNAP25 molecules to travel faster to bind Cav1.2 and staying longer on Cav1.2, which results in more insulin granules recruited to dock and fuse at the Cav1.2 sites. Finally, depleting islet SNAP23 by an shRNA virus infused into T2D Goto-Kakizaki rat pancreatic ducts greatly improved GSIS and glucose homeostasis which was superior in efficacy and more sustained (several months) than conventional treatment with sulfonylurea glybenclamide.

CONCLUSIONS

SNAP23, although fusion-competent in slower secretory cells, in the context of the beta-cell, SNAP23 acts as a weak partial fusion agonist or an inhibitory SNARE, whereby SNAP23 depletion promotes the efficiency of endogenous SNAP25 to form more fusion-competent complexes with cognate SNAREs, priming factors and calcium sensors, which serve to recruit insulin granules to Caves where exocytotic fusion occurs, thus rendering them more fusion-competent. SNAP23 depletion or blockade may be a strategy to treat T2D and also T1D, as well as other deficient secretory disorders.

O6: GENE EXPRESSION PATTERNS IN SINGLE ISLETS FROM ORGAN DONORS WITH OR WITHOUT DIABETES

AUTHORS

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PURPOSE

Type 1 diabetes (T1D) is the result of immune cell mediated destruction of insulin producing pancreatic islet beta cells. Preclinical disease progression is usually characterized by the appearance of autoantibodies in a patient's serum. Single autoantibody positive individuals (sAb+) have a lower chance of progression to overt disease, compared to multiple autoantibody positive individuals (mAb+). Changes in gene expression patterns of the islet cells at different preclinical stages may help gain a better understanding of the pathogenesis and its progression.

METHODS

We collected individual pancreatic islets by laser-capture microscopy from multiple sAb+, mAb+, and T1D organ donors. We obtained comprehensive gene expression information by microarray analyses from each islet and compared expression data from all insulitic islets (those staining positive for insulin and CD3 T-cells) within the three donor cohorts. We then obtained lists of differentially expressed genes between each donor cohort using a Student's t-test and performed data mining techniques using an online modern bioinformatics toolset.

SUMMARY OF RESULTS

Insulitic islets from sAb+ donors, when compared to T1D donors, were found to express higher levels of transcripts relating to mitochondrial structure and oxidative phosphorylation as well as genes involved in immune activity and activation. Interestingly, however, mAb+ donors, when compared to T1D donors, did not express significantly higher levels of mitochondrial genes and instead, showed a difference in expression of genes from pathways involved with immune activity and activation. When comparing sAb+ and mAb+ donors, we found the former expressed higher levels of mitochondrial genes, many of which overlapped with those differentially expressed in sAb+ versus T1D. sAb+ and mAb+ donors did not exhibit significant differences in expression of immune activity genes.

CONCLUSIONS

This data suggests that, in a timeline for insulitic islets, mitochondrial genes change expression between progression from sAb+ to mAb+ status, while mAb+ to T1D progression is marked by altered expression of immune activity and activation markers in insulitic T1D islets.

O7: ACTIVATION OF TOLL LIKE RECEPTOR PATHWAYS IN INSULITIC ISLETS OF TYPE 1 DIABETES

AUTHORS

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PURPOSE

Type 1 diabetes (T1D) is an autoimmune condition, long hypothesized to be enhanced or triggered by certain viral infections. Islet pathology of T1D is characterized by destruction of insulin producing pancreatic beta cells. The purpose of this study was to identify specific pathways with differential expression of host genes in islets to identify evidence suggesting responses to a viral infection.

METHODS

Pancreatic tissue samples were obtained from non-diabetic donors (controls), autoantibody positive donors, and donors with T1D through the Network for Pancreatic Organ donors with Diabetes (nPOD) program. Laser microdissection was used to manually isolate individual islets based on presence or absence of insulin, with T-lymphocyte RNA isolated and microarray used to assess transcriptomes. Using Student's t-tests, we analyzed expression levels of genes known to be involved in host response to viral infection. After finding increased expression of *TLR2* in T1D islets versus controls, we used anchored analysis to identify other genes with similar expression patterns. These were then cross-referenced to confirm relationships with TLR pathways.

SUMMARY OF RESULTS

TLR2 showed higher expression in T1D compared to controls ($p=8.46E-5$) and insulinitic (Insulin+CD3+) islets compared to controls ($p=3.8E-3$). Using *TLR2*, an anchored analysis yielded ~100 genes with an R value of >0.65 . Among these were several involved in the TLR signaling pathways. Increased expression in INS+CD3+ vs INS+CD3- islets was seen in a variety of genes including TLR pathway inhibitors: *LAIR1* ($p=2.77E-8$), *SIGLEC7* ($p=8.33E-6$), *SIGLEC9* ($p=1.59E-4$), and *C1q* ($p=6.11E-16$); as well as genes downstream of the TLR pathway: *CD163* ($p=2.34E-11$), *CTSS* ($p=9.89E-7$) and *IFN γ* ($p=5.9E-8$).

CONCLUSIONS

TLRs are very important for host response to infections. *TLR2* has been classically shown to have bacterial ligands; in vitro studies show *TLR2* can be activated by viral particles. Much of the TLR pathway is regulated by phosphorylation or ubiquitination and may not be upregulated at the RNA level. However, we demonstrate here that specific genes involved in *TLR2* signaling are up-regulated in T1D islets, specifically in those with signs of immune activation and residual insulin positivity (INS+CD3+). This suggests the potential involvement of host viral response pathways in the development of T1D.

O8: Fenofibrate increases very long-chain sphingolipids and prevents loss of islet sympathetic nerves in NOD mice

AUTHORS

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PURPOSE

Sphingolipid metabolism is abnormal at the onset of type 1 diabetes and is known to regulate beta-cell biology and inflammation. Fenofibrate, a regulator of sphingolipid metabolism, is known to prevent diabetes in NOD mice. In order to promote fenofibrate for use in human type 1 diabetes patients, we investigated the effect of fenofibrate on the pancreatic lipidome, pancreas morphology and blood glucose homeostasis.

METHODS

The pancreatic lipidome was analysed using mass spectrometry. Analysis of pancreas and islet volume was performed by stereology. Islet sympathetic nerve volume was evaluated by tyrosine hydroxylase staining. Effect on blood glucose homeostasis was assessed by measuring non-fasting blood glucose from age 12 to 30-weeks of age. Furthermore, we measured glucose tolerance, fasting insulin and glucagon levels and HOMA-IR.

SUMMARY OF RESULTS

We find that fenofibrate selectively increases the level of very long-chain sphingolipids in the pancreas of NOD mice. Furthermore, we find that fenofibrate causes a remodelling of the pancreatic lipidome leading to an increased amount of lysophospholipids. Fenofibrate did not affect islet or pancreas volume but lead to an increased amount of islet sympathetic nerves. Fenofibrate treated NOD mice had a more stable blood glucose which was associated with a reduced non-fasting and increased fasting blood glucose. Furthermore, fenofibrate improved glucose tolerance, reduced fasting glucagon levels and prevented fasting hyperinsulinemia.

CONCLUSIONS

These data show that fenofibrate alters the pancreatic lipidome to a more anti-inflammatory and anti-apoptotic state. The beneficial effect of islet nerves and blood glucose homeostasis suggest that fenofibrate treatment could be used as a therapeutic approach to improve blood glucose homeostasis and in the prevention of diabetes-associated pathologies.

O9: Type 1 Diabetes Development is Exacerbated Following Loss of the Sarco/Endoplasmic Reticulum Ca²⁺ ATPase

AUTHORS

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PURPOSE

Emerging data has identified a role for β -cell endoplasmic reticulum (ER) stress during the development of type 1 diabetes (T1D); however, the molecular pathways leading to β -cell ER stress in T1D have not been fully defined. ER Ca²⁺ homeostasis plays a central role in the regulation of ER health and function, while loss of ER Ca²⁺ has been shown to lead to β -cell ER stress. Intraluminal ER Ca²⁺ stores are closely regulated by activity of the sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA2) pump, which transports Ca²⁺ into the ER to maintain a steep Ca²⁺ gradient between the ER and cytosol. We and others have found that β -cell SERCA2 expression is decreased under pro-inflammatory stress conditions and in rodent and human models of T1D. Therefore, we hypothesized that SERCA2-mediated ER Ca²⁺ dyshomeostasis may contribute to T1D development.

METHODS

A mouse model haploinsufficient for SERCA2 was generated on the NOD background (NOD-S2^{+/-}) to test how loss of SERCA2 impacts T1D development. A multiple-low-dose streptozotocin (MLD-STZ) regimen was utilized in WT and SERCA2 haploinsufficient (S2^{+/-}) C57BL/6 mice to mimic β -cell loss in T1D, followed by administration of an allosteric SERCA2 activator (CDN1163). A SERCA2 knockout (S2KO) INS-1 cell line was generated using CRISPR-Cas9 technology, and a β -cell specific SERCA2 knockout mouse was generated on the C57/BL6 background to identify molecular mechanisms of how SERCA2 deficiency contributes to β -cell dysfunction in T1D.

SUMMARY OF RESULTS

Compared to wild type littermate controls (NOD-WT), NOD-S2^{+/-} mice exhibited a higher incidence of diabetes development (100.0% versus 80.0%; $p < 0.001$) and the onset of diabetes was accelerated (14-wk versus 19-wk; $p < 0.001$). SERCA2 activation using CDN1163 improved Ca²⁺ dynamics in isolated islets from both NOD-WT and NOD-S2^{+/-} mice. S2^{+/-} mice treated with MLD-STZ exhibited higher fasting glucose levels, increased circulating ratios of proinsulin/insulin (an analog for *in vivo* islet ER stress), and significantly worsened glucose tolerance compared to WT mice. Furthermore, MLD-STZ mice treated with CDN1163 exhibited improved glucose tolerance and reduced fasting glucose. Isolated islets from S2KO mice and S2KO INS-1 cells exhibited an increase in the spliced/unspliced XBP-1 ratio, impaired proinsulin processing, and impaired expression, maturation, and trafficking of prohormone convertase 1/3 and 2.

CONCLUSIONS

Taken together, our data suggests that β -cell SERCA2 loss leads to impaired proinsulin processing, activation of β -cell ER stress, and exacerbated T1D development, indicating that SERCA2 may serve as a novel therapeutic target for diabetes reversal.

O10: Role of Autophagy in β -cell homeostasis and Type I diabetes development

AUTHORS

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PURPOSE

Autophagy is a key mechanism to generate energy and facilitate recycling of damaged proteins and organelles, and is important in the adaptive response to cell stress to restore beta cell homeostasis. Loss of autophagy is associated with beta cell dysfunction and death. Accumulating evidence has implicated impairment of beta cell autophagy in the development of type 2 diabetes. However, the role of autophagy in type 1 diabetes is relatively unexplored. The purpose of our study is therefore to analyze autophagy as a function of type 1 diabetes development.

METHODS

We initially utilized immunofluorescent (IF) staining of Lamp1 (lysosomal marker), LC3 (autophagosome marker), Insulin or Pro-insulin and DAPI to analyze autophagy in both diabetic and non-diabetic NOD mice. Similarly, IF staining was performed on islets of non-diabetic human pancreas and on islets of type 2 diabetic human organ donors. All images were collected on Zeiss LSM 700 confocal microscope and analyzed using Zen software (Zeiss), ImageJ, and CellProfiler.

SUMMARY OF RESULTS

We observed a decrease of both lysosomes and autophagosomes in islets of diabetic NOD mice when compared to non-diabetic NOD mice. Further, analysis of islets in non-diabetic human pancreas showed robust islet autophagy, whereas autophagy was perturbed in islet sections of human type 2 diabetic organ donors.

CONCLUSIONS

The decrease in autophagosomes and lysosomes in diabetic NOD mice suggests that islet autophagy may be perturbed in type 1 diabetes, similar to that observed in type 2 diabetes. Currently, experiments are ongoing to determine if autophagy is altered in islets of type 1 diabetic human organ donors.

O11:

MicroRNA miR-183-5p is a regulator of β cell apoptosis and dedifferentiation in NOD mouse and T1D patients pancreatic islets

AUTHORS

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PURPOSE

MicroRNAs (miRNAs) are a class of small non coding RNAs that regulate gene expression. MiRNAs could be involved in pancreas development and β cell function, and their alteration may contribute to the development of type 1 diabetes. Aim of this study was to analyze the expression profile of miRNAs in pancreatic islets of diabetic NOD mice and T1D patients, in order to understand their possible role in β cell damage.

METHODS

MicroRNAs expression profile was evaluated using TaqMan Array Microfluidic Cards in pancreatic endocrine tissue collected using Laser Capture Microdissection(LCM) from 3 NOD.SCID, 3 NOD normoglycemic and 3 NOD diabetic mice. Individual RT-PCR validation of differentially expressed microRNAs was performed in an additional cohort of NOD mice, where islets were separately captured based on the degree of infiltration. LCM analysis was also performed on pancreatic-endocrine tissue from 3 non-diabetic organ donors (nPOD cohort) and 3 T1D recent-diabetic patients (DiViD study cohort). Differentially expressed microRNAs were modulated in MIN6 murine β -cell line treated or not with a cytokines mix (IFN γ +IL-1 β +TNF α) in order to evaluate apoptosis and microRNA target genes expression.

SUMMARY OF RESULTS

MiR-183-5p expression was found significantly lower ($p < 0.05$) in LCM captured islets from NOD diabetic vs normoglycemic mice and its expression was inversely correlated to the degree of islets infiltration. Moreover miR-183-5p was found significantly reduced in LCM islets from T1D patients vs non-diabetic control donors ($p < 0.05$).

Target gene analysis highlighted the anti-apoptotic factor Bach2 as predicted target of miR-183-5p: in line to what we observed in miR-183-5p analysis, Bach2 expression was indeed upregulated in LCM islets from both recent diabetic mice and T1D patients.

From functional analysis in MIN6 murine β cell line, miR-183-5p inhibition led to a significant increase ($p < 0.05$) of mRNA and protein Bach2 expression in MIN6 transfected with miR-183-5p inhibitor vs CTR, suggesting that miR-183-5p could directly or indirectly modulate this predicted target gene. Additionally, miR-183-5p inhibition in MIN6 prevents cytokines-induced

apoptosis ($p < 0.001$), indicating that this miRNA could modulate apoptosis under cytokine stress.

CONCLUSIONS

In T1D, islet miR-183-5p reduction is associated to β -cell protection from apoptosis through the modulation of anti-apoptotic factor Bach2.

O12: Mechanism and effects of pulsatile GABA secretion from cytosolic pools in the human beta cell

AUTHORS

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PURPOSE

Pancreatic beta cells synthesize and secrete the neurotransmitter γ -aminobutyric acid (GABA) as a paracrine and autocrine signal to help regulate hormone secretion and islet homeostasis. Islet GABA release has classically been described as a secretory vesicle-mediated event. Yet, a limitation of the hypothesized vesicular GABA release from islets is the lack of expression of a vesicular GABA transporter in beta cells. Consequentially, GABA accumulates in the cytosol. Here we provide evidence that the human beta cell effluxes GABA from a cytosolic pool in a pulsatile manner, imposing a synchronizing rhythm on pulsatile insulin secretion. Further, GABA content in beta cells is depleted and secretion is disrupted in islets from type 1 and type 2 diabetic patients, suggesting that loss of GABA as a synchronizing signal for hormone output may coincide with diabetes pathogenesis.

METHODS

We developed two complementary methods to dynamically track GABA release from human islets via high performance liquid chromatography (HPLC) and GABA biosensor cells. These methods are combined with genetic models to generate detailed mechanistic understanding of GABA release from beta cells. Islet GABA content in nPOD tissue sections from non-diabetic, type 1 diabetic, and type 2 diabetic donors was estimated by immunofluorescence staining with an GABA-specific antibody.

SUMMARY OF RESULTS

Here, we have assessed how GABA is released from human beta cells. We compared GABA release from a predominantly cytosolic pool of intracellular GABA in beta cells with that of GABA contained in vesicular membrane compartments including synaptic-like microvesicles and the larger insulin secretory vesicles. We provide evidence for a novel mechanism of cytosolic GABA efflux from human beta cells that occurs via volume regulatory anion channels (VRAC). Furthermore, the GABA-permissive taurine transporter (TauT) mediates uptake of interstitial GABA. Such dynamic GABA efflux and uptake ultimately helps to shape a pulsatile pattern of GABA release from islets. Finally, we observed that this non-vesicular GABA release has an autocrine inhibitory effect on insulin secretion in islets.

CONCLUSIONS

Our study concerns new physiology of the neurotransmitter GABA in the human pancreatic islet. Several recent high-profile papers implicate GABA as a potent paracrine controller, trophic factor, and immunomodulator in islets. Despite its well-defined importance for islet health, it has remained unclear how GABA is released from beta cells into the islet's interstitial space. We resolve this longstanding question about the nature of GABA storage and secretion, where we unambiguously show that GABA in beta cells is cytosolic, rather than vesicular, and is released via volume regulatory anion channels (VRAC). Our findings challenge a longstanding dogma, namely that beta cell GABA is stored in and released from synaptic like microvesicles.

O13:

Mechanisms that control changes in DNA methylation and hydroxymethylation induced by interferon alpha in β -cells: implications for type 1 diabetes

AUTHORS

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PURPOSE

Accumulating data support the concept of an active role of β -cells in triggering the inflammatory mechanisms that activate self-reactive T-cells in T1D. Recent evidence suggests that type I interferons mediate the cross-talk between β -cells and immune system that initiates the autoimmune response in T1D. However, the mechanisms responsible for the β -cells deleterious signals that elicit the autoimmune response are not known. We hypothesized that interferon alpha (IFN α), secreted during viral infections, can impact β -cell epigenome and trigger an inflammatory gene pattern, facilitating an islet diabetogenic microenvironment that can promote the autoimmune response. We investigated the mechanisms underlying DNA epigenetic modifications induced by IFN α in human pancreatic islets, to identify the β -cell specific pathways that initiate the inflammatory response.

METHODS

First, we analyzed the impact of IFN α on DNA methylation (DNAm) patterns, on DNA hydroxymethylation levels and on gene expression in human islets. DNAm profiles were integrated with transcriptome data in IFN α treated compared with un-treated islets. Second, we dissected the mechanisms that control changes in DNAm and 5hmC in human islets by using RNA and protein expression studies, cell reporter assays, siRNA and overexpression studies in cell lines, human islets and β -cells. Third, we generated a transgenic mouse model, IFN α -INS1^{CreERT2}, with inducible IFN α expression in β -cells and we demonstrated the activation of the same mechanisms in human islets upon IFN α expression.

SUMMARY OF RESULTS

We found that IFN α induces active DNA demethylation correlated with overexpression of inflammatory- and immune-pathway genes in human pancreatic islets. We showed that increased DNA demethylation in islets is produced via conversion of 5 methylcytosine (5mC) to 5 hydroxymethylcytosine (5hmC) by up-regulation of TET1/2/3 proteins, in particular TET2. We then demonstrated that IFN α exposure causes up-regulation of PNPase Old-35 (PNPT1), an exoribonuclease, which we found to mediate degradation of miR-26a that regulates TET2 expression. The increased miR-26a decay through PNPT1 triggered TET2 over-expression and increased 5-hmC levels in human islets and β -cells. Moreover, expression of IFN α in pancreatic β -cells of the IFN α -INS1^{CreERT2} transgenic mice triggered development of T1D, which was preceded by increased islet DNA hydroxymethylation through the PNPT1/TET2 mechanism.

CONCLUSIONS

Our results suggest a new mechanism by which IFN α regulates DNAm in β -cells, leading to changes in expression of genes in inflammatory and immune pathways. These results provide a mechanistic framework to explain how inflammatory triggers such as viral or bacterial infections, can modify the β -cell epigenome and modulate the interactions between the immune system and β -cells, to trigger autoimmunity in T1D.

O14:

Investigating the role of nuclear versus cytoplasmic hyperphosphorylated Tau in human β cells during ageing and diabetes

AUTHORS

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PURPOSE

Chromosomal region 17q21.31 has been associated with risk of developing type 1 diabetes (T1D) but no candidate genes have been identified yet, partly due to the fact that none of the genes in the region have known roles in the immune system. The same genetic signal in region 17q21.31 is also associated with increased 2-hr glucose levels following an oral glucose tolerance test and, importantly, with increased plasma proinsulin levels, thus suggesting that genetic polymorphisms in this region might act via β cells, rather than the immune system. To date, this region has been extensively studied for its association with neurodegenerative diseases, where *MAPT*, encoding the microtubule-associated protein Tau, has been identified as the causal gene. As pancreatic β cells share many similarities with neurons, we asked whether Tau and its pathogenic form, hyperphosphorylated Tau (pTau), are also expressed in β cells, and, if so, whether Tau is functionally implicated in β -cell dysfunction, in the context of ageing or diabetes. This work used a combination of immortalised human β cells, EndoC- β H cell lines, human pancreatic islets isolated from organ donors and pancreatic tissue sections from nPOD.

METHODS

A panel of total Tau and pTau antibodies were validated and optimised for use in western blotting (WB), co-immunoprecipitation (co-IP), immunocytochemistry (ICC) on cultured cells and immunohistochemistry (IHC) on FFPE tissue sections protocols. The immortalised human β cell lines, EndoC- β H1 and EndoC- β H3, were used to study the expression, subcellular localisation and co-localisation with various compartment and proliferation markers of total Tau and p-Tau variants by WB and ICC-IF. To extend observations to human β cells *in situ*, 4 μ m FFPE pancreas sections from 36 non-diabetic organ donors (BM1 <31; age range from 0 to 75y) were obtained from nPOD. Sections were stained using standard IF protocols using an antiserum raised against total Tau [HT7] and another [E178] directed against a specific form of pTau. Anti-insulin, anti-glucagon or anti-Ki67 were also included. High-resolution images of 20 islets/case were then processed using a custom Matlab script to define the subcellular localisation (cytoplasmic or nuclear) and intensity of Tau staining. To gain insights into the role of Tau and pTau variants in β cells, co-IP protocols were optimised for use with a panel of anti-Tau antibodies in rat INS-1 β cells and human EndoC- β H1 β cells. Following this, Tau-interacting proteins in β cells were identified through a series of co-IP experiments using total and pTau antibodies on lysates from both, EndoC- β H cultured cells and human pancreatic islets isolated from organ donors. Standard

sub-cellular fractionation protocols were employed to study the differences in Tau-interacting partners between the cytoplasmic and nuclear compartments. Gene ontology enrichment analysis was carried on the resulting list of proteins using publicly-available tools.

SUMMARY OF RESULTS

Using FFPE pancreas sections and a validated panel of anti-Tau antibodies, we found that both total Tau and “pathogenic” pTau variants are present in human endocrine cells from non-diabetic donors *in situ*. Tau and pTau presence were further confirmed in human islets by WB and mass-spectrometry, and in cultured EndoC-βH1 and EndoC-βH3 immortalised human β cells by WB, ICC-IF and mass-spectrometry. High-resolution confocal imaging revealed that pTau, phosphorylated at several pathological epitopes, is present in β cells under basal culture conditions, and that pTau variants localise preferentially to the nucleus, in contrast to the mainly cytoplasmic distribution of total Tau. Intriguingly, we observed that nuclear pTau is excluded from chromatin-occupied areas. Moreover, we found that pTau is significantly upregulated in β cells during mitosis, when it localises to the cytoplasm, and appears to then be cleared in cytoplasmic punctate structures during telophase. Using human donor pancreas sections, we have confirmed that both findings, the nuclear localisation of pTau variants in non-mitotic cells and pTau upregulation during mitosis, also translate to human β cells *in situ*. We note that these findings also apply to acinar cells, thus widening the relevance of these findings beyond β-cell biology. In the course of these studies we also identified a striking effect of ageing on the subcellular localisation of pTau variants. Specifically, we found that, whereas in younger individuals (<10y) pTau localises predominately to the nucleus, with increasing age, nuclear pTau levels decrease accompanied by an increase in cytosolic pTau levels. This relationship correlated directly with age ($R^2=0.4076$; $p<0.0001$) such that all individuals >35y had the majority of the pTau present within the islet localised to the cytosol. Using high-resolution confocal microscopy, it was further observed that pTau immunostaining intensity levels varied among β cells within the same islet and that insulin immunostaining levels correlated negatively with cytoplasmic pTau staining intensity. To gain insight into the role of total Tau and pTau variants in human β cells, we have optimised and carried out a series of Tau co-IP experiments in both cultured EndoC-βH1 cells and human donor islets, using either whole-cell lysates or nuclear vs. cytoplasmic fractions. Gene ontology enrichment analyses have revealed that nuclear pTau interacting partners are significantly enriched in RNA-binding proteins and spliceosome components, suggesting a novel role for pTau in control of gene expression and alternative splicing.

CONCLUSIONS

Despite being thought of as a neuronal protein, our findings confirm that Tau is present in human pancreatic β cells, in line with previous reports. Surprisingly, our findings also reveal that pTau variants, widely regarded as cytotoxic, are also present in human β cells *in situ*, even in the absence of diabetes, and in human β cells cultured under basal conditions. Furthermore, we find that, while total Tau variants are detected in the cytoplasm, pTau variants localise predominantly to the nucleus, at least in younger individuals, despite the fact that Tau lacks a nuclear localisation signal. We also find that pTau is significantly upregulated in β cells during mitosis and then cleared again during telophase, suggesting that mitotic cells possess mechanisms to clear cytoplasmic pTau, whose accumulation

causes neuronal death in neurodegenerative diseases. Although upregulation of “pathological” pTau variants during mitosis was reported in neurons two decades ago, the mechanisms that allow mitotic cells to cope with high pTau levels and the pathways that control its turnover post mitosis have not been investigated. Through co-IP experiments we have characterised the Tau-interacting partners in human β cells, thus revealing novel potential roles for Tau in the control of RNA metabolism, alternative splicing and gene expression. In future, we will investigate the effect of diabetes status, in FFPE sections, and diabetes-relevant stresses, in cultured β cells and human islets, on total Tau and pTau levels and subcellular localisation. Mechanistically, our studies will focus on understanding the changes that lead to the decrease in nuclear pTau and accumulation of pTau in the cytoplasm of aged (and, depending on findings, diabetic) β cells. Given our findings, we hypothesise that the loss of nuclear pTau and the accompanying increase in cytosolic pTau that occurs in β cells with age may be associated with reduced proliferative potential and insulin secretory dysfunction.

O15: Targeting 12-lipoxygenase protects human islets from virus-induced stress

AUTHORS

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PURPOSE

Infection with the enterovirus coxsackievirus B4 (CVB4) is suspected to precede the onset of type 1 diabetes (T1D). Our nPOD studies reveal increased 12-lipoxygenase (12-LO) staining in islets from T1D subjects that correlates with loss of insulin as compared to non-T1D donors. Additionally, 12-LO, which generates pro-inflammatory lipids, is increased in islets from auto-antibody positive donors with islets that still stain positive for insulin. We also showed that 12-LO activation leads to human beta cell dysfunction and loss of viability. ML355, a human-specific small molecule inhibitor of 12-LO, prevents inflammation-induced human beta cell damage and improves islet function. CVB4 infection activates pro-inflammatory cytokines linked to T1D pathogenesis and decreases islet insulin transcripts/protein. We hypothesized that inhibiting the inflammatory response with ML355 protects human beta cells and restores insulin secretion following exposure to CVB4.

METHODS

Primary human islets from nondiabetic donors were cultured in the presence and absence of ML355, then challenged with CVB4. Islet responses were assessed by immunohistochemical staining, glucose-stimulated insulin secretion and RNA-Seq.

