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Beta Cell Development, Differentiation & Regeneration

1. Geming Lu (City of Hope)

Abstract Title

Harmine plus Exendin-4 Protects against Cytokine-Induced Human β-Cell Death and Decreases Immunogenicity

Authors

Randy Kang, Jungeun Lee, Miguel Varela, Andrew F Stewart, Adolfo Garcia-Ocana, Geming Lu

Purpose

Type 1 diabetes (T1D) results from loss of immune tolerance and destruction of functional β-cells. Therefore, therapeutic approaches for T1D should focus on preservation and regeneration of β-cells while restoring immune tolerance. Administration of harmine (H) plus exendin-4 (E) markedly induces human β-cell expansion in vitro and in vivo. This combination therapy also enhances β-cell survival in human islet grafts in the early days post-transplantation. However, whether H+E can further enhance human β-cell survival in an inflammatory environment is unknown. Furthermore, whether this drug combination could diminish β-cell immunogenicity is also unknown.

Methods

Here, we tested whether H+E could protect human β-cells against both cytokines and ER stress. We also analyzed the single-cell transcriptome profile of human islets in the presence of cytokines and H+E and determined the levels of expression of HLA-ABCs, CXCL10, NO, ER stress markers and proteasome subunits.

Summary of Results

First, we found that H+E significantly reduced both thapsigargin (ER stress)- and cytokine-induced human β-cell apoptosis, an effect not observed with the drugs alone. Second, single-cell RNAseq analysis of human islets treated for 6h with cytokines and H+E showed that the drug combination reduced IL1β-, TNFα- and IFNgamma-mediated signaling in β-cells. Further analysis revealed that genes in the intrinsic and extrinsic apoptotic pathways were increased by cytokines and normalized by H+E. CXCL9-11, HLA-ABC and IRF1 and 2 gene expression was upregulated by cytokines and normalized by H+E in human β-cells. On the other hand, HLA-E expression was increased by H+E. Validation of these observations by qPCR, ELISA and immunostaining in human islets show that expression of ER stress markers (CHOP, GRP78, sXBP1, IRE1, ATF6), NO and CXCL10 levels, proinsulin/insulin ratio, HLA-ABC expression, and proteasome subunits 8-10 were significantly attenuated by H+E treatment.

Conclusions

Collectively, these results indicate that harmine plus exendin-4 enhances β-cell survival through regulation of inflammation and pro-apoptotic genes and decreases markers of immunogenicity. These results highlight the therapeutic potential of this drug combination for the treatment of T1D.

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

Abstract Title

Cycling Alpha Cells in Human Pancreatic Islets As a Potential Key Reservoir for Beta Cell Loss

Authors

Esra Karakose, Xuedi Wang, Peng Wang, Saul Carcamo, Deniz Demircioglu, Luca Lambertini, Olivia Wood, Randy Kang, Geming Lu, Don Scott, Adolfo Garcia-Ocaña, Carmen Argmann, Robert Serba, Dan Hasson, Andrew Stewart

Purpose

Type 1 diabetes results from inadequate numbers of insulin-producing beta cells. Although the current attempts to replenish the remaining beta cell pool in people with diabetes (whole pancreas transplantation, pancreatic islet transplantation, transplant of human stem cell-derived beta cells) are encouraging, scalability and cost issues limit the access to these therapies for the millions of people with diabetes. We previously showed that the DYRK1A inhibitors, either alone or in combination with GLP1 receptor agonists (GLP1) or TGF beta superfamily inhibitors (LY), induce beta cell replication and increase beta cell mass both in vitro and in vivo. However, the precise mechanisms of action of these regenerative drugs remain elusive.

Methods

To more deeply explore the beneficial mechanisms of action of DYRK1A inhibitors, we performed single cell RNA sequencing on four different human pancreatic islets treated with a DYRK1A inhibitor, either alone, or with GLP1 or LY. This is the first in-depth single cell transcriptomic analysis on human cadaveric islets treated with regenerative drugs.

Summary of Results

This is the first in-depth single cell transcriptomic analysis on human cadaveric islets treated with regenerative drugs. First, we confirm the presence of all previously reported cell types in human islets. More importantly, we identify a cluster of Cycling Alpha Cells as the endocrine cell type most responsive to regenerative drug treatment. Interestingly, we observed that Cycling Alpha Cell numbers diminish drastically in T1D patients, underlining the importance of this understudied cell cluster. Our in-depth analyses showed that in addition to the significant increase in the abundance of Cycling Alpha Cells, a sub-cluster of Cycling Alpha Cells gained beta cell identity with regenerative drug treatment. Our velocity and pseudotime lineage trajectory analyses confirmed this finding and also suggested that Cycling Alpha Cells serve as the primary target cell type for the regenerative drugs, and may serve as precursor cells that transdifferentiate into functional human beta cells in response to the regenerative drug treatment.

Conclusions

Collectively, these findings suggest a novel mechanism of action through which DYRK1A inhibitors are able to expand human beta cell numbers. This mechanism involves Cycling Alpha Cells which serve as a beta cell regenerative reservoir, and readily transdifferentiate into functional human beta cells upon treatment with beta cell regenerative drugs of the DYRK1A inhibitor class. Importantly, these studies raise the question as to whether “beta cell drug targeting” strategies are necessary or appropriate for human beta cell regeneration, since

alpha cells may be the principal target for “beta cell regenerative drug therapy”. These findings and potential implications will be further discussed.

3. KaLia Burnette (The University of Kansas Medical Center)

Abstract Title

Islets Encapsulated with CTLA-4-Ig and Antioxidants Restore Euglycemia in Subcutaneous Transplant Site

Authors

KaLia S. Brnette, Sheila Tsau, Veronika Kozlovskaya, Eugenia Kharlampieva, Andrew Pepper, Hubert M. Tse

Purpose

Type 1 diabetes is characterized by beta-cell-specific autoimmune destruction, leading to lifelong exogenous insulin dependence. The hepatic portal vein is the preferred site for islet transplantation however, its high flow rates present a risk for graft attrition and instant blood-mediated inflammatory reaction. There is a need to find alternative sites for islet transplantation that are highly vascularized and can elicit immunoprotection. While islet transplantation can restore euglycemia, novel strategies to delay graft rejection are needed. Our lab uses a nanothin encapsulation material consisting of poly(N-vinylpyrrolidone) (PVPON) and tannic acid (TA), an antioxidant, to delay islet allograft rejection and decrease inflammatory immune responses when transplanted under the kidney capsule of streptozotocin (STZ)-treated diabetic mice. The addition of CTLA-4-Ig, an immune inhibitor fusion protein, onto our encapsulation material significantly increased FOXP3+ regulatory and CD73+ FR4+ anergic CD4+ T cells at the graft site and delayed islet graft rejection when compared to non- and (PVPON/TA/IgG)-encapsulation. We sought to assess the ability of (PVPON/TA/CTLA-4-Ig)-encapsulation to improve islet graft survival in a pre-vascularized subcutaneous transplant site. We hypothesize that (PVPON/TA/CTLA-4-Ig)-encapsulation in combination with a deviceless subcutaneous transplant site will sustain islet allograft function by eliciting localized immunosuppression.

Methods

NOD.scid, C57BL/6, and spontaneously diabetic NOD mice will be implanted with a 2 cm 6-Fr nylon catheter for 4-weeks to generate a deviceless subcutaneous transplant site. C57BL/6 mice will be rendered diabetic with streptozotocin treatment 3 days prior to islet transplantation. The catheter will be removed at the time of transplant and 250 or 500 non- or (PVPON/TA/CTLA-4-Ig)-encapsulated syngeneic islets will be transplanted into the pre-vascularized subcutaneous space or under the left kidney capsule. To investigate the ability of (PVPON/TA/CTLA-4-Ig)-encapsulated islets to protect against autoimmune responses, transplants will also be performed in spontaneously diabetic NOD mice. The blood glucose of recipient mice will be monitored daily to assess graft function. Islet grafts will also be harvested 7 and 14 days post-transplant for immunophenotyping.

Summary of Results

We found that subcutaneous implantation of a nylon catheter into NOD.scid mice resulted in the formation of a vascularized pocket evident by CD31 staining. Syngeneic transplants of (PVPON/TA/CTLA-4-Ig)-encapsulated islets into STZ-treated NOD.scid mice successfully restored normoglycemia similar to non-encapsulated islets.

Conclusions

Subcutaneous transplantation of (PVPON/TA/CTLA-4-Ig)-encapsulated islets can successfully restore normoglycemia to mice. Future studies will investigate the ability of (PVPON/TA/CTLA-4-Ig)-encapsulated islets transplanted within the subcutaneous space to restore euglycemia in allogeneic diabetic mice.

4. Pamela Sandoval Sanchez (Florida State University)

Abstract Title

A high throughput system to generate recombinant adenoviruses for CRISPR-Cas9 mediated gene knockouts

Authors

Pamela Sandoval Sanchez, Maria Pilar Toledo, Jacob Gottlieb, Hyo Jeong Yong, Katheryn Woodford, Valerie Martino, Sarah Weiss, Isabella Bazan, Danny Alex, Yue Julia Wang

Purpose

The purpose of this project is to address the chronic health issue of diabetes by focusing on the root cause of the disease, which is a shortage of pancreatic beta cells responsible for producing insulin. Diabetes, particularly Type 1 and Type 2, is characterized by insufficient insulin production due to the destruction or dysfunction of these vital beta cells. The ultimate goal is to find a way to increase the proliferation of beta cells, thereby enhancing the body's insulin output. To achieve this, the project employs cutting-edge genetic engineering technology, specifically CRISPR-based tools. By selectively modifying key cell cycle genes in beta cells, both through loss-of-function (CRISPR knockout) and gain-of-function (CRISPRa) techniques, the project aims to stimulate beta cell proliferation and increase their overall mass. Successful execution of these genetic modifications could delay the onset of diabetes and lead to innovative therapeutic approaches for the treatment of this disease.

Methods

The project employs a robust and efficient gateway cloning method to introduce genetic modifications into pancreatic beta cells. This method is characterized by its high in vitro homologous recombination efficiency and a reliable negative selection process. To facilitate genetic engineering, the team designed the pAdEasy all-in-one vector, which incorporates the Cas9 expression cassette, the gRNA expression cassette, and a GFP fluorescent marker. Additionally, attR sites were integrated into the vector to ensure compatibility with gateway cloning. The introduction of gRNAs involves a two-step process: first, gRNA oligos are cloned into the pENTR vector, generating the pENTR gRNA entry vector through high-efficiency golden gate assembly. Subsequently, the pENTR gRNA entry vector is combined with the pAdEasy destination vector through an LR reaction, resulting in the creation of the final pAdEasy all-in-one expression vector. This method enables precise and targeted genetic modifications in beta cells to promote their proliferation, contributing to the overarching goal of improving diabetes management.
In addition to the CRISPR knockout strategy, the project is actively developing a CRISPR-mediated transcriptional activation system (CRISPRa). Unlike the CRISPR knockout approach, CRISPRa mediates targeted gain-of-function by focusing on known cell cycle activators. This gain-of-function strategy is expected to promote beta cell proliferation. By combining the CRISPR loss-of-function and the CRISPRa tools, the project aims to perform targeted genetic modulation in either direction, maximizing the promotion of beta cell replication. This multifaceted approach offers promising prospects for addressing the root causes of diabetes and enhancing diabetes management.

Summary of Results

Our newly designed system streamlines the cloning process, allowing for highly efficient in vitro reactions. Each cloning step yields over 2000 colonies per 2 µl of reaction, and further confirmation through restriction digestion shows that over 92% of the colonies contain the correct product.

Conclusions

In conclusion, this study highlights the development of an all-in-one adenoviral vector with impressive efficiency, making it a valuable tool for testing various gRNAs and facilitating whole genome screening. The innovative use of gateway assembly and ccdb in vector design significantly reduces background vectors, resulting in cleaner and more precise experimental outcomes. The importance of CRISPR genetic perturbation

research cannot be overstated, especially in the context of advancing human health. Adenoviruses, renowned for their ability to transduce hard-to-transfect cells, such as primary human islets, offer a powerful means to drive genetic modifications in challenging contexts. The creation of the all-in-one adenoviral cloning system presented in this work has the potential to not only advance our understanding of pancreatic beta cells but also to benefit functional genomic research across diverse biotechnology fields. This research represents a significant step toward addressing the complex challenges of diabetes and paves the way for innovative therapeutic approaches that can ultimately enhance the health and well-being of our species.

5. Boutheina Marnissi (University of Miami)

Abstract Title

PS02, a bifunctional RNA therapeutic to specifically modulate human β cell proliferation

Authors

Boutheina Marnissi, Daria Ivanova, Marlene Redlich, Victoria Kuznetsova, Serena Zilio, Julia Zaia, Paolo Serafini

Purpose

Increasing β cell number is an important need for the treatment of T1 diabetes. Among the different pathways and genes that repress β cell proliferation in humans, the cell cycle inhibitor p57kip2, encoded by CDKN1C, is particularly interesting. Repression of this gene because of mutation or epigenetic regulation leads to hyperinsulinism of infancy or Beckwith-Wiedemann syndrome, human diseases associated with a dramatic expansion of insulin-producing cells. Furthermore, virally induced silencing of this gene induces a strong proliferation of human β cells when transplanted in diabetic NSG mice. Unfortunately, as is common for many cycle-regulating genes and pathways, p57kip2 is not specific to β cells but is expressed in many cell types and is involved in malignant transformation and tumor progression. Because of tumorigenesis concerns, these observations prevent targeting this pathway systemically using antagonists or viral vectors. However, a temporally controlled targeted modulation of this pathway in β cells might still be viable for T1D treatment. We hypothesized that bifunctional RNA therapeutics could safely silence p57kip2 in β cells to induce their proliferation and restore insulin independence while leaving other cells and tissues untouched, thus overcoming safety concerns

Methods

Building on our recent identification of two RNA aptamers specific to mouse and human β cells, we generated PS02, a bispecific RNA therapeutic able to silence p57kip2 specifically on insulin-producing cells, and a control drug (CTRL02) by conjugating our β cell-specific aptamers to either an siRNA specific to p57kip2 or the scrambled shRNA via a RISC cleavable linker. We tested PS02 targeted silencing ability by qRT-PCR on human islets in vitro and on the human islet graft transplanted in NSG mice.
We then assessed the capacity of our bifunctional therapeutic to induce human β cell-targeted proliferation in vivo. We treated diabetic NSG mice previously engrafted with human islets (500 IEQ) with PS02 and CTRL02 intravenously three times a week for one week and determined the proliferation of β cells and other cell types by fluorescence immunocytometry. Then, we assessed the efficacy of these bifunctional therapeutics to revert diabetes in marginal mass experiments performed in STZ-treated NSG mice engrafted with human islets (500 IEQ) under the kidney capsule. Long-term experiments evaluating PS02 safety are being performed.

Summary of Results

PS02 added to undissociated human islets significantly silenced p57kip2 as assessed by qRT-PCR, whereas CTRL02 did not modulate the expression of this cell cycle inhibitor. Similarly, PS02 given i.v. decreased p57kip2 β cells, whereas we observed no modulation in α cells from the human graft nor on the other mouse tissues evaluated.
In vivo, human β cell proliferation assessment confirmed this specificity. In diabetic NSG mice engrafted with human islets, the percentage of EdU+β cells in the human graft was significantly higher than those from CTRL02-treated mice. No differences from control were observed in α cells from the same graft nor in the other

murine tissues evaluated.
We finally evaluate the effect of treatment in marginal mass experiments. All NSG mice transplanted with human islets, rendered diabetic with STZ, and treated with CTRL02 succumb to diabetes (blood glycemia higher than 600 dg/ml) within 10 days. In sharp contrast, all the mice treated with PS02 reverted to euglycemia within 14 days, and most (65%) retained glycemic control after treatment termination until nephrectomy.
We observed macroscopic differences in the graft size between the CTRL02 and PS02-treated mice, with the latter having the graft much bigger than the former. This was confirmed by the microscopic evaluation of the xenograft. Human α and β cells were detected in 8 out of 8 mice treated with PS02, whereas only one out of seven mice treated with CTRL02 still had human islet cells. Furthermore, the β/α cell ratio median of 3.17 (range 1.51-3.96) in PS02-treated mice, while it was 1.16 in the only mouse having residual human islets in the control group, suggesting that PS02-induced proliferation overcame the hyperglycemia-induced β cell death.

Conclusions

We developed and optimized PS02, a bispecific RNA therapeutic that systemically promotes β cell proliferation specifically in human β cells. This drug can be produced with an oligo synthesizer in large quantities without contamination and at GMP grade. It is not immunogenic and is RNase-resistant because of its fluorinated backbone. This is the first non-viral targeted RNA therapeutic to induce the selective proliferation of human β cells in vivo and revert T1D.

6. Teresa Mastracci (Indiana University)

Abstract Title

A translational regulatory mechanism mediated by hypusinated eukaryotic initiation factor 5A facilitates beta cell identity and function

Authors

Catharina B.P. Villaca, Craig T. Connors, Emily K. Anderson-Baucum, Spencer R. Rosario, Caleb D. Rutan, Paul J. Childress, Leah R. Padgett, Morgan A. Robertson, Teresa L. Mastracci

Purpose

Purpose: As professional secretory cells, beta cells require adaptable mRNA translation to facilitate a rapid synthesis of proteins, including insulin, in response to changing metabolic cues. Specialized mRNA translation programs are essential drivers of cellular development and differentiation. However, in the pancreatic beta cell, the majority of factors identified to promote growth and development function primarily at the level of transcription. Therefore, despite its importance, the regulatory role of mRNA translation in the formation and maintenance of functional beta cells is not well defined.

Methods

Methods: The specialized mRNA translation factor eukaryotic initiation factor 5A (EIF5A) was identified in a type 1 diabetes susceptibility locus in humans and NOD mice, which suggests that eIF5A plays a role in beta cell health. For eIF5A to be active and perform its mRNA translation function, it must be post-translationally modified by the enzyme deoxyhypusine synthase (DHPS). Therefore we generated a mutant mouse model with a beta cell-specific Dhps deletion in mouse (DhpsLoxP;Ins1-cre) to determine the role of mRNA translation during beta cell development, growth and maturation. We performed metabolic and tissue phenotyping of these animals at 1, 4, 5 and 6 weeks-of-age.

Summary of Results

Results: We discovered that the absence of beta cell DHPS in mice reduces the synthesis of proteins critical to beta cell identity and function at the stage of beta cell maturation, leading to a rapid and reproducible onset of diabetes. In particular, we identified that Ins1, Slc2a2 (Glut2), Ucn3, and ChgA are translationally regulated by eIF5A-HYP during beta cell maturation.

Conclusions

Conclusions: We have determined that without DHPS/eIF5A-HYP, beta cells lose identity/function and diabetes ensues. In short, our work has revealed a gatekeeper of specialized mRNA translation that permits the beta cell, a metabolically responsive secretory cell, to maintain the integrity of protein synthesis during times of induced or increased demand.

7. Kathrin Maedler (University of Bremen)

Abstract Title

The TEAD activator TT-10 promotes beta-cell regeneration in human and mouse islets

Authors

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Purpose

Beta-cell regenerative therapy must replace what is missing in both type 1 and type 2 diabetes (T1D/T2D); highly functional beta-cells.
Despite a small subpopulation of cells within islets with a rather progenitor-like character, which are especially capable to regenerate, human beta-cells have lost their proliferative capacity on their way towards maturation. One of the genes which controls proliferation and is lost during beta-cell differentiation is the co-transcriptional activator of the Hippo pathway Yes Associated Protein (YAP), which classically acts through transcription factor TEAD. On search of a small molecule which modulates the YAP-TEAD proliferative capacity, we found TT-10, an activator of TEAD transcription factor, which robustly induced beta-cell proliferation.

Methods

For in vitro analyses, isolated islets from C57Bl/6J mice, leptin receptor deficient db/db mice and from organ donors with and without type 2 diabetes were exposed 10-25 uM TT-10 for 48-72h. For in vivo analyses, C57BL/6J wild-type mice were daily injected with 10 mg/kg body weight TT-10 or vehicle for one week. Beta-cell proliferation (by Ki67- and pHH3-/insulin double staining), apoptosis (by TUNEL) and gene expression was analysed in isolated islets and pancreatic sections.

Summary of Results

Consistent with YAP overexpressing human islets, TT-10 profoundly induced beta-cell proliferation, compared to the untreated control group. TT-10 induced a 2.5-fold increase in proliferation in mouse and control human islets and a 3- and 4-fold increase in Ki67- and pHH3-positive beta-cells in T2D human islets. This effect was completely abolished by TEAD inhibition through genetic and pharmacological strategies. In diabetic db/db mouse islets, TT-10 inhibited beta-cell apoptosis.
While expression of most of the beta-cell functional and beta-cell marker genes remained unchanged by TT-10 treatment, MafA, Slc2A2, ABCC8, KCNJ11, Glis3 and Pdx1 were upregulated. FoxM1, which is specifically associated with beta-cell proliferation was 5-fold upregulated in human islets.
Already after 7 days of TT-10, there was a significant increase in proliferation in mouse beta-cells.

Conclusions

Our results show that TT-10, through Hippo-TEAD signalling has a strong pro-proliferative activity in human islets in vitro and in mice in vivo and suggest TT-10 as a novel compound for beta-cell regenerative therapy to restore functional mature beta-cells in diabetes.

8. Yury Kryvalap (University of Minnesota)

Abstract Title

Preclinical examination of the humanized antibody to serpinB13 in type 1 diabetes and beta-cell development

Authors

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

Purpose

Recent studies in our laboratory suggested that a combinatorial approach with a monoclonal antibody (mAb) to serpinB13, a cathepsin L (catL) inhibitor, and a suboptimal dose of antibody to CD3, is therapeutically superior to single antibody use, when these reagents are delivered into NOD mice during the late prediabetes period. We also found that inhibition of catL with our original mouse antibody to serpinB13 during embryogenesis results in protease-dependent cleavage of the extracellular domain of Notch1 receptor, and ultimately increases the number of endocrine progenitor cells with Ngn3 expression in the pancreas of normal mice. The purpose of this study was to examine the impact of our newly generated humanized antibody to serpinB13 (hB29) on type 1 diabetes and Ngn3 expression, as a readout of early endocrine lineage. Moreover, in an initial attempt to translate our findings to humans, we examined Ngn3 expression in newborn babies.

Methods

Ten-week old NOD female mice were each injected with 40 micrograms of hB29 and a suboptimal dose of 10 micrograms of anti-CD3 mAb (clone 145-2C11). Control treatments included injections with non-specific human and hamster IgG. Random blood glucose levels were assessed for 8 weeks after the last antibody injection, and mice were declared diabetic following two measurements of glucose exceeding 300 mg/dL. To assess Ngn3 expression in mice, embryonic pancreatic explants (E12.5) were incubated with hB29 or human IgG at 1.0 microgram/mL for 48 hours, and examined by flow cytometry. To assess Ngn3 expression in humans, paraformaldehyde-fixed nPOD sections from three newborn babies were treated for antigen retrieval and stained with antibodies to islet hormones, cytokeratin 18 and Ngn3. Staining was quantitatively examined using Visiopharm software

Summary of Results

We found that the combination of hB29 with anti-CD3 mAb offered a significant level of protection from diabetes, while single antibody use had no effect. We also found that in vitro exposure to hB29 led to the generation of additional endocrine progenitor cells expressing Ngn3, compared to treatment with nonspecific human IgG. Finally, our analysis of nPOD samples revealed a significant presence of Ngn3 in the human pancreas during the early days after birth. Although occasional Ngn3+ cells co-stained for insulin or glucagon, the vast majority of these cells were negative for these hormones.

Conclusions

Our data demonstrate a strong similarity between the original mouse serpinB13 mAb and our new agent, the humanized antibody, hB29. Although more work is necessary to examine whether Ngn3 is expressed at older ages, the finding of Ngn3 expression soon after birth is encouraging as it provides a rationale for developing protocols to influence the size of the endocrine progenitor pool in the pancreas, and generate additional insulin-producing cells.

Beta Cell Physiology and Dysfunction

9. Peter Thompson (University of Manitoba)

Abstract Title

Exploring prosurvival signaling in senescent human beta cells in Type 1 Diabetes

Authors

Nayara Rampazzo Morelli, Gabriel Brawerman, Peter Thompson

Purpose

Recent work has uncovered several mechanisms of beta cell stress and dysfunction that are likely to participate in the progression of Type 1 Diabetes (T1D). Among these mechanisms, beta cell senescence occurs during the presymptomatic stages of T1D as shown from previous studies on mice and human nPOD pancreas. Beta cell senescence can be targeted in nonobese diabetic (NOD) mice using small molecules that preferentially disable BCL-2 family prosurvival signaling and trigger apoptosis, and this approach is sufficient to halt disease progression in this mouse model. However, the mechanisms of prosurvival signaling in senescent human beta cells are not known. Here, we explored candidate effectors of prosurvival signaling in senescent human beta cell models to elucidate their potential as drug targets.

Methods

Studies were performed using human donor islets and EndoC-BH5 human beta cells. Adult donors islets were sourced from the Alberta Diabetes Institute or the Integrated Islet Distribution Program. EndoC-BH5 cells were obtained from Human Cell Design. Senescence was induced using a published protocol and validated at the protein level by western blotting and secretome analysis. We also performed studies using nPOD donor specimens to characterize expression of BCL-2 family proteins during T1D. Human pancreas sections were stained using immunofluorescence and analyzed by confocal imaging and quantified by ImageJ.

Summary of Results

We evaluated potential mechanisms of prosurvival signaling during senescence in human beta cell models, including GDF15 and members of the BCL-2 family. Induction of senescence led to increased BCL-2 family expression and GDF15 at both the mRNA and protein levels in human donor islets and EndoC beta cells. Inhibition of GDF15 and BCL-2 family proteins led to different effects on senescent islet/beta cell viability. Preliminary immunofluorescence and confocal imaging revealed altered expression of BCL-2 family proteins in T1D donor pancreas.

Conclusions

We explored the expression and roles of candidate prosurvival factors during senescence in human beta cell models. Our initial findings suggest that distinct mechanisms may contribute to senescent beta cell survival.

Additional studies will be required to define the contributions of these prosurvival factors and to establish whether any targets can be combined to selectively manipulate senescent beta cell survival in the context of T1D. These studies are ongoing as part of an nPOD supported project.

