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General Information

Name Badges and Conference Materials: Please pick up your name badge and conference materials on Wednesday, May 21st from noon-2:00pm in the lower lobby of the Doubletree Hotel, UAB, 808 South 20th Street, Birmingham, AL 35205. MIC participants are requested to wear their name badge during all conference activities.

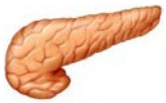
Scientific Oral Presentations: All oral presentations will take place in Heritage Room I at the Doubletree UAB hotel. Oral presentations will begin on Wednesday, May 21st at 2:00pm and conclude on Thursday, May 22nd at 6:00pm.

Poster Session: The poster session is on Wednesday, May 21st at 7:30pm and concludes at 9:30pm. Please hang posters in Heritage Room II on Wednesday between noon and 2pm. Please remove posters on Thursday, May 22nd by 10:00pm. The boards are numbered and reserved for your poster. Please see your poster number in this booklet in the poster presenter index.

Meals and Banquet: Registration fee includes meals and these will take place in the lobby and Centennial Rooms I/II at the Doubletree Hotel UAB. Please wear your name badge for all meals. Note that on Thursday, May 22nd, there will be a banquet dinner at 6:00pm.

Poster Awards: Prizes will be awarded to the presenters for what is adjudicated to be the best poster presentations – one 1st prize, two 2nd prize and three 3rd prize awards. These awards also come with supplemental travel awards to assist with the expense of attending the MIC 2014 meeting of ≤$500.00 for 1st prize; ≤$400.00 for 2nd prize; and ≤$300.00 for 3rd prize awards.

Oral Presentation Awards: Prizes will be awarded to the presenters for what is adjudicated to be the best oral presentations – a 1st, 2nd and 3rd prize award. These awards also come with supplemental travel awards to assist with the expense of attending the MIC 2014 meeting of ≤$500.00 for 1st prize; ≤$400.00 for 2nd prize; and ≤$300.00 for 3rd prize award. Further information as to how to implement these travel awards will be given to the poster prize awardees at the MIC 2014 meeting.



Cover Photo, “Human Pancreas-Shaped Mouse Islet” provided by Junqin Chen, Truman Grayson, and Anath Shalev, UAB Comprehensive Diabetes Center, University of Alabama at Birmingham.

The 7th Annual
Midwest Islet Club Meeting
May 21st-22nd, 2014
Birmingham, Alabama

The Midwest Islet Club Symposium Organizing Committee

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Christopher J. Rhodes, Ph.D.	University of Chicago
Leslie Satin, Ph.D.	University of Michigan
Anath Shalev, M.D.	University of Alabama at Birmingham
Roland Stein, Ph.D.	Vanderbilt University

Local Organizing Committee

Anath Shalev, M.D. (chair)
Meredith Preuss , Ph.D. (vice-chair)
Truman Grayson (website and administrative support)
Avni Doshi (administrative support)

Symposium Sponsors

We would like to express our gratitude to our sponsors:

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The Midwest Islet Club

The idea of the Midwest Islet Club (UMIC) began in 2007 out of a common perception by a number of established diabetes researchers in the Midwest region who felt that pancreatic islet researchers needed a forum outside of the traditional large conference format posed by the ADA annual meetings of 18,000 or more attendees. It was felt that there was a critical mass of young investigators at major research centers, in Ann Arbor, Chicago, Indianapolis, Madison, Minneapolis, Nashville, Pittsburgh, St. Louis, other cities, who would benefit from contact with one another and that more needed to be done to bring together researchers in industry and academia, on the one hand to foster collaborative and multidisciplinary research and on the other to serve as a venue for recruitment.

It was proposed to conduct annual meetings of two days' duration that would change in venue each year through major scientific and population centers each hosted by a point person at each site. The meetings are focused on basic research in islet cell physiology, biochemistry, development, growth and pathology with lesser emphasis on clinical diabetes and its current treatment (although clinical research central to islet function is encouraged). This year's host in Birmingham, Alabama is Dr. Anath Shalev, and our thanks go to Anath and her colleagues for organizing this year's MIC meeting.

The annual MIC meeting is rather unique in that students, postdoctoral fellows, and junior faculty give all of the oral and poster presentations. The only exception is the keynote address, the Lacy Medal Lecture in honor of Paul E. Lacy, M.D., who worked at Washington University in St. Louis and pioneered pancreatic islet isolation and transplantation. The Lacy Medal Lecture is given by an outstanding researcher who has made major contributions to islet research. This year's Lacy Medal Lecturer is Dr. W. Geoffrey Sharp from Cornell University Department of Pharmacology. We are pleased to have Geoff attend the 2014 MIC, a worthy recipient of the Lacy Medal.

The meeting schedule can be found in this book, together with a list of abstracts for the oral and poster presentations by the young investigators. The MIC Meeting is centered on junior investigators in the first year of their independent careers, postdoctoral fellows, graduate students, and technicians — not only to present their latest research, but also to ask questions and promote scientific discussions from the audience in a friendly, constructive environment that should spill over into the coffee breaks, meals, bar, and beyond — to form long-lasting, productive scientific collaborations in islet research. We hope that you enjoy this year's MIC meeting!

The MIC Organizing Committee

Lacy Medal Lecture

From the Artic to Bangladesh to the Islet

Geoffrey W. Sharp, Ph.D.

Dr. Sharp was born in Yorkshire, educated at Doncaster Grammar School and Nottingham University where he graduated at 19. His Ph.D. was done without a supervisor or mentor and was based on circadian rhythms. The work was performed during the first of two expeditions that he led to the Arctic island of Spitsbergen to take advantage of the 24 hour daylight and the ability to impose dark-light cycles at will.