SUMMARY OF RESULTS

Alox12 transcripts are induced by CVB4 challenge of primary human islets in the absence of ML355. Immunostaining reveals that viral protein and 12-LO exist in discrete populations in human islets. In glucose-stimulated insulin secretion assays, CVB4 decreased 1st and 2nd phase insulin secretion as well as total releasable insulin. ML355 restored the glucose-stimulated insulin secretion and total insulin in CVB4-infected islets. RNA-Seq revealed induction of chemokines and cytokines following CVB4 infection; infection in the presence of ML355 resulted in the specific reduction of inflammatory chemokines and cytokines but not type I IFN and interferon-stimulated genes.

CONCLUSIONS

CVB4 infection increases *alox12* expression in human islets. ML355 improves islet function, diminishes inflammatory but not type I IFN responses, and can restore insulin secretion during CVB4 infection of cultured primary human islets. Activation of 12-LO activity could be the key link between viral infection, inflammation, and beta cell dysfunction in T1D. Additional studies of ML355 in our diabetes model of CVB4-infection in human islet-

engrafted mice may reveal protective effects *in vivo* and identify a new opportunity to improve beta cell function during inflammation.

O16:
Loss of pancreatic iron homeostasis may contribute to the pathogenesis of Type 1 diabetes

AUTHORS

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PURPOSE

A number of epidemiological studies have demonstrated an association between high iron levels and an increased risk for developing T1D. Systemic iron levels are tightly regulated by hepcidin, a hormone that binds to ferroportin transporters on iron-rich cells (such as macrophages) to prevent the release of iron. We have shown that the gene encoding hepcidin, *Hamp*, is significantly diminished in the islets of Non-obese diabetic (NOD) compared to control NOD.B10 mice prior to the onset of destructive insulinitis. Hepcidin is expressed in the insulin secretory granules of beta cells and may play a role in maintaining iron homeostasis in the pancreas. Loss of hepcidin expression may lead to increased release of iron from macrophages that are present in the insulinitic lesion, beta cell uptake of iron, oxidative stress and beta cell death. In this study, we examine the role of iron and hepcidin expression on NOD disease and human T1D.

METHODS

Microarray analysis: Microarrays on isolated islets of NOD and NOD.B10 mice were performed using the Whole Mouse Genome Microarray Kit, 4x44K two-color arrays (Agilent).
QPCR: RNA was extracted (Trizol), cDNA was synthesized (Superscript III) and preamplified (TaqMan PreAmp Master Mix), and QPCR was performed using Taqman assays.
Human islets were obtained from the U. Alberta via the Stanford DRC and treated with IL-1b & IFNg. NOD.B10 islets were isolated and treated with activated splenocyte supernatant.
Immunohistochemistry: Frozen pancreas sections of NOD and NOD.B10 mice and nPOD patients were fixed, incubated in blocking buffer, and primary ABs for hepcidin and/or glucagon or insulin. Staining was detected with Alexa-488 or Alexa 594-conjugated secondary ABs.
Iron staining: Iron staining was performed using the Abcam iron staining kit.
Dietary iron on disease progression: NOD mice were fed standard diets containing ~250 ppm iron (Teklad 2018SX; control diet), ~400 ppm iron (LabDiet 5P04), or a custom diet containing ~450 ppm iron (Teklad 2018SX supplemented with an additional 200 ppm iron). Blood glucose levels were monitored weekly.

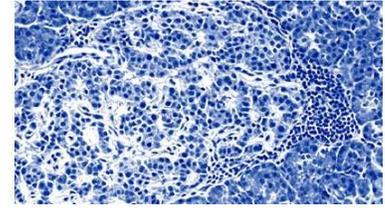
SUMMARY OF RESULTS

By microarray analysis we showed that hepcidin gene expression is significantly increased in the islets of NOD mice at 4 wks of age when the insulinitic lesions form, but by 12 wks of age, significant loss of gene expression is observed. This is confirmed by immunohistochemistry studies showing a lack of hepcidin expression in the beta cells of NOD compared to NOD.B10 mice. In a limited number of nPOD samples, we showed loss

of hepcidin expression in one of four AA+ patients studied. Short-term inflammation was found to drastically increase hepcidin gene expression in control NOD.B10 islets and in human islets. However, prolonged inflammation, led to reduced gene expression (<50% of control levels). Iron-rich macrophages were detected in the insulitic lesion of 10-12 wk old NOD mice, suggesting that the loss of hepcidin expression may allow iron to be released from these cells. Interestingly, in older normoglycemic NOD mice with highly infiltrated islets, an accumulation of iron was seen in acinar cells of certain regions of the pancreas that were devoid of islets. This is similar to what has been observed in hepcidin-KO mice. We further showed that increased levels of dietary iron could exacerbate disease in NOD mice. Disease onset was 3 weeks earlier and incidence was 2 times higher in mice that were fed higher iron diets compared to mice that were fed the low-iron control diet.

CONCLUSIONS

These data suggest that hepcidin plays an important role in maintaining iron levels in the pancreas. However, during the progression of T1D, hepcidin expression is diminished. The lack of hepcidin may allow increased export of iron from macrophages, and result in iron uptake, oxidative stress and beta cell death. This process may be further exacerbated by a high iron diet.



(LB-03)

Pancreatic islets from T2D donors are characterized by NKX6.1 nucleus-to-cytoplasm translocation and miR-184-3p reduced expression possibly contributing to β -cell dedifferentiation

AUTHORS

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PURPOSE

Type 2 diabetes (T2D) is a metabolic disease caused by several molecular mechanisms alterations, leading to β -cell dysfunction and/or loss of β -cell phenotype (dedifferentiation). MicroRNAs, small RNA molecules which modulate genes expression, have been associated with β -cell function and identity and previously correlated to islet dedifferentiation, therefore playing pivotal role in T2D pathogenesis.

Specifically, it has been previously demonstrated that miR-184-3p expression is reduced in pancreatic islets of T2D donors. Of note, our preliminary data showed that β -cell specific transcription factor NKX6.1 directly binds to miR-184-3p promoter thus regulating its expression; we also demonstrated miR-184-3p inhibition is able to protect β -cells from stress-induced apoptosis through the upregulation of its target gene CRTC1 (Creb-Regulated Transcriptional Coactivator 1).

Previous studies showed that NKX6.1 translocation from nucleus to cytoplasm is associated to the loss of β -cell identity (dedifferentiation).

Based on our previously obtained results, the aim of this study was to verify whether NKX6.1 nucleus to cytoplasm translocation in β -cells correlates to miR-184-3p reduced expression and its target gene upregulation in pancreatic islets of T2D donors.

METHODS

OCT frozen pancreatic tissue sections from n=7 non-diabetic (age: 42 ± 12.6 ; gender 4M/3F; BMI 23.14 ± 2.86 Kg/m²), n=3 recent onset T2D (age: 32.96 ± 11.72 ; gender: 3M; BMI: 33.13 ± 4 Kg/m²; disease duration: 1 ± 1.41) and n=3 long-standing T2D (age: 71 ± 12.12 ; gender: 2M/1F; BMI: 25.93 ± 4.55 Kg/m²; disease duration: $9 \pm 1,41$) donors were obtained from nPOD (Network for Pancreatic Organ Donor with Diabetes) and from University of Pisa. Pancreatic sections were subjected to double Immunofluorescence staining for Insulin, NKX6.1 and nuclei. Image analyses and colocalization (Manders' coefficient M₂) computing were performed

using Volocity software. Moreover, the same islets on subsequent serial sections were isolated through Laser Capture microdissection (LCM- Arcturus XT), in order to evaluate miR-184-3p, CRT1 and NKX6.1 expression using RT Real-Time PCR.

SUMMARY OF RESULTS

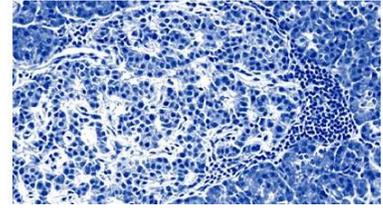
The pancreatic islets staining pattern of NKX6.1 in non-diabetic donors showed a specific nuclear signal in β -cells, while its localization in beta-cells of T2D donors is partially cytoplasmic, confirming what was previously reported. The % of colocalization coefficient DAPI/NKX6.1 nuclear is significantly reduced in T2D pancreatic islets respect to non-diabetic donors (M_2 coefficient: CTR 86.2% vs T2D 69.2%; $p < 0.0001$), demonstrating its translocation during metabolic stress. The expression analysis of miR-184-3p showed a reduction trend in T2D pancreatic islets vs controls and a concomitant increase of its target gene CRT1. Interestingly, the reduction of miR-184-3p and the increase of CRT1 expression is more pronounced in recent-onset respect to long standing T2D donors, suggesting that such mechanism occurs early in the pathogenesis of type 2 diabetes. We did not observe any difference of NKX6.1 mRNA expression between non-diabetic and T2D donors, suggesting that reduction of miR-184-3p is due to its transcription factor translocation more than to its expression reduction.

CONCLUSIONS

In conclusion, these data suggest that NKX6.1, which plays pivotal roles in the loss of β -cell phenotype/dedifferentiation, is translocated from nucleus to cytoplasm in T2D pancreatic islet β -cells; moreover its translocation is associated to the reduction of miR-184-3p expression which, in turn, leads to the increase of CRT1 in the early stages of type 2 diabetes as a mechanism of β -cell compensation and/or protection from apoptosis. These results suggest that this process may represent a new protection mechanism against β -cell apoptosis in type 2 diabetes possibly contributing to β -cell dedifferentiation.

Select the **ONE** category that best describes your research:

<input checked="" type="checkbox"/> Beta Cell Physiology and Dysfunction	<input type="checkbox"/> Novel Biomarkers
<input type="checkbox"/> Beta Cell Development, Differentiation & Regeneration	<input type="checkbox"/> Novel Technologies
<input type="checkbox"/> Bone Marrow Studies	<input type="checkbox"/> Pathology
<input type="checkbox"/> Core Lab	<input type="checkbox"/> Type 1 Diabetes Etiology & Environment
<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):



(LB-23)

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

AUTHORS

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PURPOSE

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

METHODS

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

SUMMARY OF RESULTS

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

CONCLUSIONS

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

Select the **ONE** category that best describes your research:

<input checked="" type="checkbox"/> Beta Cell Physiology and Dysfunction	<input type="checkbox"/> Novel Biomarkers
<input type="checkbox"/> Beta Cell Development, Differentiation & Regeneration	<input type="checkbox"/> Novel Technologies
<input type="checkbox"/> Bone Marrow Studies	<input type="checkbox"/> Pathology
<input type="checkbox"/> Core Lab	<input type="checkbox"/> Type 1 Diabetes Etiology & Environment
<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):

IMMUNOLOGY

O17:

Characterization of T cells Reactive to Hybrid Insulin Peptides in Type 1 Diabetes patients

AUTHORS

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PURPOSE

Using islets from nPOD T1D donors, it was recently established that T cells reactive to hybrid insulin peptides (HIPs) are found in the residual islets of type 1 diabetic (T1D) organ donors. However, a restricted panel of HIPs (8 in total) were tested. Here we investigate whether reactivity to a larger panel of HIPs could be observed in the PBMCs of T1D patients and investigated phenotype and TCR usage of HIP-reactive T cells in new onset T1D patients or control subjects.

METHODS

We used IFN- γ ELISPOT analyses on peripheral blood mononuclear cells (PBMCs) to determine if new onset T1D or control subjects displayed T cell reactivity to a panel of 16 HIPs. We used a multiplex cytokine assay to further characterize the phenotype of T cells reactive to HIPs, and TCR deep sequencing to investigate TCR usage of HIP-reactive T cells. Finally, we used a CFSE-based assay to clone T cells from PBMCs.

SUMMARY OF RESULTS

We observed that about a third of the T1D patients responded to at least one HIP, 70% of which responded to multiple HIPs whereas only one healthy control subject responded to multiple HIPs. Responses to 4 HIPs were significantly elevated in T1D patients but not in control subjects and we determined that these responses were directed towards the HIP and not the native peptide. Importantly, 3 of these 4 HIPs were not tested on T cells isolated from T1D donors. Using the multiplex cytokine assay, we determine a bias towards a Th1 phenotype upon stimulation of PBMCs with HIPs in responder patients. We isolated 8 non-redundant, antigen-specific T cell clones that are reactive to 6 different HIPs from the panel of 16HIPs. One T cell clone was isolated from the same patient on two different blood draws, indicating a high frequency of this T cell clone in the peripheral blood. The isolated T cell clones reacted to their target HIPs in the low nanomolar range and secrete IFN- γ , TNF- α and GM-CSF.

CONCLUSIONS

This work indicate that HIPs are important target antigens in human T1D subjects and suggest that investigating HIP-reactive T cells present in the islets might be an important next step to validate their role in the pathogenesis of T1D.

O18:
**The HANDEL-I Project – Human Atlas of Neonatal Development
and Early Life - Immunity**

AUTHORS

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PURPOSE

This project was created to provide a comprehensive overview of immune development in early life through procurement and study of relevant tissues and organs (namely, blood, thymus, bone marrow, spleen, lymph nodes, intestine, and lung) from pediatric donors in conjunction with HANDEL-P (Human Atlas of Neonatal Development and Early Life – Pancreas).

METHODS

A key goal of HANDEL-I is to establish a better understanding of immune system heterogeneity and cellular diversity, including defining the “normal” kinetics of innate and adaptive subset development, by obtaining requested organs from 50 individuals from age 24 weeks gestation to 10 years.

Our objective is to understand the human immune developmental program by acquiring multiple lymphoid and mucosal tissues from donors who were healthy prior to death. We propose to create a unique and essential dataset in a collaborative research project that leverages shared tissue access to provide enhanced understanding of critical knowledge gaps in human immune development.

Tissues are processed within 24 hours of procurement and assessed via genotyping, histologic analysis, immunophenotyping, and bulk and/or single cell transcriptomics. We propose to investigate:

1. Donor characteristics: Demographic, serological (HLA, CMV, and autoantibodies), and genetics will be determined for each donor
2. Immune tissue characteristics: Define the cellular niche and molecular signature of stromal tissue and lymphoid progenitors in developmental tissues, as well as repertoire diversity and phenotype of cells isolated from peripheral immune tissues.
3. Mucosal tissue characteristics: Analyze the generation and phenotype of adaptive immune responses in cells from lung and gut tissues that interact with the environment.

SUMMARY OF RESULTS

HANDEL-I has acquired and banked immune tissues from 18 individuals, age range (26 weeks gestation- 8.64 years, mean age 2.5 years). Overall, the HANDEL project has acquired 48 total cases (age range: 26 weeks gestation-10.3 years, mean age 3 years) Tissues isolated and cryopreserved in this project, as well as locally generated omics data, will serve as a resource for understanding and comparing normal immune development to disease states.

CONCLUSIONS

Data generated within this project will provide novel information on immune development and cellular phenotypes in tissues rarely assessed in the context of healthy individuals. Systems immunological approaches will be used to identify novel cellular phenotypes in correlation with patient genetics to serve as a reference for biomarker identification.

O19: Metabolic Phenotyping of Islet-Infiltrating Lymphocytes in Type 1 Diabetes

AUTHORS

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Chrys Hulbert, Dept. of Medicine, Division of Rheumatology and Immunology, Vanderbilt University Medical Center

James W. Thomas, Dept. of Medicine, Division of Rheumatology and Immunology, Dept. of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center

PURPOSE

Development of an antigen-specific intervention that targets the diabetogenic immune response without compromising systemic immunity is complicated by an expanding list of antigenic targets, including post-translationally modified peptides generated at the site of autoimmune attack in the islet. Thus, development of effective antigen-based therapy requires not only antigen identification, but also understanding of the unique antigen processing environment facilitated by the islet itself. Recently, cellular metabolism has emerged as a potent modulator of immune cell function. However, the metabolic state of areas of lymphocytic infiltrate in the islet, termed insulinitis, is not known. Given the highly vascularized nature of the islet needed to accommodate the metabolic demand associated with insulin secretion, we sought to characterize the islet's distinct metabolic environment and its influence on T-B cell interactions at the site of autoimmune attack.

METHODS

To test the hypothesis that pathogenic, islet-infiltrating lymphocytes can be characterized by a distinct metabolic phenotype, we compared the metabolic signatures of islet infiltrating lymphocytes in two transgenic mouse models. V_H125^{SD} .NOD mice develop accelerated insulinitis and diabetes, while BDC2.5.NOD mice develop accelerated insulinitis but are protected from diabetes development. These mouse models were used as examples of pathogenic and protective insulinitis, respectively.

SUMMARY OF RESULTS

Mitochondrial polarity was significantly increased in pre-diabetic V_H125^{SD} .NOD islet-infiltrating B cells, compared to BDC2.5.NOD islet-infiltrating B cells. In disease-prone V_H125^{SD} .NOD mice, mitochondrial polarity was increased in insulin-binding B cells compared to non-insulin-binding B cells in the pancreas, but not in the spleen or pancreatic lymph nodes. CD4+ T cells in diabetic V_H125^{SD} .NOD mice were characterized by decreased fatty acid oxidation compared to non-diabetic CD4+ T cell subsets in both pre-diabetic

V_H125^{SD}.NOD and BDC2.5.NOD mice. No differences in fatty acid oxidation between CD8+ T cells were identified. Assessment of hypoxic changes revealed increases in hypoxia within the islet, but not within the islet-infiltrating lymphocytes themselves in both V_H125^{SD}.NOD and BDC2.5.NOD islets.

CONCLUSIONS

These studies reveal distinctions in the metabolic programming of islet infiltrating lymphocyte subsets prior to disease development in disease-prone compared to disease-protected NOD mice, as well as metabolic reprogramming in selected lymphocyte subsets with the onset of systemic hyperglycemia. Together with the hypoxic changes identified within the infiltrated islet, but not the lymphocytes themselves, these findings suggest that tissue specific changes drive metabolic reprogramming at the site of autoimmune attack in the islet. As such, distinct metabolic features that differentiate disease prone and disease protected NOD mice are potential targets for selective therapeutic interventions in type 1 diabetes.

O20:

Interferon-gamma drives programmed death-ligand 1 expression on islet β cells to limit T cell function during autoimmune diabetes.

AUTHORS

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PURPOSE

Type 1 diabetes is caused by autoreactive T cell-mediated β cell destruction. Even though co-inhibitory receptor programmed death-1 (PD-1) restrains autoimmunity, the expression and regulation of its cognate ligands on β cell remains unknown.

METHODS

Here, we interrogated β cell-intrinsic programmed death ligand-1 (PD-L1) expression in mouse and human islets.

SUMMARY OF RESULTS

We measured a significant increase in the level of PD-L1 surface expression and the frequency of PD-L1+ β cells as non-obese diabetic (NOD) mice aged and developed diabetes. Increased β cell PD-L1 expression was dependent on T cell infiltration, as β cells from Rag1-deficient mice lacked PD-L1. Using Rag1-deficient NOD mouse islets, we determined that IFN- γ promotes β cell PD-L1 expression. We performed analogous experiments using human nPOD samples, and found a significant increase in β cell PD-L1 expression in type 1 diabetic samples compared to type 2 diabetic, autoantibody positive, and non-diabetic samples. Among type 1 diabetic samples, β cell PD-L1 expression correlated with insulinitis. *In vitro* experiments with human islets from non-diabetic individuals showed that IFN- γ promoted β cell PD-L1 expression.

CONCLUSIONS

These results suggest that insulin-producing β cells respond to pancreatic inflammation and IFN- γ production by upregulating PD-L1 expression to limit self-reactive T cells.

O21:

Type III interferons (IFN- λ s) upregulate MHC class I expression and regulate human beta cell permissiveness to Coxsackievirus infection

AUTHORS

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PURPOSE

Epidemiological data and studies of patient samples have indicated that enterovirus infections, especially those caused by Coxsackie B viruses (CVBs) are associated with type 1 diabetes (T1D). We have hypothesized that CVB infections contribute to the development of T1D by activating innate immune responses including interferons (IFNs), regulating the expression of check-point inhibitors and/or by altering the HLA class I peptidome of infected beta cells making them more vulnerable to CD8+ T cell mediated immune attack. The aims of the present study were 1) to establish a CVB infection model in human beta cell lines for mechanistic testing of these hypotheses, and 2) to use the established models to evaluate the direct effects of type III interferons (IFNs; IFN- λ s) on human beta cell MHC class I expression and antiviral defense.

METHODS

Human beta cell lines were infected with different CVB serotypes (CVB1-6), exposed to poly I:C or IFNs (IFN- α or IFN- λ 1/2), mimicking a virus-induced pro-inflammatory environment. The conditions for infection with selected CVBs (CVB1 and CVB3) were further optimized to reach a high infection efficiency, as evaluated by the detection of dsRNA, capsid or non-structural virus proteins using flow cytometry. The production of infectious viral progeny was validated using a standard plaque assay technique. Changes in cell phenotype were measured by RT-PCR and flow cytometry.

SUMMARY OF RESULTS

All CVB serotypes (1-6) infected human beta cell lines, reaching an infection efficiency up to 60%. The CVB infection cycle was completed resulting in the release of infectious CVB virions. Flow cytometry confirmed the expression of structural (VP-1) and non-structural virus proteins (viral proteases 2A and 3C) in CVB infected cells. The Coxsackie and Adenovirus Receptor (CAR) was expressed on the beta cells and blocking antibodies to the receptor attenuated CVB infection. IFN- α , IFN- λ 1 or IFN- λ 2 treatment increased the cell surface expression of MHC class I and strongly reduced cellular permissiveness to CVB infection.

CONCLUSIONS

Human beta cell lines are readily infected by all CVB1-6 serotypes. Infection is dependent on the CAR receptor, supporting the hypothesis that CAR serves as a primary entry receptor for CVBs in primary human beta cells. CVB infected beta cells are positive for structural and non-structural CVB proteins showing that viral protein components are available for antigen processing and peptide presentation. Type III IFNs increased the expression of MHC class I and activated antiviral defense. Together with previous studies demonstrating that type III IFNs are expressed by CVB infected human islets, these results highlight that both protective and potentially dangerous signals may be induced by type III IFNs when beta cells are infected with viruses linked to T1D.

O22:

HLA multimer-based characterization of islet specific CD4+ T cells that target beta cell epitopes and neo-epitopes

AUTHORS

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Sheryl Horstman – Translational Research, Benaroya Research Institute
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Sally C. Kent – Diabetes Center of Excellence, University of Massachusetts Medical School
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PURPOSE

T cells that recognize post-translationally modified peptides are present in the peripheral blood of patients with T1D. Given that, the expression and activity of enzymes responsible for modifications are upregulated in response to cellular stress, modified peptides are likely to form in stressed islets and to elicit pathogenic T cell responses. The goal of this project is to corroborate the relevance of T cell responses to modified beta cell antigens by identifying and studying T cells that recognize these peptides within disease relevant tissues.

METHODS

T cells that recognize post-translationally modified epitopes were identified by staining expanded T cell lines derived from the pancreatic lymph nodes or beta cell infiltrates using HLA class II tetramers and complementary surface marker activation assays. Resulting epitope specific T cells can be further characterized by determining their cytokine profiles and sequencing their T cell receptors, allowing a comparison of their specificity and functional characteristics with those of T cells that are found in peripheral blood.

SUMMARY OF RESULTS

To date we have confirmed the recognition of multiple post-translationally modified epitopes by T cells derived from disease relevant tissues. We have further demonstrated the feasibility of functionally interrogating those T cells and determining their T cell receptor sequences. Through our ongoing work we expect to confirm the recognition of additional modified epitopes and to confirm functional attributes shared by beta cell specific T cells that are capable of homing to disease proximal tissues.

CONCLUSIONS

Our findings support that there is an overlap between the specificity of T cells derived from disease relevant tissues (pancreatic lymph nodes and beta cell infiltrates) and peripheral blood and indicate that T cell receptors that recognize post-translationally modified epitopes constitute a detectable fraction of the T cell response in pancreatic organ donors.

O23:

Tissue-Resident T1D-Specific Memory CD8⁺ T Cells are Enriched in Pancreatic Islets and Draining Lymph Nodes of Diabetic and Auto-Antibody Positive Organ Donors

AUTHORS

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PURPOSE

Type 1 diabetes (T1D) is caused by the autoimmune destruction of insulin-producing beta cells in the pancreatic islets, resulting in insulin dependency for glycaemia control. Auto-reactive cytotoxic CD8⁺ T cells are drivers of this destruction, but the *in vivo* functional and phenotypic characteristics of T1D-specific CD8⁺ T cells remain to be clarified. Resident memory T cells (T_{RM}) are characterized by the expression of the tissue retention markers CD69 and CD103, are not found in blood, and are associated with localized allergic and autoimmune diseases of the skin, gut and lungs. T_{RM} have been previously observed in low numbers in healthy and T1D human pancreata, yet, their contribution to disease is unknown.

METHODS

We used multi-parametric flow cytometry, peptide-MHC-I tetramers, and T cell receptor (TCR) sequencing to detect and characterize islet-specific CD8⁺ T_{RM} in viable tissues (blood, spleen, islets and pancreas-draining LNs) recovered from diabetic and non-diabetic organ donors by the Human Pancreas Analysis Program (HPAP).

SUMMARY OF RESULTS

We identified islet-specific CD8⁺ T cells directly *ex vivo* in the spleen and pancreas-draining LN of T1D and non-diabetic individuals using peptide-MHC-I tetramers. These cells were antigen-experienced and expressed the T_{RM} retention markers CD69 and CD103, without evidence of recent activation. Interestingly, they appeared to be non-cytolytic, with low perforin and granzyme B expression. TCR analysis of sorted islet-specific CD8⁺ T cells (IGRP₂₆₅₋₂₇₃) from blood and LN of a long-standing T1D donor revealed oligoclonality with only partial clonal sharing between sites. In addition, CD8⁺ T cells in pancreatic islets also expressed CD69 and CD103 and, in T1D, showed increased levels of cycling and differential expression of the transcription factors eomesodermin and t-bet. Finally, analysis of tissues

from a 14-year-old donor who died of ketoacidosis due to unsuspected T1D revealed increased infiltration of the pancreas by CD8⁺ T_{RM} with heightened cycling and cytolytic properties, including cells specific for insulin and IGRP.

CONCLUSIONS

These observations suggest a pathologic role for CD8⁺ T_{RM} cells in T1D, where these cell serve as immediate effectors within pancreatic islets and long-term sentinels for auto-antigen detection in pancreas draining lymph nodes. Further work will identify the functional and transcriptional properties of these cells during health and disease, providing new insights into the pathobiology of T1D.

O24: Dextran Sulfate Ameliorates Type 1 Diabetes by Enhancing Beta Cell Survival and Mitochondrial Function and Increasing Regulatory T Cells

AUTHORS

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PURPOSE

Type 1 Diabetes (T1D) is an autoimmune-mediated disease featured by both the loss of self-immune tolerance to the beta cells and consequently their destruction leading to elevated blood glucose levels. Currently, the outlines of treating T1D focus on regeneration and preservation of residual beta cells and restoration of immune tolerance. Dextran sulfate (DS), a well-known mild anticoagulant and volume expander in clinic inhibits dendritic cell maturation and activation. Here we analyzed the effect of DS on (1) diabetes development in NOD mice, (2) beta cell survival, function and mitochondrial activity in cytokine-treated human islets and (3) the profile of immune tolerance markers.

METHODS

1. Eight week-old NOD/Ltj (NOD) mice were intraperitoneally injected with different doses of DS. Non-fasting blood glucose was measured by a portable glucometer and hyperglycemia was considered when blood glucose was ≥ 250 mg/dl for two consecutive days. At week 22, we collected plasma to measure insulin and harvested pancreases for histology staining. 2. Human islets were treated for 24h with a cytokine cocktail (IFN γ , IL1 β and TNF α) in presence or absence of DS; then, either mitochondrial function was analyzed by Seahorse, insulin secretion measured by perfusion assays or RNA collected for RNAseq analysis. 3. Non-diabetic NOD mice were treated with DS or saline for 4 weeks and lymphocytes from spleen and pancreatic lymph nodes were analyzed by flow cytometry to compare cell numbers and ratios of CD8+ and CD4+ cells, IFN γ -, IL4-, or IL17-producing CD4+ cells and Foxp3+ regulatory T cells. 4. Treg cell mitochondrial function was analyzed by Seahorse and mitochondrial mass and potential were measured with mito tracker.