10. Jason Groegler (University of Colorado Anschutz Medical Campus)

Abstract Title

The role of pH in Cathepsin D mediated HIP formation in humans and mice

Authors

Jason Groegler, Mylinh Dang, Thomas Delong

Purpose

Hybrid insulin peptides (HIPs) are autoantigens with potential implications in the onset of type 1 diabetes. HIPs are formed from the covalent linkage of an insulin peptide fragment to another granular beta cell peptide through a post-translational process. We have identified HIPs in islet samples of both NOD mice and humans along with several HIP-reactive T cells isolated from NOD mice as well as recent onset type 1 diabetic patients. Our lab recently identified cathepsin D as the protease responsible for forming HIPs targeted by pathogenic T cells. Cathepsin D has optimal activity under acidic conditions making the granular environment of the beta cell at pH5.5 a suitable location to achieve HIP formation. Although we consistently detect HIPs by mass spectrometry within islet samples from NOD mice, we detect them with less frequency in human islet samples. We suspect that mouse and human cathepsin D have different pH ranges in which optimal protease activity is observed.

Methods

Human C-peptide underwent incubation in pH buffer spanning pH 2 to pH 8, with the inclusion of either recombinant mouse cathepsin D or recombinant human cathepsin D. Following incubation, the sample were neutralized and cathepsin D was deactivated by adding the aspartic protease inhibitor pepstatin. We previously observed formation of HIPs and cleavage products in these reactions. Following these steps, mass spectrometry was employed to analyze the samples, evaluating the abundance of the relative cathepsin D cleavage product.

Summary of Results

Comparison of human and mouse cathepsin D activity showed that murine cathepsin D had a greater activity at pH 6, whereas HIP formation by recombinant human cathepsin D was significantly diminished under these conditions.

Conclusions

These results could provide an explanation for the difference in the abundance and frequency of HIPs we are able to identify in NOD mouse islets compared to human islets. Our future projects will focus on how stressors may contribute to alteration of the granular pH, and if these changes result in increased cathepsin D activity. Such a mechanism could explain the infrequent detection of human HIPs and suggest that not all beta cells will

contain HIPs and therefore protect them from autoreactive T cells, possibly providing a modification to the Eisenbarth model in which beta cells are degraded in a more stepwise fashion leading to a prolonged disease onset period.

11. Yingfeng Deng (City of Hope)

Abstract Title

Revisiting the ER stress transducer Xbp1: a novel player in beta cell survival and function

Authors

Yunqian Peng, Di Ren, Ling Fu, Chelsea Jang, Yin Wang, Qiutang Xiong, Leah Kebrom, Rodland Del Mundo, Guangyu, Zhang, Zhao V. Wang, Philipp E. Scherer, Yingfeng Deng

Purpose

Endoplasmic reticulum (ER) stress has been implicated in islet beta cell loss in type 1 diabetes, and the mechanism remains elusive. The ER stress transducer X-box binding protein 1 (XBP1) is a major regulator of the unfolded protein response (UPR). In response to ER stress, the mRNA of Xbp1 undergoes a noncanonical splicing catalyzed by Ire1a, which leads to the production of spliced Xbp1 (Xbp1s), a potent transcriptional factor. Pancreatic islet Xbp1s is found reduced in type 1 diabetes. Consistently, the loss-of-function study indicates that Xbp1s is crucial for the maintenance of beta cell identity and repression of beta-to-alpha transdifferentiation in mice. However, sustained expression of Xbp1s is found to cause apoptosis of beta cells. Therefore, it remains elusive whether and how Xbp1s as a UPR transducer can be leveraged to improve beta cell survival in T1D.

Methods

Here we revisited the work about Xbp1s in beta cell and developed an inducible model to dissect the role of Xbp1s in beta cell function and survival.

Summary of Results

Our model allows selective overexpression of Xbp1s in beta cells. We found Xbp1s induces hyperglycemia that is a result of insulin insufficiency. The decrease of insulin production is not associated with increased beta cell death, dedifferentiation, or beta-to-alpha transdifferentiation. In fact, the loss of beta cell mass by Xbp1s is reversible, and the reversal always occurs regardless of the duration of hyperglycemia caused by Xbp1s induction. In summary, we found a direct link of Xbp1 and insulin, suggesting a unique role of Xbp1s in beta cell physiology in addition to its generic role as a UPR transducer

Conclusions

Our findings suggest that Xbp1s is a potent regulator of beta cell function by facilitating the state transitions in beta cell. The interplay between Xbp1s and insulin identified here highlights that targeting Xbp1s could be a novel strategy to improve beta cell survival in T1D.

12. Farooq Syed (Indiana University School of Medicine)

Abstract Title

Targeting the Integrated Stress Response in Type 1 Diabetes: A Pre-Clinical Assessment of Therapeutic Potential

Authors

Farooq Syed, Namratha Shivani Chalasani, Sathya Sree Gudala, Carmella Evans-Molina

Purpose

Multiple lines of evidence suggest a prominent role for type 1 interferons (IFNs), particularly interferon-α (IFNα) and interferon-ɣ (IFNɣ), in T1D pathogenesis. A type I and type II IFN-inducible transcriptional signature is present in PBMCs of children with a high genetic risk of T1D even prior to seroconversion, and IFN-stimulated gene signatures are observed in biopsies of pancreatic islets from individuals with recent-onset T1D. These data suggest that IFNs have effects on both the immune and endocrine compartments, but their impact on β cell responses during T1D evolution remains incompletely characterized.

Methods

Human pancreatic islets obtained from 5 cadaveric donors from the IIDP, or the Alberta Diabetes Institute Islet Core were treated with or without pro-inflammatory cytokines (IFNα or IFNɣ) for 8 and 24 h to mimic early in vivo T1D conditions. Polyribosomal profiling (PRP) and immunoblot were performed to determine the effects of IFNs on global mRNA translation. Nanopore-based direct RNA (native) sequencing was performed to identify the temporal effects of IFNα or IFNɣ on epitranscriptomic modifications at a single nucleotide resolution. These experiments identified a prominent role in activating the integrated stress response (ISR) in the β cell in response to INFs. Next, to determine the in vivo efficacy of ISR inhibition, we utilized two preclinical mouse models of T1D: RIP-LCMV-GP mice were pre-treated with the ISR inhibitor ISRIB for 2 days prior to the LCMV injection (0.5x105 PFU), and NOD mice were treated with ISRIB from 4-6 weeks of age. In both models, vehicle- and drug-treated mice were monitored for diabetes incidence, and immunostaining for dsRNA and single-molecule RNA FISH was performed in pancreatic sections.

Summary of Results

In vitro, treatment of human islets with either IFNα or IFNɣ induced an antiviral response gene signature, increased accumulation of dsRNA, and led to time-dependent activation of the ISR (upregulation of ATF6, BIP, phospho-IRE1α, and phospho-eIF2α). ISR inhibition in human islets decreased cytokine-mediated STAT1/2 phosphorylation. PRP on human islets treated with IFNα or IFNɣ showed a global mRNA translational initiation block, which was reversed by co-treatment with ISRIB. Nanopore-based native long-read RNA sequencing of human islets treated with either IFNα or IFNɣ showed the generation of alternatively cleaved and polyadenylated mRNA transcript variants. Our preclinical in vivo studies showed that treatment with ISRIB reduced diabetes incidence by 70% in RIP-LCMV-GP mice (n=20 vehicle/21 ISRIB; $p<0.001$) and prevented mRNA translation block in islets. Furthermore, NOD mice treated with ISRIB showed improved glucose tolerance and a 48% decrease in diabetes incidence (n=31 vehicle/32 ISRIB; $p=0.046$). Immunostaining of pancreatic sections showed a decrease in the accumulation of cytoplasmic dsRNA in ISRIB-treated mice, and smFISH analysis revealed decreased β cell expression of STAT1 and MX1 in ISRIB-treated RIP-LCMV-GP and NOD pancreas.

Conclusions

Taken together, our studies demonstrate that both IFNα and IFNγ trigger activation of the ISR in β cells, leading to dysregulated global mRNA translation, increased generation of alternatively cleaved and polyadenylated transcript isoforms, and augmented development of T1D. Notably, treatment with ISRIB decreased the activation of ISR gene signatures and prevented the dysregulation of mRNA translation in in vitro and in vivo models of T1D. These data highlight that ISRIB may have potential as a novel disease-modifying therapy in T1D.

13. Denise Drotar (University of Florida)

Abstract Title

Impaired islet function and normal exocrine enzyme secretion in live tissue slices are consistent across the head, body and tail pancreas regions at type 1 diabetes onset

Authors

Denise Drotar, Ana Karen Mojica-Avila, Drew Bloss, Christian Cohrs, Cameron Manson, Amanda Posgai, MacKenzie Williams, Maigan Brusko, Edward Phelps, Clive Wasserfall, Stephan Speier, Mark Atkinson

Purpose

Beyond extensive studies noting an infiltrating T cell-mediated loss of beta-cells within the pancreas, more recent efforts have demonstrated a whole-organ and disease-associated pathology affecting both the endocrine and exocrine compartments in type 1 diabetes (T1D). While histological evidence of regional heterogeneity in endocrine cell composition (e.g., percentage of beta, alpha, delta cells) within the human pancreas is extensive, functional evidence of metabolic variance across pancreatic regions at the tissue level is scarce, especially in humans. Thus, we investigated possible differences in secretory function, as a function of pancreatic region, from endocrine (insulin, glucagon) and exocrine (amylase, lipase, trypsinogen) compartments using live pancreas tissue slice cultures, together with 3D morphometry, in recent-onset T1D, islet autoantibody-positive (AAb+), and no diabetes control (ND) donors.

Methods

Islet and acinar cell secretion was assessed using freshly generated slices obtained from 15 ND, 7 single AAb+ (1AAb+; GAD AAb+ [GADA+] n=6, insulin AAb+ [IAA+] n=1) and 5 recent-onset T1D donors (<1-year duration), acquired through the nPOD program. Kinetics of hormone release and pancreatic enzyme secretion were evaluated in slices obtained from the pancreas head (PH), body (PB) and tail (PT) regions (values averaged across 4-5 slices/region/donor) following stimulation with variable levels of glucose (1mM, 5.5mM, 11.1mM, KCl depolarization) and carbachol (10µM), respectively. Insulin and glucagon were measured from perifusates by ELISA. Pancreatic enzymes were measured by radioimmunoassay (trypsinogen) or ELISA (amylase and lipase). Functional studies were followed by whole slice three-dimensional (3D) morphometric analysis of insulin and glucagon positive volumes of the perifused slices. Statistical comparisons were carried out with one-way ANOVA between the regions within ND, or two-way ANOVA for disease groups and regions.

Summary of Results

Interestingly, our data demonstrate that insulin and glucagon secretion in response to changes in glucose concentrations are comparable in slices obtained from the PH, PB and PT in ND individuals (n=15, age range 4-34 years). Indeed, following functional assays, 3D morphometrical assessment of slices revealed similar endocrine, specifically insulin and glucagon, volumes across the different pancreas regions. As expected, islet density was also comparable across the pancreatic regions, with the majority (70-80%) of the endocrine volume consisting of insulin in all regions assessed from ND donors. Using adjacent slices from these same donors, amylase, lipase and trypsinogen were measured and demonstrated comparable acinar cell secretion

across pancreas regions, both at baseline and upon stimulation with carbachol. Next, we sought to evaluate islet and acinar cell secretion in slices from 1AAb+ and T1D pancreata. Compared to ND donors, our data show significantly reduced glucose-stimulated insulin secretion (i.e., stimulation index) in all regions at T1D onset (PH: p=0.0041, PB: p<0.0001; PT: p=0.0010), and unaltered secretion in 1AAb+. We did not detect appreciable alterations of glucose-mediated glucagon suppression in 1AAb+ or T1D donors, irrespective of the pancreas region. Despite the high degree of inter- and intra-donor variability in acinar cell secretion from tissue slices, there were no significant differences in exocrine enzyme secretion from AAb+ or recent-onset T1D as compared to ND donors, independent of pancreas region. Whole slice tissue 3D morphometry revealed significantly reduced endocrine cell mass, driven by a decrease in insulin volume rather than glucagon, in both 1AAb+ (P=0.0006) and T1D (P=<0.0001) versus ND slices. Performing secretion and morphometry assays on the same slices allowed for the investigation of the residual islet cell function. When normalized to the insulin-positive beta-cell volume, insulin release was still diminished in recent-onset T1D vs. ND slices. Interestingly, despite decreased beta-cell mass in 1AAb+, absolute insulin secretion was preserved, resulting in increased beta-cell mass-normalized insulin secretion capacity. At T1D onset, we observed slightly increased alpha-cell mass-adjusted glucagon secretion in the PT compared to ND PT; however, the difference was not statistically significant (p=0.3484 at 5.5mM baseline step and p=0.4695 at 1mM stimulation step).

Conclusions

This work provides conceptual proof that islet and acinar cell function can be simultaneously studied across the human pancreas using the tissue slice culture platform. Islet and acinar cell secretion are similar among the ND PH, PB, and PT regions. Moreover, insulin secretion is consistently impaired across all pancreas regions at T1D onset. Finally, in situ pancreatic enzyme secretion from acinar cells is unaltered across the three pancreas regions from T1D and 1AAb+ donors. Further studies are needed on pancreas slices from rare pre-T1D donors, defined as having two or more AAb, in order to appreciate the progressive loss of islet cell function across the disease stages. Although no striking inter-regional disparities were observed, this approach may provide insight into possible mechanisms that lead to the lobular heterogeneity of insulitis and beta-cell loss in organ donors with T1D, particularly when used in combination with other current and emerging methodologies, such as 3D live imaging of cellular processes (e.g., calcium dynamics) in islet and acinar cells within the same or neighboring lobules.

14. Dana Avrahami (Hebrew University)

Abstract Title

Good or bad? - Characterization of senescent beta cells in health and disease

Authors

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Purpose

Beta cell senescence is gaining increased interest as a potential pathological cellular state that could contribute to islet inflammation and dysfunction in diabetes. We and others have demonstrated accumulation of beta cells with a senescence-like signature, marked by expression of p16 (CDKN2A), during aging. Elimination of senescent cells in diabetic mice improved glycemic outcome and prevented onset of autoimmune diabetes in NOD mice and improved glycemic control in mouse models of Type 2 Diabetes (T2D), supporting removal of senescent beta cells by senolysis as a therapeutic strategy for both Type 1 Diabetes (T1D) and T2D. However, as of today, cellular senescence in human beta cells has not been defined at the molecular and functional level and it is unknown whether beta cells develop a full senescence phenotype in association with the diabetic state

or whether they acquire the senescence-associated secretory phenotype (SASP), which could negatively affect neighboring islet cells and contribute to islet dysfunction and diabetes pathology. Here, we describe the molecular profile of senescence-like beta cells from non-diabetic and diabetic donors and examine their unique response to metabolic and inflammatory stress.

Methods

To characterize senescence-like beta cells we compared the expression profile of CDKN2A positive (senescence-like) to CDKN2A negative beta cells from scRNAseq data of islets obtained from both non-diabetic (ND) T1D and T2D donors and determined their molecular profile. Importantly, these cells also express higher levels of CDKN1A and antiapoptotic genes such as BCL2L1, supporting our strategy for detecting the senescence-like beta cell population. To determine whether metabolic and inflammatory stress can trigger “stress-induced senescence” in beta cells and if senescence-like beta cells are more “primed” for acquisition of SASP, we cultured ND human islets under metabolic and inflammatory stress conditions, and subjected them to 10X single cell RNA sequencing and secretome analysis by Luminex assay.

Summary of Results

Comparing the expression profile of CDKN2A positive and negative beta cells from ND donors revealed a mature signature accompanied by a cell cycle arrest and stress-related characteristics such as increased ribosomal biogenesis, activation of the DNA damage response, and reduced levels of insulin transcripts. Interestingly, while senescence-like beta cells from ND donors are not enriched for inflammatory response or SASP gene sets, senescence-like beta cells from diabetic donors are enriched for interferon response genes. In addition, the proportion and expression level of CDKN2A among beta cells from T2D donors are elevated suggesting that beta cells from T2D donors undergo “stress-induced senescence”. Exposure of ND islets to metabolic stress conditions demonstrated that senescence-like beta cells are predisposed to stress-induced senescence based on activation of SASP and apoptotic genes and reduced beta cell identity markers. Luminex assays confirmed that islets can be induced to secrete SASP substances under glucolipotoxicity and IL-1 stress conditions, a phenotype that intensified when CDKN2A was overexpressed.

Conclusions

Our study provides a characterization of senescence-like beta cells which accumulate during aging and increase in proportion in T2D. Their expression profile defines these cells as fully differentiated, cell cycle-arrested beta cells, with an enhanced signature of cellular stress and reduced insulin expression. However, while senescence-like beta cells from non-diabetics do not present an enhanced secretory, pro-inflammatory expression signature, in diabetic donors these cells express elevated levels of interferon stimulated genes. Finally, senescence-like beta cells are predisposed to acquire SASP genes and loss of beta cell identity when exposed to stress conditions, which could both deteriorate islet cell health and function.

15. Sandra Ferreira (University of Florida)

Abstract Title

Endogenous islet GABA influences beta cell glucose sensitivity and insulin secretion

Authors

Austin Stis, Edward Phelps

Purpose

Gamma-aminobutyric acid (GABA) is a neurotransmitter synthesized and secreted by pancreatic beta cells; a unique trait shared with inhibitory neurons. The high GABA content in pancreatic beta cells indicates that it may be crucial for pancreatic endocrine function. Furthermore, islets from donors with T1D and T2D have reduced GABA content in the remaining beta cells. Many studies have attempted to elucidate the physiological role of GABA in the islet by applying exogenous GABA or manipulating GABA signaling and biosynthesis. In the central nervous system GABA is the primary inhibitory neurotransmitter. However, it is less clear whether GABA is inhibitory or stimulatory in beta cells. Surprisingly, a beta cell specific knockout that would reveal the role of GABA in the islet has not previously been generated. Here, we generated conditional knockout mice lacking the GABA-synthesizing enzymes GAD65 and GAD67 in pancreatic beta cells (β-Gad1,2-/-). Islets from these mice were used to study the contribution of endogenous islet GABA to Ca2+ signaling and insulin secretion.

Methods

β-Gad1,2-/- and Ins1-Cre control islets were evaluated for glucose stimulated insulin secretion and Ca2+ signaling. Islets were stimulated with GABA and agonists (muscimol and baclofen) and antagonists (SR95531 and CGP55845) of GABAA and GABAB receptors in 8mM glucose. β-Gad1,2-/- and Ins1-Cre control mice were evaluated for glucose and insulin tolerance under low-fat or high-fat diet for 60 days. In addition, Ca2+ responses to GABA were assessed using human islets from donors with type 2 diabetes.

Summary of Results

β-Gad1,2-/- mice have normal glucose tolerance and morphologically normal islets, indicating that GABA is not essential for preservation of beta cell mass. In perifusion analysis, β-Gad1,2-/- islets secreted 1.5-2.0x more insulin than Ins1-Cre during 16 mM glucose stimulation, consistent with endogenous GABA also having an inhibitory effect on beta cells. Mouse beta-cells express GABAAR, a Cl- channel that influences membrane potential, and GABABR, a metabotropic Gi/o-protein coupled receptor. Both receptors are inhibitory in neurons. To understand the mechanism involved in GABA modulation of beta-cells, we evaluated the effect of GABAA and GABAB receptor agonists and antagonists on whole islet cytosolic oscillatory Ca2+ signaling ([Ca2+]i). The GABAAR agonist muscimol inhibited [Ca2+]i in 8 mM glucose and the GABAAR antagonist SR95531 stimulated [Ca2+]i. These results are consistent with GABAAR being inhibitory. Surprisingly, the GABABR agonist baclofen stimulated [Ca2+]i and the GABABR antagonist CGP55845 inhibited [Ca2+]i. These results suggest an unexpected stimulatory effect of GABABR. β-Gad1,2-/- mice had normal glucose tolerance, but we suspected an insulin resistant phenotype could occur under high-fat diet. β-Gad1,2-/- mice on high-fat diet gained more weight than Ins1-Cre mice. Glucose tolerance did not differ between the groups on high-fat diet. However, high-fat diet Ins1-Cre mice presented more insulin resistance than those of β-Gad1,2-/- mice. [Ca2+]i in isolated islets from high-fat diet fed β-Gad1,2-/- mice, but not Ins1-Cre, were unresponsive to glucose and GABA. A similar effect was observed in human T2D islets. These islets also were unresponsive to glucose, and GABA did not affect [Ca2+]i. This is different from the results observed in human non-diabetic islets where GABA reduced [Ca2+]i in high glucose. Together these results indicate that T2D islets, besides having a reduced GABA content shown in prior studies, may lose responsiveness to GABA.

Conclusions

We conclude endogenous GABA in the beta cells is inhibitory and may serve to adjust the gain of glucose-sensitive secretion responses in the islet. In addition, GABAAR and GABABR affect the beta cell function differently. GABAAR had an inhibitory effect, and GABABR had an unexpected stimulatory effect. The stimulatory effect of GABABR requires further confirmation because GABABR is known to be an inhibitory GPCR. The loss of beta cell function in the high fat diet mouse model and human T2D is correlated with a loss of GABA responsiveness in these islets. Loss of islet GABA signaling may initiate as an adaptation to increased insulin demand in early stages of diabetes but, if unresolved, contributes to beta cell dysfunction.

16. Jim Johnson (University of British Columbia)

Abstract Title

Comparative analyses of human islet transcriptomes and proteomes

Authors

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Purpose

Although proteins are more directly involved in cellular functions, transcriptomic profiling has been more widely used to infer molecular mechanisms. It has been debated how well RNA expression reflects the abundance of the corresponding protein, although few studies have examined primary human tissues. Here, we aim to investigate the relationship between the transcriptome and proteome of islets.

Methods

We analyzed the islets of 90 cadaveric donors sampled from the population, including individuals with or without type 2 diabetes. RNA levels were assessed with RNA sequencing, with confirmation of selected transcripts using NanoString. The abundance of proteins was assessed by DIA-PASEF mass-spectroscopy proteomic analysis.

Summary of Results

7588 gene products were reliably detected in both transcriptomics and proteomics. Across the 96 samples, we did not detect significant RNA-protein correlations for 81% the genes, but 19% of the genes have a positive RNA-protein correlation, and 0.5% of the genes have a negative correlation. Interestingly, functional enrichment analyses suggested that the genes with non-significant RNA-protein correlation mostly encode mitochondria-related and ribosomal proteins, and genes with positive RNA-protein correlation are associated with cell surface and secretion machinery. On the other hand, the different proteins and their respective mRNAs have a moderate positive correlation (R=0.5). We also correlated RNA or protein levels with islet handling parameters, donor characteristics, and islet function. The HbA1c levels of the donors correlated with many RNAs or proteins, between which there was a small overlap. When comparing diabetic to healthy donors, gene set enrichment analysis showed that both RNA-seq and proteomics consistently enriched similar pathways, such as upregulated immune-related pathways and downregulated beta cell-related gene set.

Conclusions

In conclusion, for each gene, the variation of its RNA may not always reflect the changes of the corresponding proteins in islets, as seen from the RNA-protein correlation across samples. Transcriptome-wide RNA expressions of different genes are a moderate indicator of the relative protein levels. Both transcriptomic and proteomic changes are informative to identify altered pathways diabetic islets. These comparative analyses call for careful interpretation of transcriptomic and proteomic results and provide novel insights into islet biology on the RNA and protein levels.

17. Chaitra Rao (Indiana School of Medicine)

Abstract Title

Extracellular Vesicle PD-L1: From Cellular Dynamics to Clinical Implications in Type 1 Diabetes

Authors

Chaitra Rao, Fei Huang, Matthew B. Johnson, Zhengjie Zhou, Jennifer Nelson, Charanya Muralidharan, Xiaoyan Yi, Soumyadeep Sarkar, Bobbie-Jo Webb-Robertson, Ernesto Nakayasu, Decio L. Eizirik, Carmella Evans-Molina, Sarah May, Sarah Tersey, Yun Fang, Richard Oram, Raghavendra G. Mirmira, Emily K. Sims

Purpose

The nature of intercellular crosstalk between β cells and immune cells is a pivotal early determinant in the progression or not of type 1 diabetes (T1D). For example, surviving β cells in T1D respond to inflammation by upregulating programmed death-ligand 1 (PD-L1) that engages immune cell programmed death-1 (PD-1) to limit β cell destruction by immune cells. Extracellular vesicles (EVs) and their cargo contribute to islet intercellular communication but β cell EV PD-L1 has never been described. We hypothesized that the inflammatory milieu of T1D increases PD-L1 in β cell EV cargo and that EV PD-L1 may protect β cells against immune-mediated cell death. In this study we sought to identify the molecular determinants of PD-L1 production and extracellular vesicle shuttling in human β cells.

Methods

EV PD-L1 was analyzed in EVs emanating from EndoC-βH1 human β cells and human islets by ultracentrifugation followed by immunoblotting, flow cytometry and by Exoview®. To mimic the microenvironment of early T1D in vitro, cells were treated with IFN-α (2000 U/ml) for 24 h. The capacity of EV PD-L1 to bind PD-1 was tested in decreasing concentrations of EndoC-βH1 EVs using a PD-1/PD-L1 competitive binding assay. mRNA encoding either wild-type PD-L1 or a homozygous PD-L1 variant resulting in an in-frame deletion associated with neonatal T1D (c.682+1G>A, p.Gly177_Pro227del) was transduced in EndoC-βH1 cells using proprietary lipid nanoparticles, with Exoquick® isolation of EVs. Plasma EV PD-L1 was assayed in children with recent-onset T1D vs. age, sex, and BMI-matched nondiabetic controls.

Summary of Results

PD-L1 protein colocalized with EV-associated proteins intracellularly and was detected in β-cell EVs. IFN-α treatment of EndoC-βH1 cells and human islets yielded a 2-fold upregulation of EV PD-L1, without changing total EV concentration. Flow cytometry indicated that PD-L1 is present on the surface of EVs, and EV PD-L1 dose-dependently bound to PD-1. Plasma EV PD-L1 levels were similar in children with recent-onset T1D compared to controls. However, plasma EV PD-L1 positively correlated with circulating C-peptide in children with T1D but not controls. Finally, we studied the cell biology of a homozygous PD-L1 variant (PD-L1v) causing neonatal T1D and identified that PD-L1v transduced EndoC-βH1 exhibited: (a) decreased overall expression compared to PD-L1; (b) an intracellular pattern of distribution with reduced cell membrane localization; (c) significantly impaired interaction with PD-L1; and (d) reduced deposition into EVs.