In 1960 he left his lectureship at Nottingham University for a post-doctoral fellowship at the Middlesex Hospital Medical School in London and two years later came to the US for another post-doctoral position at the Massachusetts General Hospital. At the MGH he worked with Alexander Leaf, a great scientist, clinician and administrator, and a wonderful human being.

Dr. Sharp's research at that time was largely on the mode of action of aldosterone on transepithelial Na+ transport, with some studies on the actions of antidiuretic hormone and prostaglandins.

In 1970, he moved by accident into studies on the mode of action of cholera toxin when one of his students was about to join the NIH and go to work on cholera in Bangladesh. A few weeks after their discussions on the topic he showed that cholera toxin acted via the stimulation of adenylyl cyclase and continued to study cholera toxin for some time afterwards.

Islet studies also came about by accident! After planning a sabbatical leave in Bangladesh, the plans were dashed by the India/Pakistan war. At short notice he sought another sabbatic site. Albert Renold's Institut de Biochimie Clinique was suggested to him and though he knew nothing about islets or diabetes he applied and was accepted at the Institute.

This sabbatic lasted essentially for two years, but was extended for another 8 years as he flew to Geneva for stays of 2 – 3 weeks five or six times a year. He has studied b-cell and islet function ever since.

The Geneva connection ended in 1978 because of time constraints when he became chairman of the Physiology Department at Tufts University Medical School.

Only two years later, in 1980, he left Tufts and moved to Cornell University where he had the exciting opportunity to build a new Department of Pharmacology from scratch. Two years later he took on the additional responsibility of Director of the Division of Biological Sciences.

He has enjoyed sabbatic leaves with Albert Renold (Geneva), Yannick Le Marchand Brustel (Nice), Mark Dunne (Sheffield) and Reinhard Jahn (Gottingen).

SYMPOSIUM SCHEDULE
2014 MIC MEETING at the University of Alabama at Birmingham
Wednesday, May 21, 2014

Lower Lobby

12:00 – 2:00 pm Arrivals, Registration and Lunch.

Heritage Room I

2:00 - 2:15 pm **Welcome and Introduction: Anath Shalev** and **Meredith Preuss** (hosts)

2:15 - 3:30 pm ORAL SESSION-1 – **Developing, Regenerating & Growing New Islets – *in vivo* or *in test tubo***

(Moderator: Carmella Evans-Molina)

2:15 – 2:30 pm Corentin Cras-Méneur - Active Notch signaling controls the maintenance of the fate of the Ngn3 Progenitors through RBP-Jkappa in the developing pancreas

2:30 – 2:45 pm Iljana Gaffar - A Potential Therapeutic Role for MafA through Ectopic Expression in Pancreatic Ducts

2:45 – 3:00 pm Michael Lawrence - Differentiation of Pancreas-Derived Mesenchymal Stem Cells into Hormone-Producing Islet-Like Cell Clusters

3:00 – 3:15 pm John Wiersch - No Evidence for Bone Marrow Derived Stem Cell Contribution to Pancreatic Regeneration in a Mouse Parabiosis Model

3:15 – 3:30 am Gina M.Coudriet – Maturation Defects in NOD Macrophages Impacts Beta Cell Regeneration

3:30 - 4:00 pm Coffe/Tea Break - Lower Lobby

Heritage Room I

4:00 – 5:15 pm SESSION-2 **– Modes and Maladies of Gene Regulation in Islets**

(Moderator: Raghu Mirmira)

4:00 – 4:15 pm Brian McKenna - Identification and Characterization of Pdx1 Coregulators

4:15 –4:30 pm Justin S. Johnson - Pancreatic and Duodenal Homeobox Protein 1 Enhances Transcription of Sarcoendoplasmic Reticulum Calcium ATPase 2 in the Beta Cell

4:30 – 4:45 pm Chad S. Hunter - Ldb1-mediated transcriptional complexes during ß-cell development and function

4:45 – 5:00 pm KyungHee Hong - IL-1beta Regulates Beta-cell TXNIP Expression

5:00 – 5:15 pm Caitlin Orr - Identification of Tissue Specific Enhancer Elements in the Pancreatic Alpha Cell

5:15 – 6:00 pm Break

6:00 – 7:30 pm Dinner - Centennial Room I/II

7:30 – 9:30 pm POSTER SESSION and SOCIAL - **Heritage Room I/II**

Thursday, May 22, 2014

8:00 – 9:00 am Breakfast - **Lower Lobby**

Heritage Room I

9:00 - 10:15 am SESSION-3 – **Islet Factors, Functions and Metabolic Signals**

(Moderator: Roland Stein)

9:00 – 9:15 am Amelia K. Linnemann - Cholecystokinin is Directly Regulated by cAMP in Beta Cells

9:15 – 9:30 am Matthew J. Merrins - Phase Analysis of the Beta Cell Triggering Pathway Reveals Unexpected Feedback on Oscillatory Metabolism

9:30 – 9:45 am Kayla A. Boortz - G6PC2 Evolved to Modulate Insulin Secretion in Response to Stress

9:45 – 10:00 am Weiwei Xu - Androgen Receptor-Dependent Transcriptome Analysis in Pancreatic Beta Cells: Implications for Type 2 Diabetes Risk in Aging Men

10:00 – 10:15 am Ben A. Hall - Insulin-resistance and Decreased Beta-cell Mass in Beta-cell–specific IRS-2 Knockout Mice

10:15 - 10:45 am Coffee Break - Lower Lobby

Heritage Room I

10:45 – 12:00 pm SESSION-4 **– Oxidative and Inflammatory Stressing on Islets**

(Moderator: John Corbett)

10:45 – 11:00 am Ashley R. Burg - Superoxide Deficiency Hinders Anti-Viral Responses of Non-Obese Diabetic Macrophages during Diabetogenic Coxsackie B4 Virus Infection