SUMMARY OF RESULTS

DS treatment (10 mg/kg) significantly reduced the percentage (20%) of diabetic mice compared with saline (70%). DS treatment significantly preserved plasma insulin and beta cell mass in NOD mice compared with saline-treatment, keeping similar values to the ones present in 10-week old pre-diabetic mice. Pancreas histology showed significantly decreased immune cell infiltration in islets and beta cell death in DS-treated mice. Human islet RNAseq showed upregulation of 217 genes ($p < 0.05$, LogFC > 1) and downregulation of

353 genes ($p < 0.05$, $\text{LogFC} < -1$) in DS+cytokines compared with cytokines only treated islets. GSEA analysis showed these genes were mostly involved in reduced apoptosis, decreased inflammatory response, less chemokine production, increased oxidative phosphorylation and enhanced pyruvate metabolic activities. Seahorse studies indicated that DS protected mitochondrial function in human islets treated with cytokines. DS maintained basal OCR, maximal respiration and ATP production and these parameters were in line with upregulation of dynamic glucose-stimulated insulin secretion. DS-treated NOD mice displayed decreased IFN γ -producing CD8 $^+$ and CD4 $^+$ and a slight increase in IL4 $^+$ CD4 $^+$ T cells. Interestingly, DS treatment significantly upregulated Foxp3 $^+$ CD4 T cells *in vitro* and *in vivo*. In addition, DS treatment increased PD1 expression in T cells, which can partially explain a shift of T cell phenotype by DS. To correlate the profile of T cell subsets with human islet data, we found that DS enhanced mitochondrial activity during T cell activation, which is a metabolic feature of regulatory T cells. Although DS did not alter ECAR in both regulatory T cells and other T cell subsets, DS significantly enhanced OCR in regulatory T cells by showing increased real-time ATP production. DS also increased mitochondrial mass and potential in T cells.

CONCLUSIONS

Taken together, these results indicate that DS reduces the onset of T1D in pre-diabetic NOD mice by preserving insulin secretion and/or reducing immune cell infiltration in the pancreas. DS alleviates the metabolic impairments induced by cytokines in human islets, favors regulatory T cells and inhibits pro-inflammatory T cell subsets. DS can boost mitochondrial function that can preserve beta cell function and upregulate regulatory T cell populations. DS treatment can potentially be of great value for treating T1D.

O25:

NKG2D signaling within the pancreas decreases NOD diabetes by enhancing CD8+ central memory T cell formation

AUTHORS

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PURPOSE

The current standard of care for type 1 diabetes, insulin replacement, is cumbersome and the life expectancy of type 1 diabetes patients is still reduced compared with healthy individuals. In addition, the worldwide incidence of type 1 diabetes is increasing for unknown reasons. Therefore, novel prevention strategies are urgently needed. The main drivers of type 1 diabetes are islet-specific T cells. These cells must escape a myriad of tolerance mechanisms that control their activation in healthy individuals. Understanding these mechanisms is critical to developing better strategies to inhibit the disease. One pathway implicated in this process is signaling through the Natural Killer Group 2 member D (NKG2D) immune receptor. However, the importance of NKG2D to diabetes is unclear owing to conflicting results in studies with the non-obese diabetic (NOD) mouse model. Our published data demonstrate that these inconsistent findings are likely due to differential effects of NKG2D on diabetes in different anatomical sites. Therefore, novel experimental approaches are required to determine the role NKG2D plays in various organs during diabetes development. We demonstrate that the mRNAs encoding both NKG2D and NKG2D ligands are increased in the islets of patients with type 1 diabetes, making discerning the role of NKG2D-ligand interaction in the pancreas during diabetes development of significant interest.

METHODS

We developed a novel mouse model to determine the role of NKG2D specifically within the pancreas. Because NKG2D and its ligands are expressed on immune cells within the NOD pancreas, we could not generate a model in which there was decreased NKG2D signaling specifically in the pancreas. Therefore, we instead developed a model with increased NKG2D signaling within the pancreas. This is a NOD mouse that expresses a transgene driving expression of the NKG2D ligand RAE1 ϵ in islets (RIP-RAE1 ϵ NOD mice). We compared diabetes development and the composition of the pancreatic immune cell infiltrates in RIP-RAE1 ϵ mice with control NOD mice. Additionally, we performed studies with human T cells to complement our findings in the mouse model.

SUMMARY OF RESULTS

We found that there was a significant decrease in spontaneous diabetes in RIP-RAE1 ϵ NOD mice compared with control NOD mice. Using flow cytometry, we found similar percentages and numbers of CD4⁺ T cells, CD8⁺ T cells and NK cells within the pancreas RIP-RAE1 ϵ and control NOD mice. However, RIP-RAE1 ϵ mice contained decreased numbers of CD44^{hi}CD62L^{lo} (effector/effector memory, Tem, phenotype) CD8⁺ T cells in both the pancreas and spleen. This correlated with increased numbers of CD44^{hi}CD62L^{hi} (central memory T cell, Tcm, phenotype) CD8⁺ T cells, which is a cell population previously demonstrated to contain cells that inhibit the Teff response. This increased Tcm:Teff/Tem ratio was not observed in the CD4⁺ T cell compartment. Further, we found that NKG2D signaling in human CD8⁺ T cells also increased CD8⁺ Tcm differentiation, including a Tcm subpopulation with a Treg phenotype.

CONCLUSIONS

These data (1) demonstrate that NKG2D signaling within the pancreas is protective against NOD diabetes development, (2) indicate this protection is mediated by increased generation of a protective CD8⁺ Tcm population, and (3) demonstrate that NKG2D signaling in CD8⁺ T cells from healthy individuals increases CD8⁺ Tcm and Treg generation. We are now testing whether NKG2D signaling functions equally well in CD8⁺ T cells from type 1 diabetes patients.

O26:
**Antigen Processing and Presentation in Type 1 Diabetes:
A Role for Transpeptidation?**

AUTHORS

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PURPOSE

To identify the molecular mechanisms behind the processing and presentation events involved during the generation of CD4 T cell, diabetogenic antigens.

METHODS

Mass Spectrometry, Flow Cytometry, T Cell Stimulation

SUMMARY OF RESULTS

The major diabetogenic antigens driving the CD4 mediated T cell response in T1D have not yet been identified, however T cells specific to peptides derived from a number of the beta cell granule prohormones (chromagranin A (WE14), Insulin B chain (9-23) and IAPP) have been characterized. Both T cell stimulation data and crystallographic structures of these diabetogenic T cell receptors with the NOD MHC Class II molecule, IAg7 presenting the aforementioned peptides point to the possibility that these T1D antigens are generated through a natural posttranslational process referred to as transpeptidation. These antigens are highly immunogenic compared to the wild type sequence, presenting with up to 1e6 times more activity in stimulating the antigen specific CD4 T cells. We have begun working on elucidating the biochemical mechanism responsible for generating these peptides.

CONCLUSIONS

Posttranslational modifications and internal deletions within the proinsulin, chromagranin A and secretogranin molecules can lead to the generation of novel, diabetogenic antigens and lead to the breakdown of central tolerance.

O27: Hypo-responsive Memory T cells Predominate Following Alefacept Treatment of New Onset Type 1 Diabetes Subjects

AUTHORS

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PURPOSE

In the ITN T1DAL trial, Alefacept treatment preserved endogenous insulin production in 30% of recent onset T1D patients a year following therapy cessation. Longitudinal flow cytometric analysis, RNA-seq and CyTOF were performed to elucidate Alefacept's mechanism of action and associated responder profiles.

METHODS

Longitudinal flow cytometric analysis in blood PBMCs, RNA-seq and CyTOF in isolated CD8 T cells.

SUMMARY OF RESULTS

Treated subjects experienced marked depletion of CD2hi memory T cells and CD56hi NK cells, with preservation of CD2lo regulatory T cells (T_{REG}). Further investigation revealed an increase of PD-1+TIGIT+CD4 effector memory T cells (T_{EM}) in the spared non-T_{REG} population, which were inherently proliferative and lacked proinflammatory cytokine production after in vitro stimulation. The nature of CD4 helper T cells often determines the function and expansion of CD8 T cells. Thus, we next explored the phenotype of CD8 post-treatment and stratified patients based on treatment responses. KLRG1+TIGIT+, CD57+, CD57+KLRG1+PD1- and GRZMB+ CD8 T_{EM} populations, identified by flow cytometry, increased in treatment responders who preserved beta cell function. Transcriptome analysis of CD8 T cells identified a module of genes with similar expression kinetics as the CD8 T_{EM} populations that included genes associated with activation, exhaustion and senescence (e.g. CD38, EOMES, GRZMB, CD57, TIGIT, LAG3, KLRD1, CD160). Unbiased CyTOF analysis of CD8 cells identified two clusters whose post treatment frequencies and kinetics mirrored those of the transcript module. These represented GZMB+CD57high CD8 T_{EMRA} and EOMES+ T_{EM} expressing CXCR3, CD95, and CD27.

CONCLUSIONS

All three complementary techniques indicate a post treatment induction of activated but potentially hyporesponsive CD8 T cells in treatment responders following induction of a PD-1+TIGIT+ CD4 population.

O28: Follicular Regulatory T cells are reduced in pancreatic lymph nodes of patients with T1D

AUTHORS

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PURPOSE

The germinal center (GC) response is the foundation of the T-cell dependent antibody response, where antigen-specific B cells undergo expansion, class switching, somatic hypermutation and differentiation into plasma cells and memory B cells. The GC response is a tightly regulated process that takes place in secondary lymphoid organs such as the spleen and lymph nodes where T cells, B cells and DCs interact to promote the development of highly specific antibodies while maintaining tolerance to self-antigens. It is controlled by two subsets of CD4 T cells that are known as follicular helper (T_{FH}) and follicular regulatory T cells (T_{FR}). In human autoimmune diseases characterized by AAb production such as T1D, T_{FH} are thought to play central role in promoting autoantibody (AAb) development, while T_{FR} are considered suppressors of this process. With the aim to understand the mechanism underlying AAb development and the onset of T1D in humans, we analyzed T_{FH} and T_{FR} that reside in pancreatic lymph nodes (PLN) from patients with T1D and compared them to subjects with no T1D.

METHODS

We used a combination of antibodies specific for CD3, CD4, CXCR5, PD-1 and FOXP3 to identify T_{FH} and T_{FR} via flow cytometry, in PLN of non-diabetic donors ($n = 15$) and patients with T1D ($n = 7$). Samples derived from nPOD and the San Raffaele hospital. According to the expression of PD-1, CD4 T cells were separated into three subsets: CXCR5⁺PD-1⁻, CXCR5⁺PD-1⁺ and CXCR5⁺PD-1⁺⁺. After gating in each subset we identified T_{FH} (FOXP3⁻) and T_{FR} (FOXP3⁺). Paraffin-embedded PLN from non-diabetic ($n = 8$) and diabetic donors ($n =$

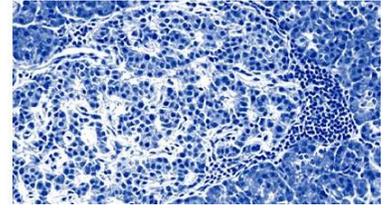
8) were stained with fluorescent labeled antibodies for CD20, CD4 and FOXP3. Follicles were identified by the expression CD20, and the number of T_{REG} and T_{FR} was estimated by counting the number of CD4⁺FOXP3⁺ cells in the T-cell zone (TCZ) and GC, respectively.

SUMMARY OF RESULTS

The frequency of PD-1⁺ T_{FR} (determined by flow cytometry) was reduced in the PLN of patients with T1D in comparison to non-diabetic subjects. Instead, the frequency of PD-1⁻ and PD-1⁺⁺ T_{FR} remained unaltered. Furthermore, no significant differences in the T_{FH} compartment were found between non-diabetic and T1D PLN, irrespective the expression levels of PD-1. In line with these observations, multiple immunofluorescence analysis showed that the number of CD4⁺FOXP3⁺ was reduced in the PLN GCs of patients with T1D as compared to controls. Interestingly, a reduced number of CD4⁺FOXP3⁺ cells was also seen in the TCZ of T1D PLN.

CONCLUSIONS

By undertaking two complementary approaches, flow cytometry and multiple immunofluorescence, we found that the frequency and number of T_{FR} is reduced in the PLN of patients with T1D as compared to non-diabetic controls. These findings indicate that perturbation in T_{FR} numbers might be responsible for the AAb development in patients with T1D.



(LB-01)

Use of Nanoparticles Containing an Insulin-ChgA Hybrid Peptide for Antigen-Specific Tolerance Induction in the NOD Mouse

AUTHORS

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PURPOSE

In Type 1 Diabetes (T1D) patients and non-obese diabetic (NOD) mice, autoreactive CD4⁺ T cells contribute to the destruction of insulin-producing β -cells in the pancreatic islets. Our lab has discovered that in NOD mice the endogenous ligand for the diabetogenic CD4⁺ T cell clone BDC-2.5 is a post-translationally modified hybrid insulin peptide (2.5HIP), consisting of a fragment from proinsulin fused to a peptide from chromogranin A (ChgA). The purpose of this study was to determine if tolerogenic poly(lactide-co-glycolide) (PLG) nanoparticles (NPs) coupled with the 2.5HIP antigen (2.5HIP-PLG) can be used to 1) prevent diabetes in a BDC-2.5 transfer model, and 2) prevent spontaneous diabetes in NOD mice.

METHODS

In the transfer model, we adoptively transferred activated BDC-2.5 T cells into NOD.*scid* mice and infused 2.5HIP-PLG NPs intravenously one day after. Splenic and pancreatic T cells from NOD.*scid* recipient mice were analyzed *ex vivo* to investigate mechanisms of tolerance induction and maintenance. In the spontaneous disease model, 2.5HIP or HEL-coupled (negative control) NPs were administered to prediabetic NOD mice either alone or in combination with a short course of low-dose (LD) recombinant IL-2 (rIL-2). Some mice were monitored for diabetes incidence whereas *ex vivo* analysis was performed on T cells from other mice, using MHC class II tetramers loaded with HEL or 2.5HIP sequences to determine if antigen-specific regulatory T cells (Tregs) were induced after treatment.

SUMMARY OF RESULTS

We have found that 2.5HIP-PLG NPs prevent transfer of diabetes with BDC-2.5 T cells by impairing the function of effector T cells (Teffs) and increasing the proportion of Tregs to Teffs. We are currently trying to understand how signaling pathways downstream of the T cell receptor are modulated in effector T cells. Importantly, we have observed that administration of

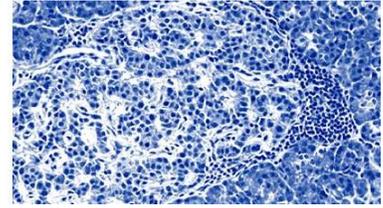
2.5HIP-PLG NPs in combination with LD rIL-2 is capable of significantly expanding 2.5HIP-specific Foxp3+ Tregs in prediabetic NOD mice. The induced Tregs persist in the pancreas for a long period of time and have an activated phenotype. Our preliminary data indicate that the combination therapy using 2.5HIP is capable of preventing/delaying the onset of spontaneous diabetes.

CONCLUSIONS

By delivering 2.5HIP on PLG nanoparticles, BDC-2.5 T cells can be tolerized providing the first evidence for tolerance induction in these cells using an endogenous ligand and not a mimotope peptide. When antigen-loaded PLG NPs are combined with LD IL-2 treatment, antigen-specific Tregs can be significantly expanded in NOD mice *in vivo*. Although the combination therapy is still being optimized it appears that expansion of 2.5HIP-specific Tregs can control autoimmune pathology in NOD mice. This robust combination therapy may have substantial implications for the development of an antigen-specific treatment for T1D patients.

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(LB-06)

Reactivity to preproinsulin and insulin-DRiP by CD8 T cells in the islets of type1 diabetes organ donors.

AUTHORS

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¹Barbara Davis Center for Childhood Diabetes University of Colorado School of Medicine, ²Vanderbilt University, ³University of Florida, ⁴City of Hope

PURPOSE

The goal of this study is to identify peptide-MHC complexes that are recognized by CD8 T cells in the islets of organ donors having type 1 diabetes (T1D).

METHODS

We isolated CD8 T cells from islets of six organ donors having T1D and identified T cell receptor (TCR) alpha and beta chain clonotypes of each single cell. To seek antigen specificity, TCR transductants were generated to test for the response to candidate peptides in the presence of antigen presenting cells expressing autologous HLA molecules. Reactivity by each TCR transductant was examined using a moderate-throughput screening system which exploits the NFAT (nuclear factor of activated T cells)-reporter and allows us to analyze up to 8 TCRs in a single culture reaction.

SUMMARY OF RESULTS

We identified TCR alpha and/or beta clonotypes from 988 CD8 T cells that were found in the islets of six T1D organ donors. A large proportion of CD8 T cells had identical clonotypes within each donor, suggesting clonal expansion upon antigen stimulation. We selected TCR clonotypes that were detected from two or more cells to generate TCR transductants for further antigen specificity analysis. We tested the TCR transductants in the presence of autologous Epstein-Barr virus-transformed B cells for the response to 100 preproinsulin (PPI) and 63 insulin-DRiP (defective ribosomal products) truncated peptide pools as well as 12 islet-derived peptides that are known to be an antigen for HLA-A2-restricted CD8 T cells. Approximately 20% of unique TCR clonotypes were reactive to PPI or insulin-DRiP peptides. We have not detected any reactivity to the known A2-epitopes outside of PPI. Of the four donors analyzed to date the frequency of CD8 T cells expressing PPI-reactive TCR clonotypes was variable (4-45%), while the insulin-DRiP-reactive TCRs were detected in a single donor (24%). Antigenic peptides were found in various regions throughout PPI, but there are "hot spots" that are frequently recognized by a large number of T cells.

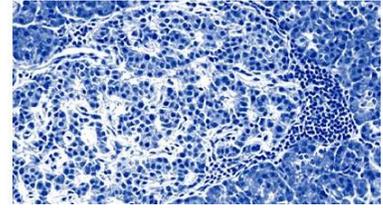
Interestingly, some of the PPI peptide regions that are determined as CD8 epitopes in our current study are overlapped with those that have been demonstrated as antigens for CD4 T cells by previous studies. Further analysis to determine HLA restriction demonstrated that HLA molecules presenting the PPI and insulin-DRiP peptides are extremely diverse across HLA-A, B, and C genes, raising a need to develop immuno-monitoring T cell assays beyond limited HLA class I restriction.

CONCLUSIONS

PPI and insulin-DRiP-reactive CD8 T cells are frequent in the islets of a certain subset of T1D donors. Our findings provide underlying evidence to use preproinsulin for antigen specific therapy. Robust biomarkers that can discriminate patients having high PPI and insulin-DRiP reactivity in the islets are needed to enrich treatment responders. Furthermore, preferential recognition of the leader region of PPI has important implication for insulin-specific therapy: i.e. whole preproinsulin rather than just insulin may need to be administered to improve tolerance induction.

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(LB-11)

Role of Dietary Insults and Lymphangiogenesis in the Initiation of T1D in NOD Mice and Human

AUTHORS

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¹ Department of IBSC

PURPOSE

Studies were aimed at understanding the initiation of T1D in mice and man. Inflammation is necessary to initiate autoimmunity. The possibility that inflammatory signals come directly from the duodenum was examined. Secondly, progression of autoimmunity requires lymphatic recirculation of T cells. Because, the islet is virtually devoid of lymphatics, neolymphangiogenesis must take place to allow the amplification of T cell activation and proliferation as well as the spreading of disease. Because lymphangiogenesis is mechanistically very well understood, it offers multiple potential therapeutic targets that could be used to limit onset and progression of disease.

METHODS

The examination of communications between duodenal content and pancreatic lymphatics was performed to address mechanistically the possibility that dietary inflammatory signals could access islet directly. Gavage, immunofluorescence and surgery were used to answer this question. Profiling of activation of islet-specific T cells in peripheral blood using single cell technology was then used to time the recirculation of CD4 T cells from islets to lymphatics to blood. Tissue immunofluorescence and single cell gene expression profiling were used to deconvolute the steps leading to lymphangiogenesis in the islets and the islet-lymphatics communication. Translational steps were taken by analyzing nPOD samples. Proof of principle therapeutic interventions were performed using surgery as well as by using inhibitors and inducers of lymphangiogenesis.

SUMMARY OF RESULTS

Communication between the duodenum, the pancreatic lymphatic system, and the islets was demonstrated for all macromolecules tested (lipids, proteins, nucleic acids). The functional impact of this communication was demonstrated for T cells recognizing lipids. In mice, activated beta cell specific cells do not recirculate before weeks 6-7. At that time point, intense lymphangiogenesis takes place near and within islets and results in the peri-

insulinitis lesion. Platelets and macrophages appear to be the drivers of this process. A fraction of islet macrophages express the master lymphangiogenesis transcription factor Prox1. Through epigenetic modifications, Prox1 drives the expression of the trophic factor of lymphatic vessels, VEGF-C, and its receptor VEGFR3. This signaling loop induces the expression of LYVE-1 and podoplanin on endothelial cells and the building of new lymphatic structures within lymphokine islands, another soluble factor expressed by macrophages during inflammation. Prox1, VEGF-C, VEGFR3, LYVE-1, podoplanin, and midkine were shown to be all expressed in the islet at a very early stage of disease. In addition, some macrophages express CCL9, LTA, and S1P to promote T cell migration and accumulation. Initial examination of nPOD samples from recently diagnosed patients seems to confirm the animal data.

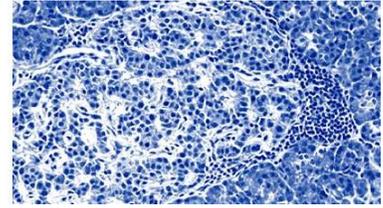
In proof of concept experiments, blockade of podoplanin with antibodies delayed the onset of diabetes as did inhibition of Prox1 activity through pharmacologic inhibition of CPT1A. To the opposite, the promotion of Prox1 activity through dietary intervention accelerated disease.

CONCLUSIONS

Original islet insult and building of islet lymphatics might constitute important therapeutically addressable steps to prevent T1D.

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(LB-18)

Development of an Immunoengineered Hydrogel Platform to Deliver CCL21 and Induce Tolerance in Type 1 Diabetes

AUTHORS

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PURPOSE

Type 1 Diabetes (T1D) is an autoimmune disease, in which T cell-mediated destruction of pancreatic β -cells leads to insulin deficiency and hypoglycemia. Defects in the regulation of central and peripheral tolerance result in escape of islet-autoreactive T cells and infiltration in the pancreas. Tumor cells can induce tolerance towards the antigens they express and escape immune destruction through autologous secretion of the secondary lymphoid chemokine CCL21 and formation of tertiary lymphoid tissues (TLOs). Recently, we found that transgenic non-obese diabetic mice (Ins2-CCL21) expressing CCL21 locally in β -cells were protected from T1D development. Here, we investigated whether local CCL21 secretion distally from the pancreas could regulate diabetogenic cells systemically and protect the pancreas, as a rationale for designing a bioengineered platform for local delivery of CCL21 to induce tolerance in T1D. Additionally, the purpose of our research was to develop natural (fibrin) and synthetic (PEG) hydrogels for local, controlled delivery of CCL21 and potentially β cell antigens.

METHODS

Seven weeks-old NOD-scid mice received transplants in the kidney capsule (KD) at a marginal dose (500 IEQ/mouse) of islets from either Ins2-CCL21 NOD or non-transgenic mice. Isolation of pancreatic islets from Ins2-CCL21 NOD mice and non-transgenic littermates, as well as transplants in the renal subcapsular space in the kidney (KD) were performed at the DRI Preclinical Cell Processing and Translational Models Core.

Splenocytes (10 million) from recently diabetic NOD mice were adoptively transferred by intravenous (IV) injection into NOD-scid recipients of CCL21 transgenic or control islets 4 weeks after islet transplantation. Diabetes was diagnosed after three consecutive blood glucose levels above 250mg/dL. Sections from formalin-fixed paraffin-embedded or OCT frozen blocks were stained and imaged following standard protocols.

To control the release of CCL21 from fibrin gels, recombinant Tg-CCL21 was engineered to contain a fibrin-binding site (transglutaminase substrate of factor XIII, Tg) and a plasmin-

cleavable linker upstream of the CCL21 protein sequence. Tg-CCL21 was obtained from Celplor Inc. and was characterized using Western Blot and ELISA. Chemotactic function of CCL21 and Tg-CCL21 was confirmed using a transwell assay. Fibrin gels (8mg/ml fibrinogen, 2U/ml thrombin, 8U/ml factor XIII, 2.5mM HEPES, 17ug/ml aprotinin) containing either CCL21 or Tg-CCL21 (500nM) were implanted subcutaneously on the back of 4 weeks female NOD mice. Implants and skin were collected 21 days after treatment, fixed in formalin and stained with stromal cell (gp38, α -SMA, Lyve-1) or lymphocyte (CD3, B220) antibodies to determine TLO formation.

SUMMARY OF RESULTS

Formation of TLOs in Ins2-CCL21 islet grafts under the kidney capsule as well as significant delay in diabetes development were observed when compared to mice transplanted with non-transgenic islets (median diabetes onset was 22.5 days in recipients of non-transgenic NOD islets vs. 75 days in recipients of Ins2-CCL21 NOD islets, $p=0.0002$). Thus, CCL21 expression in β cells was suggested to delay the onset of autoimmune diabetes when sufficient time was allowed for the formation of regulatory TLOs at the graft site. Importantly, recipient NOD-scid mice had a functional pancreas at the time of transplant; hence absence of islet infiltration in the host pancreas upon transplantation of Ins2-CCL21 islets in the kidney capsule meant that the transgenic islets also protected β -cells in the native pancreas, which suggested a systemic protective effect. This effect was not present in NOD-scid mice receiving non-transgenic islets.

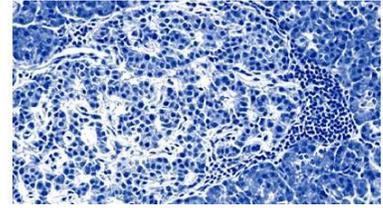
Regarding the bioengineered platform for delivery of CCL21, fibrin gels were tested *in vivo*. Fibrin gels were degraded by day 21, while mice implanted with the CCL21-containing gels revealed formation of fibroblastic reticular cell-like stromal reticula, as indicated by the presence of gp38⁺Lyve-1⁻ cells in the tissues. Hence, local CCL21 delivery in the subcutaneous site induced TLO formation.

CONCLUSIONS

Reminiscent of CCL21 secretion by tumor cells, secretion of CCL21 by β -cells either in the pancreas or in a distal site protects against diabetes on a systemic level. The local release of CCL21 from fibrin gels, implanted subcutaneously in prediabetic NOD mice, promoted the recruitment of fibroblastic reticular-like stromal cells leading to the formation of reticular networks in the skin, similar to CCL21-induced stroma in CCL21-secreting tumors and in Ins2-CCL21 NOD mice. Future studies will correlate CCL21 release kinetics to TLO formation in fibrin gels and synthetic gels (PEG) and will investigate the ability of CCL21-secreting gels to regulate autoreactive T cells after implantation in NOD mice.