Conclusions

Our data indicate that PD-L1, a pro-tolerogenic protein, is expressed in β cell EVs in response to IFN exposure. PD-L1 EV cargo has the capacity to bind to PD-1, suggesting potential for a functional interaction with PD-1 on immune cells. EV PD-L1 levels correlate with residual beta cell function in children with recent-onset type 1 diabetes, whereas a human deletion variant (PD-L1v) exhibits reduced production, altered intracellular trafficking and reduced sorting to EVs, potentially promoting the early development of T1D in the individuals harboring this variant. Collectively, the present data provide new insight into the molecular mechanisms by which β cells generate a protective response against autoimmunity in T1D and suggest that EV PD-L1 could be exploited to inhibit immune-mediated beta cell death.

18. Etienne Larger (Université Paris Cité)

Abstract Title

Persistence of beta cells secreting proinsulin in long-standing patients with minimal residual insulin secretion

Authors

Fideline Bonnet Serrano, Sylvie Daclin, Christelle Laguillier-Morizot, Corinne Zientek, Marie-Clémence Leguy, Roberto Mallone, Jean Guibourdenche, Etienne Larger

Purpose

In all forms of diabetes, beta cell failure is accompanied by a disproportionate secretion of proinsulin, a marker of endoplasmic reticulum stress. Based on histopathological studies of the pancreas, it has been suggested that proinsulin may become the main secretory product due to defective expression of prohormone convertases. We analyzed meal-stimulated proinsulin secretion in patients with minimal residual C-peptide

Methods

We included 44 patients who received a test meal (carbohydrates 60 g; lipids 5.5 g; proteins 10 g) with poorly stimulable C-peptide (peak <0.3 nM). The C-peptide (Liaison, Diasorin®, LOQ 0.01 nM) and total proinsulin assays (ELISA, Mercodia®, LOQ 0.5 pM) were carried out at T0, T60 and T120 min. C-peptide and proinsulin responses were assessed by the peak value and its ratio to the baseline value (peak/basal). Results were expressed as median [interquartile].

Summary of Results

The majority of patients included presented T1D (n=37); other etiologies were undetermined (n=4), MODY (ABBC8, HNF1B; n=2) and T2D (n=1); duration of diabetes 18 [8-29] years; GFR 105 [92-114] ml/min; HbA1c 7.6 [6.5-8.6]%; all were insulin-treated, 0.6 [0.47-0.79] U/kg/day. Peak C-peptide was <0.3 nM (0.03 [0.01-0.12]), peak/base ratio 1.8 [1-3]. Peak proinsulin was 0.8 pM (0.5-1.75); 26 had undetectable basal proinsulin (<0.5 pM; n=7) and/or poorly stimulated proinsulin (peak/basal ratio <2; n=19); 18 patients had significant proinsulin secretion (peak/basal ratio ≥ 2), i.e. 4.7 [3.1-6.6].

Conclusions

The presence of stimulated proinsulin secretion in long-standing T1D patients with minimal residual insulin secretion suggests the persistence of dysfunctional yet meal-responsive beta cells

19. Ben Giepmans (UMC Groningen)

Abstract Title

Novel in vivo zebrafish model and beta cell reporters to address whether exocrine malfunction leads to beta cell stress

Authors

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

Purpose

The trigger for autoimmune-mediated destruction of pancreatic beta cells that leads to T1D is not known. Recently it has become evident that in several situations beta cells show signs of chronic ER stress and activation of apoptotic and inflammatory pathways before the onset of T1D. However, also this trigger of this pre-symptomatic beta cell stress is unknown. Recent studies also suggest a possible involvement of exocrine tissue in the onset of T1D pathology based on a decrease in total pancreatic weight in T1D patients and pre-symptomatic donors, the presence of inflammatory cells in the exocrine pancreas and the presence of aberrant exocrine/endocrine cells.

We hypothesize that exocrine malfunction may induce beta cell ER stress, thereby contributing to beta cell dysfunction and T1D pathology. This possible cause-consequence relationship cannot directly be assessed in human pancreas and therefore we set up a zebrafish larvae model to test this hypothesis in vivo.

Methods

Exocrine malfunction can be induced in transgene fish using the nitroreductase/nifurpirinol system. In parallel, we express fluorescent ER stress and apoptosis reporters in beta cells, leading to a functional in vivo readout.

Summary of Results

Only recently we have fish present with both the inducer and the reporter expressed. In the first set of experiments the stress reporter in the beta cell lights up when we induce damage in the exocrine pancreas! Currently, larger groups of larvae and refinement of experiments and additional readouts are performed.

20. Elena Aiello (University of Siena)

Abstract Title

microRNAs as in-situ and circulating biomarkers of beta-cell glucose sensitivity and functional impairment in the progression of Type 2 Diabetes

Authors

Elena Aiello, Stefano Auddino, Daniela Fignani, Gianfranco Di Giuseppe, Eleonara Di Piazza, Laura Soldovieri, Roberto Bizzotto, Andrea Giaccari, Teresa Mezza, Guido Sebastiani, Francesco Dotta

Purpose

Type 2 Diabetes (T2D) is a complex disease due to multiple pathogenic mechanisms. At present, there is no relevant marker of progression of β-cell dysfunction. MiRNAs are involved in the control of multiple β-cell functions and may represent optimal circulating biomarkers. The aim of this study is to investigate miRNAs expression in human islets and plasma samples of fully metabolically characterized living donors stratified based on β-cell glucose sensitivity and rate sensitivity (related to first-phase insulin secretion), in order to identify in-situ and/or circulating miRNAs biomarkers of β-cell dysfunction.

Methods

Patients scheduled for partial pancreatectomy were recruited and subjected to OGTT, and hyperinsulinemic euglycemic clamp. Metabolic parameters were obtained including β-cell Glucose Sensitivity (GS) and Rate Sensitivity (RS). Based on GS and RS, three clinically relevant groups of patients were identified: group1, functional islets (GS>55; RS>300); group2, partially dysfunctional islets (GS>55; RS<300); group3, severely dysfunctional islets (GS<55; RS<300). Each patient underwent pancreatic biopsy and plasma collection. RNA was extracted from LCM-isolated islets (group1, n=16; group2, n=10; group3, n=8) and from plasma samples (group1, n=19; group2, n=19; group3, n=30). Small RNA Seq was performed and data analysed using sRNAbench. Differential Expression Analysis (DEA) among groups (padj<0.05) was performed using DESeq2. Linear Regression was carried out between miRNAs and metabolic/clinical data (p<0.01). miRNAs target gene prediction analysis was performed with TargetScanHuman7.2. EndoC-betaH1 cell line was transfected with miR-148a-3p mimic, RNA was extracted and mRNA levels of ATP6AP2 gene were evaluated through Taqman qRT Real-time PCR.

Summary of Results

In pancreatic islets, 6 miRNAs resulted DE. miR-129-5p, -25-3p, -28-3p and -148a-3p were significantly upregulated in severely dysfunctional islets. MiR-126-5p and -216a-5p resulted respectively down- and up-regulated in severely dysfunctional islets compared to intermediate dysfunctional ones. The regression analysis highlighted several miRNAs significantly associated with GS and RS and among all miR-148a-3p resulted negatively associated with GS and positively with mean glycaemia.
Target gene analysis revealed ATP6AP2 as predicted target of miR-148a-3p. This gene encodes for ATPase H+ transporting accessory protein 2, also known as (pro)renin receptor, involved in lysosomal degradative functions and autophagy. In β-cell, ATP6AP2 regulates crinophagy and insulin granules turnover. From functional analysis in EndoC-βH1 cell line, miR-148a-3p overexpression led to a significant decrease (p<0.05) of mRNA ATP6AP2 expression in EndoC-βH1 transfected with miR-148a-3p mimic vs CTR, suggesting that this miRNA could directly or indirectly modulate this predicted target gene.

In plasma samples, miR-30e-5p resulted downregulated in subjects with severely dysfunctional islets compared to those having functional ones. Regression analysis showed that miR-30e-5p is negatively correlated with fasting glycemia and positively with GS.

Conclusions

In-situ miR-148a-3p and circulating miR-30e-5p resulted DE in subjects with poor β-cell function and significantly correlated with its main parameters. In pancreatic islets, miR-148a-3p negatively regulated ATP6AP2 gene, a critical player in insulin granules homeostasis and secretion suggesting that ATP6AP2 reduction could lead to beta cell dysfunction and diabetes. However, the role of miR-148a-3p and miR-30e-5p should be further investigated to understand the mechanisms leading to β-cell dysfunction and to verify their potential as biomarkers to identify subjects at risk of rapid clinical decline and personalize intervention.

21. Elizabeth Butterworth (University of Florida)

Abstract Title

Pancreatic Endocrine Cell-Specific Iron Metabolism Linked to Innate Immunity

Authors

Elizabeth BUtterworth, Heather Kates, Dongtao Fu, Jing Chen, Wei-Jun Qian, Clayton Matthews, Martha Campbell-Thompson

Purpose

Our research goal is to characterize iron metabolism in human beta cells. During oxidative phosphorylation, iron acts as an electron carrier and is essential for all organisms. Complex organisms sequester iron during infection to starve invading microbes as a form of innate immunity. Pancreatic beta cells are highly metabolic and require a high ATP: ADP ratio to secrete insulin. Published research indicates that beta cells express the peptide hormone hepcidin (HAMP), a systemic iron regulator important for the sequestration of iron during infection. Furthermore, there is evidence that viral infection may be a triggering event for the onset of type 1 diabetes (T1D). Despite long-standing evidence that beta cells uniquely regulate iron metabolism, and the importance of iron to beta cell function, data regarding iron handling in human beta cells is scarce.

Methods

Four pancreases from non-diabetic organ donors were obtained and sectioned during the Human BioMolecular Atlas Program (HuBMAP). Tissues were formaldehyde-fixed, paraffin-embedded (FFPE), and sectioned onto slides. Four sections across each pancreas were analyzed using the Nanostring GeoMx platform to obtain whole transcriptomes for select cellular compartments. The GeoMx uses a library of photo-releasable barcoded RNA probes. Probe collection by UV light is guided by segmentation of immunofluorescence (IF) staining of insulin (beta cells), pan-cytokeratin (ductal cells), and combined CD31 and CD34 (endothelial cells). Compartment-specific barcodes were collected and quantified by Illumina sequencing. Differentially expressed genes (DEGs) were confirmed at the protein level via IF. Pancreas sections obtained from the Network for Pancreatic Organ Donors with Diabetes (nPOD) were used to define expression of iron importers (TfRC and ZIP14), exporter (FPN), storage (FTH1, FTL), and regulators (HAMP, ACO1). Confocal images of >10 islets per section were obtained. Markers were segmented by thresholding to measure mean fluorescence intensity using a Fiji custom script.

Summary of Results

There were several iron-related DEGs between the insulin compartment versus other compartments by GeoMx. These include the lipocalin-2 (LCN2) siderophore receptor SLC22A17, iron regulators HAMP and HFE, and PRNP, a ferrireductase that promotes iron uptake. Immunofluorescence revealed that PRNP and SLC22A17 were exclusively to pancreatic endocrine cells while LCN2 was localized to the duct epithelium. Despite mRNA enrichment in beta cells, HFE was co-localized with somatostatin in delta cells. Beta cells were positive for HAMP protein, but HAMP mRNA was significantly less in the insulin compartment versus the other cellular

compartments (p = 0.0499). We determined that beta cells were significantly enriched for FPN (p = 0.0157) and ferritin heavy (FTH1, p = 0.0473) and light chains (FTL, p = 0.0152).

Conclusions

Our data suggest that pancreatic endocrine cells uniquely regulate iron metabolism. Intriguingly, several DEGs have implications for immunity. HAMP is upregulated by interleukin-6 during infection to promote the sequestration of iron in cells by inhibition of FPN. LCN2 competes for iron against bacterial siderophores during infection. LCN2 is also a biomarker for chronic kidney disease and is reported to associate with matrix metalloproteinase 9 to promote tissue remodeling, which is of particular interest in the study of T1D. Further investigation is needed to determine the functional consequences of perturbing these pathways.

22. Christiana Lekka (University of Exeter)

Abstract Title

Islet expression of classical and non-classical HLA-I in Type 1 diabetes - signs of a struggle?

Authors

Christiana Lekka, Jessica Hopkinson, Jessica Hill, Fatoumata Samassa, Javier Perez-Hernandez, Christine Flaxman, Roberto Mallone, Noel Morgan, Sarah Richardson

Purpose

The upregulation of both classical and non-classical HLA class I (HLA-I) molecules in the insulin-containing islets of those diagnosed with Type 1 diabetes (T1D) has been previously reported. However, it is not clear if specific classical HLA-Is (HLA-A/B/C) are differentially regulated under these conditions. Moreover, the role of non-classical HLA-I molecules has not been extensively studied in islet cells in T1D. Here, we explore the expression of classical and non-classical HLA-I in the pancreata of people with and without T1D using multiplex immunofluorescence staining.

Methods

nPOD and Exeter Archival Diabetes Biobank (EADB) pancreas sections were stained with antibodies raised selectively against HLA-A, HLA-B, HLA-E, HLA-F, HLA-G or insulin using the OPAL platform and/ or conventional multiplex IF. Whole slide scans were spectrally unmixed and imported into Indica HALO software for image analysis using the HighPlex module.

Summary of Results

Following careful validation of HLA-A and HLA-B antibodies, immunostaining of pancreas sections confirmed that both HLA-A and HLA-B are upregulated on the insulin-containing islets of individuals with T1D. Interestingly, the elevation of HLA-B expression was significantly higher than that of HLA-A, suggesting a preferential upregulation of HLA-B in subjects with T1D. In accord with previous findings, the expression of HLA-E, HLA-F and HLA-G was minimal in the islets of individuals without diabetes whereas it was upregulated in insulin-containing islets but diminished once more in insulin-deficient islets of those diagnosed with type 1 diabetes. HLA-E was predominantly localised to alpha cells, whereas HLA-F and -G were present both in beta cells and in other endocrine cells. HLA-A and HLA-F expression was localised predominantly to the plasma membrane but this differed by comparison with the distribution of HLA-B, -E and -G-E which appeared predominately cytoplasmic. The expression of HLA-I isoforms was distinctly lobular within insulin-containing islets, such that select islets expressed more HLA-A whereas others expressed more HLA-F.

Conclusions

This study provides important insights into the relative distribution of classical and non-classical HLA-I molecules in the pancreata of individuals with and without diabetes. The differential expression of classical and non-classical HLA-I molecules among the islets of individuals with T1D could indicate differences in the

immediate islet milieu and we suggest that the balance of HLA-I isoform expression may influence the extent and pace of beta cell demise during autoimmune attack.

23. Noel Morgan (University of Exeter)

Abstract Title

Targeting of the lysine deacetylase, HDAC6, in pancreatic beta-cells as a potential means to slow the progression of type 1 diabetes

Authors

Kaiyven Afi Leslie, Christiana Lekka, Sara Richardson, Mark Russell, Noel Morgan

Purpose

Recent evidence has implicated the activation of STAT1 as a critical component of the mechanism by which proinflammatory cytokines, including interferons (IFN)-alpha and gamma, drive beta-cell demise during the development of type 1 diabetes. Activated STAT1 can exert detrimental effects on the beta-cells by promoting the expression of pro-apoptotic genes and it can also increase the visibility of beta-cells to influent autoreactive CD8+ T-cells by virtue of upregulation of MHC class I on the cell surface. Targeting of the mechanisms that promote STAT1 activation may, therefore, be a fruitful means to attenuate these actions and to improve beta-cell viability in the context of type 1 diabetes. Accordingly, trials are underway with JAK Kinase inhibitors (such as Baricitinib) since JAK kinases are central to IFN signal transduction via STAT1. In the present work, we have considered an alternative approach to intervene in this pathway by studying the activity of a cytosolic lysine deacetylase, HDAC6, which controls the acetylation of STAT1 and thereby alters its capacity for activation.

Methods

Experiments were conducted using EndoC-βH1 cells, isolated human islets and sections of fixed human pancreas. Protein expression was monitored by immunocytochemistry, Western blotting, immunoprecipitation of specific target molecules and mass spectrometric analysis of peptide composition. STAT1 reporter activity was assessed by transfection of cells with a luciferase construct under the control of an IFN-responsive promoter. Target gene transcription was also monitored by RT-PCR.

Summary of Results

Immunoprecipitation of STAT1 from human islet cells followed by probing of the immunoprecipitate with an antiserum directed against acetylated lysine residues revealed that the transcription factor is acetylated under control conditions. The extent of STAT1 acetylation was reduced rapidly upon addition of IFNγ to cultured EndoC-βH1 cells (control - 14.46±2.15AU; IFNγ - 1.94±0.13AU; $p<0.01$ by densitometry) and this correlated with enhanced phosphorylation of STAT1 at Tyr701. Inclusion of an inhibitor (BRD9757) selective for the cytosolic lysine-deacetlyase, HDAC6, resulted in enhanced STAT1 acetylation in cells treated with IFNγ (IFNγ + BRD9757: 9.5±2.5AU; $p>0.05$) and this was associated with impaired phosphorylation and attenuated activation of STAT1 (luciferase reporter assay: IFNγ: 15.08±0.84 fold activation; IFNγ + BRD9757 - 7.2±0.4 fold; $p<0.001$). Targeted knockdown of HDAC6 using siRNA approaches also resulted in impaired STAT1 activation by IFNγ when measured either by reporter assay or via the induction of a downstream target gene, Mx1.

To verify the importance of HDAC6 in human islets in situ, its expression was monitored in human pancreas samples recovered from control individuals or those with recent-onset type 1 diabetes. HDAC6 was present in the cytosol of islet cells of subjects without diabetes and was retained in the residual pancreatic beta-cells of people with recent-onset type 1 diabetes; conditions under which STAT1 levels are enhanced.

Conclusions

The present results imply that a previously unrecognised level of regulation occurs in the signalling pathways employed by IFNs to exert their influence in beta-cells. This is achieved by control of the extent of STAT1 acetylation (and, thereby, its state of activation) mediated, at least in part, by alterations in the functional activity of HDAC6. Repurposing of HDAC6 inhibitors might, therefore, represent an alternative (or additional) therapeutic approach to the use of JAK kinase inhibitors as a means to attenuate the rate of beta-cells loss during progression to type 1 diabetes.

24. Stefano Auddino (University of Siena)

Abstract Title

Sequencing of IsomiRs in Human Pancreatic Islets: Associations with Beta-Cell Function and Alterations in Type 1 Diabetes upon Inflammatory Stress

Authors

Stefano Auddino, Elena Aiello, Mattia Toniolli, Giuseppina Emanuela Grieco, Daniela Fignani, Giada Licata, Marco Bruttini, Alessia Mori, Andrea Berteramo, Claudiane Guay, Erika Pedace, Laura Nigi, Roberto Bizzotto, Caterina Formichi, Andrea Giaccari, Teresa Mezza, Romano Regazzi, Francesco Dotta, Guido Sebastiani.

Purpose

MicroRNAs (miRNAs) are key modulators of beta cell gene expression, regulating multiple pathways and functions. During miRNAs maturation, several post-transcriptional modifications could lead to the biogenesis of miRNA sequence variants, known as isomiRs, with distinct sequence and function respect to the canonical sequence. Emerging evidence suggests that the activity and expression of the enzymes involved in isomiRs biogenesis might be subjected to differential regulation upon inflammatory stress. As such, the comprehensive profiling of isomiRs has the potential to gain further insight into the underlying inflammatory processes within beta cells in type 1 diabetes (T1D). Hence, the aim of this study is to characterize the expression profile of isomiRs in human pancreatic islets and to evaluate their potential association with inflammatory stress in T1D.

Methods

IsomiRs expression profile was performed in Laser Capture Microdissected human pancreatic islet (LCM-HI) deriving from n=19 non-diabetic donors subjected to an in vivo full metabolic assessment, and in EndoC-βH1 beta cell line. To evaluate the impact of insulitis and inflammation on isomiR expression and composition, RNA was extracted from collagenase-isolated pancreatic islets from n=3 pre-insulitic (4 weeks) and from n=4 insulitic (8 weeks) NOD mice, and from human islets treated (n=3) or not treated (n=3) for 48h with IL-1β, TNF-α and IFN-γ. Small RNA-seq was performed and isomiRs/canonical miRNAs were quantified with sRNAbench pipeline.

Summary of Results

Expression profiling in LCM-HI from non-diabetic donors and EndoC-βH1 beta cell line revealed that isomiRs contribute for a consistent percentage of total miRNA expression (LCM-HI 59±2%; EndoC-βH1 44±1%), with 3'end trimming representing the most abundant class (LCM-HI 43±3%; EndoC-βH1 24±1%). We identified a signature of n=11 beta cell isomiRs consistently detected in LCM-HI and EndoC-βH1 and significantly enriched in beta-cells over other human tissues, as assessed by the analysis of an external small RNA-seq database (isomiRdb). Moreover, the regression analysis with beta cell functional parameters revealed the association of isomiR-411 with basal and total insulin secretion rate, ($p_{basal\ ISR}$ *<0.01, partial* $R_{basal\ ISR}$*=0.68 ;* $p_{total\ ISR}$ *<0.01, partial* $R_{total\ ISR}$*=0.54*), but not of the canonical counterpart (*$p_{basal\ ISR}$ =0.62, partial $R_{basal\ ISR}$= 0.02; $p_{total\ ISR}$ = 0.52, partial $R_{total\ ISR}$= 0.04*).
The profiling of pancreatic islets from NOD mice revealed a significant increase of 3'end trimmed isomiRs in NOD 8w islets respect to NOD 4w (8w 47±3% vs 4w 38±5%; p<0.01). In addition, differential expression analysis identified n=50 isomiRs differentially expressed (n=25 upregulated; n=25 downregulated; FDR<0.05 & |Log2FoldChange|>1) in NOD8w vs NOD4w islets, independently from the expression of the canonical sequence.

The increase of 3'end isomiRs was observed also in HI treated with pro-inflammatory cytokines (Ctrl 41±4%; Cyt 53±1%; p<0.01), confirming the link between 3'trimming and islets inflammation. Moreover, differential expression analysis revealed n=56 isomiRs differentially expressed (n=39 upregulated; n=17 downregulated) upon cytokines treatment, independently from the expression of the canonical sequence. Interestingly, isomiR-375 (n=3 nt trimmed at the 3'end), but not its canonical counterpart, was upregulated in NOD8w islets vs NOD4w and in HI treated with pro-inflammatory cytokines. Since miR-375 is involved in the regulation of beta cell function and mass, these data could provide insights into the impact of miRNAs 3'end trimming on beta cell function under inflammatory conditions.

Conclusions

In this study we provide (i) a comprehensive profile of isomiRs in human pancreatic islets and β-cells, highlighting their potential contribution in islet function, and (ii) a significant differential composition of isomiRs upon insulitis and human islets inflammation, thus potentially associating miRNA sequence variants biogenesis to pro-inflammatory stresses in T1D.

25. Alexandra Rippa (University of Florida)

Abstract Title

3-Dimensional Analysis of Islet and Endocrine Cell Volume in Type 1 Diabetes

Authors

Alexandra Rippa, Denise Drotar, Amanda Posgai, Seth Currlin, Maigan Brusko, Clive Wasserfall, Irina Kusmartseva, Mark Atkinson

Purpose

Previous studies have noted a loss of beta cells and reduced islet size at the onset of type 1 diabetes (T1D) as well as in those with longstanding disease. Those studies were, however, limited to 2-dimensional (2D) analysis of tissue cross-sections. We sought to readdress these notions using light sheet fluorescent microscopy (LSFM), an emerging and powerful tool that allows for imaging of tissue in three-dimensional (3D) space and enables quantitative measurements in three spatial dimensions of width, height, and depth.

Methods

Formalin-fixed human pancreatic tissues (~0.5cm3) from non-diabetic (ND), GAD autoantibody-positive (GADA), and recent-onset T1D donors (n=3/group) obtained from nPOD were cleared using a modified iDISCO protocol, stained for insulin (INS) and glucagon (GCG), and imaged using the fully automated Miltenyi UltraMicroscope Blaze™. We selected an INS-containing region (1100µm x 1100µm x 1500µm) from each donor (mean total tissue volume per group of 1.4x109mm3) for quantitative analysis using Imaris 10.0. Islets were identified by separate signals of positivity for INS or GCG, and those above a 500 µm2 area threshold were considered. The smallest islets above this threshold had ~25 µm diameter (ellipsoid axis length C ~8.5-13 µm) and 500-900 mm3 volume. The largest islet had a ~ 300 µm diameter (ellipsoid axis length C ~150-175 µm) and 7-8 x106 mm3 volume. Employing these thresholds, we analyzed 721 INS+ islets (91 in the T1D group; 322 in GADA; 308 in ND; T1D vs ND p=0.0016; T1D vs GADA p=0.0008, ND vs GADA p=ns). INS+ and GCG+ volumes were normalized to the non-endocrine tissue volume. Islets were binned by size, (very small [<10^3mm3], small [10^3-104mm3], medium [104-105mm3], large [105-106mm3], and very large [106-107 mm3]) and compared across donor groups. Data were analyzed using GraphPad Prism software and compared by two-way ANOVA or one-way ANOVA with Tukey's test for multiple comparisons, as appropriate.

Summary of Results

Our preliminary data show that T1D donors have a significantly reduced number of INS containing islets (ICI) when compared to ND or GADA groups, especially in the medium (mean value T1D = 2.6 vs ND = 29.0, p=0.01; vs GADA = 31.6, p=0.004) and large (T1D = 5.3 vs ND = 29.0, p=0.02; GADA 26.6, p=0.04) size

bins. Interestingly, we did not find significant differences in very small (T1D = 5.0 vs ND = 3.6; vs GADA = 10.6, p=ns), small (T1D = 13.3 vs ND = 32.0; vs GADA = 28.3, p=ns), and very large ICI (T1D = 6.0 vs ND = 9.0; vs GADA = 10.0, p=ns). The percent volume staining positive for INS and GCG did not differ significantly across groups (p=ns), in part, due to selection of INS containing tissue regions for this analysis. Efforts are currently ongoing to visualize T cells within the human insulitis lesion alongside INS and GCG in a three-color LSFM panel.