11:00 – 11:15 am Katarzyna Broniowska - Location, Location, Location! It Is a Key Determinant in the Biological Actions of Free Radicals on Beta Cells

11:15 – 11:30 am Ting Zhang - FoxO1 Protects Beta-Cell against Oxidative Stress

11:30 – 11:45 am Bryndon J. Oleson - DNA Damage-Mediated Activation of ATM as a Trigger for Cytokine-Induced Apoptosis

11:45 – 12:00 pm Emily K. Anderson-Baucum - The Role of Macrophage Deoxyhypusine Synthase in the Progression of Obesity-Associated Metabolic Inflammation

12:00 - 1:30 pm Lunch - Lobby and Centennial Room I/II

Heritage Room I

1:30 – 2:45 pm SESSION-5 **– Potential Means of Fixing Type-1 Diabetes**

(Moderator: Les Satin)

1:30 – 1:45 pm Zunaira Z. Chaudhry - The Proinsulin/C-peptide Ratio and HSP90: Potential Biomarkers for Early Detection of Type 1 Diabetes

1:45 – 2:00 pm J. Stewart New - Modulation of Autoimmune Diabetes By Innate-Like B Cell Derived Antibodies Against N-Acetyl Glucosamine

2:00 – 2:15 pm Robert N. Bone - Fluoroketone-based Inhibition of the Ca2+-independent PhospholipaseA2beta in Neonates Reduces Incidence of Type 1 Diabetes

2:15 – 2:30 pm Lindsey E. Padgett - NADPH Oxidase-Deficient Macrophages Display a Dampened M1 Macrophage Phenotype in Type 1 Diabetes

2.30 – 2.45 pm Stephen Harrington - Allogeneic Islet Transplantation by Encapsulation in Bio-inspired Hydrogel: the Importance of Scaffold Geometry

2:45 - 3:15 pm Coffee/Tea Break - Lower Lobby

Heritage Room I

3:15 – 4:30 pm SESSION-6 **– Pathogenesis and Potential Means of Fixing Type-2 Diabetes**
(Moderator: Colin Nichols)

3:15 – 3:30 pm Mieke Baan - FoxM1 Overexpression in Pancreatic Beta Cells Increases Pancreatic Insulin Content and Lowers Plasma Glucose

3:30 – 3.45 pm Gu Jing - TXNIP induces IAPP expression via miRNA-124a and FOXA2

3:45 – 4:00 pm Damien Demozay - Ablation of FoxO3a, but not FoxO1, specifically in beta-cells causes beta-cells failure in mice exposed to a high calorie diet

4:00 – 4:15 pm Holly A. Cyphert - Characterization of Human MafA/B Activity in Pancreatic Beta Cells

4:15 – 4:30 pm Johanne H. Ellenbroek - Long-term Ketogenic Diet Causes Glucose Intolerance and Reduced Beta and Alpha-Cell Mass but No Weight Loss in Mice

4:30 - 4:45 pm Break

4:45 – 5:45 PM SESSION-7 **– THE 7th ANNUAL LACY MEDAL LECTURE**
(Moderator: Christopher Rhodes)

Geoffrey G. W. Sharp, Ph.D. (Cornell University)
'From the Artic to Bangladesh to the Islet'

6:00 - 8:00 pm Awards Banquet - Centennial Room I/II

8:00- 10:00 pm Poster Mingling and Entertainment

Friday, May 24, 2013

9:00 am – 12:00 pm Departures

ORAL PRESENTATIONS
– Heritage Room I –

SESSION 1

Wednesday, May 21st 2:15 - 3:30 pm

Developing, Regenerating & Growing New Islets – *in vivo* or *in test tubo*

Moderator: Carmella Evans-Molina

2:15 – 2:30 pm **Corentin Cras-Méneur** - *Active Notch signaling controls the maintenance of the fate of the Ngn3 Progenitors through RBP-Jkappa in the developing pancreas*

2:30 – 2:45 pm **Iljana Gaffar** - *A Potential Therapeutic Role for MafA through Ectopic Expression in Pancreatic Ducts*

2:45 – 3:00 pm **Michael Lawrence** - *Differentiation of Pancreas-Derived Mesenchymal Stem Cells into Hormone-Producing Islet-Like Cell Clusters*

3:00 – 3:15 pm **John Wiersch** - *No Evidence for Bone Marrow Derived Stem Cell Contribution to Pancreatic Regeneration in a Mouse Parabiosis Model*

3:15 – 3:30 am **Gina M.Coudriet –** *Maturation Defects in NOD Macrophages Impacts Beta Cell Regeneration*

Active Notch signaling controls the maintenance of the fate of the Ngn3 Progenitors through RBP-Jkappa in the developing pancreas.

Corentin Cras-Méneur[*1], Megan Conlon[1], Yaqing Zhang[1], Marina Pasca Di Magliano[1], Raphael Kopan[2], Ernesto Bernal-Mizrachi[1]