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(LB-22)

Type III interferons (IFN- λ s) are expressed in the human type 1 diabetes pancreas at disease onset, upregulate HLA class I expression and protect beta cells from Coxsackievirus infection

AUTHORS

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PURPOSE

Type I and II interferons (IFNs) have been proposed to contribute to beta cell destruction in human type 1 diabetes. Type III IFNs (IFN- λ (L) 1-4) constitute a more recently described group of IFNs that are produced by both immune and parenchymal cells during inflammation and infection. They are known to induce a gene transcriptional signature similar to that induced by type I IFNs and they have been demonstrated to contribute to antiviral defense. We have previously shown that type III IFNs are expressed by human pancreatic islets infected with Coxsackievirus *in vitro*, that type III IFNs induce a type I IFN-like gene transcriptional signature and protect human islets from Coxsackievirus infection. If type III IFNs are expressed in human type 1 diabetes has previously not been investigated. Moreover, the direct effect of type III IFNs on beta cells remains unexplored. In the present study, we examined the impact of type III IFN stimulation on human beta cell HLA class I expression and analyzed the expression of genes encoding proteins involved in antiviral defense and/or frequently detected in the islets of enterovirus positive individuals with type 1 diabetes. We also investigated the expression of type III IFNs in laser captured islets from type 1 diabetes patients at disease onset.

METHODS

EndoC- β H1 cells and pancreatic islets were exposed to IFNs (IFN- α , IFN-L1 or IFN-L2) and were, in some experiments, subsequently infected with different CVB serotypes (CVB1-6). Changes in gene transcription levels were measured by RT-PCR. The cell surface expression of viral entry receptors and HLA class I, and the intracellular expression of

dsRNA and the viral proteins VP1, 2A and 3C, were analyzed by flow cytometry. Laser-capture was used to isolate islets (30-40 per donor) from recent onset type 1 diabetes patients (DiViD study) and matched controls (nPOD network). RNA was isolated, amplified and transcriptome analysis performed with Affymetrix Human 2.0ST arrays.

SUMMARY OF RESULTS

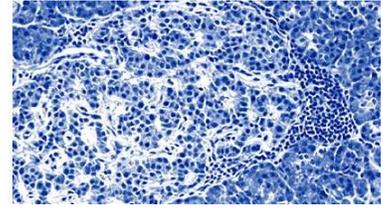
CVB1-6 infected EndoC- β H1 cells reaching an infection efficiency up to 60%, which led to the release of infectious CVB virions. Flow cytometry confirmed the expression of structural (VP-1) and non-structural (proteases 2A and 3C) virus proteins in CVB infected cells. The Coxsackie and Adenovirus Receptor (CAR) was expressed on the beta cells and blocking antibodies to the receptor attenuated CVB infection. IFN-L1 or IFN-L2 stimulation led to a strong increase in the cell surface expression of HLA class I. These IFNs also induced an IFN-inducible gene transcriptional signature including the upregulated expression of PKR, MDA5 and CXCL10, and strongly reduced beta cell permissiveness to CVB infection. The expression levels of IFN-L1 ($p < 0.001$) and IFN-L2 ($p < 0.01$) were increased in DiViD islets compared to controls. The DiViD islets were previously reported to have increased expression of HLA class I.

CONCLUSIONS

Type III IFNs are expressed in the human type 1 diabetes pancreas at disease onset. Exposure of human beta cells to type III IFNs leads to HLA class I hyperexpression, a characteristic feature of human type 1 diabetes. Type III IFNs also induce the expression of PKR, a protein commonly expressed in beta cells from enterovirus positive type 1 diabetes cases. By inducing an antiviral state, the type III IFNs have potent antiviral activity and protect beta cells from Coxsackievirus infection. Collectively, these studies highlight that type III IFNs may be important players in mediating both protective and potentially dangerous signals during the development of human type 1 diabetes.

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(LB-26)

Detection of Hybrid Insulin Peptides (HIPs) in Human Islets

AUTHORS

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PURPOSE

We recently discovered that hybrid peptides containing an insulin C-peptide fragment linked by a peptide bond to sequences derived from other proteins are present in murine beta cells. These hybrid insulin peptides (HIPs) are antigens for pathogenic CD4 T cells in the non-obese diabetic (NOD) mouse model of autoimmune type 1 diabetes (T1D). Furthermore, HIP-reactive CD4 T cells have been isolated from the islets of human donors with T1D. Here, we sought to confirm that HIPs are present in human islets.

METHODS

Islets were obtained from deceased non-diabetic human donors and proteins were extracted. Small proteins were isolated by size exclusion chromatography (SEC) and subjected to proteolytic digestion. Resultant peptides were analyzed by nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS). Spectra were searched against a standard human proteome database and a custom HIP database to identify HIP candidates. Potential HIP matches were validated by comparison of the MS/MS spectrum and chromatographic retention time of the endogenous peptides to those of synthesized peptide standards.

SUMMARY OF RESULTS

A HIP resulting from the linkage of two non-contiguous insulin C-peptide sequences was confidently identified in the islets of two different human islet donors. Several other HIP candidates were identified but remain to be validated.

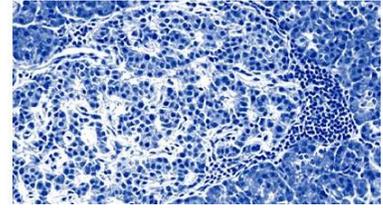
CONCLUSIONS

We demonstrate by mass spectrometry that HIPs are indeed present in human islets, providing rationale for further investigation into their role as autoantigens in T1D. Because of technical challenges associated with confidently identifying mass spectra corresponding to

HIPs, we present a set of criteria to be considered when analyzing mass spectra to reduce the likelihood of incorrectly assigning HIP sequences as interpretations. Our findings provide a foundation for future efforts to identify HIPs and highlight the potential complexity of the beta cell proteome.

Select the **ONE** category that best describes your research:

<input type="checkbox"/> Beta Cell Physiology and Dysfunction	<input type="checkbox"/> Novel Biomarkers
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Detection and characterization of antigen-specific CD8 T cells in the exocrine and endocrine pancreas of non-diabetic and diabetic donors

AUTHORS

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PURPOSE

Human type 1 diabetes is characterized by the immune-mediated destruction of insulin-producing beta cells in the pancreas. Autoreactive CD8 T cells recognizing beta cell-derived peptides are prime candidates for causing beta cell destruction. In a previous study, we have shown that in human islets from donors with type 1 diabetes but not healthy subjects, CD8 T cells dominated the lymphocytic infiltration, with some confirmed to have specificity for the beta cell autoantigen pre-proinsulin by *in situ* peptide-HLA tetramer staining. To further study the distribution and localization of pre-proinsulin specific T cells in both, endocrine and exocrine compartments, sections were stained using a pre-proinsulin (PPI) tetramer and CD8. In addition, to define the immune phenotype of autoantigen-specific CD8 T cells we included the memory marker CD45RO. Moreover, to find their exact location in the human pancreas and their position with respect to the islets we re-stained the tissue sections for insulin and glucagon. Our overall goal is to study pancreatic tissue samples not only from donors with short and disease duration but also from donors with pre-diabetes to better understand the role of antigen-specific T cells in disease pathogenesis at different time points of progression.

METHODS

Pancreatic frozen tissue sections from non-diabetic, pre-diabetic, and short and long-standing diabetic donors were obtained from nPOD. First, sections were stained with PPI tetramer overnight. The following day sections were stained with CD8 and CD45RO overnight. Sections were scanned using a Zeiss Axio.Scan Z1 slide scanner (Carl Zeiss). Afterwards, 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 done manually or with QuPath. First, cells positive for CD8, tetramer and CD45RO were determined. Then, the location in the pancreas with respect to the islets using insulin and glucagon was identified.

SUMMARY OF RESULTS

We have found PPI-positive cells localized close to the islets but also present in the exocrine tissue. The distribution of PPI positive CD8 T cells was variable between islets. In addition, the vast majority of PPI positive cells were CD45RO positive, indicating that these cells are

indeed memory T cells. Interestingly, we found many PPI-positive CD8 T cells in the pancreas tissue section from a donor with T1D (disease duration of 1 year) which had a few insulin-containing islets. Furthermore, an accumulation of cells, including PPI-positive CD8 T cells, was seen in close proximity to insulin-containing islets. Some PPI-specific CD8 T cells were also found inside the insulin-containing islets. In comparison, PPI-specific CD8 T cells were rarely seen in insulin-deficient islets in this particular tissue sample. Overall, PPI-specific CD8 T cells were found in short and long-term donors. However, insulin-containing islets were rarely seen in the long-term donors analyzed so far. Therefore, we started to include tissue samples from donors with pre-diabetes (aab+) in our analysis, in order to determine their numbers and location before disease onset.

Studies by other groups suggest that T cell behavior is highly dependent on the microenvironment of each individual islet. In addition, there are islets at many different stages of destruction in a given human pancreas that can go from insulin deficiency to a normal function and structure. Accordingly, our data suggest that PPI-specific T cell infiltration is highly dependent on the presence of insulin.

CONCLUSIONS

Our findings are of potential interest to the field, as they suggest that antigen presentation and encounter by CD8 T cells occur even in longstanding patients and as long as the antigen is present.

Select the **ONE** category that best describes your research:

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<input checked="" type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):

NOVEL BIOMARKERS

O28-2:

A type 1 diabetes genetic risk score can aid classification of nPOD cases

AUTHORS

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PURPOSE

Misclassification of diabetes is common and under-recognized due to the overlap in the clinical features of type 1 diabetes (T1D) and type 2 diabetes (T2D). nPOD is a resource of rare pancreatic samples that includes clinically defined T1D and T2D cases. Numerous analyses of these rare samples are restricted to a small sample size, and any misclassified T1D or T2D has the potential to confound these analyses. We and others have shown that a T1D genetic risk score (T1D GRS) can accurately capture an individual's genetic susceptibility to T1D and can be used to help discriminate between T1D and T2D. We aimed to test the T1D GRS as a biomarker which could inform the classification of nPOD cases.

METHODS

We calculated the T1D GRS in 117 T1D cases and 39 T2D cases from the nPOD biobank. Potentially misclassified T1D cases were identified by having a T1D GRS below a pre-defined low genetic risk threshold (<0.234 , <5 th centile of a reference T1D cohort). Potentially misclassified T2D cases were identified as cases with a T1D GRS above high-risk threshold (>0.280 , >50 th centile of T1D cases in a reference cohort). We stained $4\mu\text{m}$ FFPE pancreas sections from atypical nPOD T1D and T2D donors using standard immunohistochemical protocols with antiserum against insulin, glucagon and HLA1. We assessed sections for the presence of hallmark features of T1D.

SUMMARY OF RESULTS

Of 117 T1D cases, 13 (11% [95%CI 5.4%,16.8%]) had a T1D GRS <0.234 , higher than the 5% expected, suggesting some misclassification. 2/13 were identified as misdiagnosed, with analysis revealing features inconsistent with T1D.

Of 39 T2D cases, 3 (8% [95% CI 0%, 16%]) had a T1D GRS >0.280 . 1/3 had evidence of loss of insulin containing islets (ICIs), presence of insulin-deficient islets (IDIs) and hyperexpression of HLA1; features consistent with T1D, identifying this as a misdiagnosed donor. 46/46 T1D cases with a T1D GRS >0.28 were confirmed as T1D, with regard to their histopathology, with evidence of lobular loss of ICIs or no residual ICIs.

CONCLUSIONS

By examining cases where the T1D GRS was inconsistent with the clinical diagnosis, we were able to identify misclassified cases in the nPOD cohort. The T1D GRS may be a useful additional biomarker to help correctly classify cases and inform selection of samples for further investigation.

O29:
Unsupervised Clustering of High Parameter Mass Cytometry Data Reveals Immune Heterogeneity in T1D

AUTHORS

Leeana Peters¹, Maigan Brusko¹, Daniel Perry¹, Melanie Shapiro¹, Wen-I Yeh¹ and Todd Brusko¹

PURPOSE

Attempts to identify and characterize novel immune cell subsets involved in Type 1 Diabetes (T1D) pathogenesis have been precluded by restriction of flow cytometric parameters and subjective methods of analysis. Recent advances in both technology and bioinformatics have addressed this concern and now allow for detection of immune signatures that could be overlooked in biased gating strategies. In addition, there remains a paucity of information as to the cellular distribution and phenotype of lymphocyte subsets at or near the site of autoimmune attack. We therefore sought to apply unsupervised clustering methods to high (51) parameter Mass Cytometry (CyTOF) data from nPOD donor PLN. The potential for identification of novel cell subsets, or differentially expressed markers on existing cell subsets of interest, has important implications for biomarker discovery and the development of rational therapies for T1D.

METHODS

Cryopreserved cells from PLN of healthy controls (n=12) and T1D subjects (n=10), were stained with MAXPAR metal-chelating polymers and run on a CyTOF™ mass cytometer. Resulting FCS files were processed using the Cytobank platform and analyzed using CITRUS with a Nearest Shrunken Centroid (PAMR) model to elucidate differential clustering and compare marker expression between controls and T1D subjects.

SUMMARY OF RESULTS

CITRUS analysis in the CD4 compartment revealed two clusters with higher median expression of CCR7 and CD38, respectively (FDR corrected $p < 0.01$). The CD8 compartment contained 16 clusters with higher median expression of 14 markers (FDR corrected $p < 0.01$). These can be broken down into activation-related markers (CD28, CD27, HLADR, CD38, CD25, CD127, and CD226), chemokine receptors (CXCR3, CXCR5, and CCR6) and markers of antigen experience and tissue retention (CD45RA, CCR7, CD69, CD103).

CONCLUSIONS

Unsupervised clustering with high parameter data revealed significant heterogeneity in the CD8 compartment of T1D subjects. The differentially expressed markers present in T1D subjects as compared to controls reflect increased activation and potentially effector function. We intend to use this data in combination with sequencing and functional studies to

determine key subsets within this diverse population that may contribute to disease pathogenesis.

O30: Evidence of Incomplete Prohormone Processing in Type 1 Diabetes from Mass Spectrometry-based Proteomics

AUTHORS

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PURPOSE

The primary function of the islet is to produce mature hormones that maintain glucose homeostasis and metabolic control. It is therefore paramount that these hormones are synthesized and processed correctly and efficiently. Deficiencies in hormone processing and maturation during conditions of cellular stress have been implicated in the natural history of type 1 diabetes (T1D). Herein, we applied mass spectrometry-based proteomics assays to investigate the islet proteome as well as human sera from pre-symptomatic autoantibody positive (AAb+) and long-standing T1D patients to identify evidence of incomplete prohormone processing.

METHODS

Human islet sections from non-diabetic controls, multiple AAb+ cases, and T1D subjects from the Network for Pancreatic Organ donors with Diabetes (nPOD) program were isolated by laser microdissection (LMD). Human serum samples were obtained from the T1D Exchange Clinic Registry from individuals with varying disease duration. Islet sections were subjected to proteome profiling either as a pool of islets or at the level of a single islet by nanoscale proteomics technologies. Targeted quantification of prohormone isoforms in serum was performed by liquid chromatography selected reaction monitoring (LC-SRM).

SUMMARY OF RESULTS

Using highly sensitive mass spectrometry based proteomics techniques we were able to detect both mature hormones and the incompletely processed prohormone products such as proinsulin and pro-IAPP from *in situ* islet sections isolated by LMD. We have further developed targeted mass spectrometry assays for quantifying C-peptide and proinsulin in serum. Our assays confirm that proinsulin can be detected in control and pre-diabetic cases, and persists even in long-standing type 1 diabetic patients. In addition to uncleaved prohormone products, we have observed significant differences in the post translational modification (PTM) by phosphorylation of the pro-protein convertase, PCSK1, which may lead to altered activity.

CONCLUSIONS

Improper processing and dysfunctional hormone maturation pathways may prove to be a hallmark of type 1 diabetes. Assays that are capable of identifying and quantifying prohormone products in serum will be of great value for type 1 diabetes research. Our observations confirm the existence of prohormone products in islets and sera.

O31:

Comprehensive analysis of micro-RNA expression profiles in human islets and islet-derived exosomes during cytokine mediated β cell stress and death

AUTHORS

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PURPOSE

Type1 diabetes (T1D) is characterized by autoimmune-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 that test disease-modifying therapies in Stage 1 and 2 disease. However, there remains an urgent need to identify biomarkers with the ability to stratify heterogeneous at-risk populations within these different pre-clinical stages. In the present study, we aimed to identify signatures of islet derived exosomal microRNAs (miRNAs) that could leveraged to detect ongoing β -cell stress/death during the evolution of T1D.

METHODS

Human pancreatic islets obtained from 10 cadaveric donors (5M/5F) were treated with or without pro-inflammatory cytokines (IL-1 β and IFN- γ) to mimic *in vivo* T1D conditions. Exosomes were isolated using either an ExoQuick precipitation method or by serial ultracentrifugation and characterized using nanoparticle tracking analysis (NTA) and electron microscopy (EM and SEM). Small RNA sequencing was performed on islets and islet derived exosomes using an Ion Proton System. Partek Flow and DESeq2 package in the R statistical program were utilized for data analysis. Human mature microRNAs (miRNAs) were annotated using miRBase v 20, and those with a total read count of less than 10 were removed from further analysis. miRNAs with a fold change ≥ 1.5 and $p < 0.05$ in cytokine-treated islets and islet-derived exosomes were considered as differentially expressed (DE). Differentially expressed miRNAs were validated using independent sets of islets and islet derived exosomes using RT-PCR.

SUMMARY OF RESULTS

A total of 2794 miRNAs were profiled from islets and islet derived exosomes. After filtering for read counts, 1110 miRNAs and 890 miRNAs were retained from islets and exosomes, respectively, out of which 20 (15 upregulated and 5 down regulated) and 14 miRNAs (11 upregulated and 3 downregulated) were differentially expressed in islets and exosomes. Only 2 miRNAs (miR-155-5p and miR-146a-5p) were commonly upregulated in both islets and exosomes. These findings were validated in independent sets of cytokine-treated islets and islet-derived exosomes. Functional pathway enrichment analysis of miR-155-5p and miR-146a-5p identified pathways related to insulin signaling, ER stress, apoptosis, pancreatic secretion and antigen processing, indicating possible role of these miRNAs in β -cell stress/death. Further analysis was performed to define whether any donor characteristics were associated with miRNA expression patterns. Here, an interesting effect of sex of the donor islets was observed. Comparative analysis between male and female donor islets showed a total of 20 miRNAs in male and 29 miRNAs in female islets that were differentially expressed. Only four miRNAs were common between both sexes. Similarly, analysis of the exosomal dataset identified 7 miRNAs from male and 13 from female that were differentially expressed. Only 2 miRNAs were common between both sexes (miR-155-5p and miR-146a-5p).

CONCLUSIONS

Taken together, our analysis identified two miRNAs that were coordinately upregulated in both islets and exosomes in response to pro-inflammatory cytokine treatment in both male and female donors. Beyond these two miRNAs, we identified a strong sexual dimorphism in miRNA regulation in human pancreatic islets and islet-derived exosomes. These data highlight the importance of considering sex as a biological variable when defining new biomarker strategies for T1D.

O32:

Use of a Computational Biology Workflow to Identify and Prioritize Altered Pre-mRNA Splicing Events in Cytokine-Stressed Human Islets and Subjects with New-Onset T1D

AUTHORS

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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 islets treated with pro-inflammatory cytokines and to relate these events to those appearing in the circulation of subjects with new-onset T1D.

METHODS

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-1 β and IFN- γ) for 24 hrs. Total RNA was extracted and subjected to 2 x 75 bp paired-end RNA sequencing at a 50 million sequencing depth using an Illumina NextSeq500. 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 Δ PSI and 5% FDR were used. Differential expression analysis was performed using edgeR. We then applied a machine-learning computational model, ExonImpact, to predict the functional impact of individual skipped exon events on protein function, and a cutoff Functional Impact Score ≥ 0.5 was employed. RNA-binding protein (RBP) motif enrichment analysis was further performed on seven sequence regions around differentially spliced skipped exon events. RNA sequencing of alternatively spliced transcripts in whole blood was performed in 12 new-onset T1D subjects (age: 12 ± 4 ;

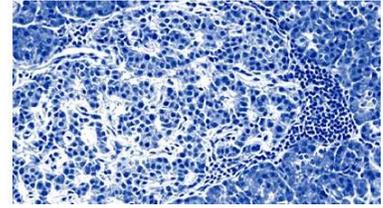
gender: 8 M/4 F; BMI: 19.15 ± 5.07) and 12 age- and sex-matched healthy controls (age: 12 ± 4 ; Gender: 8 M/4 F; BMI: 19.52 ± 2.52) using the same computational workflow.

SUMMARY OF RESULTS

In aggregate, 970 splicing events in 753 unique mRNAs were identified following cytokine treatment. The majority (44.8%) involved a skipped exon; 38.9% of events were categorized as a mutually exclusive exon; 6.3% involved a 3' alternative splice site; 5.8% involved a 5' alternative splice site, and 4.2% events resulted from a retained intron. Among this set of transcripts, gene ontology analysis revealed enrichment for biological pathways related to exocytosis, post-Golgi vesicle-mediated transport, protein transport and phosphorylation. 129 splicing events coupled with multiple isoforms were retained based on ExonImpact prediction. Notably, two events mapped to HLA-DMB, which is known to have a role in MHC Class II antigen processing and one splicing event was identified in PTBP2, an RBP with known roles in pre-mRNA splicing and interferon signaling. We next performed PCA on RBP motif enrichment scores derived from 6-mers based on a z-score calculation. We found that depletion events were enriched for G-rich motifs and inclusion events were enriched for A-rich motifs. Moreover, twelve of the predicted enriched RBPs were found to be differentially expressed at the mRNA level in response to cytokine treatment. Because mRNAs from stressed cells can be released into the circulation as cargo in extracellular vesicles, sequencing was performed at a depth of approximately 180M reads per sample in the whole blood of new-onset T1D. Our data revealed 662 differentially-expressed alternatively-spliced transcripts in subjects with T1D compared to controls; notably, 19 of these alternatively-spliced transcripts 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 islets. Future studies will test the functional impact of these splicing events on β -cell function and immunogenicity and test key events as potential biomarkers of T1D risk and heterogeneity.



(LB-07)

Expression of alternatively spliced insulin gene products in non-beta cells in human pancreatic islets

AUTHORS

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PURPOSE

Blood glucose homeostasis is tightly regulated by the endocrine cells within the islet of Langerhans. Glucagon, insulin, and somatostatin synthesis are restricted to alpha, beta, and delta cells, respectively. Yet, recent transcriptome analyses have revealed that insulin mRNA can also be detected in non-beta endocrine cells.

METHODS

We studied alternative splicing of human insulin mRNA in pancreatic islets

SUMMARY OF RESULTS

We identified the presence of an alternative insulin isoform containing the complete insulin B-chain with an altered C-terminus caused by the disruption of the original reading frame that is largely overlapping with a previously identified defective ribosomal protein (DRiP). Immunohistochemistry on pancreatic sections using antisera raised to detect alternative insulin-gene derived products shows the presence of this isoform primarily in somatostatin-producing delta cells.

CONCLUSIONS

Our data demonstrate that delta cells express the product of a spliced insulin-gene derived transcript. Although a role of this polypeptide has not yet been defined, its presence in secretory granules of somatostatin-positive cells, as determined by electron microscopy, suggests an endocrine or paracrine function. The expression of the insulin B-chain

containing immunodominant epitopes of CD4 and CD8 islet-autoreactive T-cells is stunning and worthy and may imply that a subset of delta cells is targeted too in T1D.

Select the **ONE** category that best describes your research:

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NOVEL TECHNOLOGIES

O33:

Assessment of viability, endocrine function and calcium signaling in pancreas slices generated at a remote site and first side-by-side evaluation of insulin secretion in pancreas slices and isolated islets from the same donor.

AUTHORS

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PURPOSE

The nPOD Slice Working Group has been developing the pancreas slice platform to conduct functional assessment of pancreatic cell function from organ donors, and to exploit this platform for a variety of studies that cannot be performed in isolated islets. A critical aim of the group is to make this study platform available to nPOD investigators, and thus to establish the feasibility that viable pancreas slices can be received at a remote site and effectively studied. In addition, there is a need to assess, in side by side comparisons, insulin secretion in pancreas slices and isolated islets from the same donor, to establish whether the responses observed in pancreas slices are representative of those observed in isolated islets, and to identify any differences that may exist between the two platforms, which could actually provide complementary information.

METHODS

The nPOD OPPC generated living pancreas tissue slices from nPOD donors without diabetes (n= 6), autoantibody-positive (AAb+, n= 1) and T1D (n=2, new onset and 10 years duration, respectively). On average, 60-80 pancreas slices (120 µm thick) were shipped to Miami, overnight. Upon receipt, slices were placed in culture, allowed to rest for 2 hours, and then we assessed viability, endocrine function (both beta and alpha) on perfusion studies. The perfusates were collected every minute and subsequent ELISAs for insulin and glucagon were performed. Areas under the curve (AUCs) were calculated for each case. We

performed Ca^{2+} imaging of both beta and alpha cells, after loading the cells with the membrane permeant Ca^{2+} indicator Fluo4 (2 h), in the presence of the protease inhibitor aprotinin, and after stimulation with different glucose concentrations or with substances known to alter cytosolic Ca^{2+} levels in endocrine cells. We conducted similar studies in an additional organ donor processed at the Diabetes Research Institute; for this donor, the pancreas was used to generate pancreas slices and to isolate islets with the Ricordi method.

SUMMARY OF RESULTS

Our results show that pancreas slices from all donors were viable (on average, viable endocrine and exocrine cells were 80% and 65%, respectively) upon overnight shipping from Gainesville. We did not observe differences in viability among the donors in the different donor groups. In terms on insulin secretion, the AUC of the response to 16.7 mM glucose were a mean of $1,012.3 \pm \text{SD } 137.25$ among 7 non-diabetic donors, and 414.9 in the single AAb+ donor, which appeared to be lower than in the controls. The insulin AUC values were as expected lower in the two donors with T1D, and dramatically more so in the donor with longer disease duration. Compared to the control donors, there was a 60% reduction in the insulin AUC in the donor with recent onset T1D, and a 90% reduction in the donor with 10 years disease duration. In both patients, and in the AAb+ donor, there was a delay in the insulin response. Significant alterations emerged in the glucagon responses as well, in response to the various stimuli. In performing the assessment of calcium responses, pancreatic islets in living slices from donors without diabetes can be easily distinguished from the surrounding acinar tissue because of the higher backscatter signal endocrine cells produce. Identification of remaining islets in T1D tissue can be more challenging due to the loss of backscatter signal caused by beta cell destruction and disruption of the islet architecture. Nonetheless, endocrine cells incorporated the Ca^{2+} indicator. Membrane depolarization with KCl (25 mM) increased cytosolic Ca^{2+} levels in most endocrine cells (~70%) in living slices from donors without diabetes, with/without autoantibodies. In contrast, endocrine cells from T1D donor with long duration did not respond to KCl. Similarly, glutamate, a specific stimulus for human alpha cells, elicited Ca^{2+} responses in non-diabetic and AAb+ alpha cells, but had no effect in T1D islet cells. Changing extracellular glucose levels from 3 mM to 16 mM and then lowering it down to 1 mM increased cytosolic Ca^{2+} levels in different subsets of endocrine cells in slices from non-diabetic individuals but had no effect in T1D endocrine cells. Interestingly, in T1D slices, we observed preserved responses to changes in extracellular glucose or KCl in other cell populations that also incorporated the Ca^{2+} dye (e.g. vascular and immune cells). This indicated that type T1D endocrine cells were alive but secretory cell function was severely compromised. Finally, while taking advantage of the expertise available at the Diabetes Research Institute to produce pancreas slices and isolate islets from the same donor, we performed a side-by-side comparison of insulin secretion on case HP2298, a non-diabetic donor. We observed similar response profiles from pancreas slices and isolated islets. However, reflecting the lower number of islets tested in the slices (about 30) compared to the 100 isolated islets that were studied, the absolute insulin levels were proportionally lower. In addition, we also assessed viability, endocrine function (both beta and alpha) and calcium signaling (both beta and alpha) in pancreas slices generated from this donor.