Conclusions

3D visualization of human tissue provides a powerful technology for assessing pancreas morphology across the natural history of T1D. Future studies will address possible causes of preferential loss of intermediate-sized islets in T1D, as opposed to very small, small and very large ICI.

Bone Marrow Studies

26. Stephan Ramos (Stanford University)

Abstract Title

Advancing stem cell-based therapies for diabetes through optimized bone marrow conditioning and hematopoietic chimerism

Authors

Stephan Ramos, Preksha Bhagchandani, Diego Burgos, Qizhi Tang, Audrey Parent, Everett Meyer, Seung Kim

Purpose

Type 1 diabetes mellitus (T1D) is an incurable autoimmune disease that results in the destruction of insulin producing β cells and affects nearly 10% of the global population. Islet transplantation offers an attractive and approved alternative to insulin therapy in T1D by replacing β cells. However, the widespread acceptance of this approach faces formidable challenges, including prevention of recurrent autoimmune destruction of transplanted β cells and inducing allogeneic tolerance to donor β cells without systemic immunosuppression. Mixed hematopoietic cell chimerism after hematopoietic cell transplant (HCT) is a viable option to induce tolerance to allogeneic tissues, including pancreatic islets, and correct autoimmunity. However, conditioning protocols to prepare patients for HCT often include the use of toxic drugs and/or high doses of X-ray irradiation (XRT). Reduction or elimination of conditioning XRT could advance HCT and allogeneic islet transplantation as a realistic option for patients with diabetes.

Methods

We have established a non-myeloablative conditioning regime using monoclonal antibody targeting of CD117 and CD4/CD8, to clear the hematopoietic stem cell niche and transiently deplete T cells, combined with 200 cGy XRT, that promotes durable mixed hematopoietic chimerism and allogeneic tolerance to donor-matched tissues. To reduce conditioing XRT, we systematically added clinically relevant conditioning steps including JAK/STAT inhibition with baricitinib and hematopoietic stem cell and immune cell clearance with αCD47. We quantified hematopoietic chimerism across fully major histocompatibility complex (MHC) mis-matched barriers in immunocompetent mice.

Summary of Results

Inclusion of baricitinib to αCD117-based conditioning allowed reduction of XRT to 75 cGy while achieving durable mixed hematopoietic chimerism. A combination of baricitinib and αCD47 to the conditioning regime allowed further reduction of XRT to 25 cGy.

Conclusions

Through systematic evaluation of conditioning agents, we generated mixed hematopoietic chimerism across full major MHC mis-matched barriers in immunocompetent mice with 25 cGy XRT, a significant reduction from

our prior work. Future studies aim to reduce XRT to 0-10 cGy. Clinical adoption of these methods could mitigate unwanted morbidity of allogeneic HCT and islet transplantation and advance this strategy for diabetes reversal.

Immunology

27. James DiLisio (CU Anschutz)

Abstract Title

Hybrid insulin peptide antigen-specific immunotherapy arrests functional effector differentiation of graft-infiltrating autoreactive T cells during islet transplantation

Authors

James E DiLisio, K. Scott Beard, Kaitlin Reyes, Tobias Neef, Stephen D Miller, Rocky L Baker, Kathryn Haskins

Purpose

Autoimmune diabetes is thought to be mediated by T cells that recognize islet-derived self-antigens. Autoreactive CD4 T cells that recognize post-translationally modified neoepitopes, formed by the fusion of insulin fragments to various granular cleavage products and termed hybrid insulin peptides (HIPs), have been found in both T1D patients and NOD mice. Here we examined how tolerance induction with a HIP antigen-specific immunotherapy impacts the functional effector differentiation and regulatory role of antigen-specific CD8 and CD4 T cells during islet transplantation.

Methods

Polyclonal NOD mice were monitored through blood glucose measurements for the onset of overt diabetes (>250mg/dl). Diabetic mice were then treated IV with 2.5mg of tolerogenic PLG-nanoparticles conjugated to the 2.5HIP, a dominant CD4 HIP epitope in NOD mice, before and after syngeneic islet transplant. Graft-infiltrating immune cells were isolated 9 days following transplant from both 2.5HIP-nanoparticle treated mice and control HEL-nanoparticle treated mice. Isolated antigen-specific T cells were identified using MHC-tetramers loaded with the 2.5HIP (class II) and the dominant CD8 specificity in NOD mice IGRP (class I). Tetramer+ cells from the graft were analyzed by flow cytometry and scRNA-seq to measure T cell effector function and differentiation in islet tissue during tolerance induction. Diabetic IL-10GFP-reporter NOD mice were used to examine suppressive subsets of 2.5HIP-specific T cells.

Summary of Results

Transplant recipients receiving 2.5HIP nanoparticles had reduced infiltration of antigen-specific T cells in islet grafts. Both 2.5HIP-and IGRP-specific T cells displayed reduced effector differentiation, indicated by retained TCF1 expression. TCF1- effector cells that differentiated in tolerized mice exhibited dysfunctional cytokine production upon ex vivo stimulation. When investigating regulatory populations, there was a modest increase in the abundance of graft-infiltrating 2.5HIP-specific FOXP3+ CD4 T cells. A significantly larger expansion was observed in IL-10+, CD25- 2.5HIP-specific T cells, thought to be a Tr1-like suppressive subset.

Conclusions

Antigen-specific immunotherapy targeting HIP T cell neoepitopes during islet transplantation arrested functional differentiation of graft-infiltrating antigen-specific CD4 and CD8 T cells. Simultaneously, the therapy expanded antigen-specific regulatory populations of CD4 T cells including IL-10+ Tr1-like cells. These preclinical tolerance studies may help inform how antigen-specific therapies function in humans.

28. Richard Musca (University of Florida)

Abstract Title

Broad Immunophenotyping in nPOD spleen and pLN via spectral flow cytometry

Authors

Richard Musca, Travis Hill, Trevor Rogers, James McNichols, Daniel Perry, Ryan Merritt, Leeana Peters, Maigan Brusko, Todd Brusko

Purpose

The nPOD Immune Core designed and optimized a spectral flow cytometry panel to enumerate a broad range of both common and rare immune cell populations in single cell suspensions from immune tissues, enabling nPOD investigators to select cryopreserved specimens sufficient for analysis of their cell population of interest. These data are collected immediately after tissue processing and visualizations made available through the nPOD portal (https://portal.jdrfnpod.org/explore). Proportional cell subset changes within tissues in subjects with autoantibodies and/or diabetes may reflect disease relevant shifts meriting further investigation via detailed phenotyping.

Methods

Tissue was obtained from 34 total donors (43% female, average age 21.7 (range 4-37.4)).
Splenocyte and pancreatic lymph node (pLN) cell suspensions were generated in the nPOD Immune Core via non-enzymatic dissociation, and one million cell aliquots were stained with a panel consisting of viability dye and 17 antibodies targeting canonical cell subset defining markers spanning both innate and adaptive cell subsets. Cell populations identified include: Neutrophils (CD16+CD15+), eosinophils (CD15+CD16-), basophils (CD123+CD117-), mast cells (CD117+CD123-), T cells (CD3+), B cells (CD19+CD20+), plasma cells (CD19-CD20+), natural killer cells (CD56+CD3-), hematopoietic stem cells (CD34+), dendritic cells (HLA-DR+CD14-CD16-), and monocytes (HLA-DR+CD14/CD16+). A minimum of 100,000 gated live cells were collected per tissue on a spectral flow cytometer (Cytek Aurora). Autofluorescence extraction methods enhanced spectral unmixing in tissue specimens. Cell populations were defined by manual hierarchical gating (FlowJo), semi-supervised automated cell classification (scyan), and unsupervised self-organizing maps (FlowSOM). Analysis of cell subset proportions across subject groups was performed in Graphpad Prism with 2-Way ANOVA and Tukey's multiple comparison testing.

Summary of Results

Cell subset proportions were enumerated in both pancreatic lymph nodes and spleen, reported here as (population mean frequency (% of total live cells)): in spleen [B cells (36.9%), T cells (28.5%), Neutrophils (10.3%), Natural killer cells (5.4%), Eosinophils (2.9%), Monocytes (2.9%), Plasma cells (2.8%), Dendritic cells (0.9%), Hematopoietic stem cells (0.09%), Basophils (0.03%), Mast cells (0.02%)], and in pLN [T cells

(62.6%), B cells (27.8%), Natural killer cells (0.95%), Plasma cells (0.8%), Neutrophils (0.6%), Dendritic cells (0.4%), Monocytes (0.26%), Eosinophils (0.12%), Hematopoietic stem cells (0.04%), Mast cells (0.04%), Basophils (0.01%)].

The above immunophenotyping data was collected from donors across four subject groups (n, mean age in years (age range); type 1 diabetes (T1D) (n=6, 12.93 (11.95-25.06)), autoantibody positive (n=6, 23.46 (20.00-29.77)), no disease (ND)(n=13, 21.22 (13.21-37.38)), and pending (n=10, 19.18 (4.06-29.3)).

Multiple comparison tests revealed a statistically significant increase in the frequency of B cells present in single AAb+ donor splenocytes compared with ND (p=0.0002), and with T1D splenocytes when compared to AAb+ (p=0.0459). B cells were also found to be significantly elevated in pLN lymphocytes of T1D subjects as compared to ND subjects (p=0.029). Additionally, a significant increase in eosinophils in AAb+ splenocytes compared to ND (p=0.0218), with a similar trend in Aab+ subjects compared to T1D (p=0.0753).

Conclusions

Proportional shifts in total B cells within both spleen and lymph nodes in both autoantibody positive and T1D donors likely reflect preferential expansion of distinct B cell subsets.
Eosinophilia has been noted in peripheral blood and in the pancreas in T1D. Their increased presence in autoantibody positive donor splenocytes may indicate a role in disease pathogenesis, or as a signal of ongoing inflammatory processes.
These data provide the nPOD community with access to a broad array of immunophenotyping data, enabling comparisons with biobanked materials, and provide insights for future efforts to discern the roles of innate and adaptive subsets in the pathogenesis of T1D.

29. Melanie Shapiro (University of Florida)

Abstract Title

Human Leukocyte Antigen Risk Loci for Type 1 Diabetes Associate with T Cell Receptor Motif in Peripheral Blood and Pancreatic Lymph Nodes

Authors

Melanie Shapiro, Puneet Rawat, Michael Widrich, Keshav Motwani, Daniel Perry, Milena Pavlović, Leeana Peters, Amanda Posgai, Michael Haller, Desmond Schatz, Mark Atkinson, Clive Wasserfall, Geir Sandve, Victor Greiff, Todd Brusko

Purpose

Despite significant progress in defining genetic loci that contribute to type 1 diabetes (T1D) risk, via genome-wide association studies (GWAS), our understanding of the impact for such variants on immune function remains limited. Human leukocyte antigen (HLA) haplotypes are known to alter thymocyte development and peripheral immune tolerance, suggesting that T1D risk HLA may alter the T cell receptor (TCR) repertoire allowing for autoreactive TCR persistence in genetically-predisposed individuals.

Methods

We performed deep TCRβ sequencing on peripheral blood (PB) samples and microarray-based precision medicine genotyping of 793 unrelated living participants from the University of Florida Diabetes Institute (UFDI) biobank (no-diabetes [ND], n=502; islet autoantibody positive [AAb+], n=56; T1D, n=235). A machine learning algorithm, Deep Repertoire Classification (DeepRC), was used to identify a TCR motif significantly enriched in the PB of T1D subjects. Genetic ancestry was inferred using Admixture software for projection analysis on the 1000Genomes reference. A continuous T1D genetic risk score (GRS2) was calculated to determine associations with the frequency of an identified TCR motif. Quantitative trait loci (QTL) analysis was performed to detect associations between TCR motif frequency versus 240 T1D risk variants. Observations in PB were tested in TCRβ-sequenced sorted conventional CD4+ T cells (Tconv), regulatory CD4+ T cells (Treg), and CD8+ T cells from pancreatic lymph nodes (pLN) as well as spleen from 75 nPOD donors (ND, n=29; islet AAb+, n=4; T1D, n=37; T2D, n=3; other diabetes, n=2) in order to understand matters of cell type and tissue specificity.

Summary of Results

GRS2 was significantly associated with the TCR prediction motif frequency in PB of European ancestry ND individuals (r=0.329, p<0.0001) and, to a lower magnitude, in persons with T1D (r=0.133, p=0.048). HLA-DRB1*0301-DQA1*05:01-DQB1*02:01 (p=1.62e-11), HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 (p=1.43e-9), HLA-DQA1*05:05-DQB1*03:01 (p=5.03e-6), and HLA-DQA1*03:0X-DQB1*03:01 (p=2.31e-5) haplotypes were significantly associated with a TCR motif frequency independent of disease status such that risk haplotype correlated with an increased motif score. A T1D-associated variant tagging the XL9 super enhancer (p=9.41e-11), known to regulate HLA-DRB1 and HLA-DQA1 expression, and an intergenic HLA-DRA1-DRB1 variant (p=4.82e-5) were likewise associated with a PB motif score. Of these findings, the protective allele of the XL9 variant (p=5.88e-3) and HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 (p=4.46e-3) were significantly associated with decreased TCR motif frequency in pLN CD8+ T cells.

Conclusions

T1D HLA risk genetics may confer selection pressure for a TCRβ motif enriched in PB and CD8+ T cells in pLN of individuals with T1D. Efforts to understand the antigenic specificity of TCR containing an enriched motif are in progress. We propose that the TCR motif score may complement existing biomarkers to track disease progression in individuals at-risk for T1D.

30. Tim Tree (Kings College London)

Abstract Title

Single Cell Profile of Islet-specific Responses in Type 1 Diabetes

Authors

Tim Tree

Purpose

The argument for a role for T cells in type 1 diabetes is compelling. T cells dominate the insulitis; selected HLA gene polymorphisms confer the major genetic risk; and anti-T cell therapies have shown success in halting disease extension. HLA class II genes confer the highest disease risk of any gene polymorphism; this strongly implicates CD4 T cells as disease initiators/drivers, because of their requirement for HLA class II molecules for antigen presentation. However, whereas islet-specific T cells can be detected in the peripheral blood of most individuals regardless of disease status, detailed phenotypic studies have suggested subtle differences between the phenotype of these cells in those with and without T1D but detailed unbiased profiling

of these cells remains challenging. Development of assays that can enumerate and characterise both islet-specific effector (Teff) and Treg cells would provide powerful analysis tools which could be employed within INNODIA to increase our understanding at each stage disease: from initial loss of tolerance, progression to clinical disease and the rate of beta-cell loss following diagnosis, as well as providing valuable insights into the efficacy and specificity of immunotherapies.

Methods

We have developed an activation-induced marker (AIM) assay for the detection, isolation and phenotypic characterisation of antigen-specific CD4 T cells incorporating unbiased single-cell transcriptional profiling, detailed cell surface phenotyping by CITE-Seq and TCR use via full-length paired TCRA/B sequencing using the 10x Genomics platform. In theory, this assay makes no assumption about the phenotype of cells isolated and can identify cells with a variety of effector and regulatory functions. Using this technology, we characterised islet-specific (cells responsive to proinsulin or a pool of proinsulin and IA-2 peptides) and compared this to vaccine-specific CD4 T cells from individuals with 24 individuals with new-onset T1D (<100d from diagnosis) and compared this to cells isolated from 12 age and sex-matched individuals with a family history of T1D but with no evidence of islet autoimmunity (islet AAB-ve).

Summary of Results

Single-cell transcriptional analysis revealed islet-specific T cells displayed a distinct transcriptional profile compared to vaccine-responsive T cells in both T1D and AAB-ve groups including an increase in populations of FOXP3 Tregs and cells with a cytolytic profile consistent with a regulatory phenotype. Unbiased clustering analysis of islet-specific T cells revealed cells with a variety of effector and regulatory functional profiles in both T1D and AAB- cohorts.

Comparison of islet-specific responses between cohorts revealed individuals with T1D displayed a significant expansion of cells with a highly proinflammatory phenotype (in particular clusters characterised by expression of IIL-17 family cytokines) whereas individuals in the AAB- cohort displayed an increased number of cells characterised by markers of immune regulation including subpopulations of FoxP3 Tregs.

Analysis of islet-specific T cells from individuals who developed T1D at different ages (range 2-18y) revealed distinct transcriptional profiles were associated with younger and older ages of diagnosis including a higher expression of transcripts associated with a highly proinflammatory phenotype (IL21, TNF, IFNG) in those diagnosed young and a more regulatory phenotype in those diagnosed later (FOXP3, IL32).

Finally, we observed distinct clonotypic expansions in individuals with and without T1D and were often restricted by the phenotype of responding cell. Re-expression of these paired TCR A and B genes is ongoing and will determine if structural differences in TCR contribute to the phenotype of responding cells.

Conclusions

In conclusion, islet-specific T cells display a distinct phenotypic profile which differs significantly in those with and without T1D and are related to the age of disease onset. Ongoing analysis seeks to link different profiles of islet-specific immunity with disease development.

31. Leeana Peters (University of Florida)

Abstract Title

The type 1 diabetes-associated BACH2 risk variant rs729298038 restricts T cell receptor clonal diversity in CD4+ regulatory T cells and drives CD8+ effector phenotype in pancreatic lymph nodes

Authors

Leeana Peters, Maigan Brusko, Richard Musca, Travis Hill, Melanie Shapiro, Todd Brusko

Purpose

Genome-wide association studies have implicated multiple genome wide loci conferring risk for type 1 diabetes (T1D) development, with notable enrichments in immune-related genes. Despite the identification of candidate genes, there remain considerable knowledge gaps regarding the impact of T1D risk loci on immune cell phenotype and function. Of note, the candidate gene BACH2 has been putatively identified as a transcription factor containing a single nucleotide polymorphism (SNP) conferring risk for T1D (rs729298038G>A; OR=1.18; MAF=0.18). The risk variant has previously been linked to reduced enhancer accessibility and expression of BACH2 in naïve human T cells, while naïve CD8+ T cells derived from a mouse model possessing an orthologous mutation displayed reduced features of stemness and enhanced effector phenotype. Interestingly, Bach2-/- mouse models have shown impacts on both central and peripheral T cell phenotypic alterations, with reduced thymic Treg frequency and increased peripheral T cell activation observed. There is currently a lack of data regarding the impact of this locus on central and peripheral T cell phenotypes and function in humans with and at risk for T1D.

Methods

We sought to examine the impact of BACH2 risk on both peripheral tissue cellular phenotypes and thymic output from organ donors derived from the HANDEL and nPOD tissue programs. We employed high-parameter mass cytometry (n=12 control (mean age 16.9; 33% female), n=10 T1D, mean age 19.3, 30% female) and flow cytometry as a validation method (n=18 control, n=6 T1D) to examine immune cell phenotypes derived from pancreatic lymph nodes (pLN). We utilized a custom Affymetrix array for genotyping organ donor samples which provides coverage of genotypes at 985,971 unique loci. Additionally, we assessed T cell receptor excision circle (TREC) levels in splenic naïve T cells from age-matched pediatric donors via qPCR as an established surrogate marker of thymic output. Lastly, we assessed T cell receptor (TCR, TRB) repertoire clonality by comparing Shannon entropy within sorted pLN CD8+ T cell and Treg subsets.

Summary of Results

When segregated by BACH2 rs729298038 genotype, heterozygous (AG) donors possessed a greater frequency of CD8+ T cells co-expressing effector T cell associated chemokine receptors CXCR3 and CXCR5 as compared to homozygous protective (GG) donors ($p=0.0165$). In support of a shift toward a Tc1 effector phenotype, pseudotime analysis of mass cytometry data indicated subjects carrying risk at BACH2 possessed higher mean CD8+ T cell pseudotime values, an indicator of more terminally differentiated effector T cells, as compared to homozygous protective donors ($p=0.0362$). Moreover, bulk TCR (TRB) repertoire analysis revealed increased receptor clonality in sorted pLN CD8+ T cells ($p=0.0340$) and Tregs ($p<0.0001$) from heterozygous donors. In addition to alterations in peripheral tissues, we also examined alterations in thymic output by assessing TRECs within splenic naïve T cells. We observed a trend ($p=0.08$) towards increased thymic output in the form of TRECs in heterozygous donors relative to homozygous protective donors.

Conclusions

These data support a role for the T1D-associated risk allele of BACH2 in controlling both central and effector profiles of CD8+ T cells and Tregs in organ donor tissues. Ongoing efforts involve corroborating these findings in isogenic systems by gene-targeted deletion and SNP-editing in primary human autoreactive T cells. These findings derived from peripheral tissues in organ donors provide novel insights on how genetic risk variants alter immune regulation in the context of T1D and may offer novel opportunities for therapeutic targeting of this pathway to restore immune tolerance.

32. Miguel Medina-Serpas (University of Florida)

Abstract Title

Spatial transcriptomic profilling and cell type identification of human donor paired pancreas and pancreatic lymph nodes reveals shared and unique tissue specific immune responses at distinct stages of T1D.

Authors

Miguel Medina-Serpas, Gregory Golden, Maigan Brusko, Trevor Rogers, Richard Musca, Michael Betts, Klaus Kaestner, Ali Naji, Mark Atkinson, Todd Brusko

Purpose

Spatial transcriptomic technologies enable in situ whole transcriptome detection thereby preserving biologically relevant signals typically lost in disaggregated single cell approaches – rare cells, stromal cell populations, and cell:cell interactions. This is advantageous in studying the histopathology of type 1 diabetes (T1D), where both immune and pancreatic cells have been implicated in initiation and progression of insulitis, and where striking islet and lobular heterogeneity has been described within the pancreas of T1D organ donors. To investigate key features of disease pathogenesis, we acquired formalin-fixed paraffin-embedded (FFPE) sections from paired pancreas and pancreatic lymph node (pLN) of 16 total donors (Mean Age: 20.25 y.o.[(SD: +/- 6.12)(Range: 10 – 31 y.o.)], 37.5% Female) and employed the Visium Spatial Gene Expression Array (10x Genomics). Resultant spatial transcriptome data was linked with annotated single cell RNAseq data from matched tissues and individuals to bolster cell type annotation.

Methods

We analyzed a cross-sectional cohort comprised of non-diabetic controls (n = 6), at-risk subjects presenting with a single (n = 3) or multiple (n = 2) autoantibodies (AABs), and T1D (n = 6) subjects with variable disease duration (mean+SD: 4.67 yr +3.20 yr). The Visium Spatial Gene Expression Array contains defined capture areas (6.5mm x 6.5mm or 11.5mm x 11.5mm capture areas), composed of a grid of 55µm diameter capture spots containing a probe-based amplification system . Spatial gene expression libraries were pooled at equimolar ratios and sequenced at minimum to 25,000 reads/ capture spot according to manufacturer protocol. Low quality capture spots were identified and removed based on total number of reads, total number of detected genes, and <10% mitochondrial gene content. Gene counts were normalized and scaled for interdonor differential expression analysis. Visium capture spots are. To overcome this technical limitation, in silico cell deconvolution was performed using a single-cell resolution reference, and tissue functional regions (e.g. islets, follicles) were annotated.

Summary of Results

In silico cell deconvolution of pancreas spatial data revealed an immune cell signature within at-risk and T1D donors compared to non-diabetic controls, with greater enrichment detected among donors that present with multiple AABs and/or closer to disease onset. Furthermore, we identified increased inflammatory chemokine expression (CXCL12, CCL2) (LogFC $\geq$ 0.25), with significant enrichment of CCL2 ($p = 0.0245$, Wilcoxon rank sum test) in the pancreatic islets of the aforementioned subject groups. Differential expression analysis of inflammation-associated genes revealed significant increases in TNFRSF1A, TNFAIP3, and IFNGR2 expression ($p \leq 0.05$, Wilcoxon rank sum test) in the pancreatic islets, with parallel increases observed in downstream signaling gene networks. Finally, preliminary analysis of paired pLN data via cell deconvolution has revealed enrichment of cell-type specific gene expression programs at specific stages of T1D. Notably, we identified relative enrichment of Tcm/Treg GEX programs in non-diabetic controls, as well as effector CD8+ T-cell programs in at-risk and T1D subjects. Differential expression analysis of pLN revealed upregulation of various chemotactic (CXCL13, CCL19, CCR7) (LogFC $\geq$ 0.25) and immune modulatory genes (ZAP70, TNFRSF4), and significant upregulation of TXNIP ($p = 0.036$, Wilcoxon rank sum test) within the T-cell zone of the pLN.

Conclusions

Collectively, we demonstrate the potential of emerging technology amd bioinformatic tools to unvocer unique insights in the immune histopathological features of T1D. Application of in silico cell deconvolution to pancreas spatial data identified a distinct immune cell signature is enriched in at-risk and T1D donors presenting with multiple islet-specific autoantibodies relative to non-diabetic controls. We further identified a global and compartment-level inflammatory chemokine signature in donor pancreata which follows a similar pattern of enrichment to the identified bulk immune signature which may represent a non-specific mechanism contributing to pancreatic immune infiltration in T1D. Lastly, we identified cell-type specific GEX programs and disease stage patterns of enrichment within matched donor pLN which we intend to leverage in resolving specific transcriptional phenotypes from the bulk pancreas signature. Ongoing work includes validating ST signature genes and pathways in serially prepared tissue sections using high parameter single cell resolution probe and protein staining technologies which we expect will provide novel leads for new therapeutic targets for therapeutic interventions in T1D.

33. Miranda Chávez (University of Kansas Medical Center)

Abstract Title

Metabolic Blockade of Glycolysis Diminishes Autoreactive CD4 T cell Differentiation in Type 1 Diabetes

Authors

Miranda Chávez, Hubert Tse

Purpose

Type 1 diabetes (T1D) is a result of insulin-secreting pancreatic β-cell destruction by autoreactive CD4 and CD8 T cells. There is no cure for T1D, but determining the role of immunometabolism in promoting autoreactive CD4+ T cell effector responses may provide a novel target in delaying T1D. Proinflammatory Th1 CD4 T cells prefer glycolysis for maintaining effector responses to mediate β-cell destruction, while immunosuppressive Treg cells prefer oxidative phosphorylation. We hypothesize that metabolic inhibition of glycolysis will diminish proinflammatory Th1 CD4 T cell responses and delay β-cell destruction in T1D.