[*] Corresponding Author
[1]. University of Michigan, Ann Arbor, MI, USA
[2]. Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Notch signaling is known to control the earliest steps of pancreatic determination. The four membrane-bound Notch receptors mediate short-range cell-cell communications allowing neighboring cells to adopt different fates during embryonic development. The implication of this signaling pathway in later stages of pancreatic organogenesis remains vastly unexplored though.
In the presumptive pancreas, undetermined progenitors express Ptf1a, then Pdx1. During early organogenesis, a subset of cells escapes Notch activity, expresses Ngn3 and commits to the endocrine lineage. A previous publication demonstrated that Presenilins, a core component that proteolytically process notch and allow its activation, still played a critical role in the maintenance of the fate of these progenitors after Ngn3 is expressed.
Although Presenilins have multiple targets, we hypothesized that the maintenance of the endocrine fate was under the control of Notch signaling through the sequestration of the central Notch effector, RBP-Jkappa. Notch activity would be necessary to take the protein away from the competing Ptf1a complex and therefore prevent a conversion to the acinar fate.
Using genetic mice models with the conditional elimination of the individual Notch receptors in the Ngn3 progenitors and with lineage-tracing, we were able to demonstrate that Notch2 was the main contributor to the maintenance of the endocrine fate after Ngn3 is expressed.
Once the Notch receptors bind their ligands, they are cleaved by the Presenilins, allowing the intracellular domain to translocate to the nucleus and form a transcription factor complex with RBP-Jkappa. The conditional elimination of RBP-Jκ in Ngn3 progenitors provided additional insights into the molecular mechanisms involved in the control of early endocrine fate. In the absence of RBP-Jkappa, Notch signaling is ineffective, though lineage-tracing indicated that in this model, Ngn3 progenitors could still properly give rise to endocrine cells. Unexpectedly, a number of Ngn3, RBP-Jkappa deficient cells also seem to fail to properly differentiate, express very few markers of pancreatic differentiation in the adult and form pre-malignant masses.
These results dissect out the necessary molecular events that take place in early endocrine progenitors and demonstrate that, downstream of Notch, RBP-Jkappa must be unavailable in order to maintain the endocrine commitment.

A Potential Therapeutic Role for MafA through Ectopic Expression in Pancreatic Ducts

Iljana Gaffar*, John Wiersch, Ping Guo, Krishna Prasadan, Xiangwei Xiao, Chiyo Shiota, Zewen Song, George Gittes

Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania

Background: Limiting factors such as availability of a sufficient number of transplantable islets have led researchers to look for non-beta cell sources for the generation of new beta cells for diabetes therapy. Pancreatic ducts have been shown to undergo transdifferentiation into islet cells in vitro, both in rodents and in humans; however, previous in vivo studies have been controversial. Here, we show for the first time that Adeno-Associated Virus (AAV) mediated duct-specific ectopic expression of key factors can induce transdifferentiation of ductal cells into functional beta cells.

Methods and Results: Here we describe the expansion and conversion of adult mouse pancreatic ductal cells into insulin producing cells following Adeno-Associated Virus (AAV) mediated ectopic expression of pancreas-specific transcription factors;AAV carrying the expression construct is infused into the pancreatic duct following laparotomy. Specifically, we used a Sox9-promoter driving MafA alone (Sox9-MafA), or a combination of MafA with Ngn3 or Pax6 in adult mouse pancreatic ductal cells. With all constructs we observed rapid onset MafA gene expression, specifically in pancreatic ducts. When we infused either MafA and Ngn3, or MafA and Pax6 combinations into the pancreatic duct using a Sox-9 promoter, we saw pancreatic duct specific expression of those genes, resulting in insulin expression in duct cells. When the mice were treated with alloxan to induce diabetes prior to viral mediated gene expression, blood glucose levels were significantly elevated. However, after two weeks of ectopic expression of either MafA-Ngn3 or MafA-Pax6 gene expression combinations, we found that the blood glucose level in alloxan-treated mice returned to normal ranges. Interestingly, we found that if MafA is not included in the combination of expression vectors, the blood glucose in alloxan-treated mice remains high.

Conclusion: Our results suggest that gene therapy by ectopic expression of combinations of MafA-Ngn3 or MafA-Pax6 genes in pancreatic ductal cells using Adeno-Associated Virus mediation is a viable alternative for diabetes treatment. Our study also identified a critical role for MafA in the formation of pancreatic ductal cell derived insulin-positive cells.

Differentiation of Pancreas-Derived Mesenchymal Stem Cells into Hormone-Producing Islet-Like Cell Clusters

Faisal Kunnathodi[1], Nofit Borenstein[1], Mazhar Kanak[2], Rauf Shahbazov[1], Katheen McGlynn[3], Morihito Takita[1], Marlon F. Levy[4], Bashoo Naziruddin[4], Michael C. Lawrence[1]

[1] Islet Cell Laboratory, Baylor Research Institute, Dallas, TX
[2] Institute of Biomedical Studies, Baylor University, Waco, TX
[3] Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX
[4] Annette C. and Harold C. Simmons Transplant Institute, Baylor University Medical Center, Dallas, TX

Mesenchymal stem cells have been shown to have the capacity to protect islet cells from immune assault and improve islet engraftment. In this study, we isolated human mesenchymal stem cells from pancreatic tissue obtained from organ donors and chronic pancreatitis patients undergoing pancreatectomy. The pancreatic-derived mesenchymal stem cells (PMSCs) were positive for expression of surface markers CD29, CD73, CD90, and CD105; negative for CD14, CD34, and CD45; and capable of adipocyte and chondrocyte differentiation. Moreover, PMSCs formed cell aggregates in culture which had capacity to differentiate into glucose-responsive insulin-producing cells. Differentiation of PMSC aggregates into insulin-producing cells correlated with sequential induction of NFATc2, neurod1, neurogenin3, Nkx2.2, and MafA gene expression and was blocked by inhibition of calcineurin and NFAT signaling. Upon co-transplantation of a marginal dose of human islets into kidney capsules of streptozotocin (STZ)-treated diabetic nude mice, PMSCs restored normal blood glucose concentrations for up to 30 days. Surprisingly, upon nephrectomy, and thus removal of the human islet graft, the mice remained normoglycemic and maintained an intraperitoneal glucose tolerance test profile comparable to non-STZ-treated controls. Immunohistochemistry analysis revealed the presence of human insulin- and glucagon-producing islet-like cell clusters scattered throughout the pancreas of STZ-treated mice 30 days after human islet/PMSC co-transplantation. Overall, the data indicate that human PMSCs have the capacity to 1) differentiate into insulin-secreting islet-like cell clusters *in vitro* and 2) migrate from an islet/PMSC co-transplant site to the native damaged/inflamed pancreas in STZ-treated mice to differentiate into hormone-producing islet-like cell clusters and restore islet endocrine function.