CONCLUSIONS

Our results support the feasibility of generating pancreas slices that remain highly viable after shipment and can be utilized for a series of experiments involving live tissue. Furthermore, we observe alteration in the responses from T1D pancreata, and provide initial evidence that insulin secretion profiles in pancreas slices mimic the profile in isolated islets.

O34: Long-term culture of human pancreatic slices as a model to study real-time islet regeneration

AUTHORS

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PURPOSE

The slicing and *in vitro* culture of live pancreatic tissue is a powerful emerging technology that allows for the study of physiological responses in tissues that retain near-intact histological architecture. However, current culture conditions for human pancreatic slices (HPS) have only been tested for short-term culture. The study of potential regeneration phenomena would require a culture setting whereby viability and function are preserved for many days. Here, we describe studies to develop long-term culture conditions HPS.

METHODS

Since the pancreas is a highly vascularized organ, a key to long-term culture and tissue viability is to provide an adequate source of oxygenation. COMSOL modeling of oxygenation throughout 120 μm -thick slices confirms that their oxygen requirements are not met with the current trans-well culture standard. Here we cultured HPS atop perfluorocarbon (PFC)/silicone membranes, which are designed for the direct aeration of the tissue, enhance oxygenation and minimize oxygen diffusion gradients.

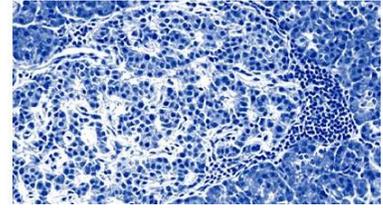
SUMMARY OF RESULTS

Using PFC/silicone dishes, we show for the first time high survival, robust insulin and glucagon secretion during perfusion as well as calcium imaging of islets in HPS, at baseline and at day 10 after the initial sectioning. Slices from $n=3$ independent donors cultured on PFC/silicone dishes exhibited better long-term viability (75.44 ± 2.02 vs. 59.78 ± 2.57 %, $P = 0.0002$), less tissue degradation (5.78 ± 0.5 vs. 3.1 ± 0.48 sq mm cross-sectional area, $P = 0.0015$), higher glucose stimulation index (2.11 ± 0.13 vs. 1.59 ± 0.08 , $P = 0.0008$) and stronger calcium imaging responses compared to those cultured in trans-wells. Of note, the stimulation index of slices cultured for 10 days in PFC/silicone dishes was comparable to

that obtained immediately after isolation from the same donors (2.11 ± 0.13 vs. 2.13 ± 0.14 , $P = 0.91$, n.s.). Trans-well-cultured HPS, in contrast, showed a statistically significant deterioration in glucose-mediated stimulation at day 10 post-slicing. Our data support our mathematical modeling prediction and that PFC/silicone dishes afford robust long-term culture and function. Moreover, based on our preliminary results using murine tissue, we present an adenovirus-based reporter system designed to dynamically monitor new islet cell formation in HPS.

CONCLUSIONS

Refined conditions for the long-term culture of HPS, coupled with our method to lineage trace specific cell populations for the detection of potential beta cell neogenic events, are expected to be of great impact for the study of human pancreatic regeneration.



O-35

Assessment of cell viability and endocrine hormone secretion in human pancreases obtained from nPOD organ donors by implementing fresh tissue slices technique

AUTHORS

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PURPOSE

In recent years tissue slicing has demonstrated to be a great investigative tool for the understanding of the exocrine and endocrine cell biology in pancreatic islets. Moreover, pancreas tissue slices facilitate the investigation of glucose sensitivity, insulin release and calcium dynamics within the pancreas. The main goal of our study was to assess acute cell viability and glucose-stimulated insulin release in every human pancreata received by nPOD program utilizing fresh tissue slicing techniques.

METHODS

Small pieces of pancreatic tissue from non-diabetic, T1D and autoantibody-positive non diabetic donors were sliced (~120µm thickness) using a fully automated Leica VT1200 S vibratome. To assess slice cell viability, tissue slices were stained using a sensitive two-color fluorescence cell viability assay that allows discrimination between live and dead cells. Taking advantage of the islets' natural scattering properties, slices were then imaged using a Leica TCS SP8 confocal microscope. To assess glucose-stimulated insulin release, viable fresh tissue slices were placed in a perfusion system and exposed to different media. First, slices were incubated in low glucose 3mM media to determine basal insulin secretion, followed by incubation in high glucose 16.7mM media to stimulate insulin release. Then, slices were incubated in 1mM glucose (recuperation period) and finally in high glucose 3mM + 60mM KCl media to strongly depolarize beta cells and obtain a more robust insulin secretion. Insulin content in obtained perfusates were measured using commercially available ELISA kits.

SUMMARY OF RESULTS

To date, we evaluated acute cell viability and hormone secretion in 20 nPOD organ donors (*non-diabetic n= 15; T1D n= 3; AAb+ No diabetes n= 2*). Twelve out of 15 non-diabetic pancreases produced a peak after the first high glucose stimulus (16.7mM) followed by a higher peak when infused with 3mM glucose + 60mM KCl. Likewise, AAb+ cases displayed a similar response, with robust peaks after perfusion with high glucose concentrations. This suggests pancreatic islets were viable and responsive to glucose stimulation. Contrarily, in all 3 T1D cases we did not observe any defined peaks followed by the 16.7mM glucose or 3 mM glucose + 60mM KCl stimulus despite having good tissue viability. Cell imaging analysis of tissue slices and histological evaluation of pancreatic tissue sections of all three T1D cases demonstrated an extremely low presence of insulin producing cells, confirming that insulin release was lacking or untraceable using commercially available insulin ultra-sensitive ELISA kits. Although hormone secretion displayed similar dynamic responses in non-diabetic and autoantibody positive without diabetes donor groups, great variability was observed among cases. Most likely, this variation could be attributed to intrinsic differences between organ donors. Factors like age, race, diet, etc. may play a significant role in cell function and hormonal secretion. Further studies with larger sample size are needed to determine the exact parameters influencing hormonal release by pancreatic tissue slices.

CONCLUSIONS

In conclusion, our results on acute viability and glucose-stimulated insulin release from fresh pancreatic tissue slices suggest that the fresh slices technique can be applied consistently to the pancreata obtained from organ donors. Moreover, the acquired data could provide essential information about islet cell function for every pancreas recovered by nPOD and processed under this protocol. In addition, acute tissue viability measured at the time of organ processing could serve as a standard parameter for tissue quality characterization.

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<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):

O36:
Multiplexed in Situ Imaging Mass Cytometry Analysis of the Human Endocrine Pancreas and Immune System in Type 1 Diabetes

AUTHORS

Yue J. Wang, Daniel Traum, Jonathan Schug, Long Gao, Chengyang Liu, Mark A. Atkinson, Alvin C. Powers, Michael D. Feldman, Ali Najj, Kyong-Mi Chang, and Klaus H. Kaestner

PURPOSE

The interaction of the immune system with endocrine cells in the pancreas is crucial for the initiation and progression of T1D. Imaging mass cytometry (IMC) enables multiplexed assessment of the abundance and localization of more than 30 proteins on the same tissue section at one μm resolution. Our purpose was to develop an IMC panel for the analysis of the immune cells/islet interactions in the human pancreas during the progression to T1D.

METHODS

We developed a panel of 33 antibodies that allows for the quantification of key pancreatic cell types including exocrine cells, islet cells, immune cells, and stromal components. We employed this panel to analyze 12 pancreata obtained from donors with clinically diagnosed T1D and 6 pancreata from non-diabetic controls.

SUMMARY OF RESULTS

We have established and validated an antibody panel, including a combination of 33 different markers and an imaging analysis pipeline, that facilitates large-scale quantitative analyses of human pancreata from control donors as well as donors with different durations of T1D. With an array of protein expression data, we simultaneously identified major pancreatic and immune cell types and quantified complex cellular interactions during T1D progression. In the pancreata from donors with T1D, we simultaneously visualized significant alterations in islet architecture, endocrine cell composition, and immune cell representation.

CONCLUSIONS

We demonstrate the utility of IMC to investigate complex events on the cellular level that will provide new insights on the pathophysiology of T1D. We found dramatic changes in islet architecture, endocrine cell number and immune cell number in the T1D pancreata and observed molecular changes indicating altered cell identity and cellular signals in the histopathology of T1D.

O37:

Human pancreas tissue slices from nPOD organ donors provide a comprehensive and novel insight into T1D pathogenesis

AUTHORS

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PURPOSE

The cellular and molecular mechanisms of type 1 diabetes (T1D) pathogenesis in the human pancreas are still poorly understood. One reason for this lack of knowledge has been the absence of approaches enabling the study of human pancreas cell physiology during T1D development. Recently, pancreas tissue slices have been shown to enable the in situ study of human pancreas cell biology for the first time. By combination of the nPOD organ procurement program, the new human pancreas tissue slice platform and distinct imaging, biochemical and cell biological approaches, the nPOD Slice Working Group has aimed to establish a platform which allows thorough collaborative investigation of pancreas cell biology from organ donors with and without T1D. Following this objective we hope to provide a tool to acquire novel and unprecedented insight into disease pathogenesis.

METHODS

Human pancreas from non-diabetic, T1D and autoantibody-positive non diabetic donors were processed according to nPOD SOPs at the OPPC at the University of Florida (UF), Gainesville. Additionally, small pieces of tissues were sliced to 120µm thick live sections using a vibratome. Slice cell viability after preparation was assessed by live/dead staining. Subsequently, slices were perfused in slice chambers attached to an automated perfusion system to assess glucose-stimulated hormone release by exposure to different media. Hormone slice content and perfusates were measured using commercially available ELISA kits. In addition, slices from each donor were sent to the University of Miami (UM) for live cell imaging and additional measurements of hormone secretion, as well as to the Paul Langerhans Institute Dresden (PLID), Germany, for detailed 3D morphometry.

SUMMARY OF RESULTS

Slices were obtained from 20 nPOD organ donors, including 15 organs from non-diabetic (ND), 2 non-diabetes autoantibody-positive (AAb+), and 3 type 1 diabetes (T1D) donors. Insulin secretion was measured from all donors. Additionally, glucagon secretion, live cell Ca²⁺ imaging of endocrine cells and detailed 3D morphometric analysis of the slices were assessed in a number of cases of each donor group. Pancreas tissue slices from ND donors

demonstrated robust insulin secretion and an inducible increase in cytosolic Ca^{2+} levels of most endocrine cells in response to stimulation by elevated glucose or high KCl. In slices from the limited number of AAb⁺ donor organs examined so far these responses were in the normal range. However, slices prepared from T1D donor organs lacked a defined insulin secretion profile and showed no inducible cytosolic Ca^{2+} activity in endocrine cells. Moreover, slices from T1D donor organs demonstrated an altered profile of glucagon release and no cytosolic Ca^{2+} activity in response to alpha cell specific stimuli. Finally, T1D slices exhibited a reduced islet density with mostly small, monohormonal islet clusters and individual cells, as well as a strong decrease in alpha, beta and delta cell mass in comparison to slices from ND donor organs.

CONCLUSIONS

Human pancreas tissue slices prepared from ND, AAb⁺ and T1D donor organs procured by nPOD, facilitate the collaborative analysis of numerous aspects of pancreas cell physiology within the same organ/organ donors. This approach enables the correlation of hormone secretion with studies on cell biology and detailed pancreas morphology, providing a thorough and comprehensive characterization of human pancreas biology. Utilizing this program within the nPOD Slice Working Group, our preliminary results underline a massive morphological and functional deterioration of beta and alpha cells after T1D onset. The ability to demonstrate known (and novel) alterations of endocrine cells using the pancreas slice platform validates this approach to study donor pancreas from defined stages of T1D disease development. Future studies of pancreas slices will provide a detailed understanding on the mechanisms underlying the natural history of T1D.

O38: 3D morphometry of human pancreas tissue slices obtained from nPOD organ donors

AUTHORS

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PURPOSE

The use of human pancreas tissue slices is a valuable tool to assess both exocrine and endocrine cell function in their natural environment. As no enzymatic digestion of the tissue is necessary, this approach enables the investigation of islet cell function in their natural environment, which makes it an extremely valuable methodology for the study of T1D pathogenesis. We here demonstrate how pancreas tissue slices, subsequent to assessing islet cell secretory function, can be additionally used to quantify 3D morphometry of the human pancreas within the same tissue sample, which allows direct correlation of morphology and function of pancreas donor organs.

METHODS

Human pancreas tissue from non-diabetic, autoantibody-positive and T1D donors was processed according to the SOP's by the nPOD/OPPC at the University of Florida (UF) in Gainesville. Tissue slices were generated with a thickness of 120 µm and viability was evaluated by immunofluorescence staining. After perfusion for the assessment of hormone secretion at UF, slices were fixed and shipped to the Paul Langerhans Institute Dresden, Germany (PLID). To assess 3D slice morphology, slices were stained for endocrine and immune cell markers and whole slice volume imaging was performed on a Zeiss LSM 780 NLO equipped with an automated stage. Image processing and analysis was accomplished semi-automatically using Fiji and MorphoLibJ.

SUMMARY OF RESULTS

Image analysis was performed on donor organs procured by nPOD, including organs from non-diabetic (ND) autoantibody positive (AAb+), and type 1 diabetes (T1D) donors. Detailed 3D analysis of endocrine cells within the slice volume were determined for each case to assess endocrine cell volume and islet composition of beta, alpha and delta cells. As image resolution allows for a quantification down to single hormone positive cells within the total slice volume, islet/cluster count, size distribution and contribution to total endocrine volume were additionally analyzed and compared between the different donor groups. Preliminary data comparing T1D and non-diabetic donor organs confirmed a strongly reduced endocrine cell volume and islet density, as well as a vastly increased number of small islet clusters, individual

cells and monohormonal islet clusters in T1D cases. Interestingly, the observed relative decrease of all endocrine cell volumes was found to be greatest in the alpha cell fraction, followed by the loss in beta and delta cell fraction.

CONCLUSIONS

In conclusion, volumetric morphometry of pancreas tissue slices from nPOD donors is a valuable tool to compare numerous aspects of pancreas morphology, including endocrine cell volume, islet composition, endocrine cell density, islet cluster size, and more. This method is advantageous over analysis of thin histological sections as 3D analysis allows for a more detailed analysis of alterations in endocrine and exocrine cytoarchitecture of the pancreas. Assessment of additional structures like blood and lymphatic vessels, or immune cell infiltration will provide a unique 3D understanding of the human pancreas. Most importantly, and in contrast to other histological approaches, pancreas tissue slices allow acquisition and correlation of morphological information to cell functional from the same tissue, providing an unprecedented insight into T1D pathogenesis.

O39:

A Multiplexed Assay Panel for Detecting Autoantibodies Implicated in Human Type 1 Diabetes and Celiac Disease

AUTHORS

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¹*Meso Scale Discovery (MSD)*

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PURPOSE

Simultaneous detection of autoantibodies implicated in type 1 diabetes (T1D) is critical for early diagnosis and prediction of the disease. We report the development of a research-use only multiplexed assay panel that can simultaneously detect autoantibodies against insulin (IA), Islet antigen-2 (IA2), glutamic acid decarboxylase 65 (GAD65), zinc transporter 8 (ZnT8), and transglutaminase (TGM2). MSD's U-PLEX[®] platform was used for the development and validation of the multiplexed immunogenicity assays for the detection of the five autoantibodies.

METHODS

Each protein was conjugated with biotin label and SULFO-TAG[™] label separately. In the assay protocol, each biotin conjugated protein was first coupled to a unique U-PLEX linker. All five linker-coupled biotin proteins were then combined with the five SULFO-TAG conjugated proteins. This multiplexed protein solution was incubated with samples and controls. During this step, the autoantibodies in the sample bridged specifically to their corresponding biotin and SULFO-TAG proteins. The mixture was then added to U-PLEX plates, where the unique U-PLEX linker guided the protein-autoantibody immune-complexes to bind to a unique spot on the plate. The assay time for this protocol is three hours.

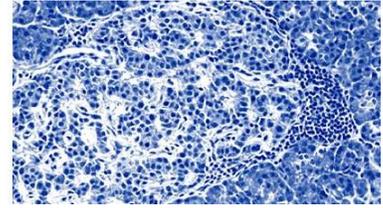
SUMMARY OF RESULTS

A multiplexed assay panel was developed and validated for the simultaneous detection of the antibodies against IA, IA2, GAD65, ZnT8, and TGM2 autoantibodies in a bridging assay format. Extensive assay analyses were conducted to determine robustness to variables such as sample volume, incubation time, plate shaking speed, and temperature stability. Method validation involved testing of samples as well as establishing limits of detection, lower limits of quantitation, cut-points, assay specificities, and reproducibility. The intra-run precision determined by testing two levels of positive controls was <10% CV for all five assays; the inter-run assay precision was determined to be <15% CV for all assays. Assay accuracy, calculated as the ratio of the measured concentration of the positive controls to the expected concentrations, was determined to be within 80-120% for all assays. Samples from four separate groups [controls (30), T1D positives (30), T1D and celiac positives (8), and celiac positives (3)] were tested to evaluate the sensitivity and specificity of the multiplexed assays. Using a lab-established cut-point, the specificity and sensitivity, respectively, for each assay was determined to be: IA (97%, 61%); IA2 (87%, 63%); GAD65

(93%, 58%); ZnT8 (90%, 63%); and TGM2 (83%, 73%). Consistent with the presence of two or more T1D specific autoantibodies being a strong predictor for the development and progression of T1D in at-risk individuals, 73% of T1D samples were positive for more than one autoantibody.

CONCLUSIONS

We have developed and validated a research-use only multiplex assay panel for the detection of antibodies against IA, IA2, GAD65, ZnT8, and TGM2 in human serum samples. This panel can simultaneously detect the five autoantibodies in only 30 μ L of sample within three hours. This panel offers great precision and accuracy, and does not involve the use of radioactive reagents.



(LB-04)

An Insulin-Specific B Cell Deletional Immunotherapy, AKS107, for Prevention of Type 1 Diabetes

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PURPOSE

Type 1 diabetes mellitus (T1D) is an autoimmune disease in which insulin-producing β -cells within pancreatic islets are destroyed by an autoimmune attack coordinated by islet antigen-specific polyclonal T effector lymphocytes (T_{eff}) that have escaped control of immune tolerance. There is growing evidence that insulin-specific B cells play an essential pathogenic role as antigen-presenting cells (APCs) in T1D that results in the activation of insulin-specific autoreactive T_{eff} cells. The role of insulin-specific B cells may be responsible for triggering the process of β -cell damage in humans and NOD mice. Antigen-specific immunotherapies (ASIs) that specifically delete insulin-specific B cells early in the pre-diabetes process at the first signs of insulin autoantibody (IAA) titers are a logical approach to prevent T1D. Therefore, we developed an ASI, AKS-107, designed to target such insulin-specific B cells. AKS-107 is expressed as an Ig Fc fusion molecule containing a metabolically inactivated form of insulin that retains insulin's native conformation such that the only possible *in vivo* binding site is the B Cell Receptor (BCR) of insulin-specific B cells. Upon binding the BCR, the Fc portion can mediate cytotoxicity of the B cell *via* antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). Also, in the event that AKS-107 is processed and presented as antigen to insulin-specific T_{eff} cells, the dominant insulin epitope, B chain 9-23 a.a. (B_{9-23}), is modified at the cysteine 16 position to an alanine residue that is known to avoid activation of pathogenic T_{eff} cells in NOD mice and human T1D subjects. We present the pharmacology of AKS-107, showing that the specific targeting of insulin-reactive B cells is associated with therapeutic efficacy in preventing T1D in NOD mice, along with a feature of restored immune tolerance.

METHODS

- The capacity of AKS-107 to bind insulin-specific polyclonal BCRs was demonstrated (i) in a competition assay with ^{125}I -labeled recombinant human insulin (RHI) to bind anti-insulin antibodies (IAA) contained in pooled human IAA+ sera from pre-diabetic subjects *via* radioimmunoassay (Barbara Davis Center for Diabetes NIH/NIDDK Autoantibody/HLA

Core Lab), and (ii) *via* specific binding to human insulin-specific BCR B cells *via* ELISpot assay.

- The lack of glucose-lowering activity of AKS-107 was demonstrated with insulin hormone receptor binding and phosphorylation (DiscoverX, Fremont, CA) assays using insulin-responsive human IM-9 cells, as well as *in vivo* using glucose pharmacodynamics in BALB/c mice.
- The insulin-specific B cell deletion activity of AKS-107 was demonstrated using insulin-reactive B cells from NOD 125Tg(H+L) mice that contain ~30%-80% insulin-specific B cells of the total splenocyte population: (i) *in vitro* cytotoxicity assay using co-cultures of Tg125 splenic B cells (5×10^5) and rat cytotoxic alveolar macrophages (AM; 2.5×10^4) for 3 days, washed, and insulin-specific B cells measured *via* FACS (anti-B220, anti-IgM, and RHI- μ Beads followed by anti- μ Bead mAb); (ii) *in vivo* insulin-specific B cell analysis after AKS-107 treatment (single *i.p.* dose 2 mg/kg) analyzed *via* flow cytometry of labeled splenocyte suspensions applied to a Miltenyi MS magnetic column in which the total percentage of B cells was determined in flow-through fraction and insulin-specific B cells determined in eluted fraction.
- The non-stimulatory effect on T_{eff} cells of the A16 mutation in the insulin B₉₋₂₃ epitope in AKS-107 was confirmed using an insulin B₉₋₂₃ specific T-cell hybridoma cell line (5KC-3-4; mouse IAg7). NOD bone-marrow-derived dendritic cells (1.7×10^4) and 5KC-3-4 T cells (10^5) were cultured with or without AKS-107 and native insulin (AKS-120 or RHI) for 16 h and T cell activation was assessed by IL-2 production in culture medium *via* ELISA.
- The *in vivo* efficacy of AKS-107 was tested in wt NOD mice and in VH125 NOD mice, which are a cross-breed between wt NOD and Tg125 NOD mice (fixed heavy chain with anti-insulin affinity) that develop T1D more aggressively than wt NOD mice and with a measurable concentration of insulin-specific B cells in tissue and blood.

SUMMARY OF RESULTS

To confirm that AKS-107 retained intact native insulin epitope conformation, the binding affinity of AKS-107 to circulating IAAs in human serum was used as a proxy for binding the insulin-specific BCRs on B cells from which the IAAs originated. RHI and AKS-107 inhibited ¹²⁵I-labeled RHI from binding to IAAs *via* radioimmunoassay, in which AKS-107 had a similar affinity to that of RHI, demonstrating a high probability of targeting the majority of anti-insulin B cell clones in T1D subjects. The inability of AKS-107 to activate the insulin hormone receptor is critical to its therapeutic use, which was confirmed by the lack of binding to and the lack of receptor kinase stimulation of the insulin-responsive cell line, IM-9 (up to 150 nM), indicating that it is at least 100x less active than RHI. Furthermore, a 2 mg/kg *i.p.* injection to BALB/c or *i.v.* injection to cynomolgus monkeys (Biomere, Worcester, MA) demonstrated that AKS-107 did not lower blood glucose levels. AKS-107 serum half-life was 4.5 days in NHPs and 1 day in mice. The insulin-specific B cell deletional activity was demonstrated in co-cultures of insulin-specific BCR B cells from Tg125 NOD mice and rat cytotoxic AMs in which 80 nM AKS-107 reduced B220+ insulin-specific B cells by >98% with an EC₅₀ ~ 70 pM. In addition, treatment with AKS-107 (2 mg/kg, 2/wk for 2 wks) almost completely depleted peripheral blood insulin-specific B cells in VH125 NOD mice while preserving the rest of the B cell population. Similar results were obtained with splenic B cells. The avoidance to activate pathogenic insulin-specific T_{eff} cells by AKS-107 was demonstrated by its failure to stimulate the insulin B₉₋₂₃-reactive T-cell hybridoma cell line

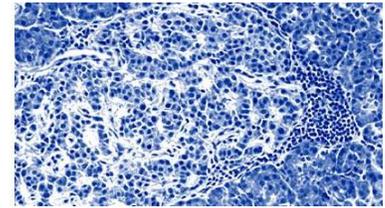
(5KC-3-4) in addition to inhibiting the ability of active insulin to stimulate these cells. Finally, AKS-107 was effective in preventing diabetes in VH125 NOD mice (Akston Biosciences, Beverley, MA), and in female wt NOD mice when treated at 6 weeks of age in two blinded studies performed at two different institutions (Akston Biosciences and Prof. Mark Atkinson, U of FL, Gainesville, FL). Additional studies in wt NOD mice demonstrated that the protective effect of AKS-107 was maintained after cessation of treatment at 16 wks of age through at least 50 wks, a characteristic of restored immune tolerance. Additional analyses of the cellular mechanisms conferring such immune tolerance are underway.

CONCLUSIONS

Based on these promising pharmacology findings, Akston has assembled a consortium of public and private charitable organizations, including the NIDDK, the Helmsley Charitable Trust, and the Sanford Health Research foundation, and is in the process of filling its preclinical funding gap and commitment for the first-in-human Phase I trial for its AKS-107 program. Akston has initiated discussions with regulatory authorities, is conducting GMP manufacturing activities, and intends to complete its IND-enabling GLP pharmacology studies in 2020. The first-in-human study seeks to demonstrate AKS-107 safety, early efficacy, and initial proof-of-principle in adult IAA+ T1D subjects.

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(LB-10)

Targeted delivery of therapeutics in T1D

AUTHORS

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Transplantation Research Center, EBRC
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PURPOSE

The purpose of this study was to assess the formation of tertiary lymphoid tissue in the pancreata of NOD mice and human T1D patients. We also tested the efficacy of our method of targeted delivery of nanoparticles to the pancreata and pancreatic lymph nodes (PLNs) of NOD mice.

METHODS

Four human T1D pancreata received from Network for Pancreatic Organ Donors with Diabetes (nPOD) were sectioned and examined for the presence of high endothelial venules (HEVs) and immune cells. We also synthesized a novel form of nanoparticles coated with MECA79 mAb, which detects peripheral node addressin (PNAd) molecules on the surface of HEVs (Please refer to JCI 2018, Bahmani et al).

SUMMARY OF RESULTS

The expression of pNAD, a marker of HEVs and sign of tertiary lymphoid tissue, was examined in the pancreata of four human T1D organ donors, obtained through nPOD. Immunohistochemical staining of the pancreas tissues from all four T1D donors (average T1D duration = 7.5 years; range = 5-10 years) was positive for PNAd. Similarly, HEV formation was observed in the pancreata of NOD mice, especially those around 8 to 12 weeks of age. The pancreata of younger NOD mice lacked HEVs. Next, we demonstrated that MECA79-coated nanoparticles home to pancreata and PLNs. We are the first group to target PLNs and pancreata following systemic injection, as previous delivery methods of therapeutic agents to LNs have relied on subcutaneous injection. Recently, we have demonstrated that the delivery of anti-CD3 to the heart allograft in a transplantation model markedly increases its survival. Utilizing our targeted delivery method of anti-CD3 treatment in NOD mice has yielded promising early results in reversing T1D in NOD mice. This delivery platform allows not only for

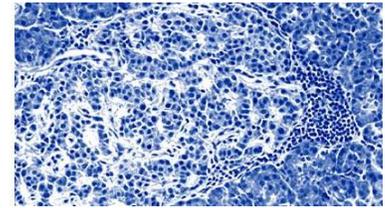
the delivery of immune therapeutics, but also for islet growth factors. Finally, we arrived serendipitously at a striking finding that various PLNs have marked differences in their distribution of HEVs and lymphatic vessels, as well as compartmentalization of immune cells. These findings could be extremely important for nPOD investigators, as they attempt to identify specific clones of T cells from a mixture of heterogeneous PLNs.