Methods

Systemically diminishing glycolysis with 2-deoxyglucose (2-DG) can decrease autoreactive CD8 T cell islet infiltration. However, the effects of 2-DG on CD4 T cell diabetogenicity is unknown. Utilizing 2-DG, we performed an adoptive transfer of diabetogenic CD4+ T cells from NOD.BDC-2.5 or NOD.BDC-6.9 mice into NOD.scid mice. Recipient mice were systemically treated with 30 mM 2-DG in the drinking water beginning 4 days prior to adoptive transfer and monitored for hyperglycemia for 80 days post-transfer. To define the molecular mechanisms of 2-DG on autoreactive CD4 T cell effector responses, NOD.BDC-6.9 splenocytes were in vitro stimulated with their cognate hybrid insulin peptide (HIP; insulin C-peptide and islet amyloid polypeptide) with or without 2-DG.

Summary of Results

NOD.scid mice treated with 2-DG had a significant delay in diabetes onset when transferred with either BDC-2-5 (n=14, p=0.0284) or BDC-6.9 (n=17, p=0.0249) CD4+ T cells compared to vehicle-treated groups. We wanted to define the molecular mechanism of protection, flow cytometry analysis from our in vitro studies revealed a decrease in T cell proliferation, T cell activation, and Th1 lineage commitment as shown by Ki67, CD25, and T-bet expression, respectively. Effector responses were also significantly dampened as IFN-γ synthesis was decreased in our 2-DG treatment group compared to HIP only controls ($p<0.05$).

Conclusions

Diminishing glycolysis in autoreactive T cells delays diabetes onset and Th1 effector responses. Future studies will continue to investigate the effects of 2-DG on the metabolic profile of CD4 T cell differentiation, CD4 T cell effector responses, and immune cell trafficking. Studies will also include understanding the effects of 2-DG on antigen presentation by dendritic cells to CD4 T cell in a co-culture assay.

34. Rachel Friedman (University of Colorado Anschutz Medical Campus)

Abstract Title

Efferocytosis pathways in islet myeloid cells suppress autoimmunity during type 1 diabetes

Authors

Kristen Wells, Jennifer Whitesell, Robin Lindsay, Sambra Redick, Alan Derr, Mason Tarpley, David Harlan, Sally Kent, Rachel Friedman

Purpose

In type 1 diabetes (T1D), islet beta cells are destroyed by T cell-mediated autoimmunity. Evidence from mouse models of T1D and human samples suggest that myeloid cells play a role in modulating T cell pathogenesis, with most data pointing to a pathogenic role for myeloid cells in T1D. Our goal was to identify mechanisms by which islet myeloid cells influence the T cell response during T1D.

Methods

Using the non-obese diabetic (NOD) mouse model of T1D, we analyzed the effect of signaling through the efferocytosis receptor Mertk in islet myeloid cells on the autoreactive T cell response. To do so, we inhibited Mertk with a small molecule inhibitor and analyzed antigenic interactions between T cells and myeloid cells in the islets by 2-photon microscopy, and T1D disease progression.

In human samples, we quantified Mertk-expressing cell numbers in islets of non-diabetic and T1D donors by immunofluorescent staining of pancreas sections. To further understand the function of myeloid cells in human islets, we analyzed scRNA-seq data from non-diabetic and T1D donors. However, the phagocytic nature of the myeloid cells complicated the analysis. Since T1D results in the loss of beta cells, the local transcriptional milieu surrounding islet myeloid cells changes profoundly. As a result, phagocytosed “passenger transcripts” represented the most differentially expressed genes between disease states. Passenger transcripts were described by Lantz et. al. (PMID: 32868786), with 20% of macrophage transcripts being derived from cells phagocytosed from the local environment. Thus, to analyze myeloid cell phenotypic differences, we developed a computational pipeline to subtract out passenger transcripts. For each sample, our pipeline models the environmental transcript distribution, estimates the contribution of environmental transcripts to the myeloid cell profile, and subtracts the environmental genes from the myeloid cells. We then analyzed differentially regulated genes to determine how myeloid cell phenotype and functions were changing with T1D.

Summary of Results

We show that Mertk signaling in islet macrophages suppresses the autoreactive T cell response in NOD islets. Mertk signaling prevented rapid progression of T1D by reducing the sensitivity of T cell scanning for cognate antigen in the islets. In human pancreas samples Mertk-expressing myeloid cells were increased in the remaining insulin-containing islets of T1D donors compared to islets of non-diabetic donors, suggesting that they may protect islets from autoimmune destruction as seen in the NOD mouse model.

Transcriptional analysis of islet myeloid cells from non-diabetic and T1D human donors showed that the differentially expressed genes in islet myeloid cells provided more insight into the cellular context of the pancreas than the myeloid cell phenotype, with Insulin (beta cell gene) and CEL3A (exocrine tissue gene) as top differentially expressed genes. Thus, separating phagocytosed passenger transcripts from myeloid-derived transcripts is important for accurately analyzing myeloid cell function and phenotype.

Our novel pipeline for filtering out passenger transcripts specifically removed differentially expressed genes that were environmentally derived (ex. INS, CEL3A), but did not alter myeloid cell genes (ex. HLA-DQB1). This pipeline enabled us to identify an enhancement in efferocytosis and suppression of antigen processing and presentation in islet myeloid cells from T1D donors with a short disease duration.

Conclusions

Islet myeloid cells play a role in restraining T cell-mediated autoimmunity and protecting islets from destruction through a Mertk-dependent mechanism. These data further suggest that human islet myeloid cells play a role in restraining autoimmunity in the islets during T1D progression by inhibiting antigen processing and presentation in response to efferocytosis pathways.

35. Gregory Golden (University of Pennsylvania)

Abstract Title

Development of an autologous lymphocyte and dispersed islet co-culture system

Authors

Gregory Golden, Chengyang Liu, Kevin Amses, Jacob Hamilton, HPAP Consortium, Ali Naji, Michael Betts

Purpose

While T1D predisposition may start early in life with the appearance of islet-specific autoantibodies (AAb), T1D often takes years to progress as the immune memory response to β-cell autoantigens develops. Evidence suggests T cells play a critical role in disease development as cytotoxic CD8+ T cells infiltrate islets and T1D susceptibility genes lie within major histocompatibility complex (MHC) Class II and Class I genes. To study β-cell-specific T cell responses, investigators typically stimulate peripheral blood T cells with putative autoantigenic peptides to induce T cell expansion and derive auto-reactive T cell clones. However, in vitro expansion induces non-physiological differentiation and functional changes of antigen-responsive cells, and use of individual selected antigenic peptides will not represent the full array of potential autoantigens expressed by islet cells. Moreover, T cells from blood may not represent those in pancreatic tissue or pancreas-draining lymph nodes. Here, we report the initial development of an autologous co-culture system to directly study islet-reactive T cell responses against pancreatic islet cells. To study islet-reactive T cell responses from human donors, we hypothesized that co-culture of islet cells with autologous pancreatic lymph node (pLN) lymphocytes would induce T cell activation.

Methods

We developed a collagenase digestion method for islet cell disaggregation that yields single islet cells while preserving surface antigen expression and endocrine function. As an initial unbiased strategy to define the presence of islet-reactive T cells in the co-cultures, we assessed proliferative responses to directly measure antigen recognition and activation of T cells. pLN-derived lymphocytes from AAb+ and T1D donors (n= 6) proliferated robustly (as measured by CFSE dilution via flow cytometry) in response to a 4-day co-incubation with dispersed islet cells compared to the control condition without islet cells.

Summary of Results

We observed both CD4+ and CD8+ T cell responses against autologous islets, with CD4+ T cells proliferating more robustly in each donor. Highly proliferating CD4+ T cells exhibited a bifurcating phenotype typical of an antigen-specific T cell response, where T cells either exhibited stem-like properties and or more terminally differentiated phenotypes. Co-stimulation of TCR signaling via anti-CD28/49d antibodies was required for T cell proliferation, suggesting that TCR binding of the antigen-MHC complex is the dominant activating signal. Further, inhibiting antigen presentation via MHC blocking antibodies, particularly for MHC-II, greatly inhibited T cell proliferation, suggesting that CD4+ T cell responses are primarily responsible for T cell proliferation. Ongoing efforts in this co-culture system include CITEseq strategies that permit single cell TCR sequencing, thus allowing deep phenotyping and clonal analysis of expanded T cells.

Conclusions

Overall, autologous co-culture of dispersed islets and pLN lymphocytes induces robust T cell responses that require antigen presentation. The co-culture system allows for islet-reactive T cell identification and phenotyping in a short-term assay, potentially giving new insights into autoimmune T cell responses in T1D development.

36. Sally C. Kent (University of Massachusetts Chan Medical School)

Abstract Title

Islet-derived CD8+ T cells from donors with type 1 diabetes (T1D) have increased cytotoxic potential as compared to those from donors without diabetes by scRNA-Seq.

Authors

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

Purpose

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

Methods

Upon receipt, isolated islets were doubly handpicked to exclude acinar and ductal tissue, then islets (180-500 islets) were immediately processed for transcriptome analyses. For one sample, nPOD 6578, live pancreas slices were digested with collagenase-P and single islets were hand-picked and processed immediately for transcriptome analysis. Two platforms for microfluidic single cell capture and scRNA-Seq library construction were used: inDrops and 10x Genomics (3' RNA sequencing; 5' RNA sequencing for nPOD 6578 islets). Doublets, empty droplets, and dead cells were removed. All samples were then combined, harmonized, and cell types were identified using a pancreatic islet reference dataset and analysis with celltypist.org. One cluster was identified as T cells expressing multiple T cell specific genes. T cells were identified in islets of 5 donors with T1D (disease duration: nPOD 6578- demise at onset of T1D and other samples: 0.42 to 8 years) from nPOD, Dr. Alvin C. Powers (Vanderbilt University), and from the Alberta IsletCore. T cells were identified in islets of donors without diabetes from 12 donors were that received from IIDP, Prodo Labs, and from the Alberta IsletCore.

Major considerations for analyzing these data sets include (1) the infrequency of the immune cell types as compared to the abundance of the islet hormone-producing cells, (2) that immune infiltrated islets are generally rare and analyses from each donor is limited to 3,000 (inDrops) or 10,000 (10x Genomics) cells, and (3) methods were optimized for recovery of beta cells. In the context of the much more abundant islet cells with hormone mRNA species, analyses of the relatively infrequent immune cells using scRNA-Seq poses several technical and analytic challenges. CD4 transcripts were not well detected with 98% of T cells identified as CD8+ T cells.

Summary of Results

While gene expression heterogeneity was seen both within the sample set of donors without diabetes and within the sample set of donors with T1D, a group of genes of interest was identified. In this 'snap-shot', islet-derived CD8+ T cells from donors with T1D possessed cytolytic potential to a greater extent than did those from donors without diabetes (mean scores for module of GZMA, GZMB, GZMM, GZMH, PRF1, NKG7, GNLY; Wilcoxon rank sum test with continuity correction; $p = 0.005$). In addition, significantly increased transcripts

for CD7 and MIF were detected from islet-derived CD8+ T cells from donors with T1D as compared to those from donors without diabetes indicating T cell activation and potential regulation of the microenvironment via macrophage function ($p = 0.0002$ and $p = 0.0003$, respectively).

Conclusions

With detection of greater transcription of genes for cytotoxic proteins in the CD8+ T cell from the islets of donors with T1D as compared to those without diabetes, these data indicate an ongoing CD8+ mediated response in the islets of donors with T1D. While this is a widely held assumption, these data offer support for this. In ongoing experiments, with 5' scRNA-Seq + T cell receptor (TCR) sequencing, transcriptionally active cytotoxic CD8+ T cells with TCR sequencing and subsequent epitope identification will aid our understanding of the pathological process in islets of donors with T1D.
These studies were supported by grants UC4DK116284 and DK104218-04.

37. Devan Moodley (Abata Therapeutics)

Abstract Title

Functional Characterization of Regulatory T cell (Treg) Therapy for the Treatment of Type 1 Diabetes (T1D)

Authors

Grace Voorhees, Eugene Antipov, Juliana Barrios, Jeremy C. Burns, Ellen Cahir-McFarland, Katie Callow, Bethan Chilton, Leonard L. Dragone, Jefte M. Drijvers, Yuan Feng, Michelle Fleury, Stefan Herrera, CJ Ives, Matthias John, Enoch Kisubika, Josh Lengieza, Conor O'Malley, Devan Moodley, Elissa Murphy, Niranjana Nagarajan, Timothy Nelson, Joanna Pizzo, Richard M. Ransohoff, Lawrence Schweitzer, Christina Strange, Andrea Van Elsas, Yizhou Wang, Stephanie Woodall, Fang Xia, Yanbo Zhang, Richard Zhou, Jiang Zhu, Geetha Mylvaganam

Purpose

Abata Therapeutics is dedicated to developing novel targeted Treg cell therapies for the treatment of intractable autoimmune conditions such as Type 1 diabetes (T1D). In recent years, regulatory T cell (Treg) therapy has emerged as a potential intervention to restore immune tolerance and halt progression of T1D. Abata Therapeutics is currently developing an autologous TCR-engineered Treg cell therapy product targeting proinsulin for the treatment of T1D. Here, we present the in vitro functional characterization of representative drug product, highlighting key biological and polypharmacological features that support Treg mediated immune modulation.

Methods

Using single cell RNA sequencing (scRNAseq), DNA methylation analysis, flow cytometry and multiplexed cytokine-based assays, TCR engineered Tregs were evaluated for purity, lineage commitment, phenotypic stability, and function. Functional characterization of our T1D representative drug product was assessed utilizing engineered and expanded Tregs from healthy and T1D donors. Specifically, we measured direct and bystander suppressive capacity of representative drug product against activated effector CD4 T cells as well as direct modulation of matured dendritic cells (DC).

Summary of Results

Our Treg engineering platform yields highly pure and stable Tregs with a transcriptome mirroring that of natural, thymically educated Tregs found within the peripheral blood mononuclear cell (PBMC) compartment as well as a stable Treg profile in the presence of a highly inflammatory milieu. TCR+ Tregs engineered from both healthy and T1D subjects demonstrated robust antigen-specific and bystander suppression against autologous islet reactive CD4 T cells. Moreover, when interrogating DC-Treg crosstalk, a key interaction

critical to limiting antigen priming of autoreactive T cells, antigen activated Tregs demonstrated greater deactivation of mature DCs as compared to polyclonal Tregs derived from a T1D donor. scRNAseq analyses further support a distinct DC transcriptional signature in the presence of TCR+ Tregs. Collectively, these polypharmacological mechanisms underscore the potential high therapeutic impact of our Treg cell therapy in treating T1D.

Conclusions

Abata has developed a proinsulin targeted TCR engineered Treg cell therapy with high purity, lineage stability, and robust function. Restriction to human class II expressing antigen presenting cells (APCs) and target peptide in the inflamed tissue and draining lymph node affords TCR engineered Tregs the opportunity to fine-tune the autoimmune response, resulting in both direct and broad infectious tolerance. Taken together, TCR engineered Tregs hold the potential of establishing tissue residence, halting beta cell destruction, recalibrating the immune response, and offering hope for an improved quality of life for those affected by T1D.

38. Geetha Mylvaganam (Abata Therapeutics)

Abstract Title

Unveiling Novel T-cell Receptors (TCRs) for Enhanced Treg Cell Therapy in Type 1 Diabetes (T1D)

Authors

Timothy Nelson, Juliana Barrios, Jeremy C. Burns, Ellen Cahir-McFarland, Katie Callow, Yuan Feng, Michelle Fleury, CJ Ives, Matthias John, Josh Lengieza, Devan Moodley, Elissa Murphy, Niranjana Nagarajan, Lawrence Schweitzer, Andrea Van Elsas, Grace Voorhees, Yizhou Wang, Yanbo Zhang, Richard Zhou, Jiang Zhu, Geetha Mylvaganam

Purpose

Abata Therapeutics is dedicated to developing novel targeted Treg cell therapies for the treatment of tissue-specific autoimmune disorders. Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin producing beta cells. Regulatory T (Treg) cell therapy offers a promising therapeutic avenue to restore immune tolerance in T1D patients by limiting ongoing immune activation and beta cell destruction. Here, we developed a proprietary innovative TCR discovery pipeline that facilitated the identification of novel MHC class II restricted islet reactive TCRs against two critical autoantigens in T1D, proinsulin and GAD65. These novel islet reactive TCRs have the potential to redirect Tregs to the sites of tissue inflammation, provide targeted immune suppression, and promote tissue repair resulting in targeted immune tolerance in T1D.

Methods

Antigen-specific CD4+ T cells were enriched in vitro, in silico reconstructed, and screened in a human T cell line and in primary human CD4+ T cells enabling ranking of candidate TCRs based on high functional avidity, Treg functional characteristics and on-target reactivity against lead antigens. Primary Tregs engineered with candidate TCRs against our lead target antigen, proinsulin, were subjected to in vitro suppression assays to further confirm their functional rank.

Summary of Results

Our robust end-to-end TCR discovery pipeline allows for the isolation of antigen-specific T cells, in silico TCR analysis, functionalization and screening that identified novel proinsulin and GAD65 specific TCRs. Ranking of TCRs based on TCR functional avidity facilitates further functional testing in a primary human Treg assay, providing an additional filter for candidate TCRs based on avidity and function within Tregs, as well an assessment of on-target specificity and screening against potential alloreactivity. This process enables efficient ranking of high functioning TCRs leading to the nomination of a candidate TCR for use in IND-enabling studies. Furthermore, downstream antigen-specific Treg functional suppression assays confirm TCR avidity ranking observed in the Treg activation assays.

Conclusions

In summary, we isolated, functionalized, and validated a novel set of candidate TCRs specific for two immunodominant islet autoantigens. Our robust end-to-end TCR discovery platform enabled rapid identification and tiered selection of lead candidate TCRs that exhibited high function, on target specificity and low cross reactivity. A candidate TCR targeting proinsulin was selected for use in the development of a TCR-engineered Treg cell therapy, for the treatment of T1D.

39. Victoria Kuznetsova (University of Miami)

Abstract Title

Upregulation of PDL-1 on endogenous pancreatic islet β cells by saRNA-Aptamer chimera

Authors

Victoria Kuznetsova, Daria Ivanova, Boutheina Marnissi, Luciana Mateus, Joana Almaca, Silvio Bicciato, Paolo Serafini

Purpose

T cell exhaustion and the interaction between programmed cell death protein 1 (PD1) and PD1 ligand 1 (PDL1) play a crucial role in the progression of Type 1 Diabetes (T1D). Residual functional islets from patients with a long history of T1D express PDL1; patients with autoreactive antibodies undergoing PD1 inhibition as cancer treatment develop fulminant autoimmune diabetes; the length of the honeymoon phase correlates with circulating exhausted T cells. In mice, transgenic or viral expression of PDL1 on allogeneic islet grafts prevents their rejection. This aligns with the observations in oncology and viral infection settings that PD1/PDL1 interaction gradually modulates T cell differentiation, increases coinhibitory receptors, reduces their effector function, and plays a role in peripheral tolerance. While in these settings, the use of checkpoint inhibitors restored the anti-tumor and anti-viral immunity and revolutionized the treatment of these diseases, the opposite (induction of PDL1 to inhibit autoimmunity) has not been therapeutically explored. This study aims to develop and test a bifunctional RNA therapeutic to modulate PDL1 expression on β cells in vivo. We hypothesize that our smart drug will upregulate PDL1 specifically on β cells, modulate the immune landscape in the pancreatic environment, and induce exhaustion on autoreactive T cells, potentially halting or reversing autoimmunity in T1D.

Methods

We generated a bifunctional RNA therapeutic (hereafter called PS03) by conjugating mouse and human β cell-targeting RNA aptamers with small activating RNA (saRNA) for upregulating PDL1.
We first identified and empirically tested saRNA specific for PDL1 using previously described algorithms and immortalized cell lines. Then, we conjugate the one with the largest effect size to optimized RNA aptamer 1-717 and m12-3773 by RISC cleavable loop. We evaluated the resulting construct by adding it to MIN6 cultures and assessing PDL1 expression by flow cytometry and qRT-PCR. To test PS03 specificity in vivo, we intravenously injected NOD female mice and evaluated PDL1 expression by quantitative fluorescence immunocytometry on the pancreas and other tissues. We then evaluate the effect of PS03-driven PDL1 upregulation on the immune system. First, we employed living slices from female NOD pancreas to evaluate its effect on β cells and islet infiltrating leukocytes. Then, we evaluate the phenotype of pancreatic T cells from NOD mice treated TIW for 4 weeks with PS03 or vehicle by multicolor flow cytometry. Finally, we assessed the effectiveness and safety of PS03 by treating 15-week-old NOD mice TIW for six weeks.

Summary of Results

We evaluated PS03 on MIN6 and observed a significant upregulation of the PDL1 starting at 24 hours and peaking three days after adding the culture media. Similarly, we observed upregulation of PDL1 on the living slice islets that were treated with PS03 for 48 hours. This PDL1 upregulation correlated with the inhibition of Ca2+ influx of islets infiltrating leukocytes, suggesting inhibition of TCR signaling. In vivo, we observed specific PDL1 upregulation on endogenous β cells of NOD mice, with a peak at 5 days after treatment and

persistence observed at day 10 after administration. In sharp contrast, we did not observe PDL1 upregulation in acinar and alpha cells and all other tissues evaluated.
We then evaluated the phenotype of autoreactive T cells after prolonged PS03 treatment on β cells and observed a three-fold increase in the exhaustion-associated phenotype population compared to untreated controls. Finally, a long-term treatment of female NOD mice with a still unoptimized PS03 formulation significantly delayed T1D onset and prevented it in 40% of mice.

Conclusions

In summary, PS03 is a new RNA therapeutic that, given systemically, provides effective and β cell-specific PDL1 upregulation and protects NOD mice from T1D, likely by blocking autoreactive T cells' function and inducing their exhaustion.

40. Jessie Barra (University of Florida)

Abstract Title

Combinatorial genetic engineering strategy for antigen specific immune protection of stem cell-derived beta cells

Authors

Jessie Barra, Rob Robino, Roberto Castro-Gutierrez, Leonardo Ferreira, Holger Russ

Purpose

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

Methods

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

Summary of Results

We demonstrate uniform overexpression of EGFRt within human pluripotent stem cells (hPSC) while maintaining normal expression of pluripotent cell markers compared to wild type control cells (WT). Directed differentiation of WT and EGFRt hPSCs into sBCs displayed comparable efficiency in the generation of insulin positive cells (40-50%). We can successfully transduce Tregs with the EGFR CAR with greater than 80% efficiency. CAR-Tregs are stable and continue to express key Treg markers including FOXP3, HELIOS, and CTLA-4. EGFR CAR-Tregs are activated specifically when co-cultured with EGFRt-expressing hPSC or sBCs. Stimulated EGFR CAR-Tregs suppress effector T cell proliferation and dendritic cell responses during in vitro co-culture assays. Employing a novel transplantation model, we show that EGFR CAR-Tregs prevent T cell mediated immune destruction of EGFRt sBC grafts in vivo.

Conclusions

We have successfully integrated a truncated form of EGFR into a human stem cell line to serve as a unique target for CAR-Treg activity at the site of beta cell engraftment. We observe efficient transduction of EGFR CAR into human Tregs, and CAR-Tregs are robustly activated when co-cultured with EGFRt overexpressing cells. Based on these promising results, we predict that the use of CAR-Tregs combined with the genetic engineering of a unique ligand for the CAR on sBCs represents a novel way to suppress immune responses in a highly localized manner, ultimately providing long-term sBC graft survival without systemic immunosuppression.

41. Etienne Larger (APHP)

Abstract Title

Insulitis and exocrinitis in autoantibody-positive non-diabetic individuals: role of HLA genotypes

Authors

Marc Diedisheim, Roberto Mallone, Daniele Dubois-Laforgue, Etienne Larger

Purpose

Type 1 diabetes (T1D) is characterized by the presence of autoantibodies on a genetic background largely determined by HLA class II haplotypes. Stage 1 of the disease is characterized by the presence of autoantibodies and normoglycemia. We quantified pancreatic immune infiltrates at stage 1 T1D, according to HLA

Methods

We analyzed CD3+ lymphocyte infiltration of both endocrine and exocrine pancreas in 117 sections from 30 non-diabetic individuals positive for at least one autoantibody from the Network for Pancreatic Organ Donors with Diabetes (nPOD). HLA haplotypes were classified as at risk (DQ2/DQB1*02:01 and/or DQ8/DQB1*03:02), protective (DQ6/DQB1*06:02) or neutral (other HLA-DQ alleles).

Summary of Results

Among these 30 individuals (median age 25 years, IQR 21-39; median BMI 24 kg/m², IQR 21-30), 23 were single autoantibody-positive and 7 were positive for 2 autoantibodies. β-cell mass was normal in all. HLA-DQ allele distribution was similar to that of autoantibody-negative non-diabetic nPOD donors, and differed from that of nPOD donors at stage 3. Insulitis was identified only in one case. CD3+ lymphocyte densities in islets did no correlate with HLA status or autoantibody number, and correlated with densities in the exocrine pancreas

Conclusions

These autoantibody-positive donors had normal β-cell mass and no significant insulitis, suggesting an underlying autoimmune process progressing very slowly even in the presence of HLA genetic risk

42. Jacob Hamilton (University of Pennsylvania)

Presenter of the Abstract

Jacob Hamilton

Abstract Title

A high-parameter flow cytometry panel for profiling natural killer cells in T1D

Authors

Jacob T. Hamilton, Gregory J. Golden, Maria Betina Pampena, HPAP Consortium, Michael R. Betts

Purpose

Many investigations of immune cells in T1D have focused on T cells that eliminate beta cells or B cells that produce autoantibodies. Recent studies have suggested that the involvement of components of innate immunity may provide a more complete picture of the autoimmune process, in either a regulatory or destructive role. Natural Killer (NK) cells are known to have both adaptive and innate characteristics and may contribute to our understanding of T1D immunopathology. We have shown that donors with T1D have a higher proportion of cytotoxic CD56-dim CD16-negative (CD56dimCD16-) NK cells in pancreatic lymph nodes (pLN) compared to non-diabetic control (ND) donors. Conversely, T1D pLN showed an decreased proportion of regulatory CD16-negative NK cells. Here we report the development of a flow panel for in-depth analysis of NK immunophenotype in tissues including pLN, mesenteric LN (mLN), and spleen of individuals with T1D.