No Evidence for Bone Marrow Derived Stem Cell Contribution to Pancreatic Regeneration in a Mouse Parabiosis Model

John Wiersch, Xiangwei Xiao, Chiyo Shiota, Ping Guo, Iljana Gaffar, Zewen Song, Krishna Prasadan, George Gittes

Affiliation: University of Pittsburgh / Children's Hospital of Pittsburgh, Department of Surgery, Division of Pediatric Surgery, Pittsburgh, PA

Background: Several studies in the literature have investigated the ability of circulating stem cells to differentiate into pancreatic cells in mice, however all have been dependent on lethal irradiation and bone marrow transplantation to establish blood cell chimerism. In our study, we utilized parabiosis to establish blood cell chimerism without the significant adverse effects of irradiation.

Methods: Wild type mice were parabiosed to mTmG mice of the same genetic background. The mTmG mouse cells show red fluorescence at baseline detectable by microscopy and flow cytometry. After parabiosis, any fluorescent cells detected in the wild type mouse's pancreas are derived from circulating stem cells. Robust blood chimerism was confirmed 2 weeks after parabiosis by flow cytometry. At that time, the animals received either a partial pancreatectomy or pancreatic duct ligation (PDL) to cause pancreatic injury and incite regeneration. Mice were harvested four weeks after surgery and the pancreas was analyzed histologically.

Results: Nearly all mice developed 50% blood chimerism two weeks after parabiosis. Analysis of the pancreas two weeks after parabiosis showed no fluorescent pancreatic cells. No bone marrow derived cells were present in the partial pancreatectomy or PDL mice pancreata four weeks after surgery.

Conclusion: Bone marrow derived circulating stem cells do not contribute to pancreatic regeneration following partial pancreatectomy or PDL in a parabiosis model.

Maturation Defects in NOD Macrophages Impacts Beta Cell Regeneration

Gina M. Coudriet[1], Angela Criscimanna[2], Farzad Esni[2], and Jon D. Piganelli[1]

Children's Hospital of Pittsburgh of UPMC [1]Department of Pediatrics, Division of Immunogenetics, [2]Department of Surgery. Pittsburgh, PA, USA.

In Type 1 Diabetes (T1D), self-reactive $CD4^+$ T cells drive the autoimmune destruction of pancreatic beta cells by activating macrophages to a cytotoxic phenotype (M1) hindering beta cell repair potential. It has been shown that endogenous beta cell growth can occur if the inflammation is tempered. In fact, a more controlled inflammatory response appears to be necessary to induce the regenerative process, characterized by a transition of macrophage phenotypes from inflammatory (M1) to anti-inflammatory (M2). Macrophages from the autoimmune NOD mouse are known to have a number of maturation defects. Therefore, we hypothesize that in autoimmunity, defective macrophages fail to transition from the M1 to M2 phenotype upon beta cell destruction, which is necessary to initiate the repair program. We adoptively transferred NOD.*scid* mice with BDC-2.5-TCR-Tg splenocytes to cause T1D. Mice receiving a $CD4^+$ T cell-depleting antibody (GK 1.5) had a restricted inflammatory response and delayed T1D onset. Ten days after CD4 depletion, we observed low levels of M1 macrophage genes (*inos*, *ccl2*, *il6*, and *tnfalpha*) in pancreatic tissue, while M2-macrophage gene expression (*arg1*) was maintained. However, control mice demonstrated a persistent M1 phenotype ($IL\text{-}6^+$ and $TNFalpha^+$ macrophages) overwhelming the $IL\text{-}10^+$ M2 macrophage lineage. Beta cell maintenance is also achieved through beta cell expressed growth factors, which is facilitated through the help of alternatively activated M2 macrophages. Indeed, in pancreatic injury we observed that autoimmune mice have decreased growth factor expression, including hepatocyte growth factor (HGF), which was reduced compared to non-autoimmune mice. These macrophage defects may render beta cells unable to initiate replicative programs. Therefore, we propose that addition of exogenous growth factors, such as HGF, to the islet will provide the necessary cues from macrophages for beta cell growth and function.

SESSION 2

Wednesday, May 21st 4:00 – 5:15 pm

Modes and Maladies of Gene Regulation in Islets

Moderator: Raghu Mirmira

4:00 – 4:15 pm **Brian McKenna** - *Identification and Characterization of Pdx1 Coregulators*

4:15 –4:30 pm **Justin S. Johnson** - *Pancreatic and Duodenal Homeobox Protein 1 Enhances Transcription of Sarcoendoplasmic Reticulum Calcium ATPase 2 in the Beta Cell*

4:30 – 4:45 pm **Chad S. Hunter -** *Ldb1-mediated transcriptional complexes during ß-cell development and function*

4:45 – 5:00 pm **KyungHee Hong** - *IL-1beta Regulates Beta-cell TXNIP Expression*

5:00 – 5:15 pm **Caitlin Orr -** *Identification of Tissue Specific Enhancer Elements in the Pancreatic Alpha Cell*