CONCLUSIONS

The pancreata from NOD mice and Human T1D patients contain ectopic HEVs, a marker of tertiary lymphoid tissue. Targeted delivery of nanoparticles to pancreata and PLN can be achieved. However, the PLNs that drain the tail and head of a given pancreas could harbor strikingly different phenotypes.

Select the **ONE** category that best describes your research:

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(LB-13)

Transcriptional heterogeneity of beta cells in the intact pancreas

AUTHORS

Lydia Farack^{1,+}, Matan Golan^{1,+}, Adi Egozi¹, Nili Dezorella², Keren Bahar Halpern¹, Shani Ben-Moshe¹, Immacolata Garzilli¹, Beáta Tóth¹, Lior Roitman¹, Valery Krizhanovsky¹, Shalev Itzkovitz¹

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⁺ *These authors contributed equally.*

PURPOSE

Pancreatic beta cells have been shown to be heterogeneous at multiple levels. However, spatially interrogating transcriptional heterogeneity in the intact tissue has been challenging.

METHODS

Here, we developed an optimized protocol for single-molecule fluorescence *in situ* hybridization in the intact pancreas to facilitate the detection of individual transcripts in the intact mouse and human pancreas.

SUMMARY OF RESULTS

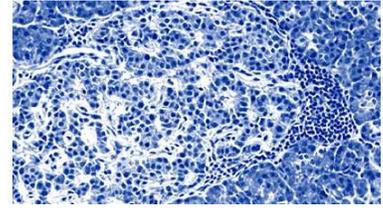
Using an optimized protocol for single-molecule transcript imaging in the intact mouse pancreas we identified a sub-population of “extreme” beta cells with elevated mRNA levels of insulin and other secretory genes. Extreme beta cells contain higher ribosomal and proinsulin content but lower levels of insulin protein in fasted states, suggesting they may be tuned for basal insulin secretion. They exhibit a distinctive intra-cellular polarization pattern, with elevated mRNA concentrations in an apical ER-enriched compartment, distinct from the localization of nascent and mature proteins. The proportion of extreme cells increases in db/db diabetic mice, potentially facilitating the required increase in basal insulin. We identified a similar pattern of transcriptional heterogeneity in human pancreatic slides.

CONCLUSIONS

Our results thus highlight a sub-population of beta cells prone for basal insulin secretion that may carry distinct functional roles along physiological and pathological timescales. Our technology, facilitating highly quantitative single cell interrogation of beta cells in intact fixed human pancreatic tissues may reveal new dynamic features of beta cell heterogeneity and immune response along the development of diabetes,

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<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):



(LB-20)

Tissue-Engineered Stromal Reticulum to Study Lymph Node Fibroblastic Reticular Cell Networks in Autoimmune Diabetes

AUTHORS

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PURPOSE

The purpose of this project is to develop a physiologically relevant *in-vitro* model of the Fibroblastic Reticular Cell stromal network that recapitulates the native lymph node to study Fibroblastic Reticular Cells (FRCs) in Type 1 Diabetes (T1D). The lymph node (LN) is a secondary lymphoid organ where immune reactions are orchestrated leading to both immunity and tolerance. FRCs are the main stromal component of the LN paracortex (the T cell area). Besides forming the LN scaffold, FRCs expand/contract the LN to make space for the proliferating T cells during immune responses. This process is mediated by gp38 interaction with CLEC-2 on dendritic cells. Whether FRC abundance, contractility and reticular network porosity is affected by T1D and whether these changes lead to defective interactions with T cells is unknown. Importantly, FRCs are able to present tissue-restricted antigens to autoreactive T cells leading to their deletion, which maintains peripheral tolerance. Recent evidence suggests that in T1D, expression of the insulin antigen by FRCs in the pancreatic LN is decreased. However, it is not known whether reduced antigen expression in FRCs leads to defective interactions and potential reduction in the deletion of autoreactive T cells. Using the FRC engineered reticulum we determined whether FRC contractility is affected in T1D by comparing engineered reticula formed using FRCs from T1D mouse models to reticula formed using FRCs from control mice that do not develop diabetes. FRC interactions with beta cell autoreactive T cells will also be determined using this FRC engineered reticulum. This work will help identifying novel targets for T1D therapies.

METHODS

LN sections were obtained from 4 and 12 week-old C57BL/6 (B6) mice and from age-matched non-obese diabetic (NOD) mice, which start developing diabetes at 12 weeks of age. LN sections were fixed in formalin, paraffin-embedded and stained for alpha-smooth muscle actin (α -SMA), gp38 and DAPI. Images (z-stacks) were obtained with a Leica SP5 confocal microscope and analyzed using ImageJ to quantify the reticulum pore size. To isolate FRCs, LN sections were digested with a Dispase II, DNase I and Collagenase P enzyme mix. FRCs were obtained by FACS sorting live CD45⁻ CD31⁻ gp38⁺ cells and expanded on tissue culture

plates. After expansion, FRCs were harvested and seeded on collagen type I scaffolds to determine their ability of forming an *in vivo*-like reticular network. FRC phenotype (gp38 expression) was assessed both before and after culture in collagen scaffolds by flow cytometry. Commercially available sponges (Advanced Biomatrix) and 2% collagen gels were compared as FRC scaffolds. 24 hours after FRC seeding, scaffolds were fixed in formalin and stained with Phalloidin (F-actin) and DAPI (nuclear counterstain). Images were obtained and analyzed as explained before. FRC contractility in collagen scaffolds was measured by quantifying the size of FRC-seeded 2% collagen gel disks at 225k cells/gel 48 hours after fabrication.

SUMMARY OF RESULTS

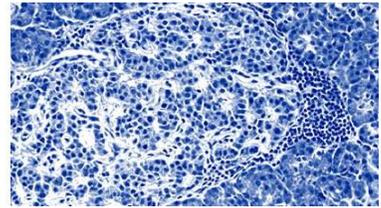
The average pore size of FRC reticula in native skin draining LNs from 4-week-old but not from 12-week-old B6 mice ($5.42 \pm 1.99 \mu\text{m}$) was significantly smaller than age-matched NOD mice ($7.03 \pm 2.58 \mu\text{m}$, $p < 0.0001$). Isolated FRCs were able to create reticular networks on both collagen scaffolds analyzed, but the reticula pore sizes were larger (collagen sponges: 42.7 ± 16.3 ; collagen gels: 22.6 ± 13.9) than LN reticula. Culture of FRCs in collagen scaffolds increased their gp38 expression (MFI 2D: 121720 ± 6432 ; 3D: 271299 ± 8282) after culture in collagen scaffolds. Collagen gel-seeded FRCs from B6 mice contracted collagen gels more (40%) than FRCs from age-matched NOD mice (10%).

CONCLUSIONS

We found that FRC reticula in skin-draining LNs of high risk 12-week-old NOD mice have larger pore sizes than FRC reticula in healthy B6 models. In addition, we found that FRCs from both B6 and NOD mice can be cultured on collagen scaffolds to form a 3D reticulum that mimics FRC reticular organization in LNs, although scaffold optimization is needed to tailor pore size to the LN values we measured. Larger reticular structures of 12-week-old NOD FRC reticula in LNs correlated with reduced contractility of FRCs isolated and cultured in 3D collagen scaffolds compared to B6 FRCs. These results confirm our hypothesis that there are functional differences in NOD-derived FRCs that may impact FRC immunological functions. Future studies will utilize the 3D engineered FRC platform we developed to determine whether defective contractility of NOD-derived FRCs affects their interaction with antigen-specific T cells.

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LB-27

Identification of a Small Molecule Blocking Antigen Presentation in Type 1 Diabetes

AUTHORS

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PURPOSE

Type 1 Diabetes (T1D) is a disease affecting almost three million Americans; every year 30,000 new cases are diagnosed, 50% of whom are children. There is currently no curative therapy for T1D and the only available treatment is insulin replacement. Substantial recent data demonstrate a strong association between the MHC Class II molecule DQ8 and the development of T1D. Indeed DQ8 has been shown to present antigenic peptides (such as InsB:9-23) in a manner that drives activation of CD4+ T cells in T1D patients. The goal of this study is to identify and optimize a small molecule that binds to DQ8 molecule and blocks the continued activation of DQ8 restricted T cells.

METHODS

A virtual screening of a library of 150,000 small molecules for blocking HLADQ8 pockets was conducted on a structure obtained in molecular dynamics simulations of the complex with the peptide InsB:9-23. The virtual screening yielded 50 top scoring small molecules (SM1-SM50), which were then first tested *in vitro* using a DELFIA immunoassay developed

by our group. The 50 compounds were then tested functionally in a mixed lymphocyte reaction (MLR) containing a murine T cell line expressing a human TCR specific for the InsB: 9-23 – DQ8 complex. Our best candidate small molecule was finally tested *ex vivo* using DQ8 transgenic mice and human peripheral blood mononuclear cells (hPBMCs) isolated from DQ8 patients at early stage of the disease (within 6 months of diagnosis). Cytokines production was evaluated using a Luminex assay and T-cell proliferation was measured by CFSE with flow cytometry analysis.

SUMMARY OF RESULTS

Of the 50 small molecules screened, 3 compounds (SM13, SM36, and SM45) showed > 50% inhibition of InsB: 9-23 to HLA-DQ8 *in vitro* (both in the DELFIA immunoassay and in the *in vitro* MLR). Their potency was evaluated by testing the inhibition of InsB: 9-23 binding to HLA-DQ8 using decreasing concentrations of the 3 small molecules (from 0.2 to 0.0125 mM). Only SM36 inhibited InsB: 9-23 binding to HLA-DQ8 in a dose dependent manner, with approximate IC_{50} of 0.05 mM; therefore we selected it for further *ex vivo* analysis. SM36 was able to significantly inhibit T cell activation in splenocytes isolated from transgenic DQ8 mice immunized with InsB:9-23, significantly decreasing the production of pro inflammatory cytokines such as IL-2 and IFN- γ . Moreover, SM36 significantly inhibited the ability of InsB: 9-23 to stimulate T-cell activation in PBMCs from DQ8-T1D patients at early stage of the disease. Also, SM36 decreased T-cell proliferation in CFSE-labeled DQ8 splenocytes and DQ8 hPBMCs incubated with InsB: 9-23. Interestingly, the production of IL4 and IL10 was not significantly decreased both in DQ8 splenocytes and DQ8 hPBMCs indicating a specific suppression of cytokines by SM36.

CONCLUSIONS

Through a combination of virtual and *in vitro/ex vivo* screenings we identified a new compound able to block InsB: 9-23 binding to the HLADQ8 pocket and its presentation to T-cells. These data set the stage for the development of peptide blocking compounds as a novel therapeutic approach for treating T1D. We believe our small molecule approach would prevent the ongoing immune response responsible for the destruction of insulin producing beta cells, preventing disease progression, preserving beta cell function, and reducing or eliminating the need for insulin replacement therapy in patients with T1D.

Select the **ONE** category that best describes your research:

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OTHER

O40: Acinar-to-Ductal Metaplasia Assay of Human Acini Cell Culture

AUTHORS

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PURPOSE

Our objective is to develop and optimize a medium-throughput 3D cell culture assay that models human acinar-to-ductal metaplasia (ADM) to provide a platform for mechanistic and therapeutic studies of pancreatic cell transdifferentiation. Completion of this project will provide a more clinically relevant ADM model by using human rather than mouse primary pancreas cells.

METHODS

Fresh human pancreatic acinar clusters were obtained from organ donors through the Integrated Islet Distribution Program and the University of Florida nPOD program. Cell viability upon arrival and at the experimental endpoint was confirmed using Calcein AM stain. Acinar clusters were seeded in 50% Matrigel matrix and cultured in 96-well plate. Brightfield microscopic images were acquired, and ductal morphologies were manually counted daily for quantification of ADM transformation. RNA was isolated to evaluate changes in acinar and ductal gene expression during ADM.

SUMMARY OF RESULTS

Over the course of 6 days, approximately 40% of acinar clusters successfully transdifferentiated into ductal-like cells in Matrigel. Grape-like acinar clusters changed into a thin, circular structure with a hollow lumen. Real-time quantitative PCR analysis showed a significant decrease in acinar genes *Amy2a*, *CPA2*, and *CPA1* and a concurrent increase in ductal genes *Krt 7* and *Krt19* by day 6 of culture, suggesting a shift from acinar to ductal cellular identity. Viability of human explant culture was confirmed by a positive fluorescence stain (Calcein AM) of acini and/or ductal cells at day 0 and day 6.

CONCLUSIONS

Acinar plasticity from human pancreas tissue can be evaluated in culture using a medium throughput platform (96-well plate). Matrigel culture is sufficient for primary acinar cells from digested human pancreas to transdifferentiate into ductal-like cells over the course of 6 days. This platform is usable for morphological and gene expression analysis to quantify ADM transformation for small sample sizes, which expands data acquisition and limits costs. Optimization of a medium-throughput protocol of human ADM assay can have numerous laboratory and clinical applications.

O41:

Purinergic signals stimulate activation of human islet leukocytes in-vitro and in ex-vivo living pancreatic tissue slices

AUTHORS

Jonathan Weitz^{1,2}, Carol Jacques-Silva^{1,2}, Joana Almaça^{1,2}, Mirza Muhammed Fahd Qadir^{2,3}, Juan Dominguez-Bendala and Alejandro Caicedo^{1,2,3} on behalf of the nPOD Pancreas Slice Group.

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PURPOSE

The pancreatic islet is composed of endocrine cells but it also contains vascular and immune cells, which play an important role in the islet in health and disease. The human islet macrophage function has not been extensively investigated. Studies on human islet macrophages have been mainly limited to histological examinations of fixed human pancreas sections. It is not known what stimuli macrophages respond to, or how they contribute to either normal islet physiology or diabetes pathogenesis. Their phenotype, physiology, and function have not been investigated, mainly because culturing islets induces leukocyte depletion in extended culture. We are exploring the use of human pancreatic tissue slices in order to determine the physiology of local tissue leukocytes in an *ex vivo* tissue preparation, which allows for paracrine communication between immune cells and the local microenvironment.

METHODS

Living human pancreas tissue slices were prepared from non-diabetic donors (n= 3) received at the Diabetes Research Institute. Using a combination of *in situ* cytolabeling with fluorescent conjugated antibodies and Ca²⁺ imaging (Fluo-4), we tracked immune cell behavior and recorded Ca²⁺ responses of human pancreas tissue macrophages (CD45+, CD14+) and lymphocytes (CD45+, CD14-). Confocal imaging with a Leica SP5 microscope was used in order to track changes in intracellular Ca²⁺ as well local movement of islet leukocytes. We observed Ca²⁺ responses to various receptor agonists in local tissue immune cells. To confirm the expression of receptors which were identified by pharmacological agonists, we FACS sorted islet macrophages, lymphocytes and endocrine cells from freshly isolated human islets (n= 5 donors). After confirmation by physiological and genetic approaches, we tested the effect of receptor agonists on acutely cultured human islets by measuring changes in cytokine and chemokines in the culture supernatant.

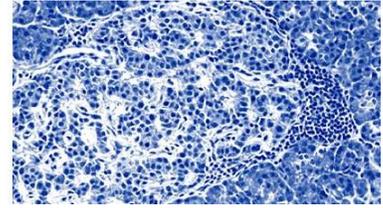
SUMMARY OF RESULTS

Our group has previously shown that islet resident macrophages respond to purinergic signals (such as ATP) released from the pancreatic beta cell (Weitz et al., 2018). In agreement with this data we found that human islet leukocytes showed increased Ca²⁺ responses to purinergic stimulation including agonists for P2Y2, P2Y6, and P2X7 receptors.

Expression of these purinergic receptors were confirmed in FACS sorted cells. Activation of beta cells with high glucose induced increases in Ca^{2+} responses in local immune cells. The Ca^{2+} responses in the local immune cells were blunted in the presence of the beta cell inhibitor Nifedipine (L-type calcium channel blocker). This suggests that like the murine model, human islet macrophages may sense purinergic signals from the beta cell. However, in contrast to murine islet macrophages, we found that human islet resident macrophages express a different repertoire of CD surface markers, genes involved in tissue remodeling, as well as cytokine secretory products.

CONCLUSIONS

Using live human pancreas slices we are able to track and identify endogenous immune cell populations using in-situ cyto-labeling techniques, as well as record physiological Ca^{2+} responses. Based on these data, we propose that inhibiting purinergic signaling in the islet could modulate the inflammatory status of the islet, thus could provide an intervention point to improve islet function and insulin secretion. In addition to their potential involvement in inflammation, we have identified macrophage cluster of identification markers and gene expression signatures which identify the local islet macrophage as a likely contributor to tissue homeostasis.



(LB-09)

Status Of Vitamin B12 Level Among Type 2 Diabetes Mellitus Patients On Metformin Treatment Attending Diabetic Clinic Of The Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

AUTHORS

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PURPOSE

Metformin is a standard therapy, most commonly prescribed oral anti-hyperglycemic agent for individuals with type-2 diabetes (T2DM). Many studies also documented the association between long-term metformin use and low vitamin B12 levels among individuals with T2DM; however, metformin-induced vitamin B12 deficiency (VBD) prevalence estimates showed large variation among studies. To the best of our knowledge, studies on the association between long term metformin use and low vitamin B12 levels among individuals with T2DM are not found in Ethiopia.

our objective was To determine the status of vitamin B12 level and risk factors associated with its deficiency in patients with type 2 diabetes mellitus on metformin treatment attending diabetes clinic of the Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

METHODS

Institutional based cross-sectional study was conducted from March 12/ 2017 to May 15/2017 at diabetes clinic of TASH. Convenient sampling method was used to include a total of 121 T2DM patients with and without metformin treatment. Blood samples were collected and Vitamin B12 levels were determined by Cobas e411 analyzer by electro chemiluminescence immunoassay. Neuropathy Total Symptom Score-6 questionnaire (NTSS-6 scores) was used to compare severity of Peripheral neuropathy (PN) in both groups. VBD was defined as serum concentration of <200 pg/dl. NTSS-6 scores >6 is considered to have PN.

SUMMARY OF RESULTS

From the total of 121 study participants serum B12 levels were low(<200pg/ml) in 15(21.1%) T2DM patients with metformin (n=71) as compared to 2 patients (4.0%) without metformin treatment(n=50). Mean B12 level with metformin was found to be 331.58 pg/ml(± 134.48) as compare to those without metformin 482.23pg/ml(± 235.24), the difference was stastically significant with p value <0.001. The factors that interfered with serum B12 levels were duration of diabetes, dose and duration of metformin use. Vitamin B12 levels and NTSS-6 scores were not correlated (Spearman's rho =0.021, P=0.819).

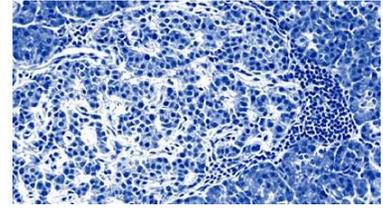
CONCLUSIONS

Among patients with T2DM treated with metformin had low serum B12 levels than patients not treated by metformin with significant effect of metformin dose and duration on B12 levels. The deficiency was not associated with peripheral neuropathy.

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<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):

abst



(LB-19)

Coxsackievirus-infected human beta cells display selected viral sequences on their HLA Class I molecules

AUTHORS

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PURPOSE

Despite increasing evidence for an association between Coxsackievirus B (CVB) and type 1 diabetes (T1D), very little is known about the immune response mounted against CVB infection in terms of epitopes targeted by T cells. This is particularly relevant for developing CVB vaccines aimed at testing whether T1D prevention can be achieved. Such vaccines should incorporate immunogenic epitopes and avoid those (if any) that may be potentially cross-reactive with homologous beta-cell sequences. The current study aimed at characterizing the HLA Class I (HLA-I) peptidome of CVB-infected beta cells.

METHODS

The ECN90 human beta-cell line was infected with either CVB1 or CVB3. HLA-I complexes were purified, and the eluted peptides identified by liquid-chromatography/tandem mass spectrometry. An *in silico* search for potential HLA-A2 and –A3 binding peptides was performed in parallel. HLA-A2- and HLA-A3-restricted peptides were retained by confirming their HLA binding *in vitro* and their recognition by circulating CD8+ T cells from CVB seropositive healthy donors, using combinatorial HLA-I multimer assays.

SUMMARY OF RESULTS

ECN90 beta cells were permissive to CVB1 and CVB3, and a robust and reproducible infection protocol was established. Despite a sizable presentation on the HLA-I molecules of beta cells, the repertoire of HLA-I-bound CVB peptides was focused on a limited number of sequences, mostly within the VP2 protein and with significant overlap between CVB1 and

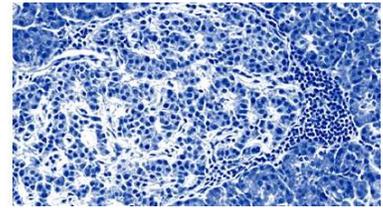
CVB3. Moreover, most of the peptides are well conserved among the 6 CVB serotypes. The HLA-I peptidomics pipeline yielded 18 unique CVB peptides, among which 15 peptides and length variants thereof were predicted to bind HLA-A2 or –A3. This list was complemented by 32 peptides predicted *in silico*. A total of 43 candidates were retained after *in vitro* validation of HLA-A2/A3 binding, including 4 CVB-homologous beta-cell peptides: 11 from the HLA-I peptidomics and 32 from the *in silico* pipeline; 28 HLA-A2-restricted and 15 HLA-A3-restricted. Several of these candidates were subsequently validated for CD8+ T-cell recognition.

CONCLUSIONS

The HLA-I peptidome obtained from CVB-infected beta cells and *in silico* predictions will allow to track the cognate CD8+ T cells in peripheral blood and local tissues and to study potential cross-reactivity with beta-cell peptides, thus informing the selection of the most suitable sequences to incorporate into vaccine design.

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<input type="checkbox"/> Immunology	<input checked="" type="checkbox"/> Other (list): virus



(LB-21)

Regulation of glucagon release using re-aggregated pure human pancreatic islet alpha cells as a model

AUTHORS

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PURPOSE

The regulation of glucagon secretion by pancreatic islet α -cells under physiological and diabetic conditions remain elusive. We aimed to develop an *in vitro* model for investigating the function of human α -cells.

METHODS

Highly purified α -cells from islets of deceased donors were re-aggregated in the presence or absence of β -cells in culture, evaluated for glucagon secretion under various treatment conditions, and compared to that of intact human islets and non-sorted islet cell aggregates.

SUMMARY OF RESULTS

The pure human α -cell aggregates maintained proper glucagon secretion capability in response to changes in ambient glucose concentration, with maximal secretion in the absence of glucose and a slight but similar degree of inhibition under all elevated glucose concentrations tested (ranging from 2.0 mM to 28.0 mM). The presence of human islet β -cells significantly inhibited α -cell glucagon release under high concentrations of glucose. Insulin alone at a concentration 500-fold, but not 5-fold, higher than that released by the β -cells under 16.8 mM glucose significantly inhibit glucagon release. Glibenclamide, a K_{ATP} -channel inhibitor, did not show a significant impact on glucagon release by the α -cell aggregates while it significantly stimulated insulin release by the β -cell aggregates.

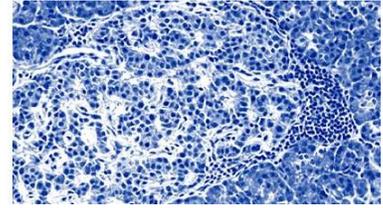
CONCLUSIONS

This study validated the use of isolated and then re-aggregated human α -cells as a model for investigating α -cell function and dissecting its potential role in diabetes development. The results ascertained that fluctuations in glucose concentration ranging from 2.0 mM to 28.0 mM and Glibenclamide had an insignificant direct influence on human α -cell glucagon

secretion. Higher doses of insulin were required to achieve sufficient inhibition of glucagon release in the absence of β -cells, suggesting that other factors released by β -cells or α - and β -cell-cell contact are critical for glucose-inhibited glucagon secretion.

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<input type="checkbox"/> Immunology	<input checked="" type="checkbox"/> Other (list): Alpha Cell Physiology and Dysfunction



(LB-25)

Ability of Type 1 Diabetes associated enteroviruses to cause chronic infection in pancreatic cells and use of antiviral drugs to eradicate such infection

AUTHORS

*Anni Honkima, Amir-Babak Sioofy-Khojine, Jutta Laiho, Sami Oikarinen, Heikki Hyöty
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PURPOSE

Type 1 Diabetes (T1D) is an immune mediated disease in which insulin producing pancreatic beta-cells are selectively destroyed leading to disruption in insulin synthesis and subsequent secretion with clinical manifestations of hyperglycaemia. Enteroviruses have been linked to T1D in many studies and the most plausible mechanism seems to be a persistent infection in pancreatic beta cells being responsible (at least partially) for the disease. Most commonly Coxsackie B group viruses have been linked to the disease, and Coxsackievirus B1 (CVB1) seems to be associated with the increased risk of T1D reported in many studies recently. The purpose of this study was to assess the ability of CVB1 to establish persistent infection in a pancreatic cell line and to evaluate the efficiency of two antiviral drugs, Pleconaril and Ribavirin to abrogate that persistency. These drugs are currently used in the clinical trial named DiViDtrial (PI Prof. Knut Dahl-Jørgensen, University of Oslo, Norway, EudraCT No. 2015 003350-1). The aim of the trial is to treat patient newly diagnosed T1D and to monitor if the treatment can preserve the beta-cell function.

METHODS

We have established two persistent infection model, by two strains of CVB1 (prototype ATCC strain and a field isolate), in pancreatic epithelial cell line named PANC-1. The virus have been replicating for 18 months in serial passages of two persistent infection models by ATCC and a clinical isolate of CVB1 verified by RT-PCR, virus plaque titration assay, immunofluorescent staining, immunohistochemistry, and in situ hybridization. Established persistent models were subcultured in 12 well plates and the tested drugs were added into DMEM supplemented with 10 % FBS and 0.5 % penicillin-streptomycin. The treatment medium was renewed three times a week and the cells were passaged once a week. Drug treatment continued for 8 weeks and cells were monitored for an additional period of 4 weeks after the termination of antiviral treatment. Regularly collected supernatant

and cell fractions were analyzed by RT-qPCR to monitor the quantity of the viral RNA and identify the efficiency of the treatments.

SUMMARY OF RESULTS

Pleconaril, a well known pocket factor replacing agent of Picornaviruses, was able to eradicate persistent CVB1 infection from pancreatic cells. However, its effect varied between the two CVB1 strains. It completely cured persistent infection caused by the ATCC strain of CVB1. The virus protein and RNA disappeared after two months treatment and the cells remained virus negative after the cessation of the treatment. Pleconaril inhibited also the replication of the field isolate of CVB1 in PANC-1 but the initial beneficial effect was lost after 2 weeks treatment possibly due to the accumulation of resistant mutants of the virus. The efficacy of Ribavirin could not be reliably studied since it was cytotoxic for persistently infected cells.

CONCLUSIONS

Antiviral drugs could offer clinical benefit in targeted patient groups suffering from acute or chronic CVB infection. Pleconaril is a good candidate for clinical trials aiming at evaluation of possible beneficial effects of antiviral drugs in type 1 diabetes and other chronic diseases that have been connected to CVB infections. Possible effect of Ribavirin could not be studied reliably.