Methods

We developed a 25-color flow cytometry antibody panel to analyze the phenotype of NK cells in the tissues of organ donors with T1D or non-diabetic donors with beta cell antigen autoantibodies (AAb+). ND donors were used as controls. Freshly purified mononuclear cells derived from pLN, mLNs, and spleen were stained and subsequently acquired within 5 days on a BD FACSymphony A5 Instrument.

Summary of Results

This flow cytometry panel is able to detect a large number a regulatory and cytotoxic NK cell phenotypes. Preliminary data show that spleen and mLNs of T1D donors have a trend towards a higher proportion of perforin and granzyme B double positive NK cells compared to control and AAb+ donors. Interestingly, only T1D and AAb+ donors show a trend towards a higher proportion of proliferating (Ki67+) NK cells in the spleen and mLNs compared to the pLNs. No definitive trend is seen in residency markers (CD69 and CD103), memory-like NKs (NKG2C and CD57), and inhibitory receptors (PD1).

Conclusions

High parameter flow cytometry allows for deep characterization of NK cells, a cell subset that has evidence of differential phenotype in T1D yet is understudied. Additional data need to be collected on more donors as they become available. However, this NK flow cytometry panel shows promise for elucidating the role of NK cells in the progression of T1D.

Preferred Presentation Format

Poster Presentation

Research Category

Immunology

43. Rachel Bonami (Vanderbilt University Medical Center)

Abstract Title

The complex role of somatic hypermutation in supporting insulin recognition by B lymphocytes in at-risk type 1 diabetes participants

Authors

Lindsay Bass, Tommi Taylor, Mason Forchetti, Steen Scaglione, Alaina Skellett, Anika Mahajan, Daniel Moore, Scott Smith, Rachel Bonami

Purpose

Purpose: Insulin autoantibodies are among the best predictive type 1 diabetes (T1D) biomarkers, yet the molecular basis for B cell receptor (BCR) recognition of insulin in humans has not been well-studied. We therefore developed new approaches to identify and characterize insulin-binding B cells to address this knowledge gap.

Methods

Methods: We used single-cell RNA-seq/BCR-seq/CITE-seq and advanced hybridoma technology to identify insulin-binding B lymphocytes from the peripheral blood of n = 11 or n=9 Type 1 Diabetes TrialNet Pathway to Prevention participants who were positive for ≥ 2 islet autoantibodies and thus at high risk for diabetes. These participants were categorized as Stage 1 or Stage 2 based on normal or impaired glucose tolerance test results at the time of blood draw, respectively. Clonally expanded B lymphocytes were identified in BCR-seq data based on n ≥ 3 BCRs per clonotype (with 2,000-5,000 cells profiled per participant).

Summary of Results

Summary of Results: Clonally expanded B cells expressed an autoreactive-prone CD21lo CD27+ phenotype and were primarily identified in a specific transcriptionally defined memory B cell cluster. Recombinant expression of these clonally expanded BCRs revealed that ~50% recognized insulin. Sequence analysis of insulin-binding BCRs captured using single-cell or hybridoma technology revealed they ranged from 0-11% somatic hypermutation. Germline reversion of mutated anti-insulin BCRs revealed dichotomy in the functional importance of identified mutations. For one BCR, germline reversion of the single amino acid mutation present in the participant-derived BCR eliminated insulin recognition, whereas in another, germline reversion of all 22 mutations present in the participant-derived heavy and light chain resulted in only modest diminution of insulin binding.

Conclusions

Conclusions: These data highlight clonally expanded, CD21lo memory B cells as a reservoir for insulin-reactive B lymphocytes. Further, we show extensive somatic hypermutation of BCRs may not always substantially enhance insulin autoantigen recognition, and rather may simply be a byproduct of a chronic autoimmune response.

Novel Biomarkers

44. Daniela Fignani (University of Siena; Umberto di Mario Foundation)

Abstract Title

Comprehensive Small RNA-seq profiling of blood circulating extracellular vesicles in islet autoantibody-positive and autoantibody-negative individuals within the DIPP Cohort

Authors

Daniela Fignani, Eirka Pedace, Mattia Toniolli, Stefano Auddino, Elena Aiello, Giuseppina Emanuela Grieco, Giada Licata, Mikael Knip, Jorma Toppari, Riitta Veijola, Noora Nurminen, Jutta E Laiho, Heikki Hyöty, Guido Sebastiani, Francesco Dotta, HEDIMED Investigator group

Purpose

Extracellular Vesicles (EVs) are particles with a size of ~ 40 to 160 nm in diameter, emerging from cytosolic multivesicular endosomes (MVEs) and then actively secreted in the extracellular space, thus commonly found in many biological fluids including plasma. EVs are important mediators of tissues crosstalk through the transfer of a series of biologically active molecules including non-coding RNAs, including microRNAs (miRNAs). miRNAs are small non-coding 19-22 nucleotides-long RNAs which are able to negatively regulate the mRNA expression; several studies have demonstrated their involvement in Type 1 Diabetes (T1D).

Therefore, the aim of this study was to evaluate the vesicular small RNAome in plasma samples obtained from the Finnish Type 1 Diabetes Prevention and Prediction Study (DIPP) cohort.

Methods

EVs from 200 µl of plasma were isolated using Size Exclusion Chromatography (SEC) and analyzed in terms of concentration and morphology using Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy (TEM). CD81 expression was evaluated using Western Blot (WB) analysis. Plasma samples were obtained from n=26 non-diabetic autoantibody-negative subjects (islet AAb-neg; age: 7±4,1) and n=26 non-diabetic autoantibody-positive subjects (islets AAb-pos; age: 7±4,1) matched for age and sample collection time, and all carry HLA risk alleles for T1D. Small RNAs content was evaluated using Small RNA sequencing by adopting the Qiaseq Small RNA library preparation kit (Qiagen), with minor modifications. After sequencing, QC analysis was performed on FastQ using FastQC and then analyzed with sRNAbench. After low counts filtering, PCA analyses were performed in order to evaluate a clusterization of samples. Differential expression analysis was applied using DESeq2 package (Wald test; p-adj <0.05). Specificity analyses of miRNAs expression in cells and tissues were performed in multiple small RNA-seq datasets obtained from isomiRdb; from these we retrieved the specificity score (Z-score), to measure the enrichment of miRNAs in different cells type.

Summary of Results

EVs isolated from plasma samples showed a cup-shaped morphology and a concentration of 7.9±1.3^{x}109 EVs measured using TEM and NTA, respectively. Additionally, we confirmed the presence of CD81 tetraspanin. Small RNA-seq revealed that vesicular small RNAome is composed by 5 different relevant small RNA species: miRNAs (2,7%), tRNAs fragments (2,9%), mRNAs fragments (3,9%) rRNAs fragments (25,9%), and other RNAs (64,1%). PCA analyses using different small RNA species there isn't a clear separation between the two group. The differential expression analyses revealed the let-7f-5p is upregulated while miR-125a-5p is downregulated in EVs of AAb-pos vs AAb-neg. From the dataset mentioned above, we found that let-7f-5p is enriched in CD4+ T lymphocytes while miR-125a-5p is enriched in mast cells.

Conclusions

For the first time, we identified two miRNAs differentially expressed in EVs isolated from plasma obtained from AAb-pos vs AAb-neg individuals recruited in DIPP study; In this cohort, let-7f-5p, a CD4+ T lymphocytes enriched miRNA, resulted upregulated, while miR-125a-5p, a mast cell enriched miRNA, was downregulated in EVs from Aab-positive vs negative individuals and may both represent putative biormarkers of ongoing phenomena involved in the progression to seroconversion or T1D onset.

45. Julius Nyalwidhe (Eastern Virginia Medical School)

Abstract Title

Pre-Validation of Early Detection Biomarkers for Type 1 Diabetes

Authors

Julius Nyalwidhe, Ian Oduor, Marta Satin-Smith

Purpose

Type 1 diabetes (T1D) occurs as a result of impaired immune tolerance or dysfunction, leading to the autoimmune destruction and death of insulin producing beta cells that are found in the pancreatic islets of Langerhans. This destruction leads to insulin deficiency and the consequent development of the disease. A key critical unmet need in the management of the disease is the identification of reliable biomarkers that discriminate individuals who will from those who will not progress to T1D. Importantly, these markers should be detectable in biological fluids that are obtained using minimally invasive methods. Our objective is to pre-validate a panel of serum based prognostic and diagnostic markers for early detection of T1D.

Methods

Used targeted Liquid Chromatography Parallel-Reaction Monitoring Mass Spectrometry (LC-PRM-MS) method for highly selective, sensitive, and high-throughput absolute quantification of peptides from multiple proteins that we discovered to be differentially regulated in proteomics studies using pancreatic tissue lysates and serum samples from patients with non-T1D cases, prediabetic autoantibody positive (Aab+) cases, and T1D cases. Orthogonal biochemical assays have been developed utilized to verify mass spectrometry data.

Summary of Results

Developed a robust LC-PRM-MS assay for detecting and quantifying peptides that are associated with the initiation and progression of T1D using pancreatic tissue lysates and serum clinical samples. We are currently using the results and datasets to generate receiver operating characteristic (ROC) curves to determine the performance (sensitivity and specificity) of our putative markers individually and in multiplexed panels.

Conclusions

Our pre-validation preliminary data show robust differential expression of a panel of peptides in disease stratified clinical samples from patients with non-T1D cases, prediabetic autoantibody positive (Aab+) cases, and T1D cases. These molecules may have a role in the pathogenesis of T1D and are targets for further development of diagnostic and prognostic markers for the T1D using biological fluids obtained using minimally invasive methods.

46. Carla Di Dedda (San Raffaele Diabetes Research Institute, IRCCS Ospedale San Raffaele)

Abstract Title

Targeting the glucose transporter GLUT1 to control T cell activation

Authors

Carla Di Dedda, Isaac Snowhite, Alexandra Amaya, Carlos Blaschke, Janine Sanchez, George Burke, Paolo Monti, Alberto Pugliese

Purpose

Beta cell replacement, through pancreas or islet transplantation, represents a therapeutic option for patients with severe T1D. However, while allo-rejection can be efficiently controlled by immunosuppressive regimens, the long-lived autoreactive memory T cells can reactivate after the encounter with the graft antigens and initiate the recurrence of T1D. The glucose transporter GLUT1 is upregulated in T cells upon activation as part of the metabolic switch from oxidative phosphorylation to glycolysis. This project aims to test pharmacological GLUT1 blockade as a potential strategy to control T cell activation.

Methods

Ex- vivo and in vitro studies were performed using PBMCs from healthy donors (HD), patients with T1D (T1D), and patients with T1D who received a simultaneous pancreas-kidney (SPK) transplant. These were sub-divided into three groups: normal glucose-tolerant recipients without autoantibodies on follow-up (SPK-neg) or who experienced autoantibody conversions (SPK-Conv), and recipients who developed recurrence of autoimmunity and T1D (T1DR). We characterized T cell subsets involved in T1DR and assessed whether GLUT1 expression is associated with autoimmune reactivation. Pools of class I and class II dextramers were used to characterize autoreactive T cells among SPK patients. Moreover, using the small molecule GLUT1 inhibitor WZB117, we studied phenotypic, differentiation, and metabolic changes of T cells activated under GLUT1 blockade in vitro.

Summary of Results

The results showed that all SPK groups had higher frequencies of CD4+ Tem cells compared to HD and T1D patients, while CD8+ Tem cells were increased among T1DR patients compared to HD, T1D, and, importantly,

compared to SPK-Neg patients. There was also a trend toward increased Tscm cells, whose expression of GLUT1 was consistently higher compared to other T cell subsets. Although the frequency of autoreactive T cells did not differ in T1DR patients, their phenotype was more activated and less exhausted compared to SPK-Neg and SPK-Conv, and this was associated with an upregulation of GLUT1 expression. This upregulation was not observed within the total CD4+ and CD8+ T cell populations. In vitro studies showed that GLUT1 blockade reduced T cell proliferation in HD, T1D patients, and SPK-Neg recipients, causing greater effects on cells from T1D and SPK patients, compared to HD. Other key changes that consistently occurred in T1D and SPK patients after GLUT1 blockade were reductions in the size of naïve, Tcm, and Tscm subsets, accompanied by a downregulation of several activation markers. Only T1D patients showed decreased GLUT1 expression following WZB117 treatment, especially in Tscm and Tcm subsets.

Conclusions

GLUT1 was highly expressed in autoreactive T cells from patients experiencing autoimmunity recurrence post-transplant, but not in T cells with irrelevant reactivity. GLUT1 expression levels were also consistently higher in Tscm compared to other T cell subsets, a population that was reported to be a major driver of islet autoimmunity. This, together with the demonstrated ability of GLUT1 blockade to inhibit T cell activation and proliferation in vitro, provides a rationale for further investigation of GLUT1 blockade approach for selectively targeting autoreactive T cells, preventing or inhibiting their activation, minimizing off-target effects, and enhancing the specificity of therapeutic interventions.

47. Guido Sebastiani (University of Siena)

Abstract Title

Circulating miR-150-5p, a B-lymphocyte associated miRNA, is increased selectively in plasma of young children (<7y) with recent onset type 1 diabetes

Authors

Mattia Toniolli, Giuseppina Emanuela Grieco, Marco Bruttini, Stefano Auddino, Elena Aiello, Alessia Mori, Daniela Fignani, Giada Licata, Andrea Berteramo, Erika Pedace, Laura Nigi, Caterina Formichi, Chantal Mathieu, Noel Morgan, Guido Sebastiani, Francesco Dotta

Purpose

The age at which type 1 diabetes mellitus (T1DM) is diagnosed differs according to genetic predisposition, presence of islet autoantibodies, clinical presentation of the disease and the decline in beta-cell function. Younger individuals typically exhibit a more severe clinical presentation together with a more rapid disease progression. This heterogeneity has led to the hypothesis that multiple endotypes of T1DM exist. Histopathological evidence has defined one such endotype (T1DE1) as being characterized by an early age of onset (below 7 years of age), a substantial infiltration of CD20+ B cells in the pancreas, and a pronounced and rapid loss of beta-cell function. However, it remains uncertain how the recognition of this endotype can be leveraged for specific interventional therapies and how it can be readily identified in clinical practice. Circulating microRNAs have demonstrated consistent associations with T1DM onset and some have also been linked to disease progression, particularly the decline in beta-cell function. As such, microRNAs hold promise as potential biomarkers for tracking T1DM progression and/or staging and/or personalized intervention. Consequently, the objective of this study is to establish whether specific circulating microRNAs are associated with the T1DE1 endotype.

Methods

Small RNA sequencing was carried out on plasma samples collected during the initial visit (v1) from two independent cohorts of individuals recently diagnosed with T1DM (within 4 weeks of onset). These cohorts were recruited as part of the European INNODIA consortium and consisted of n=115(sex: 58F/57M; mean age: 12.4±7.7 years; age range: 2-38y) and n=147 subjects (sex: 55M/92F; mean age: 11.9 ± 7.9 years; age range: 1-42y). T1DM individuals underwent programmed follow-up visits at 3- (v2), 6- (v3), and 12-months (v4). During these visits, they were tested for autoantibodies, fasting and mixed-meal tolerance test (MMTT)

glycaemia, C-peptide levels and HbA1c. T1DM individuals were stratified based on their age at onset (<7 or ≥7 years), and miRNA differential expression analysis was conducted using the DeSeq2 Wald test (P-value adjusted using FDR<0.05). Differences in clinical parameters were evaluated using monovariate regression analysis. Detected miRNAs were validated using miRCury LNA reverse transcription and droplet digital PCR (ddPCR). Additionally, publicly available small RNA sequencing datasets from control (n=28), celiac disease (n=46), and asthmatic cohort individuals (n=462) with an age range similar to INNODIA T1DM cohorts, were re-analyzed to verify the disease specificity of the detected microRNAs or their association with age.

Summary of Results

Stratification based on an age cutoff of 7 years revealed that in the first T1DM cohort, there were n=18 individuals aged <7 and n=97 individuals aged ≥7, while in the second cohort, there were n=30 individuals aged <7 and n=117 individuals aged ≥7.
One particular circulating miRNA, miR-150-5p, was found to be selectively and significantly elevated in plasma of T1DM individuals aged <7 in comparison to those aged ≥7 in both cohorts (log2 fold change: 0.65; adjusted p-value (Padj)=0.0008 and log2 fold change: 1.18; Padj: 9.6x10^-8). Additionally, miR-150-5p displayed a slight negative correlation with the age at onset in both cohorts (first cohort: R = 0.41, p-value = 5.6x10^-6; second cohort: R=-0.36, p-value: 5.7x10^-6). ddPCR confirmed the upregulation of miR-150-5p in T1D subjects <7 with respect to those ≥7 years, in both cohorts (first cohort p-value = 0.00048; second cohort p-value = 0.0066, non-parametric Wilcoxon test).
As expected, T1DM individuals aged <7 exhibited reduced fasting C-peptide levels, a lower fasting C-peptide/glucose ratio, and a diminished MMTT AUC C-peptide over the 12 months of follow-up. They also showed an increase in MMTT AUC glucose. Furthermore, at baseline, T1DM individuals aged <7 displayed higher IAA titers (first cohort p-value = 0.024; second cohort p-value = 0.019) and lower GADA (first cohort p-value = 0.015; second cohort p-value = 0.00033) and ZnT8A (first cohort p-value = 0.0054; second cohort p-value = 9.6x10^-5) titers in both cohorts compared to those aged ≥7 at the time of onset.
Notably, circulating miR-150-5p did not exhibit any association with age (<7 , ≥7y) in non-diabetic control individuals or in individuals with celiac disease or asthma. Additionally, in these cohorts miR-150-5p did not correlate with age in a linear regression model, thus excluding any association with age.

Conclusions

Plasma circulating levels of miR-150-5p, a microRNA associated with early B-cell development and activation, are increased in T1DM subjects aged <7 years (endotype T1DE1). Interestingly, this phenomenon was not observed in control subjects, or in individuals with celiac disease or asthma. This microRNA could be potentially used to categorize individuals with the T1DE1 endotype and, in doing so, shed light on additional mechanisms underlying the heterogeneity of T1D.

48. Marlene E. Redlich (University of Miami)

Abstract Title

Generation of RNA Aptamers specific for FXYD2γa on human pancreatic β cells

Authors

Marlene E. Redlich, Cheyenne Stringfellow, Victoria Kuznetsova, Boutheina Marnissi, Daria Ivanova, Paolo Serafini, Decio L. Eizirik

Purpose

Type 1 Diabetes (T1D) is characterized by decreased functional β cell mass (BCM). However, the kinetic of this decrease remains unknown, and it is not yet possible to evaluate the effects of therapy on beta cell mass. There is thus an urgent need to develop noninvasive probes and techniques for imaging human β cell mass in vivo.

Among the targets preferentially expressed on beta cells, the previously identified integral membrane protein FXYD2γa isotype is of particular interest: it is expressed in human β cells but not in other tissues; its expression co-localizes with insulin-positive cells and decreases during T1D progression. Developing probes against this marker is technically challenging because of the short (8 aa-long) extracellular domain.
We hypothesized that RNA aptamers can be selected against this protein. RNA aptamers, or synthetic antibodies, have been identified against hapten and other small molecules using Systemic Evolution of Ligands by Exponential Enrichment (SELEX) and optimally designed selection methods. RNA aptamers can efficiently deliver therapeutics and imaging reagents to specific targets due to high affinity to the desired target, lack of immunogenicity, and simple manufacturing. The core objective of this project is thus to identify monoclonal aptamers tailored specifically for FXYD2γa.

Methods

To identify monoclonal aptamers specific to FXYD2γa, we employed SELEX and Cell-SELEX. We started from a 1015 random RNA aptamer library (C0) and used peptides corresponding to the extracellular domains of FXYDγa and FXYDγb as positive and negative selectors, respectively. To avoid the selection of irrelevant aptamers, we employed magnetic beads conjugated to the peptides via an amide covalent bond during the initial selection cycles (C1-7) and performed an additional selection using handpicked human islets (C7i). Libraries C0, C3, C7, and C7i were sampled for high throughput (HT) sequencing and tested for binding against relevant and irrelevant beads by flow cytometry. We then performed bioinformatics analysis of the polyclonal aptamers with Aptani to select putative monoclonal aptamers. We selected 12 candidates based on their frequency in the different cycles. These candidates were produced enzymatically by PCR and T7 RNA polymerase reaction, Cy3-labeled, and used as a probe against dissociated human islets by flow cytometry and against human pancreatic tissues by immunofluorescence microscopy.

Summary of Results

During SELEX, we observed an increase in DNA recovery during cycles, suggesting a promising aptamer enrichment. This was supported by the flow cytometry analysis against the relevant and the irrelevant beads that showed increased specific binding of libraries from cycle C0 to cycles C3, C7, and C7i. Our bioinformatic analysis confirmed this finding since library complexity, defined as unique sequences/total sequences, decreased during selection. Furthermore, we observed a steady increase in the frequency of the most abundant clones found in C7i during selection. We chose 12 putative monoclonal aptamers based on their frequency in C7i, frequency variation among cycles, and sequence similarities for empirical evaluation against human islets. Fluorescence immunocytometry and flow cytometry indicate that most of the selected aptamers preferentially recognize β cells, with minimal binding to α and acinar cells.

Conclusions

Our results confirmed that, with the proper selection strategy, RNA aptamers can be selected against small and challenging targets. More importantly, we identified monoclonal aptamers against FXYDγa that selectively recognize human β cells. Since aptamers with different specificities have already been successfully used in positron emission tomography, these aptamers may help measure beta cell mass to determine T1D progression and the efficacy of new therapeutics.

Novel Technologies

49. KaLia Burnette (The University of Kansas Medical Center)

Abstract Title

Islets Encapsulated with CTLA-4-Ig and Antioxidants Restore Euglycemia in Subcutaneous Transplant Site

Authors

KaLia S. Brnette, Sheila Tsau, Veronika Kozlovskaya, Eugenia Kharlampieva, Andrew Pepper, Hubert M. Tse

Purpose

Type 1 diabetes is characterized by beta-cell-specific autoimmune destruction, leading to lifelong exogenous insulin dependence. The hepatic portal vein is the preferred site for islet transplantation however, its high flow rates present a risk for graft attrition and instant blood-mediated inflammatory reaction. There is a need to find alternative sites for islet transplantation that are highly vascularized and can elicit immunoprotection. While islet transplantation can restore euglycemia, novel strategies to delay graft rejection are needed. Our lab uses a nanothin encapsulation material consisting of poly(N-vinylpyrrolidone) (PVPON) and tannic acid (TA), an antioxidant, to delay islet allograft rejection and decrease inflammatory immune responses when transplanted under the kidney capsule of streptozotocin (STZ)-treated diabetic mice. The addition of CTLA-4-Ig, an immune inhibitor fusion protein, onto our encapsulation material significantly increased FOXP3+ regulatory and CD73+ FR4+ anergic CD4+ T cells at the graft site and delayed islet graft rejection when compared to non- and (PVPON/TA/IgG)-encapsulation. We sought to assess the ability of (PVPON/TA/CTLA-4-Ig)-encapsulation to improve islet graft survival in a pre-vascularized subcutaneous transplant site. We hypothesize that (PVPON/TA/CTLA-4-Ig)-encapsulation in combination with a deviceless subcutaneous transplant site will sustain islet allograft function by eliciting localized immunosuppression.

Methods

NOD.scid, C57BL/6, and spontaneously diabetic NOD mice will be implanted with a 2 cm 6-Fr nylon catheter for 4-weeks to generate a deviceless subcutaneous transplant site. C57BL/6 mice will be rendered diabetic with streptozotocin treatment 3 days prior to islet transplantation. The catheter will be removed at the time of transplant and 250 or 500 non- or (PVPON/TA/CTLA-4-Ig)-encapsulated syngeneic islets will be transplanted into the pre-vascularized subcutaneous space or under the left kidney capsule. To investigate the ability of (PVPON/TA/CTLA-4-Ig)-encapsulated islets to protect against autoimmune responses, transplants will also be performed in spontaneously diabetic NOD mice. The blood glucose of recipient mice will be monitored daily to assess graft function. Islet grafts will also be harvested 7 and 14 days post-transplant for immunophenotyping.

Summary of Results

We found that subcutaneous implantation of a nylon catheter into NOD.scid mice resulted in the formation of a vascularized pocket evident by CD31 staining. Syngeneic transplants of (PVPON/TA/CTLA-4-Ig)-encapsulated islets into STZ-treated NOD.scid mice successfully restored normoglycemia similar to non-encapsulated islets.

Conclusions

Subcutaneous transplantation of (PVPON/TA/CTLA-4-Ig)-encapsulated islets can successfully restore normoglycemia to mice. Future studies will investigate the ability of (PVPON/TA/CTLA-4-Ig)-encapsulated islets transplanted within the subcutaneous space to restore euglycemia in allogeneic diabetic mice.

50. Peter Duinkerken (University Medical Center Groningen)

Abstract Title

Automated identification of structures in large-scale electron microscopy data of nPOD donors

Authors

Peter Duinkerken, Ahmad Alsahaf, Jacob Hoogenboom, Ben Giepmans

Purpose

Large-scale electron microscopy (EM) of nPOD pancreas using ‘nanotomy’ has yielded a valuable database of (non-)diabetic tissue at biomolecular resolution (www.nanotomy.org/OA/nPOD/). While the large-scale representations contain a wealth of information, the size and greyscale nature makes it a challenging task even for experts to fully comprehend and or analyze at the nanoscale. We aim to develop energy-dispersive X-ray (EDX) imaging of complete cross sections of Islets to extract nanoscale features that may be relevant in (pre)diabetic donors versus control donors.

Methods

Large-scale EM combined with EDX imaging, known as ColorEM, of nPOD pancreas. This add-on in EM imaging can be compared to fluorescence imaging in light microscopy. Spectral analysis is subsequently conducted to decompose the rich dataset.

Summary of Results

Large-scale ColorEM of pancreas allow various subcellular features to be visualized through color-coded representations of elemental distributions. By selecting different elemental combinations various biological features, such as endocrine granules, exocrine granules, lysosomes and nuclei are automatically highlighted.