Identification and Characterization of Pdx1 Coregulators

Brian McKenna, Roland Stein

Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN

The transcription factor Pdx1 makes critical contributions to the identity and proliferative capacity of newly forming beta-cells. In differentiated beta-cells, Pdx1 takes on a new role to preserve the mature cell phenotype by maintaining the cellular machinery required for glucose-stimulated insulin secretion in addition to repressing the alpha□cell program. ChIP-chip and ChIP-seq experiments coupled with microarray have demonstrated that Pdx1 directly regulates expression of over one thousand genes in the mature beta-cell. Like many other transcription factors, Pdx1 relies on its ability to recruit coregulator proteins to mediate its transcriptional action. However, to date only a handful of Pdx1 coregulators have been identified. In light of this, we hypothesized that the list of known Pdx1-interacting factors represents a mere subset of the coregulators recruited to mediate Pdx1 action. We therefore set out to identify additional Pdx1 coregulators. Using the RE-CLIP co-immunoprecipitation technique coupled with mass spectrometry, we identified a substantial number of novel Pdx1-interacting factors with a wide range of cellular functions. Among these factors were multiple members of the ATP-dependent chromatin-remodeling complex, Swi/Snf. At its core, the complex harbors either of two mutually exclusive enzymatic ATPase subunits, Brg1 or Brm, which utilize the energy generated from ATP-hydrolysis to mobilize histones and modulate transcription. We show Brg1 serves as a critical coactivator of Pdx1 targets such as *Insulin II, MafA* and *Glut2*, whereas Brm appears to have indirect, yet opposing function, repressing *Insulin II*, *Glut2,* and *MafB.* Using the Proximity Ligation Assay (PLA) method, we examined PDX1:SWI/SNF complex formation in normal and T2DM human pancreas sections. Strikingly, in T2DM samples we observed nearly twice as many islet beta-cells devoid of PDX1:BRG1 complexes, and >50% reduction in the average number of PLA signals/ins+ cell. Together, these findings illustrate the Swi/Snf complex is critical to Pdx1 action in beta-cells and the loss of complex formation may play a key role in the altered PDX1 activity associated with beta-cell dysfunction observed in T2DM.

Pancreatic and Duodenal Homeobox Protein 1 Enhances Transcription of Sarco-endoplasmic Reticulum Calcium ATPase 2 in the Beta Cell

Justin S. Johnson*[1], Tatsuyoshi Kono[2], Wataru Yamamoto[3], Xin Tong[3], Matthew J. Merrins[4], Leslie S. Satin[4], Patrick Gilon[5], and Carmella Evans-Molina[1,2,3,6,7]

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While the homeobox transcription factor Pdx-1 is known to play an indispensable role in normal beta cell development and secretory function, recent data also implicate Pdx-1 in the maintenance of endoplasmic reticulum (ER) function. Beta cell ER function is dependent on the activity of the Sarco Endoplasmic Reticulum Ca^{2+} ATPase 2b (SERCA2b) pump to maintain a steep Ca^{2+} gradient between the cytosol and ER lumen. In diabetic rodent and human islets, our data demonstrate loss of Pdx-1 expression that occurs in parallel with alterations in SERCA2b expression and activity, and *in silico* analysis of the SERCA2b promoter revealed multiple putative Pdx-1 binding sites. Here, we hypothesized that Pdx-1 loss under glycotoxic and inflammatory conditions leads to dysregulated SERCA2b expression and concomitant alterations in ER Ca^{2+} homeostasis. To test this hypothesis, adenoviral overexpression of Pdx-1 in NIH-3T3 cells increased SERCA2b mRNA and protein levels, while siRNA-mediated knockdown of Pdx-1 in INS-1 cells caused a 60% reduction in SERCA2 protein. Similar results were observed in islets isolated from Pdx-1 haploinsufficient mice. To assess the impact of Pdx-1 loss on ER Ca^{2+}, INS-1 cells were transduced with an ER-targeted D4ER adenovirus as well as Pdx-1 siRNA, and fluorescence lifetime imaging microscopy was performed. ECFP donor lifetime increased significantly from 1.66 ± 0.022 ns under control conditions to 1.80 ± 0.020 ns with Pdx-1 knockdown, consistent with reduced ER Ca^{2+}. In parallel, Fura 2-AM imaging showed that cytosolic Ca^{2+} levels significantly increased after Pdx-1 loss, consistent with a leak of ER Ca^{2+}. To determine if Pdx-1 regulates SERCA2b transcriptionally, luciferase assays were performed in NIH-3T3 cells. Co-transfection of human Pdx-1 with a SERCA2 promoter luciferase construct led to a three-fold increase in promoter activity relative to control, and direct binding of Pdx-1 to the proximal SERCA2 promoter was confirmed by chromatin immunoprecipitation. To test whether SERCA2b could reverse the ER stress phenotype of Pdx-1 deficiency, Pdx-1 haploinsufficient mice were fed a 45% high fat diet for 8 weeks. Islet SERCA2b levels were further reduced compared to wild-type littermate controls, and Pdx-1 haploinsufficient islets had increased expression of spliced XBP1, consistent with activation of ER stress signaling. Restoration of SERCA2b reduced spliced XBP1 levels to that of wild-type controls. These results indicate that SERCA2b is a transcriptional target of Pdx-1, and further suggest that Pdx-1 deficiency under diabetic conditions may be a causal factor in dysregulated ER Ca^{2+} homeostasis in beta cells.