Select the **ONE** category that best describes your research:

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<input type="checkbox"/> Immunology	<input checked="" type="checkbox"/> Other (list): Antiviral studies

PATHOLOGY

O42: Nanoscale Deep Proteome Profiling of Pancreatic Islets from Presymptomatic Autoantibody Positive and T1D Subjects

AUTHORS

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PURPOSE

Clinical studies indicate that initial signs of β -cell dysfunction (e.g., a decline of first phase insulin release) can, depending on the age of onset, occur as much as 4-6 years before a clinical diagnosis of T1D. Unfortunately, very little knowledge exists regarding the exact mechanisms underlying early β -cell dysfunction. Such information would prove useful for identifying novel therapeutic targets that could potentially arrest disease development. To identify molecular signals of β -cell dysfunction, we performed an *in situ* molecular characterization of the pancreatic islet proteome from archived pancreatic tissues from both pre-symptomatic autoantibody positive (AAb+) cases and T1D cases utilizing ultrasensitive nanoscale proteomics technologies.

METHODS

Human islet sections from non-diabetic controls, multiple AAb+ cases, and T1D subjects from the Network for Pancreatic Organ donors with Diabetes (nPOD) program were isolated by laser microdissection (LMD). Islet sections were subjected to proteome profiling either as a pool of islets or at the level of a single islet by nanoscale proteomics technologies.

SUMMARY OF RESULTS

In an initial study comparing human islets from pre-symptomatic AAb+ subjects and age/sex matched controls (n=6 for each group), pools of ~100 islet sections per donor were analyzed and ~2500 proteins quantified. Despite large donor-to-donor variations, ~200 proteins were observed with significant alterations in abundance. This revealed potential dysregulated pathways of early phases of β -cell dysfunction including those of oxidative stress response, unfolded protein response, and mitochondrial dysfunction. A subsequent experiment compared islets from a T1D and a control case at the single islet level (n=9 islets per case) where ~2400 proteins were quantified and ~300 proteins observed with significant alterations in abundances. Again, the dysregulated pathways include translation, antigen presentation, and NF-KappaB signaling. Furthermore, we have performed a deep proteome profiling involving a pool of 10 islet sections from AAb+ donors with 6311 islet proteins identified, including important islet cell transcription factors such as PDX1, NKX2.2, NKX6.1, ARX, ISL1, and PAX6.

CONCLUSIONS

Together, proteomic characterization of LMD islets from AAb+ and T1D subjects revealed important pathway information relevant to β -cell dysfunction that potentially precedes the signals of islet inflammation that are detectable in islets from donors with T1D. Expansion of this study will include more cases along the spectrum of risk for T1D and will provide a valuable molecular resource for the broader diabetes community.

O43:

***In-situ* detection of IL-6 in human pancreatic tissue sections**

AUTHORS

Sakthi Rajendran, Florence Anquetil and Matthias von Herrath

PURPOSE

Type 1 diabetes (T1D) is a chronic autoimmune disease in which insulin-producing beta cells are destroyed by the immune system. Due to scarcity of pancreas tissues originating from patients with T1D, knowledge about the cytokine milieu in human T1D is very limited. Interleukin-6 (IL-6) is a pleiotropic cytokine with varying effects on immune and non-immune cells, including islet cells. Recent evidences from *in-vitro* and rodent studies show that IL-6 promotes beta-cell survival by reducing oxidative cell stress. The aim of this project is to characterize and quantify the expression of IL-6 in islets of human pancreatic tissue sections, obtained from the network of pancreatic organ donors (nPOD).

METHODS

HUVEC cells were either untreated or stimulated with 100 ng/ml LPS and Brefeldin A for 24 hours, fixed with acetone and stained for IL-6. Next, FFPE sections of human tonsils were stained using different antigen retrieval methods and concentrations of the anti-IL-6 antibody. Human pancreas sections from 3 non-diabetic, 3 T1D and 3 T2D cases were stained for Insulin, Glucagon and IL-6.

SUMMARY OF RESULTS

We observed an upregulation of IL-6 staining in HUVEC cells upon stimulation with LPS, thus validating the anti-IL-6 antibody. We have successfully optimized a multicolor immunofluorescence imaging strategy to precisely locate and quantify the expression of IL-6 in islet cell types of FFPE pancreas sections. IL-6 expression was observed mostly on beta cells and alpha cells in non-diabetic controls, T2D cases and in insulin-containing islets of T1D cases. In insulin-deficient islets of T1D cases, IL-6 expression was found on alpha cells.

CONCLUSIONS

We will quantify the expression levels and source of IL-6 between non-diabetic, auto-antibody positive and donors with T1D to obtain a snapshot of the immunological changes that occur during T1D progression. Future findings from this project will answer key biological questions regarding the role of IL-6 in the pathogenesis of T1D.

O44:

Expression of Inducible Nitric Oxide Synthase (iNOS) in Islet and Exocrine Cells in Pancreatic Biopsies from Living Donors with New-onset Type 1 Diabetes and in Cadaveric Pancreatic Sections from nPOD with and without Disease

AUTHORS

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PURPOSE

Exposure of human islets to proinflammatory cytokines in culture results in the generation of nitric oxide (NO), following up-regulation of inducible nitric oxide synthase (iNOS). NO in the presence of other oxidants may, therefore, contribute to beta cell destruction during type 1 diabetes. The goal of our study was to determine if pancreatic iNOS is expressed in new-onset type 1 diabetes and during longer duration of disease and to identify its cellular sources.

METHODS

Pancreatic biopsy sections from 6 new-onset living volunteers within 3-9 weeks of diagnosis of type 1 diabetes (T1D) from the DiViD Study were triple-immunostained for iNOS, insulin and glucagon. Cadaveric pancreatic sections from nPOD (4 autoantibody-negative non-diabetic cases, 3 autoantibody-positive non-diabetic cases and 5 T1D cases with a disease duration ranging from 4-11 years) were immunostained similarly. All islets and 10 random fields from the exocrine regions were examined, analyzed and enumerated for the expression and cellular sources of iNOS.

SUMMARY OF RESULTS

In most islets from all 4 study group, iNOS expression within the islet cells was often selective and eccentric. It was present not only in beta and alpha cells but also in some islet cells negative for insulin and glucagon. iNOS cells were also observed in the peri-islet and intra-islet regions of some islets and were likely to be immune cells, such as macrophages.

In the new-onset group, 4/6 cases showed moderate staining for iNOS. iNOS was present irrespective of the presence of residual beta cells. In the exocrine region, iNOS-positive cells were rare.

2/4 non-diabetic, autoantibody-negative nPOD cases showed moderate staining for iNOS in islet cells; iNOS cells were sparsely distributed in the exocrine region in all 4 cases. Moderate staining in islet cells was also observed in 2 autoantibody-positive non-diabetic cases (nPOD 6267 and nPOD 6310) and was weakly expressed in nPOD 6167. iNOS cells in the exocrine region were sparsely distributed in all 3 cases.

In the 5 diabetic cases from nPOD (6088, 6211, 6045, 6262, 6220), 3/5 cases showed weak to moderate staining for iNOS in islet cells. All 5 cases showed prominent iNOS-positive cells in the exocrine region. iNOS-positivity was also present in some ductal cells.

CONCLUSIONS

In new-onset cases, variable cellular expression of iNOS was observed in islet cells; iNOS cells were only sparsely distributed in the exocrine region.

A similar pattern of expression in islet cells was observed in autoantibody-negative and -positive non-diabetic cases. However, iNOS cells in the exocrine region were more prominent in autoantibody-positive non-diabetic cases than in autoantibody-negative non-diabetic and new-onset cases.

In the long-term diabetic cases, the greater intensity iNOS immunolabelling in islet cells and exocrine areas than in new-onset cases may suggest a pathogenic role for NO during long-term diabetes.

The heterogeneous expression of iNOS in islet cells may be dynamic.

Its presence in non-diabetic autoantibody-negative cases and in adjacent non-beta cells raises new questions regarding the true role of NO in beta cell destruction during type 1 diabetes.

O45:
**Neutrophil infiltration and tissue damage
in the exocrine pancreas
of pre-symptomatic and symptomatic type 1 diabetes**

AUTHORS

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PURPOSE

Human type 1 diabetes (T1D) is a predictable autoimmune disease characterized by the presence of islet-specific autoantibodies that can be detected months or even years before the clinical onset, a stage recognized as pre-symptomatic T1D. In addition to adaptive immunity, it is now clear that innate immunity is abnormal in both pre-symptomatic and symptomatic T1D patients. Our group demonstrated that neutrophils might play a key role in this innate pathway. Although often neglected, it has been reported that not only endocrine irregularities but also exocrine subclinical abnormalities, such as lymphocyte infiltration, exocrine atrophy and reduced volume, exist in the pancreas of T1D patients. New recent data suggest that exocrine dysfunction is present already in the pre-symptomatic stage. It remains to be elucidated the underlying pathological mechanism leading to this whole pancreas damage in T1D.

METHODS

Immunofluorescence analysis of neutrophils was performed on 47 pancreas sections from symptomatic T1D, autoantibody-positive pre-symptomatic and non-diabetic organ donors collected from nPOD and DiViD cohorts. Whole slide acquisition was performed by confocal microscopy. Histological reports from nPOD organ donors and from biopsies performed on T1D living donors were collected. Statistical analysis considering important potential confounding factors was performed to test whether exocrine pancreas damage is more frequent in pre-symptomatic and symptomatic T1D than in non-diabetic organ donors.

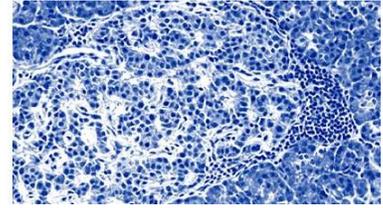
SUMMARY OF RESULTS

It was observed that a higher number of neutrophils is present in the pancreas of pre-symptomatic and symptomatic T1D subjects as compared to non-diabetic organ donors. A fraction of pancreas-infiltrating neutrophils releases Neutrophil Extracellular Traps (NETs), pathogenic component of several autoinflammatory disorders. Despite T1D is a β -cell-specific disease, neutrophils and NETs were found infiltrating both endocrine and exocrine pancreas. Tissue-residing NETs in the exocrine pancreas, are known to contribute to plug

formation, duct obstruction and pancreatitis. We therefore hypothesized that pancreatitis/exocrine damage are key features of T1D. To test this, analysis of data collected from both the nPOD datashare and DiViD samples reveals a higher presence of histological signs of chronic pancreatitis (e.g. immune cell infiltration, plug formation, duct obstruction and fibrosis) in both pre-symptomatic and symptomatic T1D subjects as compared to non-diabetic organ donors.

CONCLUSIONS

Convincing data support the hypothesis that T1D is a disease of the whole pancreas, in which the loss of functional beta-cell mass is most clinically apparent. Our results demonstrate that both NETs and histological signs of chronic pancreatitis are present in the pancreas of pre-symptomatic and symptomatic T1D subjects. We therefore hypothesize that T1D subclinical pancreatitis might be NET-driven and play a key role in T1D exocrine-endocrine “pancreatopathy”.



(LB-02)

nPOD-Kidney (nPOD-K): A new adventure into mapping the pathology of Diabetic Kidney Disease

AUTHORS

Florence Anquetil, Vivek Das, Allie Roach, Claire Gibson Bamman, Matthias von Herrath, Johnna D. Wesley, Anil Karihaloo

PURPOSE

Diabetes is associated with long-term complications that are associated with high morbidity and mortality. Among them, diabetic kidney disease (DKD) is the most common yet poorly understood. Chronic diabetes alters the parenchyma of the kidney that eventually leads to end-stage kidney disease. Kidney biopsies are relatively rare; typically, a diagnosis of diabetic nephropathy (DN) is based on clinical presentation rather than histopathological assessment of a needle biopsy. However, while useful, such a biopsy rarely accurately represents the whole kidney and is often accompanied by sampling error. We have tried to overcome this by the initiation of the nPOD-Kidney (nPOD-K) pilot project. In this pilot, large sections of kidney comprising an average of 250 glomeruli per section are available and currently being analyzed. The program has thus far collected and analyzed 36/48 kidneys. We aim to validate the cohort and then use it to build a virtual time-line of disease progression, validate high-profile pathways published in the literature from non-clinical studies, and potentially uncover new disease mechanisms of DKD.

METHODS

Two- μ m renal FFPE sections from the nPOD-K cohort were analyzed using 'gold-standard stainings' of renal histology (kidney-specific cells) and renal endpoints (i.e., fibrosis, tubular injury, basement membrane thickening). QuPath was used to perform image analysis and to evaluate the whole spectrum of glomeruli.

SUMMARY OF RESULTS

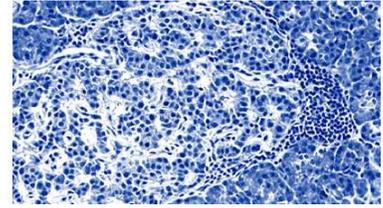
We optimized multiple brightfield and immunofluorescent stainings, followed by in-depth whole-slide image analyses using QuPath. We are now able to automatically identify kidney compartments and define the disease stage of each glomerulus using computer learning. Two-thirds of the cohort demonstrates characteristic features of diabetic kidney disease (mesangial expansion, Kimmelstiel-Wilson nodules, loss of podocyte, basement membrane thickening, hyalinosis) along with arteriosclerosis, tubular injury, and immune infiltration that is published findings.

CONCLUSIONS

This cohort provides a new opportunity to better understand DN physiopathology by analyzing larger areas of the kidney at various stages of disease. As previously observed in the nPOD pancreas cohort, all stages of DKD can be detected in diseased kidneys, allowing us to evaluate the evolution of the disease.

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(LB-08)

Comprehensive histopathology of insulinitis in young-onset Type 1 diabetes: To 'B' or not to 'B' – that is the question...?

AUTHORS

*Sarah J Richardson*¹, Irina Kusmartseva*², Pia Leete¹, Myriam Padilla², Mingder Yang², Marika Bogdani³, Gail Deutsch⁴, Christine Flaxman¹, Mark Atkinson² and Noel G Morgan¹.*

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PURPOSE

Worldwide, fewer than 600 Type 1 diabetes pancreata have been described in the literature or are accessible within tissue biobanks. Less than 80 of these are from individuals who were <10y at symptomatic onset and had a short duration (<1y) of disease. Among these, the majority were collected >30 years ago and emanate from autopsy cases having only limited information regarding fixation and tissue processing. Due to welcome improvements in the diagnosis and clinical management of Type 1 diabetes, deaths close to diagnosis are now very rare in young children, highlighting the value and importance of these archival samples. In the present study, we examined pancreas sections from two different historical collections, the Exeter Archival Diabetes Biobank (EADB n=40) and Seattle Children Hospital (n=6). Specifically, we optimized a triple chromogen immunohistochemical staining method to assess these tissues for insulinitis and beta cell mass in a blinded manner, using a state-of-the-art image analysis platform.

METHODS

Serial sections of pancreas from the two biobanks were triple-immunostained for insulin/ glucagon/ CD45; CD8/CD20/ glucagon; and CD4/CD20/glucagon. Sections were scanned using an Aperio CS2 system and analyzed in a blinded manner using Halo 2.1 image software with Cytonuclear and Tissue classifier modules. Sections were annotated to identify inflamed insulin-containing islets (ICIs; >15 CD45+ cells); ICIs with <15 CD45+ cells; inflamed Insulin-deficient islets (IDIs; >15 CD45+ cells) and IDIs with <15 CD45+ cells. The total numbers of CD45+, CD8+ T, CD4+ T and CD20+ B cells in each of the islet categories was calculated along with the total number of CD45+ cells found within the acinar tissue, remote from islets.

SUMMARY OF RESULTS

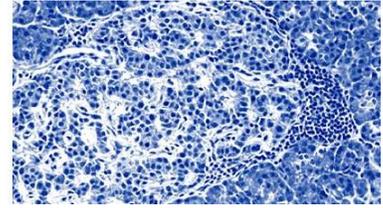
Staining for CD45, insulin and glucagon was successful in all 46 cases, allowing for assessment of CD45 positive immune cells in a total of 7178 islets, of which 1906 (26.6%) contained insulin and 9.7% were defined as insulitic. The numbers of CD20+ B cells, CD4 and CD8+ T cells were also calculated in the 30 cases which had not been post-fixed in mercuric chloride. High numbers of CD20+ B cells were identified in a subset of these cases. Prior to unblinding and by using an average of <3 B cells/ ICI (Group 1) versus >3 B cells/ICI (Group 2) as criteria, the cases were split into two groups. Group 1 (n=10) had a median age at diagnosis of 19y (range 4-40y); a median of 31.6% residual ICIs (range 12.2-65.1%) of which 15.6% (range 5.8 – 52.2%) were defined as insulitic. The mean number of CD45+ cells and CD20+ B cells/ ICI were 9.8±2.6 and 0.21±0.01 respectively. By contrast, the subjects in Group 2 (n=20), had a median age at diagnosis of 5y (range 0.92-17y); All were under 11y, except 1 case (17y) which had evidence of pancreatitis. The group had a median of 13.7% residual ICIs (range 0-38.2%) with 77.9% (range 19.1 – 100.0%) were defined as insulitic. The mean number of CD45+ cells and CD20+ B cells were 61.1±7.8 and 28.6±6.9 respectively. Group 1 differed significantly from Group 2 in all of these criteria. The percentage IDIs that were defined as inflamed was also increased in Group 2 versus Group 1, 3.7±0.8 v 1.1±0.5 (p=0.02). Finally, significantly higher numbers of CD45+ cells/ mm² were observed in acinar tissue of Group 1 compared to Group 2, 176.1±20.8 versus 67.11±0.03 (p=0.008).

CONCLUSIONS

We demonstrate that pancreata collected 30-40 years ago from two independent sources can be utilized to investigate the immunopathology of T1D using state-of-the-art image analysis platforms. Importantly, this dataset confirms, strengthens and expands our previous observations that individuals with young onset Type 1 diabetes (<13y) have a different profile of insulinitis from those diagnosed over the age of 13y. This is characterized by aggressive infiltration of >50% of residual ICIs, the presence of high numbers of CD8+ T and B cells and low numbers of residual ICIs at diagnosis.

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(LB-17)

Tertiary Lymphoid Organ-like Structures as Sites for in Situ Perpetuation of Human Type 1 Diabetes

AUTHORS

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PURPOSE

Tertiary lymphoid organs (TLOs) are found mainly in tissues with long-standing inflammation as a result of infection, autoimmunity or cancer (1,2). TLOs show structural and functional similarities to the lymph node and contribute to disease progression. Although TLOs have been detected in several human autoimmune diseases, such as rheumatoid arthritis and Sjögren's syndrome (3), they had not been detected in human type 1 diabetes (T1D).

In nPOD donor samples with extensive insulinitis, we have identified TLO-like structures that are characterized by accumulations of inter-mixed T and B-cells and in rare cases B-cell follicles; the presence of a fibroblastic reticular network, and high endothelial venules (presented at last nPOD meeting). Work from our lab (4,5) and others on NOD mice suggest that the main events that occur in the pancreatic lymph node, such as antigen presentation, plasma cell differentiation and autoantibody production, also take place in the TLOs and hence that they promote disease progression. Whether this is also the case in human T1D has not been investigated to date. Our aim was to investigate the existence of specific immune cell subtypes (plasma cells, regulatory T cells, memory T cells, follicular dendritic cells) and proliferating cells) in the TLO-like structures, which could indicate a function in disease development.

METHODS

Immunofluorescence stainings were performed on cryosections of pancreas with insulinitis using markers for regulatory T cells (Foxp3), plasma cells (CD138), memory T cell (CD45RO) and follicular dendritic cell markers (CD21) in combination with antibodies specific for basement membrane and interstitial matrix components. The presence of proliferating cells in TLO-like structures were identified by Ki67 staining. The sections were analyzed by confocal microscopy.

SUMMARY OF RESULTS

The complexity of TLO-like structures in different T1D donor samples was variable, probably because their formation is a dynamic process and they can disappear once the antigen source is resolved. The follicular dendritic cell network, which is the stromal component of B-cell follicle, was detected in a few inflamed islets. Plasma cell number was increased in T1D donors compared to the healthy donors and they were found both associated with TLO-like structures or scattered through the interstitial matrix in close proximity to the islets. Whether these plasma cells are antigen specific remains to be determined. High numbers of memory T cells were detected in the TLO-like structures; however, few Foxp3⁺ cells were detectable. Proliferating leukocytes Ki67⁺ were present in all TLO-like structures.

CONCLUSIONS

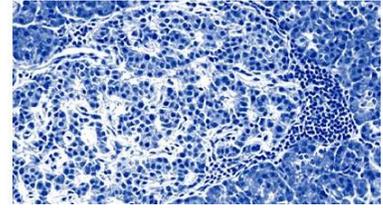
The presence of proliferating leukocytes, plasma cells and memory T cells in the pancreas samples with extensive insulinitis suggests that TLO-like structures might contribute to in situ perpetuation of human T1D.

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<input type="checkbox"/> Immunology	<input type="checkbox"/> Other (list):



(LB-30)

Extracellular Matrix of Pancreatic Lymph Node During Development of Type 1 Diabetes

AUTHORS

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PURPOSE

Pancreatic lymph nodes (PLNs) play an important role in type 1 diabetes (T1D) since they are the sites where islet cell specific antigen presentation occurs, effector T cells are generated and where antibody production takes place. The T cell compartment and the inter-follicular regions of lymph nodes contain a reticular fiber network composed of reticular fibroblast cells (RFCs) that ensheath the reticular fibers (RFs), unique extracellular matrix (ECM) structures that have been characterized in our lab (Sixt et al, 2005; Lokmic et al., 2008). The RFs of lymph nodes constitute the structural backbone of the organ, but also support the migration and survival of T- and B-cells, and act as conduits for the transport of soluble antigens and molecules (Sixt et al, 2005) including immunoglobulins produced in the germinal centers (Thierry et al., 2018). Recently, the expression of some ECM components of PLNs such as hyaluronan have been reported to change during development of T1D (Bogdani et al, 2014). However, it is not clear whether the amount and/or organization of other ECM components of RFs are also altered. In the present study we compare the expression patterns of ECM molecules in PLNs of healthy and autoantibody positive (aAb+) and T1D donors and correlate this data with the localization of different immune cell subtypes and stromal cell markers.

METHODS

Triple immunofluorescence staining was performed on PLN cryosections from control (n=10), autoantibody positive (n=11), and type 1 diabetic (n=17) donor samples using antibodies specific for basement membrane molecules (laminin α 4, α 5 chain, pan-laminin), interstitial matrix proteins (collagen type III), immune cell markers (T-, plasma cells), fibroblastic reticular cells (PDGFR β , smooth muscle actin (SMA), desmin, ERTR7) and a high endothelial cell (HEV) marker (Meca79). The sections were analyzed using confocal microscopy.

SUMMARY OF RESULTS

Like all lymph nodes, the PLN is covered by a thick interstitial matrix rich in collagen type III that penetrates the parenchyma forming the trabeculae. The RF network defines different immune cell niches, with the T cell compartment being characterized by 1-2 μm thin RFs, compared to the interfollicular region where the RFs are thicker ($>2\mu\text{m}$) and densely organized, while in B cell follicles RFs are sparse and individual follicular dendritic cells provide a structural and functional niche for the B-cells. By contrast, germinal centers (GC) of secondary follicles show extensive staining for ECM molecules. The medullary cords also contain a dense network of RFs, which may provide a niche for the localization of plasma cells. In general, RFs of PLNs have a collagen type I/III core that is surrounded by a continuous BM layer which is rich in laminin $\alpha 5$, but also shows weak staining for laminin $\alpha 4$. However in the subcapsular and medullary sinuses, fibrillar collagen core of RFs is surrounded by discontinuous staining for BM components. PDGFR β^+ RFCs ensheath the RFs throughout the PLN, with desmin $^+$ cells being widely distributed in the T cell zone of paracortex and in the inner part of medullary cords, while SMA $^+$ cells dominate the interfollicular zone and the periphery of medullary cords. HEVs are surrounded by a laminin $\alpha 4$ and $\alpha 5$ positive BM and PDGFR β^+ /SMA $^+$ /desmin $^-$ stromal cells. We have not detected obvious changes in the expression pattern of BM or interstitial matrix molecules or stromal cell markers in the aAb+ and T1D donors compared to healthy donors, rather individual differences were detected which may correlate with the health status of the donor.

CONCLUSIONS

Our data show that the composition and organization of the ECM and stromal cells defines different immune cell compartments in the human lymph node as it does in the mouse lymph node and, hence, provides structural and functional niches for immune reactions. Investigation of further sections from donors at different stages in the development of T1D would be permit assessment of whether lengthen of T1D duration, affects the ECM of RFs and/or RFB markers.

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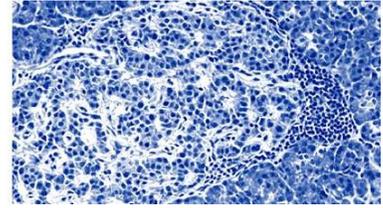
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(LB-29)

Human Herpesvirus-6 glycoprotein B is more frequently expressed in human pancreatic islets from donors with type 1 diabetes

AUTHORS

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PURPOSE

The aim of this study was to investigate the presence of HHV-6 in pancreatic tissue sections from non-diabetic donors, auto-antibody positive donors (AAB+), and donors with T1D and further explore whether there is any association between HHV-6 presence and MHC class I hyperexpression or CD8 T cell infiltration inside the islets.

METHODS

The presence of HHV-6 DNA and protein in the pancreata was examined by PCR, and Indirect Immunofluorescence assay, respectively, followed by imaging using high-resolution confocal microscopy.

SUMMARY OF RESULTS

HHV-6 genomic sequence (U67) was found in the pancreatic tissues of non-diabetic subjects (3 out of 4), AAB+ (4 out of 5) and diabetic donors (6 out of 7). Consistently, HHV-6 glycoprotein B (gB) was detected in the exocrine tissue of subjects with and without diabetes. Interestingly, gB protein was more frequently expressed in the islets of T1D donors. Lastly, out of 20 islets with high gB expression, only 3 islets (15%) showed MHC class I hyperexpression. Furthermore, no correlation was found between gB protein expression and CD8 T cell infiltration on a per-islet basis in any of the groups.

CONCLUSIONS

Our observations indicate that HHV-6 is present in the pancreatic tissue sections of both non-diabetic and diabetic subjects, but with a higher expression inside the islets of subjects with diabetes. The possible role of HHV-6 (and other viruses) as a contributory factor for T1D should therefore be further investigated.

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T1D ETIOLOGY & ENVIRONMENT

O46:
Detection and localization of enteroviral mRNA in the pancreas of patients with T1D

AUTHORS

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PURPOSE

The viral genome has been difficult to detect in the pancreas and more sensitive and reliable methods are required to establish the possible correlation of enteroviral infection with Type 1 Diabetes (T1D). This study has established a single molecule (sm)FISH based method to detect the presence of very low copies of the virus genome in tissue samples from T1D patients, using a previously designed panel of fluorescently labeled oligonucleotide probes, each of 17-22 nucleotides in length with a unique sequence to specifically bind to the enteroviral genome of the picornaviridae family.

METHODS

Co-staining of small RNA probes by smFISH was performed in parallel with classical immunostaining for CD45 and CD68 and insulin of 14 control, 4 Auto-antibody (AAb)+ and 21 T1D pancreata in duplicates of the well-characterized nPOD cohort.

SUMMARY OF RESULTS

Massive enteroviral RNA with more than 10 fully infected cells throughout the pancreas was detected with high sensitivity and specificity in none of the control pancreata, but in 1 out of 4 AAb+ and in 10 out of 21 T1D pancreata. The mean number of infected cells in the pancreas was significantly increased in T1D and AAb+ pancreata, compared to controls (182, 24 and 4 viral mRNA+ cells, respectively).