Conclusions

Combining ColorEM with nanotomy provides elemental context on a large scale, allowing subcellular features to be visually localized at a glance. Highlighting biological features has the potential to train a model that in retrospect can highlight similar features in already recorded EM datasets, such as available in the nPOD nanotomy database.

51. Jacob Enriquez (University of Chicago)

Abstract Title

Engineering Functionalized Lipid Nanoparticles for Targeted Beta-Cell Therapeutics

Authors

Jacob R. Enriquez, Zhengjie Zhou, Jennifer B. Nelson, Fei Huang, Brian Xi, Yun Fang, Sarah A. Tersey, Raghavendra G. Mirmira

Purpose

Increasing evidence suggests that β-cells actively promote autoimmunity in type 1 diabetes (T1D) by producing aberrant proteins that act as neoantigens to stimulate the immune system. Therefore, blocking the early stress responses in β-cells that produce neoantigens could delay diabetes onset. Although potential drugs affecting β-cells have been identified, these drugs are delivered systemically and can affect multiple cell types. In this

study, we sought to develop a targeted delivery approach to supply biological therapeutics directly to β-cells using nanomedicine technology.

Methods

We identified candidate self-assembled lipid nanoparticles (LNPs) by establishing a library of more than 60 LNP combinations containing the following lipids: lipid grafted polyamidoamine (PAMAM) dendrimer (G0-C14); cholesterol; polyethylene glycol (PEG) 2000; 1, 2-Distearoyl-sn-glycero-3-phosphoethanolamine-PEG (DSPE-PEG), and dioleoylphosphatidylethanolamine (DOPE). After selecting the most stable and encapsulation-efficient LNP (Candidate-9, C9), we customized the C9-LNP via the addition of a functionalized peptide, eGLP1, which targets glucagon-like peptide-1 receptor (GLP-1R) — a cell surface receptor enriched on β-cells. To determine the capability of the eGLP1-functionalized LNP (eGLP1-LNP) in vitro, we treated human cell lines or mouse islets with eGLP1-LNPs containing mScarlet mRNA and measured fluorescence after 24 hours. To test in vivo targeting specificity, we injected eGLP1-LNPs encapsulating Cy5-eGFP mRNA intraperitoneally into C57BL/6 mice or non-obese diabetic (NOD) mice (a model of autoimmune diabetes). We assessed cargo uptake by in vivo imaging after 24 hours.

Summary of Results

Using the optimized eGLP1-targeting LNP, we saw enrichment of fluorescent mScarlet in cultured human β-cells (EndoC-βH1) and isolated mouse islets —but not in HEK-293 cells—indicating β-cell specificity and activity of mRNA cargo. In isolated mouse islets, there was a 20% increase in mScarlet expression using the eGLP1-targeting LNP compared to an untargeted LNP. With the delivery of eGLP1-LNPs into C57BL/6 mice, we observed at least a two-fold enrichment in Cy5 fluorescence in the pancreas compared to the liver or other major organs. The eGLP1-LNP resulted in greater specificity than the untargeted LNP, with at least 1.5-fold more fluorescent enrichment in the pancreas. Lastly, when we injected eGLP1-LNP into NOD mice, only the 6-week-old NOD mice exhibited increases in Cy5 fluorescence, whereas 9-week-old NOD mice displayed lower or similar fluorescence levels compared to untargeted LNPs.

Conclusions

eGLP1-functionalized nanoparticles show improved specificity for the pancreas and practical ability to deliver functional mRNA cargo. Our results also suggest that the effectiveness of eGLP1-LNP delivery likely depends on the state of T1D progression and severity. Based on these and ongoing studies, we anticipate the eGLP1-LNPs may be effective vehicles to deliver nucleic acid therapeutics to β-cells in human islets and mouse models of T1D.

52. Barbara Ehall (Medical University of Graz)

Abstract Title

Automated detection of insulin-, glucagon- and somatostatin granules in electron micrographs using a convolutional neural network

Authors

Barbara Ehall, Lea Bogensperger, Dagmar Kolb, Clemens Harer, Thomas R Pieber

Purpose

Increasingly, evidence is mounting for the role beta cells play in triggering their autoimmune destruction in type 1 diabetes. To investigate the role of insulin granules, we trained a convolutional neural network (CNN) on transmission electron microscopy (TEM) images to detect mature insulin granules as well as glucagon and somatostatin granules. We applied the trained CNN onto whole islet electron micrographs obtained using STEM (scanning transmission electron microscopy) of C57BL/6 mice and further on also non-diabetic NOD mice. Our goal was to generate an automated, high throughput system for detecting granules in endocrine islet cells to make quantification of electron micrographs possible.

Methods

The TEM data set consisted of 32 TEM images of C57BL/6 mice with pixelwise annotations to indicate glucagon, insulin and somatostatin granules. A subset of 23 images were used to train the CNN, where a pre-trained DeepLabV3 model with a ResNet-50 backbone was used to obtain semantic masks. Subsequently, individual granule instances for all three semantic classes were extracted from the masks using a post-processing pipeline based on the marker-based watershed algorithm. This allows for a more in-depth analysis including automated granule counting.
While the CNN and the post-processing algorithms were trained and calibrated exclusively on the training images, they were then evaluated on separate nine test images.
The proposed pipeline can further be applied to STEM images by histogram matching and adapting the resolution. First, the transfer to STEM images of C57BL/6 mice was evaluated, followed by analysing STEM images of NOD mice.

Summary of Results

We successfully trained a CNN on TEM images to automatically detect insulin-, glucagon- and somatostatin-containing granules. When applied on STEM images of whole islets (of C57BL/6 mice), the CNN and post-processing pipeline detected around 90% of all mature insulin granules with slightly lower accuracy for glucagon and somatostatin granules due to the lower number of these events in the training set. We present a side-by-side comparison of whole islet electron micrographs of one C57BL/6 and one NOD mouse in addition to the generated masks.

Conclusions

The presented CNN-based method of granule detection makes quantification in whole islet electron micrographs possible and generates masks and data tables for each granule type. In the future, we want to apply it on electron micrographs of NOD islets in various stages of beta cell destruction and further on apply the presented method on the available nPOD nanotomy dataset.

53. Giovanna Bossi (Immunocore Ltd)

Abstract Title

Development of a β-cell targeted Immune modulating PD-1 Bispecific Agonist to suppress immune cells and treat Type 1 Diabetes

Authors

Adam P. Curnock, Matthew W Becker, Mollie K Huber, David X Overton, Veronica Gonzalez, Fiona McCann, Susan Aungier, Nitha Mulakkal, Andrece Powell, Todd M Brusko , Edward A. Phelps, Tara M Mahon, Giovanna Bossi

Purpose

Localized immune-mediated destruction of beta cells is a hallmark of type 1 diabetes. To-date, the majority of therapeutic approaches has involved systemic immunosuppression which carries risks particularly in the young population. We sought to develop a tissue targeted localised immunomodulatory molecule, called ImmTAAI, to protect beta cells from T cell attack but preserving the systemic immune response.

Methods

To create a β-cell specific ImmTAAI, an affinity-enhanced T cell receptor (TCR) specific for the peptide-HLA-A*02 complex of pre-pro-insulin PPI15-24 was fused to an agonistic PD-1 antibody. The impact of the β-cell bound PD-1 ImmTAAI was evaluated using a variety of assays that quantify TCR signalling, antigen-specific T cell effector and functional responses in co-culture experiments. To test the targeting to β-cells in tissue, live human pancreas slices obtained through nPOD were incubated with CF647 labeled PD-1 agonist ImmTAAI together with AF568 labeled anti-ENTPD3 to label beta cells. Furthermore, slices from a nPOD donor with T1 diabetes at onset were treated with PD-1 ImmTAAI and the mobility of T cells was tracked by time-lapse microscopy.

Summary of Results

In co-culture experiments, the ImmTAAI was able to suppress TCR signaling at picomolar concentrations and demonstrate modulation of inflammatory cytokine secretion in addition to protection of β-cell killing by autoreactive CD8+ T cell clones. Importantly, this PD-1 agonist ImmTAAI did not inhibit T cells when free in solution. The molecule is active only in the presence of β-cells since no binding or activity was detected with antigen negative cell lines. Confocal microscopy of live pancreas tissue slices from non-diabetic and diabetic nPOD donors showed that PPI PD-1 ImmTAAI co-localized strongly with ENTPD3-labeled beta cells demonstrating specific binding to β-cells within the islets in a physiologically relevant setting. Finally, treatment of pancreatic slices from nPOD donor 6578, a T1 diabetic donor at onset, resulted in an increase of T cell mobility within the islets suggesting PD-1 ImmTAAI-mediated reduction of β-cell-T cell interaction.

Conclusions

We have generated a TCR bispecific inhibitory ImmTAAI molecule that binds specifically to β-cells to suppress autoreactive T cells and potentially to prevent pancreatic islets damage. Importantly, the molecule is inactive when free in solution and therefore has the potential to deliver localized immune suppression while avoiding systemic immunosuppression. These features make PD-1 agonist ImmTAAI molecules an attractive and novel approach to potentially treat T1D.

54. Nick Thomas (Royal Devon and Exeter Hospital)

Abstract Title

Pre-symptomatic adult type 1 diabetes case detection: type 1 diabetes genetic risk guided Islet autoantibody screening in a general population.

Authors

Nicholas Thomas, Anita Hill, Diane Fraser, Timothy McDonald, Richard Oram

Purpose

The FDA recently approved the first therapy for altering the clinical course of early-stage type 1 diabetes (T1D). However identifying individuals with asymptomatic T1D requires islet autoantibody screening. Measuring genetic risk for T1D identifies individuals with high T1D risk potentially allowing cost effective targeted islet autoantibody testing. Most screening studies of T1D have been performed in children, we aimed to assess the validity of using a T1D genetic risk to guide early T1D detection in adults.

Methods

The EXTEND study includes stored serum collected a median 10 years ago, in 7368 adults without diabetes. We calculated a T1D genetic risk score (T1DGRS) in all and selected the highest decile (≥13.3)(High-T1DGRS, n=711) and 726 controls selected irrespective of T1DGRS from the remaining participants. In these n=1437 we measured islet autoantibodies using a multiplex assay (GADA/ZNT8/IA-2A together) with positives confirmed by individually measuring GADA/ZNT8/IA-2A.

Summary of Results

Multiplex islet-autoantibodies were present in 3.0% (43/1437) and confirmed in 82% (36/44). Confirmed ≥1 positive Islet-autoantibodies were present 2.3 times more frequently in the high-T1DGRS group (3.5% (25/711) than controls (1.5% (11/726)[p=0.02]. Stage 1 T1D (defined as ≥2 positive Islet-autoantibodies) was detected in 1% (7/711) of the high-T1DGRS group but not in controls [p<0.01]. Separately of 510 islet-autoantibody confirmed adult onset T1D cases, 49% (248/510) had elevated T1DGRS (≥13.3) consistent with our high-T1DGRS group.

Conclusions

For the first time we show capturing T1D genetic risk can help detect early adult-onset T1D who may benefit from novel therapies. Screening 10% of adults at highest genetic risk could capture over half of future cases.

Type 1 Diabetes Etiology & Environment

55. Khyati Girdhar (Boston College)

Abstract Title

Deciphering the mechanism of a human gut commensal in the acceleration of Type 1 Diabetes Pathogenesis

Authors

Khyati Girdhar, Clarissa Howard, Alessandro Pezzella, Tomáš Hudcovic, Juan J. A. Henao, Mike Kiebish, Martin Schwarzer, Emrah Altindis

Purpose

Type 1 diabetes (T1D) is a chronic autoimmune disorder characterized by the destruction of pancreatic β-cells, resulting in insulin deficiency and subsequent hyperglycemia. While the gut microbiome has been associated with T1D development, there is no study indicating a causal link. Insulin protein epitopes, particularly the B-chain 9-23 epitope (insB:9-23), are the primary targets of the T-cells, stimulating an autoimmune response against β-cells. In our prior work (Girdhar et al., 2022, PNAS), we identified a bacterial peptide mimic of the insulin B-chain 9-23 epitope (insB:9-23), hprt4-18, derived from the N-terminal of the hypoxanthineguanine phosphoribosyltransferase protein in Parabacteroides distasonis, a human gut commensal. Hprt4-18 activates both human and NOD mice T-cells specific to insB:9-23 in. Upon reanalyzing the DIABIMMUNE microbiome data, we demonstrated that early-life exposure to this peptide increases the risk of seropositivity. Notably, colonization of female NOD mice's gut microbiome with P. distasonis accelerated T-cell infiltration into islets and elevated T1D incidence. The purpose of this study is to unravel how P. distasonis induces inflammation within islets or redirects T-cell infiltration.

Methods

NOD/ShiLtJ (NOD) mice (#Jackson Laboratory) were maintained and bred in the Boston College Animal Care Facility (Protocol No.#B2019-003 and 2019-004). P. distasonis bacteria were orally gavaged daily for four weeks to 3-week-old female NOD mice at a concentration of 108 CFU/mouse). Germ-free (GF) NOD mice experiments were performed at the Institute of Microbiology of the Czech Academy of Sciences. Mice were gavaged with a single dose of P. distasonis, and pancreata, intestines, and serum were harvested at 12 weeks of age. Intestinal intraepithelial lymphocytes were collected from female NOD mice (12 weeks) as described previously (Girdhar et al. 2023, Microbiome). Cells were incubated in fluorescently labeled antibodies (TCRβ, CD4, CD8, CD44, CD62L, CD19, CD11c+ CD11b+ F4/80) for 15 min in staining buffer followed by washing and analysis using flow cytometry (BD FACSAria III sorter). Gene expression analysis was performed by qPCR. Metabolite analysis was performed using LC-MS at the BPG Bio facility. The data were analyzed using FlowJo 10.0, GraphPad, and R software.

Summary of Results

Given the pivotal role of the intestines in homing and interacting with microbes, we investigated the direct effects of P. distasonis colonization on intestinal intraepithelial lymphocyte (IEL) composition. Remarkably, P. distasonis colonization led to a 1.72-fold reduction in CD4+, a 2.3-fold reduction in effector CD4+CD44+CD62L-, a two-fold reduction in central CD4+CD44+CD62L+ T-cell, and a 1.85-fold reduction in B-cell populations, indicative of decreased immune cell composition in the intestines. Interestingly, the gene expression of gut permeability markers, including Occludin, ZO-1, and Claudins family proteins, remained unchanged upon P. distasonis colonization. Additionally, P. distasonis did not induce an increase in the IgA response to fecal microbes upon colonization. However, P. distasonis colonization induces changes in serum metabolite composition; specifically, a 1.6-fold increase in a gut microbial molecular metabolite trimethylamine N-oxide (TMAO) was observed. Notably, NOD Germ-free (GF) mice colonized with P. distasonis exhibited increased severe insulitis, indicating that this effect is independent of other gut microbes. Upon analysis of the serum metabolite composition of P. distasonis-colonized GF mice in comparison to those administered saline, no significant increases in any metabolite were observed. This suggests that the escalation of severe insulitis in NOD mice is autonomous, independent of TMAO or other metabolites.

Conclusions

This study aims to unravel the mechanisms of P. distasonis-induced inflammation within islets, building upon our previous findings. In this study, we focus on elucidating the effects of P. distasonis-colonization on the IELs, gut permeability, and direct effects of the bacterium on GF NOD mice. The observed reduction in crucial immune cell populations in the small intestines suggests that P. distasonis colonization does not stimulate an unspecific inflammation in the gut. Notably, P. distasonis colonization did not alter the expression of gut permeability genes, indicating that insulitis induced by P. distasonis is independent of changes in intestinal barrier function.

The absence of an increased IgA response to fecal microbes suggests no specific impact of P. distasonis on mucosal humoral immunity. Moreover, the increase in TMAO expression indicates the potential role of a proinflammatory metabolite. Notably, the independent induction of insulitis in NOD Germ-free (GF) mice colonized with P. distasonis highlights its direct role in influencing T1D autonomously. In conclusion, P. distasonis colonization orchestrates intricate changes in immune cell infiltration to islets without compromising gut barrier integrity or gut immunity. Our findings provide robust support for the molecular mimicry mechanism hypothesis, highlighting the intricate role of P. distasonis in modulating intestinal immunity and influencing T1D development. By shedding light on these mechanisms, our research endeavors to provide a comprehensive understanding of P. distasonis's immunological impact on T1D pathogenesis.

56. Erin Templeman (University of Exeter)

Abstract Title

Sex differences in pre-stage 1 T1D Risk and Progression: Insights from the TrialNet Pathway to prevention study

Authors

Erin Templeman, Lauric Ferrat, Elizabeth Rideout, Nicholas Thomas, Lowri Allen, Maria Redondo, Carmella Evans-Molina, Jay Sosenko, Richard Oram, Emily Sims

Purpose

In contrast to most autoimmune diseases, male sex is a risk factor for type 1 diabetes (T1D). This raises the hypothesis that either immune, metabolic, or other differences between sexes may impact risk of T1D or progression through stages of T1D. We aimed to assess the risk and rate of progression for individuals in the TrialNet natural history study.

Methods

We studied 135,791 pediatric (<18 years old) and 99,795 adult relatives of people with T1D screened for autoantibody (AB) positivity in the TrialNet Pathway to Prevention (50% and 66% female, respectively). We estimated Kaplan-Meier survival curves per strata, and tested differences between strata with log-rank tests. Risk is stated as estimated 5-year risk [95% CI] for females and males respectively. Cox-proportional hazard models determined the effect of sex on the outcome, T1D, after adjusting for confounders.

Summary of Results

The proportion of individuals who screened positive for ABs was higher in males (females: 0.05 [0.049 – 0.051], males: 0.054 [0.053 – 0.056], $p < 0.001$). Of these individuals, males were more likely to screen positive for multiple ABs (females: 0.018 [0.017 – 0.018], males: 0.026 [0.025 – 0.027], $p < 0.001$). The impact of sex on screening was most prominent in children (pediatric: AB positive females: 0.051 [0.049 – 0.052], AB positive males: 0.058 [0.056 – 0.06], $p < 0.001$; adults: AB positive females: 0.049 [0.047 – 0.051], AB positive males: 0.048 [0.045 – 0.05], $p = 0.4$).

Overall risk of progression to T1D in autoantibody positive individuals was higher in males (females: 32% [30 – 34%], males: 42% [39% - 44%], $p < 0.001$). This was particularly true in single AB positive individuals (5 year risk in females: 14% [12 – 16%], males: 21% [18 – 23%], $p < 0.001$), whereas males and females with stage 1 T1D (at least 2 ABs) (females: 38% [33 – 42%, males: 38% [33 – 42%]) or stage 2 T1D (AB and dysglycaemia) (females: 57% [54 – 60%], males: 59% [62 – 56%]) had similar 5 year T1D risk.

After adjusting for the presence of a first-degree relative with T1D, age, and stage of T1D, the risk of T1D remains significantly increased in all individuals (hazard ratio = 1.21, $p < 0.0001$) where males were more likely to progress to T1D in children and adults.

Conclusions

Male relatives of people with T1D are more likely to screen positively for AB and are more likely to screen positive for multiple AB. Males have higher progression risk if single AB positive but have similar risk compared to females when presenting with multiple ABs. Antibody positive males have an increased risk of progression to T1D compared to females, regardless of age. We do not currently have islet biology or immune system explanations for this difference, but it is interesting that the clearest difference in risk between sexes occurs before stage 1. This analysis suggests the importance of incorporating sex in assessment of risk progression, especially early in the natural history of T1D.

57. Luciana Mateus Gonçalves (University of Miami)

Abstract Title

Changes in islet vasculature during the pathogenesis of type 1 diabetes

Authors

Luciana Mateus Gonçalves, Mirza Muhammad Fahd Qadir, Maria Boulina, Madina Makhmutova, Elizabeth Pereira, Joana Almaça

Purpose

In type 1 diabetes (T1D), the autoimmune destruction of insulin-producing beta cells leads to impaired glucose homeostasis and chronic hyperglycemia. However, not only beta cells are affected, but significant anatomical and functional alterations of the islet vasculature are also detected in pre-symptomatic stages. The aim of this study was to identify the cellular and molecular mechanisms underlying these vascular defects. Because pericytes comprise the islet vascular network along with endothelial cells and are crucial for vascular stability and function, we hypothesized that changes in islet pericyte phenotype and function accompany the development of T1D.

Methods

We compared the density, phenotype and function of pericytes in islets from non-diabetic organ donors (ND, n=9), with those from single autoantibody positive donors (GADA+, n=7) and recent onset type 1 diabetic donors (T1D duration 0-4 years, n=8) by immunohistochemistry and calcium imaging of living pancreas slices from nPOD. Living slices were incubated with a membrane permeant calcium indicator (Fluo-4), and pericytes and endothelial cells were visualized with a fluorescent antibody against the pericyte marker NG2 (neuron-glial antigen 2) or with a fluorescent lectin, respectively.

Summary of Results

Our data show that islet pericyte and capillary responses to vasoactive stimuli were impaired early on in T1D. In particular, vascular responses to changes in extracellular glucose levels or to the sympathetic agonist norepinephrine were abolished in GADA+ and T1D donors, but blood vessels remained responsive to the vasoconstrictor endothelin-1. We further found that islet vascular dysfunction was associated with a switch in the islet pericyte phenotype towards a myofibroblast-like cell. Using publicly available RNA sequencing (RNA-seq) databases, we found that transcriptional alterations related to endothelin-1 signaling, vascular and extracellular matrix (ECM) remodeling were hallmarks of pancreata from GADA+ donors.

Conclusions

Our data show that pericytes are dysfunctional at early stages of islet autoimmunity. Because islets need their vasculature to function properly, vascular dysfunction can compromise hormone release into the circulation and contribute to the development of T1D.

58. Harshraj Shinde (University of Kansas Medical Center)

Abstract Title

Transcriptomic Dynamics in NOD Mice During the Transition from a Pre-Diabetic to a Diabetic State

Authors

Harshraj Shinde, Joseph M. Feduska, KaLia Burnette, Hubert M. Tse

Purpose

Type 1 diabetes (T1D) arises from the immune attack on insulin-producing pancreatic β cells. As established by prior research, the main culprits behind β cell destruction are CD4+ and CD8+ T cells. T1D could be the product of a confluence of genetic factors and external agents, like viruses, which may act as triggers for the disease. The development of T1D still lacks comprehensive understanding. NOD (Non-Obese Diabetic) mice are valuable animal models as they naturally develop T1D. To investigate the genes and metabolic pathways associated with the progression of T1D, we conducted RNA sequencing on RNA isolated from the islets of NOD mice at 6 weeks, 10 weeks, 14 weeks, and 20 weeks of age. Our study illustrates the chronological transcriptional signatures of T1D in the NOD mouse, ranging from 6 weeks of age (pre-diabetes) to 20 weeks of age (diabetes).

Methods

We conducted RNA sequencing on RNA isolated from the islets of NOD mice at 6 weeks, 10 weeks, 14 weeks, and 20 weeks of age. Our study illustrates the chronological transcriptional signatures of T1D in the NOD mouse, ranging from 6 weeks of age (pre-diabetes) to 20 weeks of age (diabetes). The quantification of the expression of transcripts of RNA sequencing data were performed using the tool salmon. Using the "clust" method for automated clustering on 34,426 transcripts

Summary of Results

The quantification of the expression of transcripts of RNA sequencing data were performed using the tool salmon. Using the "clust" method for automated clustering on 34,426 transcripts, we identified 10 clusters, each exhibiting unique expression patterns across different age groups of NOD mice. Cluster 1, which encompasses 715 co-expressed genes, represents the rise in gene expression as the transition occurs from the pre-diabetes stage to the diabetes stage. The pathway analysis of cluster 1 shows Th17 cell differentiation and T cell receptor signaling as most significant pathways. Cluster 9 of 624 co-expressed genes, signifies the decrease in gene expression as the transition advances. The pathway analysis of cluster 9 highlights chemical carcinogenesis and steroid hormone biosynthesis as the most significant pathways.

Conclusions

The candidate genes and metabolic pathways identified in this study offer a foundation for the future investigation of T1D and the markers associated with its disease progression.

59. Yuval Dor (The Hebrew University - Hadassah Medical School)

Abstract Title

Modeling heterogenous islet inflammation

Authors

Liza Zamashanski, Shani Peleg, Maya Israeli, Roy Novoselsky, Roni Cohen-Fultheim, Chunhua Dai, Klaus Kaestner, Shalev Itzkovitz, Erez Levanon, Alvin Powers, Agnes Klochendler, Yuval Dor

Purpose

One little understood hallmark of type 1 diabetes is the heterogenous pattern of islet inflammation, whereby intact islets are observed adjacent to islets that are heavily inflamed. We have generated a mouse model that mimics early T1D-associated insulitis. We are interrogating this system to understand the molecular processes that may lead to heterogenous islet destruction.

Methods

We have established a mouse model for deficient RNA editing, using knockout of the RNA editing gene Adar in pancreatic beta-cells to model the interferon-mediated inflammatory response associated with early Type I diabetes (T1D). We used immunofluorescent staining of pancreatic sections to examine inflammation heterogeneity. To assess the interferon response at a single cell level, we performed single-cell RNA-sequencing and achieved spatial resolution using single molecule RNA in situ-hybridization.

Summary of Results

We found that deletion of the RNA editing enzyme Adar in beta cells causes an accumulation of double-stranded RNA (dsRNA), which triggers a massive interferon response followed by insulitis and beta-cell destruction, eventually leading to diabetes. Strikingly, despite efficient disruption of RNA editing in beta cells, islet inflammation is asynchronous. Some islets are infiltrated and destroyed within few days, while others remain intact for a long time.
Using single cell RNA sequencing and RNA in situ hybridization, we found that only a small subset of beta cells mounts an interferon response. These cells are concentrated in individual islets, which are targeted for

rapid infiltration and destruction. With time, more islets turn on an interferon response and are destroyed. Interestingly, preliminary experiments indicate that hyperglycemia induced by an insulin receptor antagonist accelerates the onset of insulitis and amplifies its severity. This suggests that beta cell metabolic activity is a determinant of the heterogenous islet interferon response and consequent insulitis.