Ldb1-mediated transcriptional complexes during b-cell development and function

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Transcription factors are critical for regulating genes involved in pancreatic endocrine cell fate determination and mature beta-cell function. Islet biologists are developing strategies to produce transplantable beta-cells *de novo* that improve diabetes outcomes. Success will require a clear understanding of endogenous gene regulatory mechanisms that drive functional beta-cell development. This includes continued studies of islet-enriched transcription factors plus increased appreciation for interacting coregulators. We have shown that the LIM-homeodomain Islet-1 (Isl1) transcription factor is required for late islet endocrine cell development and survival, which largely requires interaction with the LIM domain binding protein 1 coregulator, or Ldb1 [1-3]. In other tissues, Ldb1 and Isl1 form complexes with additional factors to impact target gene transcription, yet very few binding partners have been described in beta-cells. To address this, I utilized a crosslinked-immunoprecipitation/mass spectrometry approach, termed ReCLIP [4], to enrich and identify proteins directly interacting with Ldb1 and Isl1. Mass spectrometry datasets revealed numerous interacting candidates that may participate in beta-cell Ldb1::Isl1 complexes, including a class of Single-Stranded DNA Binding Proteins (e.g. SSBP2-4). SSBPs are coregulators that potentiate Ldb1::LIM factor target gene *trans*-activation and complex stability in other tissues [5, 6]. However, nothing is known of SSBP expression or activity in pancreatic islets. Western blotting and immunofluorescence analyses demonstrated that at least SSBP3 appeared to be enriched in beta-cell lines, developing endocrine cells and in mouse islets. Future studies will enlist beta-cell lines and pancreas tissue to test the significance of SSBP3 (and other) coregulators to the regulation of Ldb1 or Ldb1::Isl1 target genes, including *MafA*, *Glut2*, *Glp1r* and *Insulin*. I will also examine SSBP occupation of target promoters by ChIP, perform reporter gene experiments testing SSBP activity, plus analyze SSBP impact on Ldb1::LIM stability in beta cells. This project will further elucidate the components and activity of Ldb1::Isl1 complexes, which are required for beta-cell development, maturation and function.

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4 Smith, A.L.*, et al.* (2011) ReCLIP (reversible cross-link immuno-precipitation): an efficient method for interrogation of labile protein complexes. *PLoS One* 6, e16206
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IL-1beta Regulates Beta-cell TXNIP Expression

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In type 1 diabetes the autoimmune reaction and the release of proinflammatory cytokines (interleukin (IL)-1beta, tumor necrosis factors (TNF) alpha, and interferon (IFN) gamma) leads to beta-cell destruction and apoptosis. Recently, we identified thioredoxin-interacting protein (TXNIP) as an important regulator of beta-cell apoptosis and found that its deletion prevented type 1 and type 2 diabetes. TXNIP is highly induced by glucose via the carbohydrate response element binding protein (ChREBP), but the effects of cytokines on TXNIP expression have remained largely unknown. We therefore cultured INS-1 beta-cells with a cocktail of IL-1beta (1 ng/ml), TNFalpha (5 ng/ml) and INFgamma (5 ng/ml) under low (5 mM) and high (25 mM) glucose conditions and measured TXNIP by quantitative real-time RT-PCR. Cytokine cocktail treatment increased TXNIP expression at 5 mM, but not at 25 mM glucose. Interestingly, we found that this discrepancy was due to IL-1beta, which up-regulated TXNIP mRNA at 5 mM glucose, but down-regulated TXNIP at 25 mM glucose. To investigate the molecular mechanism involved in this surprising IL-1beta effect at high glucose, we performed luciferase assays using INS-1 beta-cells transfected with a TXNIP promoter-driven reporter plasmid containing different truncations and mutations. Consistent with the observed decrease in TXNIP mRNA, a 6.7-fold reduction in TXNIP promoter activity was observed with IL-1beta treatment at 25 mM glucose. Mutation of the E-box ChREBP binding site in the TXNIP promoter blunted this effect, indicating that IL-1beta may inhibit TXNIP expression through ChREBP. Indeed, IL-1beta treatment resulted in a decrease in ChREBP nuclear localization and ChREBP binding to the TXNIP promoter at 25 mM glucose as assessed by nuclear fractionation and ChREBP chromatin immunoprecipitation (ChIP). Taken together, these results reveal for the first time that cytokines have differential effects on pro-apoptotic beta-cell TXNIP expression and that the effects are dependent on the concomitant glucose concentration.

Identification of Tissue Specific Enhancer Elements in the Pancreatic α Cell

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Gene expression profiles preliminarily define the identity of specific cell types. Physiologically alpha cells of the islets of Langerhans are known primarily for the secretetion of the hormone glucagon. While the significance of glucagon is relatively well understood, the transcriptional network that defines the alpha cell remains unclear. The mechanism by which the alpha cell maintains its unique identity is dependent on cell-type specific gene expression. DNA enhancer elements found throughout the genome, aiding in the expression of many genes, regulates this mode of cell-type specific expression. The identificiation of such elements for alpha cells will allow an improved understanding of the mechanisms that define the character of these cell types. To identify these elements, we have performed ChIP-seq analysis for known markers of DNA enhancer elements in alpha cell lines. ChIP of mono-methylated Histone 3-Lysine 4 has revealed approximately 75,000 putative enhancer elements in alpha cells. These 75,000 elements were reduced to 1,200 putative enhancer elements that show association with evolutionarily conserved DNA sequences and are not found is control cell types. Of these 1,200 putative elements, 300 were found within regulatory range of genes enriched in alpha cells versus beta cells. From this final subset of putative enhancers, 30 have been screened in a luciferase expression assay for selective enhancer activity in alpha cell lines versus non alpha cell lines**.** Finally these elements were scrutinized for alpha cell specific expression in zebrafish. In elucidating DNA elements that are able to drive alpha cell specific transcription will be able to develop novel tools for the study of these cells in vivo. Furthermore the identification and characterization of these tissue specific enhancer elements will allow for better understanding of transcription factor activity that defines an alpha cell.