Together with the significant increase in viral mRNA in AAb+ and T1D pancreata, there was a confirmed reduction in the number of insulin+ islets/ pancreatic section (207, 147 and 82 β -cell containing islets in controls, AAb+ and T1D pancreata, respectively) together with an increase in intra-islet CD45+ lymphocytes/ CD68+ macrophages (0.02, 0.14 and 1.33 CD45+ cells/ islet in controls, AAb+ and T1D pancreata, respectively) as well as in the exocrine pancreas.

Clear single molecule viral mRNA staining allowed the identification of virus localization in the pancreas. While several virus+ cells were detected inside the islets as well as the islets periphery, most of the virus-containing cells were found scattered within the exocrine pancreas, with rare co-localization or proximity with ductal or CD45+/CD68+ cells. We observed a significantly higher number of virus+ cells in the pancreas, which were not

localized to islets in T1D and AAb+ pancreata, compared to controls (169, 14 and 3 viral mRNA+ cells, localized in the exocrine pancreas of T1D, AAb+ and control pancreata, respectively).

CONCLUSIONS

A specific smFISH based detection method could clearly confirm the significant correlation of viral RNA with T1D. Virus infected cells were localized scattered throughout the exocrine pancreas, and may lead to β -cell destruction in a paracrine manner through their specific sensitivity to the inflammatory cascade.

O47:

Monogenic Diabetes and Integrative Stress Response Genes are Differentially Expressed in Multifactorial Type 1 Diabetes with a Distinctive Pattern in Autoantibody Positive Human Pancreata

AUTHORS

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*Co-Senior Authors

PURPOSE

We hypothesize that gene expression levels for the ever-expanding cohort of nonredundant mutations linked to monogenic diabetes would be altered in human pancreas tissues from organ donors with multifactorial/polygenic type 1 diabetes (T1D) and in autoantibody positive (AAB+) donors considered to have pre-T1D. Our preliminary data led to studies on the potential importance of the integrated stress response (ISR) in T1D pathogenesis.

METHODS

To this end, we have tested the expression of 42 genes causative in monogenic diabetes and >30 genes associated with the ISR employing real time qPCR, immunofluorescence, and *in situ* hybridization studies on high quality pancreatic tissues from unaffected ("control"; n~30), T1D (n~30), AAB+ (n~24) and T2D (n~20) organ donors from the nPOD repository.

SUMMARY OF RESULTS

We identified ~20 monogenic diabetes genes involved in immunity, β -cell function and development, as well as endoplasmic reticulum (ER) function/stress that show significantly altered gene expression in T1D compared to control human pancreata ($p < 0.05$ - < 0.0001). These data highlight the potential importance of the ER stress response leading to our identification of an additional ~22 genes that span the three major arms of the ISR. Immunofluorescence data for proteins corresponding to select monogenic diabetes genes demonstrate a distinct pattern of protein localization in T1D pancreata compared to control. We have also identified a subset of genes that are differentially expressed in AAB+ and T2D compared control pancreata ($p < 0.05$ - < 0.0001).

CONCLUSIONS

In support of our original hypothesis, we demonstrated that nonredundant, monogenic diabetes genes are differentially expressed in T1D pancreata. Our results clearly show that a large cohort of proteins known to be associated with the ISR are, in fact, differentially expressed in the T1D versus control pancreas. Expanding these studies into a population of AAB+ donor pancreata, we identified a subset of genes whose expression is uniquely altered

in this pre-T1D group. Our results will hopefully provide a new avenue of research for the entire T1D community to further explore potential mechanisms underlying disease pathogenesis or as new targets for preventative therapies.

O48:

Live human pancreas tissue slices as a platform for investigating the immunopathological processes of type 1 diabetes

AUTHORS

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PURPOSE

A major goal of type 1 diabetes (T1D) research is the development of state-of-the-art devices that recreate the human islet microenvironment to help elucidate the molecular and cellular mechanisms of beta cell loss due to autoimmunity and to provide a platform for testing potential new drugs. Here, we explore the potential use of live human pancreas slices to serve as a genuine and native three-dimensional microenvironment for studies of the islet—immune cell interface within the context of human T1D. In this system, exogenous, engineered islet-reactive T cells can be introduced into histocompatible non-diabetic human pancreas tissue to simulate the beta cell destruction observed in T1D. Further, endogenous tissue-resident or pathological immune cells can be studied in slices to identify translatable difference between donors at varying levels of T1D risk.

METHODS

Live human pancreas tissue slices were prepared from non-diabetic and autoantibody-positive donors co-developed by the nPOD/OPPC at the University of Florida and Dresden University. Engineered human CD8⁺ T cell avatars were introduced into live pancreas slices and imaged by time-lapse confocal microscopy on a Leica SP8 confocal equipped with an automated stage and incubation system. The T cell avatars express eGFP and the alpha and beta chains of a TCR recognizing the beta cell antigen islet antigen glucose-6-phosphatase catalytic subunit 2 (G6PC2, also known as IGRP) in the context of HLA-A*0201 (A2), a globally common human MHC class I allele. Cell viability/apoptosis in exocrine and islet tissue was dynamically tracked by SYTOX Blue dead cell stain and NucView 530 Caspase-3 substrate. Exogenous T cell motility was tracked by eGFP and the position of islets/beta cells by reflected light. Endogenous T cells were stained and tracked in live tissue slices with anti-CD3-APC.

SUMMARY OF RESULTS

The motility of exogenous T cell avatars in human pancreas slice tissue was tracked over 15 hours in nondiabetic donors (n = 2). Lacking any applied chemotactic stimulus, the T cells' motility qualitatively appeared to be directionally random, pending a more detailed cell tracking analysis. However, individual T cells did occasionally infiltrate periphery and core of islets and slow their migration speed. This is consistent with published results reporting random diabetic T cell migration in NOD mouse pancreas until cognate antigen is encountered. The rate of endocrine and exocrine cell apoptosis was quantified and compared to negative controls without T cells, or positive controls where apoptosis was artificially introduced by staurosporin. When endogenous CD3⁺ cells were examined in live pancreas slices from a GAD autoantibody-positive donor (n = 1), one islet was found with >18 CD3⁺ T cells forming an apparent focal insulinitis. To our knowledge, this is the first image ever taken of live, endogenous human immune cells attacking live insulin-producing beta cells *in situ*.

CONCLUSIONS

Using live human pancreas slices, engineered T cells or T cell lines are able to be exogenously introduced and their interactions, migration, and effector functions within the human pancreas tissue can be visually tracked dynamically. Features such as *in situ* islets, peri-islet basement membrane, vasculature, and endogenous immune cells remain intact. These initial successes are guiding our development of pancreas slices for further detailed three-dimensional studies of islet—immune cell interactions relevant to human T1D. This technology has strong potential as a fully human platform for understanding the etiopathology of T1D and testing interventional therapies that act in the local islet immune microenvironment.

O49:

The JDRF nPOD-Virus Group: Association of Type 1 Diabetes with Viral Markers as Triggers of Disease

AUTHORS

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PURPOSE

Over the last years, the nPOD-Virus group has coordinated investigations that have provided epidemiological evidence for an association of type 1 diabetes (T1D) with enteroviruses (EV). Recent data have shown that certain genes involved in the response to viruses are risk factors for T1D. Moreover, EV infections in beta cells trigger innate responses (inflammation, ER stress), dysfunction, impair insulin secretion (in vitro and in vivo) and even beta cell death, which may contribute in the initiation of chronic islet autoimmunity in genetically predisposed individuals.

With the aim of providing robust evidence for the presence or absence of viral markers in the pancreas of T1D donors, pancreatic tissue sections from 21 new additional donors were examined in 2 different laboratories. Viral markers at the protein (VP1) and RNA level (dsRNA), indicators of an anti-viral immune response (HLA-I, MxA, PKR and MDA5) and immune infiltration (CD45, CD3 and CD8) were investigated.

METHODS

Pancreatic sections from the head, body and tail from 10 controls, 7 T1D, and 4 autoantibody positive (AAb+) donors were distributed to nPOD-Virus consortium laboratories. Frozen sections (405 in total) were stained for insulin, glucagon, HLA-I, MxA, PKR, MDA5, dsRNA, CD45, CD3 and CD8 by immunofluorescence at Helmholtz-Zentrum in Munich (Germany). Paraffin sections were stained for insulin/glucagon, VP1 and HLA-I by immunohistochemistry at the University of Exeter Medical School (Exeter, UK).

SUMMARY OF RESULTS

In the islets, 6 out of 10 of the non-diabetic controls presented a normal HLA-I expression pattern while the rest had a slight elevation. One control, number 6413, was found to have a

higher level of HLA-I expression. Of the 4 AAb+ cases, 1 was normal, one had elevated HLA-I and 2 presented hyper-expression. All T1D cases had insulin containing islets in multiple regions of the pancreas and all but one presented HLA-I hyper-expression. Accordingly, exocrine HLA-I expression was also high in the majority of T1D donors. Lastly, HLA-I expression in T1D cases correlated with the presence of MX1, PKR, VP1, dsRNA as well as insulin content. However, in agreement with previous findings, signs of acute and widespread infection were not found. Ongoing analysis will determine if there is also a correlation between viral markers and the number of CD45⁺, CD3⁺ and CD8⁺ cells in both islets and exocrine pancreas.

CONCLUSIONS

The data obtained from 21 donors recently incorporated to nPOD indicates that HLA-I hyper-expression either in islets and/or in regions of the exocrine pancreas is associated with the detection of viral markers like PKR, dsRNA, VP1 and MX1. These “viral signature” was more prevalent in T1D cases than in non-diabetic controls, confirming previous findings by the nPOD-Virus group. The data may suggest that EV markers in the tissue and specifically in the islets are associated with abnormal pancreatic expression of HLA-I, a defining feature of T1D; and provide robust evidence that T1D is associated with a low level, persistent, EV infection of pancreatic beta cells.

O50:

High frequency detection of enteroviruses in spleen and peripheral blood leukocytes of people with diabetes of short and long duration

AUTHORS

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PURPOSE

To positively identify enteroviruses and other viral agents in spleen samples of the nPOD biobank and in peripheral blood leukocytes of diabetic adults.

METHODS

We used an integrated approach for EV detection based on the pre-enrichment of virus in cell culture before searching for the viral genome and antigens. To maximize cell susceptibility to different EV types, a mixture of 4 cell lines collectively expressing the key EV receptors was used. The cell mix expressed the following markers: CAR, DAF, integrins VLA2 and ITGB3, ICAM-1, PSGL-1, PVR, SCARB2. Gene amplification assays targeting conserved regions of all EV types were employed. PCR amplicons of sufficient intensity were sequenced to get hints on the involved EV species. Results were confirmed by staining infected cell cultures with pan-EV antibodies (9D5 and 6-E9/2) and group-specific EV antibodies. Other common viruses possibly involved in autoimmunity and diabetes were investigated by molecular assays. Sixty-five nPOD spleen samples, 20 blood samples of long-standing diabetic people, and 20 samples of healthy non-diabetic controls were analyzed.

SUMMARY OF RESULTS

Among nPOD spleen samples of organ donors, EV positivity was 71% in T1D cases vs. 18% in non-diabetic controls and 11% in AAb+ cases. The association of EV infection with T1D of variable duration was significantly higher than that found in non-diabetic controls and AAb+ cases. Of interest, AAb+ donors had significantly higher levels of C-peptide compared to non-diabetic controls. Comparable results were obtained with blood leukocytes of T1D and LADA cases of adult diabetes (1 to 48 years duration). EV positivity was found in 65% of cases vs. 5% non-diabetic controls. Partial sequences indicated that different EV groups were represented: Cox-A, Cox-B, EV-C, Rhinovirus. CMV, EBV, HHV6, Parvovirus B19 were detected in only a few cases without significant differences between diabetics and controls.

CONCLUSIONS

The rate of EV detection in spleen and in peripheral blood leukocytes was significantly higher in T1D and LADA cases vs AAb+ non-diabetic subjects, non-diabetic donors from the nPOD biobank, and local non-diabetic controls. Sequencing and immunostaining confirm that different EV species may be present, suggesting that diabetes may be associated with a variety of EV types. These data confirm that T1D and, possibly, other forms of diabetes are significantly associated with low level EV infection that can be detected in spleen and in blood leukocytes.

O51: Antibodies to the RNA-replicase of enteroviruses: possible cross-reactivity with human proteins

AUTHORS

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PURPOSE

Enteroviruses (EV) use a virally encoded RNA-replicase to carry out the synthesis of an RNA product directed by an RNA template. The EV replicase (3Dpol) is about 50-kDa and is essential for RNA genome expression and replication. Thus, expression of this enzyme is necessary in both acute and chronic infection. To test whether 3Dpol could serve as an additional marker of EV infection in the pancreas of diabetic subjects and in cell cultures infected with virus obtained from diabetic people, we generated mouse monoclonal antibodies against the 3Dpol of CV-B3, a representative EV type.

METHODS

Upon hybridoma selection by ELISA using 3Dpol as antigen, the reactivity of positive clones was tested by immunofluorescence in cultured cells acutely infected with CV-B3 or cells persistently infected with EV isolates obtained from T1D cases. Linear epitopes of MAbs were mapped in the 3Dpol using peptide microarray technology. Four linear epitopes were identified. The 3D-reactive MAbs were tested by a) staining cell lines infected with a large variety of EV types; b) staining cells persistently infected with virus derived from diabetic subjects; c) bioinformatics analysis to explore their reactivity with all reported EV types; d) microarray tests for investigating their reactivity with 18,000 members of the human proteome.

SUMMARY OF RESULTS

The 3Dpol MAbs produced no staining of uninfected cell lines and of cells infected with control viruses (Adeno-C, HPeV1, HIV1). 3D-01, 02, 03, 05 produced granular cytoplasmic fluorescence in cells acutely-infected with coxsackieviruses-B. Bioinformatics analysis revealed that MAbs 02 and 05 were reactive with EV members of the B, A, D, and C species, whereas MAbs 01 and 03 were more specific to members of the B species. Surprisingly, when tested on normal human pancreas, 3D-01 and 3D-03 produced selective staining of islets. 3D-01 recognized insulin-producing cells. Tests with microarrays covering over 80% of the human proteome showed that: a) 3D-01 recognized CALCOCO2, an autophagy receptor and mediator of viroplasm (Mohamud et al., 2018); b) 3D-03 bound to IGHG3 (constant domain of human IgG3 heavy chains) that is differentially expressed in the pancreas of T1D patients (Planas et al., 2009), c) 3D-05 bound to C6orf118 (mitochondrial localization; possible RNA binding/ribonuclease activity). In contrast, 3D-02 did not recognize any protein that was represented in the microarrays used for this investigation.

CONCLUSIONS

Due to lack of reactivity with the human proteome, and strong specificity for different EV types, MAb 3D-02 was chosen for selectively staining the viral replicase in tissues of diabetics and in EV-infected cell cultures. In principle, structural components and enzymes of viruses could trigger autoimmune responses of possible pathogenic significance. The above results start delineating potential human targets cross-reacting with the EV replicase.

(LB-05)

Coxsackievirus B (CV-B) transactivate Human Endogenous type W expression in macrophage cells: a potential link between enteroviruses and T1D pathogenesis.

AUTHORS

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PURPOSE

Human endogenous retroviruses (HERVs), known to represent 8% of the human genome, have been associated with several autoimmune diseases. Most of HERV elements have lost their protein-coding capacity, unless they are transactivated under pathological conditions. In particular, HERV-W, which has been involved in the pathogenesis of Multiple Sclerosis (MS), can be transactivated by environmental viruses such as Epstein-Barr virus (EBV) or Herpes simplex virus type 1 (HSV-1). The envelope protein of HERV-W (pHERV-W-Env) displays pro-inflammatory, autoimmune and TLR4-driven cytopathic properties that had initially been demonstrated in an MS perspective and have subsequently revealed to be relevant for Type 1 Diabetes (T1D; Levet S et al.. *JCI Insight*. 2017).

Coxsackievirus B (CV-B) are enteroviruses that have been associated with T1D etiology long ago but were not systematically detected and never proved to explain T1D pathogenesis. Here, we present data supporting the concept that HERV-W could represent a missing link between environmental factors such as enteroviruses, playing a role on a “hit and run” mode transactivating HERV-W copies in human DNA, and T1D pathogenesis.

METHODS

CV-B4 strains (CV-B4 E2 and CV-B4 JBV) were inoculated to primary cultures of human macrophages at different multiplicities of infection (MOI) and analyzed at different time points by qRT-PCR.

SUMMARY OF RESULTS

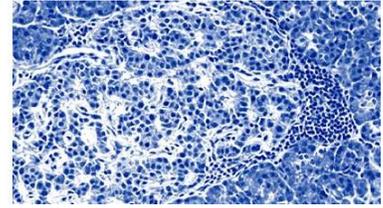
Our results confirmed a quite significant upregulation of HERV-W-Env mRNA induced by these coxsackievirus strains, even at low MOI, compared to mock-infected cells. Kinetics indicated a significant transactivating effect from 16h to 48h post-infection. Analyses of the pHERV-W Env protein expression are in progress.

CONCLUSIONS

Taken together, our results indicate that HERV-W-Env could be transactivated by enteroviruses such as CV-B4 E2, which can be detected in pancreata from T1D patients. These results support a link between enteroviral infection and T1D, through the transactivation of HERV-W-Env, an endogenous retroviral protein with pro-diabetogenic features.

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(LB-12)

Stool Enterovirus Precedes Risk of Islet Autoimmunity: The TEDDY Study

AUTHORS

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PURPOSE

Viruses have been implicated in islet autoimmunity leading to type 1 diabetes (T1D).

METHODS

The Environmental Determinants of Diabetes in the Young (TEDDY) study evaluated the association between fecally shed viruses, islet autoimmunity (IA) and T1D in two nested matched case-control analyses: IA, 1:1, n=383 case-control pairs, median (IQR) age at appearance of IA =21.8 (13.3-33.1) months; and, T1D, 1:1, n=112 case-control pairs, median age (IQR) at T1D diagnosis =29.5 (19.3-41.7) months. Stool samples were collected monthly beginning at age three months. The fecal virome of these samples was analyzed by direct sequencing and after cultivation on cells. Viral associations with IA and T1D were

tested in conditional logistic regression models, matched for clinical site, gender, and family history of T1D. Full adjusted model included HLA, known T1D SNPs, probiotics and timing and season of sampling.

SUMMARY OF RESULTS

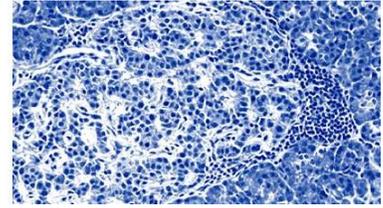
Species A and B *Enterovirus* were detected in stool samples in 40.7% and 36.6% of cases before IA and 38.1% and 37.1% of paired controls, respectively. Species B *Enterovirus* (EV-B) positivity in 2+ consecutive samples including prolonged shedding of the same EV-B virus determined from RNA sequence homology (15.7% cases, 8.1% controls), showed an independent association with children developing IA (full adjusted OR=3.0, 95%CI=1.4-6.5, p=0.005). Single-sample (OR=0.8, 95%CI=0.5-1.2) or multiple non-consecutive (OR=0.7, 95%CI=0.4-1.3) EV-B positive children with no evidence of prolonged shedding did not differentiate in development of IA compared to children negative for EV-B.

CONCLUSIONS

In the largest known study to analyze the whole virome in T1D genetically at-risk children, prolonged EV-B shedding is linked with subsequent development of IA in early life.

Select the **ONE** category that best describes your research:

<input type="checkbox"/> Beta Cell Physiology and Dysfunction	<input type="checkbox"/> Novel Biomarkers
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(LB-15)

Antibodies Bind a Hybrid Insulin Peptide (HIP) Target in T1D

AUTHORS

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PURPOSE

Our lab discovered unique post-translationally modified antigens whereby derivatives of insulin C-peptide form peptide bonds with endogenous granule peptides to form HIPs in mouse β -cell extracts and human islets. Diabetogenic CD4 T cell clones (NOD mice) and CD4 T cells from T1D patients are activated by such HIP ligands and imply that HIPs may also serve as epitopes for humoral immunity. The primary goal of our study is to identify and characterize antibodies targeting HIPs among T1D individuals and evaluate their utility as biomarkers.

METHODS

Cognizant of the HIPs capable of eliciting an antigenic response in CD4 T cell lines from T1D individuals and NOD mice, we generated antibody probes reflecting derivatives of the same HIP epitopes. Purified, recombinant proteins were systematically refolded to achieve epitope conformation. A sensitive and specific ECL-based HIP assay was adapted from the hallmark ECL-IAA protocol.

SUMMARY OF RESULTS

The key determinant for the HIP ECL was design and implementation of a putative HIP protein intermediate as the target antigen probe to measure both prevalence and specificity of anti-HIP antibodies. Sera from a new onset T1D cohort using a proinsulin(PI)/IAPP HIP probe showed the presence of anti-PI/IAPP antibodies. However similar prevalence was measured among control and T2D sera samples even when the data was stratified according to HLA genotype. Anti-PI/IAPP antibodies are present in ~90% of all individuals although the majority are at low titers. These HIP antibody epitopes are conformationally

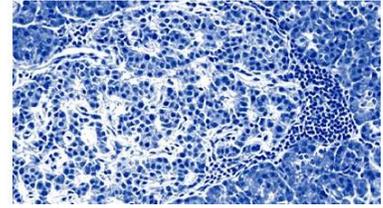
specific as they are preabsorbed only with refolded PI/IAPP, but not alternate or random HIPs or linear PI/IAPP protein. Screens of prediabetic retrospective sera typically display a peak of anti-PI/IAPP antibodies prior to that of IAA autoantibodies.

CONCLUSIONS

The majority of individuals have HIP/IAPP-specific antibodies in their circulation, irrespective of T1D disease state or risk. Although pervasive, and the first antibodies in T1D targeting HIP epitopes, they are not predictive of disease. As the appearance of such HIP antibodies typically precedes insulin autoantibodies in prospectively followed at risk prediabetic individuals, it is possible that antibodies to other HIPs may serve as earlier biomarkers for ensuing disease.

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(LB-16)

Transgenic substitution with Greater Amberjack *Seriola dumerili* fish insulin 2 in NOD mice reduces beta cell immunogenicity

AUTHORS

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PURPOSE

An abiding issue in the biology of T1D is the nature of the primary immunologic trigger(s) for β cell destruction. Insulin itself is a compelling candidate, with the mechanism involving developmental failure to recognize some epitope of the insulin molecule as self. Amino acids B:9-23 of the beta chain of insulin have been identified as the epitopes of insulin in this regard. Fish (e.g. Greater Amberjack) insulins are known to differ from human and mouse insulins by 2 aa in this region. Before the availability of recombinant human insulin, mammalian and sometimes fish insulins were used in the treatment of T1D. We hypothesized that β -cells synthesizing fish insulin would be less immunogenic in a mouse model of T1D.

METHODS

Transgenic NOD mice in which Greater Amberjack fish (*Seriola dumerili*) insulin was substituted for the insulin 2 gene were generated (mouse *Ins1*^{-/-} mouse *Ins2*^{-/-} fish *Ins2*^{+/+}). In these mice, pancreatic islets remained free of the cellular stigmata of autoimmune attack. To determine whether such reduction in immunogenicity is sufficient to protect β -cells from autoimmunity upon transplantation, we transplanted fish *Ins2* transgenic (expressing solely *Seriola dumerili* *Ins2*), NOD, or B16:A-dKO islets under the kidney capsules of 5 weeks old female NOD wildtype mice.

SUMMARY OF RESULTS

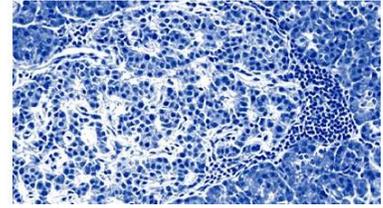
The B:Y16A B-chain substitution has been previously shown to be protective of T1D in NOD mice. NOD mice receiving *Seriola dumerili* transgenic islet transplants showed a significant ($p=0.004$) prolongation of their euglycemic period (by 6 weeks; up to 18 weeks of age) compared to un-manipulated female NOD (diabetes onset at 12 weeks of age) and those receiving B16:A-dKO islet transplants (diabetes onset at 12 weeks of age). Additionally, the replacement of mouse insulin genes with Greater Amberjack insulin reduces the apparent immunogenicity of the islets in T1D-prone NOD mice, but does not completely halt an ongoing immune response.

CONCLUSIONS

Modification of the B9:23 epitope on the insulin gene in transplanted β -cells can potentially improve their survival in recipients with extant T1D. However, while the congenital substitution of the B9:23 insulin epitope alone is sufficient to prevent autoimmunity in the NOD mouse, it is not sufficient to fully suppress assault in NOD mice congenitally sensitized to insulin. These data support the concept that specific amino acid sequence modifications can reduce insulin immunogenicity. Additionally, our study shows that alteration of a single epitope is not sufficient to halt an ongoing autoimmune response. Which, and how many, T cell epitopes are required and suffice to perpetuate autoimmunity is currently unknown. Such studies may be useful to achieve host tolerance to β -cells by inactivating key immunogenic epitopes of stem cell-derived β -cells intended for transplantation.

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(LB-24)

The JDRF nPOD-Virus Group: Targeted Proteomic Analysis to Uncover the Role of Viruses in T1D

AUTHORS

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PURPOSE

As part of the JDRF nPOD-Virus group, our ongoing studies using disease stratified nPOD pancreata demonstrate the presence of enteroviruses and proteomic signatures consistent with enterovirus infections. With the objective of improving identification rates and providing robust evidence for viral infections in the pancreata, a new targeted strategy has been used for selecting nPOD cases, tissue sections and specific tissue slices prior to mass spectrometry analysis. In this approach, tissues sections were selected based on positive VP1 signals by IHC on FFPE tissues, positive signals for HLA Class I hyper-expression and insulin using fresh frozen OCT embedded tissues. Fresh frozen OCT embedded tissues slices adjacent to those that are positive to VP1, Insulin, and HLA Class I hyper-expression and corresponding controls have been utilized for extensive proteomic analysis.

METHODS

20 μ M pancreatic tissue slices from fresh frozen OCT embedded tissue sections from the head and tail from 18 new nPOD cases that included 8 controls, 3 autoantibody positive (AAb+) and 7 T1D, donors were selected and obtained from the nPOD Biorepository based on detection of insulin and HLA-I in fresh frozen OCT embedded tissue by immunofluorescence at Helmholtz-Zentrum in Munich using (Germany) and preliminary IHC detection of VP1 and HLA-I using formalin fixed paraffin embedded tissues at the University of Exeter Medical School (Exeter, UK). The tissue slices were used to isolate and purify proteins prior to processing for LC/MS/MS on an Orbitrap Fusion Lumos Mass Spectrometer using different scanning methods including data dependent acquisition (DDA), data independent acquisition (DIA), and parallel reaction monitoring (PRM) followed by qualitative and quantitative comparative analysis.

SUMMARY OF RESULTS

Our data demonstrate the utility of using 20 µM pancreatic tissue slices from fresh frozen OCT embedded tissue sections for comprehensive qualitative and quantitative proteomics analysis. Potential enterovirus peptides are also identified in a number of the nPOD cases and these are currently undergoing validation in targeted LC/MS-PRM analysis. Comparative proteomic analysis of the cases using data dependent acquisition methods (DDA) reveal the upregulation and activation of pathways that are associated with viral infections and may be supportive and complementary to the data obtained by immunostaining for specific viral infection markers.

CONCLUSIONS

Our current data provides additional evidence that T1D pancreata are infected and potentially harbour various enterovirus proteins; and pathways that are associated with the infection are also activated in infected tissues. Ongoing studies include targeted studies to validate the serotypes of the identified viruses using affinity purification, LC retention time and MSn fragmentation analysis using stable isotope labelled synthetic peptides and verification with orthogonal other biochemical assays.

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