Conclusions

The asynchronous and heterogenous insulitis seen in mice with Adar-deficient beta cells is reminiscent of observations in the pancreas of recently diagnosed T1D patients. We propose that the underlying mechanism involves a positive feedback loop, wherein potential metabolic cues within a few neighboring beta cells modulate their transcription landscape, promoting the accumulation of dsRNA structures. This results in the production of type 1 interferon, rapidly leading to massive, islet-wide interferon response followed by infiltration and islet destruction. While our findings indicate that beta-cell metabolic activity regulates the onset of interferon response and islet inflammation, it remains unclear why and when a given islet is drawn into this cycle, both in Adar-deficient mice and in human T1D.

60. Liza Zamashanski (The Hebrew University of Jerusalem)

Abstract Title

Impaired RNA editing in beta or alpha cells models differential sensitivity of these cell types in Type I diabetes

Authors

Liza Zamashanski, Shani Peleg, Ehud Knebel, Chunhua Dai, Roni Cohen-Fultheim, Klaus Kaestner, Erez Levanon, Alvin C. Powers, Agnes Klochendler, Yuval Dor

Purpose

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

Methods

We have generated a mouse model for deficient RNA editing, using knockout of the RNA editing gene Adar in pancreatic beta and alpha-cells. We have used fluorescent immunostaining of pancreatic sections and standard functional assays to characterize islet inflammation and alpha and beta-cell phenotype following Adar disruption. We have FACS-sorted mutant alpha and beta-cells and performed RNA-sequencing to assess the interferon response triggered by disrupted RNA editing in each cell-type.

Summary of Results

Disruption of Adar specifically in insulin-producing beta-cells of young mice causes massive islet inflammation, beta-cell destruction and diabetes. Remarkably, alpha cells are spared amidst this detrimental inflammation, reproducing beta-cell specific death typically associated with autoimmunity in T1D. Moreover, targeted inactivation of Adar in alpha-cells does not provoke an immune response or any metabolic changes. Consistently, while Adar-deficient beta-cells elicit a robust interferon response, Adar-deficient alpha cells show only a minimal response. Experiments in isolated pancreatic islets and in-vivo demonstrate that, analogous to the effect of glucose flux in beta-cells, palmitate in alpha-cells enhanced the interferon response induced by Adar disruption via calcium signaling. Finally, we demonstrate that Adar-mutant alpha cells exhibit cell-autonomous reduction in glucagon levels. This is in contrast to Adar-deficient beta-cells, which lose insulin in an inflammation-dependent manner.

Conclusions

Alpha cells exhibit a remarkably reduced sensitivity to RNA editing disruption compared with beta cells. The exquisite specificity of the beta-cell innate immune response and destruction in response to RNA editing disruption is reminiscent of the beta-cell-specific attack in T1D. This phenomenon may shed light on the unique involvement of beta cells in autoimmune diabetes.

61. Agnes Klochendler (Hebrew University of Jerusalem)

Abstract Title

Disrupted RNA editing in beta cells mimics early-stage type 1 diabetes

Authors

Udi Knebel, Shani Peleg, Chunhua Dai, Roni Cohen-Fultheim, Benjamin Glaser, Erez Y. Levanon, Alvin C. Powers, Agnes Klochendler, Yuval Dor

Purpose

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

Methods

We have generated a mouse model for deficient RNA editing, using knockout of the RNA editing gene Adar in pancreatic beta and alpha-cells. We have used fluorescent immunostaining of pancreatic sections and standard functional assays on isolated islets to characterize islet inflammation and beta-cell phenotype following Adar disruption. To elucidate the molecular basis for islet inflammation in mutant mice, we have performed bulk RNA-sequencing on FACS-sorted beta-cells.

Summary of Results

Defective RNA editing in mouse beta-cells strikingly recapitulates key features of early T1D: a strong interferon response triggered by dsRNA, causing massive insulitis; disrupted expression programs in beta-cells culminating in beta-cell death and diabetes, while alpha-cells remain unaffected. Furthermore, we discovered that the interferon response in both mutant and wild-type beta-cells depends on cellular metabolic activity, specifically through calcium signaling. As a result, enforced hyperglycemia in Adar-mutant mice enhanced the inflammatory phenotype.

Conclusions

Our findings indicate that deficient editing of dsRNA in islets leads to heterogenous islet inflammation and destruction, resembling features of early-stage T1D. The results also suggest a potential vicious cycle whereby metabolic stress in damaged islets enhances the interferon response, further boosting inflammation and islet cell dysfunction towards diabetes.

62. Amber Luckett (University of Exeter)

Abstract Title

Genome wide association studies of HLA stratified type 1 diabetes identifies differences in pathways to disease progression.

Authors

Amber Luckett, Carolyn Mcgrail, Gareth Hawkes, Stephen Rich, Michael Weedon, Kyle Gaulton, Richard Oram

Purpose

Type 1 diabetes is characterised by the development of autoantibodies such as GADA and IAA. The DR3-DQ2.5 (DR3) HLA haplotype is associated with GADA-first type 1 diabetes, whilst DR4-DQ8.1 (DR4) is associated with IAA-first type 1 diabetes. There are not sufficient numbers of individuals with either GADA or IAA defined to perform a Genome Wide Association Study (GWAS), but given the association with DR3 and DR4 respectively, we aimed to test if there was evidence for differential genetic associations when a large GWAS case-control cohort is stratified by DR3/DR4 status.

Methods

We performed the first large-scale GWAS for type 1 diabetes stratified by DR3 (n=1598 cases, n=1826 controls) and DR4 (n=1909 cases, n=1267 controls) status from the Type 1 Diabetes Genetics Consortium. Genetic variants were imputed using the TOPMed Imputation Server. We performed analyses adjusted and unadjusted for the type 1 diabetes genetic risk score. We also compared associations in DR3 vs. DR4 carriers for known type 1 diabetes variants.

Summary of Results

We identified 3 associations with both DR3 and DR4 type 1 diabetes in the INS, PTPN22 and Class 2 HLA-DQB1 loci, at genome-wide significance (P<5x10-8). When adjusted for SNPs in the 67 SNP Type 1 Diabetes Genetic Risk score model (T1D-GRS2), we found differences of 2 SNPs and 8 SNPs at borderline genome-wide significance (P<5x10-6) for DR3/DQ2.5 and DR4/DQ8.1 stratified type 1 diabetes, respectively. We identified one type 1 diabetes associated variant, CENPW with opposite effects when stratified by HLA status. The effect of other known type 1 diabetes associated variants was similar in both the DR3 and DR4 cohorts.

Conclusions

We have performed the first GWAS for type 1 diabetes stratified by DR3 and DR4 status. The majority of previously identified type 1 diabetes associated variants have a similar impact on risk when stratified by HLA status. Our finding of a difference in CENPW risk contribution, highlights there may be potential differences in progression to type 1 diabetes (dependent on DR3/DR4 status) which may elucidate pathways that are differentially important to autoimmunity initiation. In future work, these could be examined experimentally in individuals with known IAA or GADA-first autoimmunity. We are currently expanding our analysis in 8 additional cohorts to identify further associations.

63. Miri Stolovich-Rain (The Hebrew University - Hadassah Medical School)

Abstract Title

Extensive elimination of acinar cells during normal postnatal pancreas growth

Authors

Miri Stolovich Rain, Ori Fridlich, Shira Azulai, Agnes Klochendler, Shira Anzi, Judith Magenheim, Ilan Stein, Fatima Mushasha, Benjamin Glaser, Eli Pikarsky, Danny Ben – Zvi, Yuval Dor

Purpose

While programmed cell death plays important roles during morphogenetic stages of development, post-differentiation organ growth is considered an efficient process whereby cell proliferation increases cell number. However, there is little evidence regarding the extent of cell death during postnatal development of most organs, largely because of the difficulty in quantifying dying cells that are rapidly cleared. We designed experiments to assess if cell proliferation can fully account for the observed increase in pancreas size during postnatal life of mice and humans.

Methods

Organ growth is a function of the change in cell size and cell number, and cell number is determined by proliferation and death rate. Thus, a measurement of organ weight, cell size and proliferation rate should allow inference of cell death, assuming that extracellular matrix volume is negligible. We obtained samples of the human and mouse pancreas during early postnatal development, measured organ size, acinar cell size, acinar cell proliferation and acinar cell death, and calculated the expected vs observed rate of organ growth.

Summary of Results

We demonstrate that early postnatal growth of the pancreas unexpectedly involves massive acinar cell elimination. Measurements of cell proliferation and death in the early postnatal human pancreas relative to the actual increase in cell number predict that each 100 dividing cells give rise to only 112 live progeny. Using mouse models, we show that death is associated with mitosis, through a failure of dividing cells to generate two viable daughters. In p53-deficient mice acinar cell death and proliferation are reduced, while organ size is normal, suggesting that p53-dependent developmental apoptosis triggers compensatory proliferation to robustly achieve proper organ size.

Conclusions

Postnatal pancreas growth involves surprisingly extensive acinar cell turnover. We propose that excess cell turnover facilitates robustness to perturbations and supports maintenance of tissue architecture.

64. Anna Lang (University of Miami)

Abstract Title

Targeting alpha cells to prevent hypoglycemia in type 1 diabetes

Authors

Anna Lang, Julia Panzer, Alejandro Caicedo

Purpose

Type 1 diabetes is characterized by a targeted immune attack of pancreatic beta cells. Their destruction results in dysregulated glucose homeostasis. One defense against this is the pancreatic alpha cell and their ability to secrete glucagon, which lowers blood glucose levels. However, in Type 1 diabetes, the alpha cell becomes blind to changes in glucose levels, due in part to the loss of inhibitory signals released by the beta cells and the increased need for glucagon secretion. Hypoglycemia is a life-threatening event for patients with Type 1 diabetes. Therefore, the goal of this project is to determine how to reset the pancreatic alpha cell to respond to a lowering in glucose before hypoglycemic events occur in patients. We hypothesize that stimulating alpha cells with inhibitory compounds will restore the alpha cells' response to hypoglycemia in Type 1 diabetes.

Methods

We therefore wanted to determine how to rescue the alpha cell's response to changes in glucose concentration by using alpha cell antagonists and determine glucagon secretion in both non-diabetic and Type 1 diabetes isolated islets. We used dynamic perifusion assays to determine hormone secretion in response to changes in glucose concentrations and to inhibitory stimuli (serotonin, GABA, and somatostatin).

Summary of Results

In non-diabetic islets, stimulation with serotonin, GABA and somatostatin all caused a decrease in glucagon secretion followed by an increased level of secretion compared to baseline. This effect is also observed with application of high glucose concentrations. Interestingly, in Type 1 diabetic islets, the application of the alpha cell antagonists causes a dramatic increase in glucagon secretion followed by sustained elevated glucagon levels.

Conclusions

We conclude that application of inhibitory stimuli does allow the alpha cell to recover and subsequently to secrete glucagon. Indeed, we show that providing alpha cells with inhibitory stimuli restores glucagon secretion in Type 1 diabetes islets. However, future studies are needed to determine the mechanisms involved.

65. Vikash Chandra (Helsinki University)

Abstract Title

The type 1 diabetes gene IFIH1 regulates anti-viral responses differentially in pancreatic alpha and beta cells

Authors

Sophia Forsskahl, Hazem Ibrahim, Jouni Kvist, Solja Eurola, Timo Otonkoski, Vikash Chandra

Purpose

IFIH1 encodes the melanoma differentiation associated protein 5 (MDA5) which is responsible for the detection of cytosolic dsRNA virus to initiate an antiviral response in infected cells. Recent evidence suggests that direct enteroviral infection of the islet may be an important trigger for T1D pathogenesis, where insulin-producing beta cells are primarily destroyed by an autoimmune response. Loss-of-function variants of IFIH1 confer protection against T1D, while gain-of-function variants causes type I interferonopathy. Notably, MDA5 is predominantly expressed in the alpha cells in the human islet. This study aims to elucidate the differential role of MDA5 in the inflammatory response to dsRNA in human alpha vs. beta cells.

Methods

We generated IFIH1-knock-out (IFIH1KO) iPSC lines using CRISPR-Cas12a and differentiated them to stem cell derived islets (SC-islets). To better understand the islet cell type specific responses, we performed single-cell RNA sequencing (scRNA-seq) on SC-islets after 24h treatment with PolyI:C, a synthetic double-stranded RNA. Additionally, we also generated a gain-of-function IFIH1 knock-in iPSC line carrying the mutation E444G which has been shown to activate type I interferon signaling.

Summary of Results

Our results suggest that loss of MDA5 does not compromise the generation of glucose responsive SC-islets. However, treatment with PolyI:C revealed STAT1 mediated and MDA5 dependent induction of CXCL10 in the SC-islets. By employing scRNA-seq we found only a small subset of alpha and beta cells with high level of CXCL10 expression following PolyI:C treatment albeit STAT1 was up-regulated throughout all cell types. Notably, reactome pathway analysis confirmed decreased interferon signaling enrichment in IFIH1KO alpha ($p=5.2E-25$) and beta ($p=3.8E-27$) cell clusters. In parallel with this decrease, eukaryotic translation elongation pathway enrichment was remarkably up-regulated in IFIH1KO alpha ($p=4.8E-18$) but not in beta cell clusters. Additionally, the gain-of-function IFIH1E444G hiPSCs showed spontaneous high interferon signaling from early stages of differentiation. This could be suppressed by TYK2 inhibition.

Conclusions

These observations suggest that MDA5 is needed for the full execution of the interferon antiviral response of islet cells. Our model enables to further elucidate the differences in the antiviral responses between alpha and beta cell.

Pathology

66. Yi-Chun Chen (University of British Columbia)

Abstract Title

In situ single cell analysis indicates PC1/3 mis-expression and morphological changes in alpha cells during the course of T1D

Authors

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

Purpose

In type 1 diabetes (T1D), loss of beta cells and hyperglycemia have a deleterious impact on the islet microenvironment and alpha cells. It is known that the prohormone convertase enzyme PC1/3 can be mis-expressed in alpha cells upon glucose dysregulation. Here we aimed to provide an in-depth alpha cell morphological characterization, including the analysis of glucagon (GCG) and PC1/3 in non-diabetic (ND), autoantibody-positive (AAb+), type 1 diabetic (T1D) and type 2 diabetic (T2D) donors using confocal imaging and high-dimensional image analysis.

Methods

FFPE-pancreatic sections from 20 sex-, BMI-, and age-matched ND donors, 7 Aab+, 8 short-duration T1D (<5y; sT1D), 9 long-duration T1D (>15y; lT1D) and 6 T2D donors were examined (mean age 33.5±13.2 years). Confocal microscopy images from islets stained for GCG and PC1/3 (up to 40 islets/donor) were analyzed using QuPath. Islets were detected and two different alpha cell populations were classified: GCG+PC1/3+ and GCG+PC1/3-. Alpha cell area, cytoplasm and nuclear size, as well as whole islet GCG+ area were calculated. The proportion of GCG+ cells, PC1/3 and GCG staining intensity, and colocalization of PC1/3 with GCG at the single-cell level were also assessed.

Summary of Results

Cytoplasmic area was reduced in GCG+ cells (cell mean 93.2±2.3 µm2 vs. 8.7±2.5 µm2 vs. 73.6±2.5 µm2; ND vs. sT1D vs. lT1D), while nuclear area remained comparable among all donor groups. Islet GCG-positive area was increased in donors with T1D (22.9±1.6% vs. 41.6±3.4% vs. 39.8±2.8%; ND vs. sT1D vs. lT1D). The proportion of GCG+PC1/3+ cells was increased in sT1D, as well as T2D donors (34.1±3.0% vs. 58.6±5.0% vs. 53.9±3.1%; ND vs. sT1D vs. T2D). GCG+PC1/3- cell proportion was also increased in T1D donors (3.5±0.7% vs. 9.7±1.9% vs. 14.6±3.0%; ND vs. sT1D vs. lT1D). Average GCG staining intensity in alpha cells (GCG+PC1/3+ and GCG+PC1/3- cells) remained comparable across all donor groups. However, GCG staining intensity was significantly elevated in GCG+PC1/3+ cells. Moreover, we observed reduced PC1/3 expression in GCG+PC1/3+ alpha cells in lT1D donors (21.5±1.5% vs. 11.0±0.8%; ND vs. lT1D). Interestingly, colocalization of GCG with PC1/3 was increased in T1D donors (0.62±0.01 vs. 0.82±0.03 vs. 0.73±0.31; ND vs. sT1D vs. lT1D), hinting towards enhanced glucagon processing via PC1/3.

Conclusions

Alpha cells undergo functional and morphological changes during the course of T1D. We found increased GCG expression in PC1/3 expressing-alpha cells from T1D donors, and propose that increased GCG and PC1/3 colocalization may result in alternative processing of proglucagon to glucagon-like peptide 1 (GLP-1). A better understanding of PC1/3 expression and function in alpha cells in T1D may facilitate the identification of

additional prohormone biomarkers and inform the design of new therapeutic approaches that target alpha cell adaptation.

67. Yu Shen (Johns Hopkins University)

Abstract Title

3D Mapping of Human Pancreas for Studying Microanatomical Structures Influenced by Type 1 Diabetes at Cellular Resolution

Authors

Yu Shen, Mia Grahn, Won June (Kevin) Cho, Bridgette Kim, André Forjaz, Casey Grubel, Maria Beery, Irina Kusmartseva, Pei-Hsun Wu, Mark Atkinson, Denis Wirtz, Ashley Kiemen

Purpose

The prevalence of type 1 diabetes (T1D) is expected to increase worldwide due to improvements in testing and healthcare access [1]. T1D is an autoimmune disease associated with the malfunctions of the pancreas' endocrine component. For patients with T1D, the immune system attacks the insulin-producing beta cells in the islets of the pancreas and subsequently leads to uncontrolled blood glucose levels. The beta cell reduction in the islet is associated with the interactions among multiple anatomical structures of the pancreas, including the pancreatic acini, stroma, vasculature, and immune components. Determining the interplay between these structures and their role in T1D pathogenesis is critical for optimizing T1D treatments. However, current research is limited to the spatial contributions of a single or few anatomical components at a time [2]. As a result, we aimed to utilize a novel 3D tissue mapping platform, CODA [3], to study the multi-faceted spatial interactions of multiple structures at once.

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Methods

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

Summary of Results

Currently, we analyzed one sample from a newly diagnosed diabetic donor and one sample from a non-diabetic donor. The complete anatomical structures of two cm^3-sized human pancreas samples were reconstructed, and eight microanatomical components were distinctively segmented in the reconstruction model. Furthermore, the anatomical changes of the diabetic pancreas were quantified and compared to the healthy pancreas based on the volumetric compositions of each tissue type. Comparing two pilot samples, we discovered a total increase in bulk composition of islets, pancreatic ducts, vasculatures, fat, extracellular matrix, and nerves, and decreases in acinar content. The increase in the composition of islets of Langerhans can be caused by obtaining samples from different parts of the pancreas. This discovery emphasizes the need for investigating microarchitecture and

molecular variations in pancreatic compositions with the onset of diabetes. Cellular changes associated with immune inflammations were quantified based on the subcellular segmentation of alpha, beta, and delta cell compositions of healthy and diabetic islets, and the inflammation map revealed an elevation in immune content in the diabetic pancreas.

Conclusions

Integrating various histological staining results using CODA allows collective evaluation of the anatomical environment in T1D. The spatial analysis of diabetic and nondiabetic pancreases at tissue and cellular levels provides a comprehensive understanding of the disease progression in T1D patients. Current results indicate that advances in artificial intelligence systems can facilitate the discovery of autoimmune and pancreatic pathogenesis of T1D.

68. Ery Petropoulou (Helmholtz Center Munich)

Abstract Title

Dissecting the phenotype of HLA-I low, elevated and high islets before and after Type 1 Diabetes onset

Authors

Ery Petropoulou, Puñet Valls, José Zapardiel-Gonzalo, Teresa Rodriguez-Calvo

Purpose

High HLA-I expression is considered one of the hallmarks of T1D. Previous studies aiming to decipher its role showed that it is strongly associated with insulin-containing islets in T1D donors, and that interferons are powerful drivers of its expression. However, the magnitude and distribution of HLA-expression in the pancreas and in the T1D timeline have not been fully characterized. Here, using mean islet HLA-I fluorescence intensity as surrogate for expression, we systematically phenotyped and classified the islets according to HLA-I expression, immune infiltration, and beta cell content.

Methods

We stained human pancreatic tail sections for HLA-ABC, CD3, CD8, Insulin, Proinsulin, Glucagon and CD45. First, we acquired whole-slide images and analyzed entire pancreatic sections from 47 donors (10 non-diabetic-ND, 7 single autoantibody positive-sAAb+, 7 double autoantibody positive-dAAb+, 5 with recent–onset T1D-roT1D (disease duration <1 year), 7 with short duration T1D-sdT1D (1 to 5 years), and 6 with long duration T1D-ldT1D (>5 to 12 years). From the above, we generated quantitative results from more than 10000 islets at all disease stages. To avoid introducing bias by handpicking islets, we categorized the islets into high (H), elevated (E) and low (L) by setting distinctive intensity thresholds. Second, high resolution confocal images were acquired from 5 islets per HLA-I category and insulin cellular distribution was classified into clusters or spots based on pixel size, intensity, and spatial proximity. Overall, we extracted data regarding beta cell and alpha cell content, insulin cluster/spots numbers, area, and intensity, as well as density of CD45+, CD3+CD8-, and CD3+CD8+ cells. All these parameters were correlated to the level of HLA-I expression.

Summary of Results

Our data shows that the highest level of HLA-I expression is found in insulin-containing islets (ICIs) and poor insulin-containing islets (pICIs) from dAAb+ and T1D donors; its expression dramatically decreases when beta

cells are lost. Interestingly, beta cells of HLA-I elevated and high islets of T1D donors have comparable insulin cluster numbers, area, and intensity to ND donors, whereas beta cells of T1D HLA-I low islets have significantly decreased values for these parameters. Furthermore, HLA-I high islets are actively targeted by the immune system. Specifically, the number of CD45+, CD3+CD8- and CD3+CD8+ cells is higher in the HLA-I high islet and peri-islet areas of dAAb+ and T1D donors compared to low HLA-I islets.

Conclusions

In conclusion, HLA-I hyperexpression is a true hallmark of T1D that can be already detected in dAAb+, but is no longer present when beta cells are lost. The remaining beta cells of the elevated and high HLA-I islets of T1D donors seem to retain their cellular insulin content, since they have similar numbers of insulin clusters and comparable cluster area (size) and intensity to ND donors. On the other hand, the beta cells of HLA-I low islets of T1D donors show a significant decrease in all these parameters, pointing to malfunction in insulin synthesis or processing. Our data also demonstrates that islet HLA-I expression correlates with immune infiltration, indicating that these are synergistic events during T1D pathogenesis. Moreover, peri-islet areas are more heavily infiltrated than intra-islet areas, suggesting that the islet periphery is an active site of immunological crosstalk. Altogether, this indicates that the inflammatory environment of insulitis may enhance HLA-I expression and the presentation of beta cell antigens through HLA-I molecules. This information is critical to understand disease pathogenesis and progression, and to inform mechanistic studies directed towards identifying which beta cell antigens might be presented in the context of HLA-I. Based on these data, we hypothesize that strategies aiming at decreasing HLA-I expression may delay or avoid disease progression and preserve beta cell mass, alone, or in combination with strategies focusing on eliminating immune-mediated beta cell destruction.

69. Katy Murrall (University of Exeter)

Abstract Title

Leveraging knowledge from the human pancreas using AI-based image analysis on routine histological stained tissue from archival and contemporary biobank collections

Authors

Katy Murrall, Sephanie Hunter, Christine Flaxman, Lydia Russell, Matthew Palmer, Isabel Burn, James Shaw, Noel Morgan, Sarah Richardson

Purpose

The human pancreas is relatively inaccessible in living subjects, and its architecture and cellular composition are not readily assessed. This is problematic for studies of the immunopathology of type 1 diabetes (T1D), particularly in childhood, where fewer than 80 pancreata are available globally from children <10y with a short disease duration (<1y). Most of these were recovered between 30-50 years ago and are held within the Exeter Archival Diabetes Biobank (EADB). Consequently, much of the published research using more contemporary pancreas biobanks includes data from only a few young children with recent-onset T1D, who may experience a different disease course from those who are older at onset.
The pancreas grows exponentially during early postnatal life, with expansion of both endocrine and acinar mass requiring extensive pancreatic restructuring. The initial signs of islet autoimmunity observed in individuals at-risk of developing T1D often coincide with this expansion. This study aims to characterise the restructuring of the human pancreas throughout the life course, with a particular focus on control, islet AAb+ and T1D individuals (≤10y). Routinely stained tissue sections from multiple biobanks were studied using high-throughput AI-based image analysis pipelines.

Methods

AI-based image analysis pipelines (Indica HALO) were established using H&E and/or dual immunostained pancreas sections (anti-chromogranin A/cytokeratin 19) from within the EADB, nPOD, and MRC-QUOD (Total donor n = >200) biobanks. The leveraging of existing H&E and endocrine marker-stained sections aids conservation of these precious resources and expands the number of donors in which analysis can be performed. In donors with multiple pancreas regions available for analysis, comparisons were made between and within donors. Outputs include endocrine/exocrine area, islet size and density, % fat, fibrosis and the proportion of clustered islets.

Summary of Results

Analysis of the QUOD biobank enabled the study of 16 different regions throughout the pancreas. These were analysed to reveal that endocrine cell area, islet size and density vary significantly across the pancreas; thereby replicating and expanding earlier studies using fewer pancreas samples. Study of donors without diabetes aged between 0-2y (to correspond with early AAb seroconversion) and those aged 3-8y (for later AAb seroconversion) revealed a significant difference in endocrine and acinar area; endocrine:acinar ratio; islet density and the clustering of islets across these closely aligned age ranges. Analysis of donors with T1D revealed a significantly reduced endocrine area and islet density vs age-matched controls, and the reduction in islet density was most profound in donors diagnosed in their teens.

Conclusions

We have established a series of validated image analysis pipelines that can be used on contemporary and archival pancreas biobank sections prepared with routine histological stains (eg. H&E). This has allowed us to combine analyses from multiple biobanks, providing for an expansion of the number of donors across different age and disease categories, and producing an unprecedented analysis of the changes in pancreatic architecture occurring through life. These analyses will complement ongoing studies which are employing more targeted immunostaining to assess the abundance and distribution of specific cell populations in the human pancreas.