SESSION 3

Thursday, May 22nd 9:00 - 10:15 am

Islet Factors, Functions and Metabolic Signals

Moderator: Roland Stein

9:00 – 9:15 am **Amelia K. Linnemann** - *Cholecystokinin is Directly Regulated by cAMP in Beta Cells*

9:15 – 9:30 am **Matthew J. Merrins** - *Phase Analysis of the Beta Cell Triggering Pathway Reveals Unexpected Feedback on Oscillatory Metabolism*

9:30 – 9:45 am **Kayla A. Boortz** - *G6PC2 Evolved to Modulate Insulin Secretion in Response to Stress*

9:45 – 10:00 am **Weiwei Xu** - *Androgen Receptor-Dependent Transcriptome Analysis in Pancreatic Beta Cells: Implications for Type 2 Diabetes Risk in Aging Men*

10:00 – 10:15 am **Ben A. Hall** - *Insulin-resistance and Decreased Beta-cell Mass in Beta-cell–specific IRS-2 Knockout Mice*

Cholecystokinin is Directly Regulated by cAMP in Beta Cells

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The hormone cholecystokinin (CCK) plays an important role in digestion and satiety. CCK is turned on in pancreatic beta cells in obesity and transgenic overexpression protects beta cells from streptozotocin-induced death. Similarly, CCK knockout results in increased apoptosis. Therefore its upregulation in obesity plays a critical role in beta cell mass preservation. However, the direct mechanisms of CCK transcriptional regulation in the islet are unknown. In the beta cell, cAMP plays a critical role in glucose metabolism and insulin secretion making it a key player in obesity response. Furthermore, in neuroendocrine cells of the gut, cAMP can activate *Cck* transcription. The combined cyclic AMP response element (CRE)/ 12-O-tetradecanoylphorbol-13-acetate (TPA) response element (TRE) in the *Cck* promoter is necessary for this activation. We now show that expression of *Cck* is rapidly increased by a cAMP analog (8-CPT-cAMP) in INS-1 cells (>7-fold within 4 hours). There is a delayed increase in expression of the CCK A receptor (*Cckar*) suggesting *Cck* autoregulation. *Cck* is also upregulated by cAMP in both mouse and human islets. *Cck* upregulation by cAMP is not glucose dependent, although the response is blunted (to ~4-fold) in the presence of low glucose (2.3 mM). Basal *Cck* is at least partially dependent on cAMP as treatment of INS-1 with Sulprostone, an inhibitor of cAMP signaling, causes a >50% reduction in *Cck* transcript levels.

Since cAMP regulates many genes through direct binding of the transcription factor CREB to the CRE, we analyzed binding of activated CREB (phosphorylated at serine 133) at the *Cck* gene promoter through chromatin immunoprecipitation (ChIP). As expected, levels of p-CREB in the cells are highly upregulated after cAMP treatment. Preliminary analysis demonstrates a recruitment of both RNA polymerase II and p-CREB to the CRE within 4 hours of cAMP treatment. Interestingly, this same CRE/TRE element is also required for c-Jun and c-Fos mediated activation of *Cck* in neuroblastoma cells, and the enzyme that catalyzes CREB phosphorylation ($p90^{RSK}$) also catalyzes Fos phosphorylation. Considering the massive increase in insulin production – and thus ER stress – after cAMP treatment, it is likely that stress also plays a role in *Cck* regulation. In line with this, expression of the ER stress response gene *Chop* is upregulated after cAMP treatment, yet cell viability is not altered. Treatment of INS-1 with GLP-1, which also signals through cAMP, increases *Cck* ~2 fold yet does not upregulate *Chop*. Taken together, we demonstrate that *Cck* is directly regulated by cAMP to protect beta cells from ER stress induced apoptosis. Furthermore, we can now hypothesize that GLP-1 upregulates CCK in obesity and enables preservation of beta cell mass.

Phase Analysis of the Beta Cell Triggering Pathway Reveals Unexpected Feedback on Oscillatory Metabolism

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Introduction and Objectives: Stimulus-secretion coupling in pancreatic beta cells, the process by which glucose catabolism drives oscillatory ATP/ADP, action potentials, and insulin secretion, involves the coordinated interaction of key ionic and metabolic variables. Our goal was to take advantage of new optical probes developed by our lab and others to monitor glycolytic and mitochondrial activity in real time to systematically evaluate the relationship between metabolism, islet electrical activity, and insulin secretion.

Methods: Patch-clamp measurements of islet electrical activity were conducted simultaneously with optical measurements of glycolysis (PKM2 activity; PKAR), mitochondrial redox potential (NADH/ flavin fluorescence), cytosolic ATP/ADP (Perceval), cytosolic calcium (Fura2), ER calcium (D4ER), and insulin secretion (ZIMIR).

Results: In 10 mM glucose, beta cell electrical activity was evident as oscillations between silent and active phases having a period of 3-5 minutes. The silent phase (V_m @ -60 mV) progressed as expected, with a slow buildup in glycolytic activity followed within 30 s by rises in mitochondrial redox potential and ATP/ADP; calcium and insulin secretion during this time were both low and stable. Unexpectedly, triggering of the active phase (V_m ≥-40 mV), which was accompanied by sharp increases in intracellular calcium and secretion, immediately suppressed glycolytic PKM2 activity, which fell monotonically throughout the active phase. Mitochondrial redox potential also fell at this time, although with a significant delay relative to glycolysis. Persistent membrane hyperpolarization with diazoxide (V_m @ -73 mV) stimulated glycolytic and mitochondrial flux at glucose concentrations ≥10 mM, while depolarization with diazoxide/KCl (V_m @ -40 mV), which caused a calcium increase, was inhibitory.

Conclusions: The pattern of mitochondrial changes observed during islet action potential firing is consistent with a short-lived calcium activation of mitochondrial dehydrogenases that was quickly overcome by short-circuiting of the mitochondrial membrane potential. Furthermore, the fall in PKM2 activity, NADH/Flavin, and ATP/ADP throughout the active phase suggests that while persistently high ATP/ADP triggers the firing of action potentials, it may not determine the active phase duration. Supported by R01DK46409 (L.S.).

G6PC2 Evolved to Modulate Insulin Secretion in Response to Stress

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Glucose-6-phosphatase (G6Pase) exists as a multi-component enzyme system that catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate (Pi). G6Pase is composed of a glucose transporter, a G6P/Pi transporter and a catalytic subunit, of which there are three isoforms, G6PC, G6PC2, and G6PC3.

G6PC2 is expressed exclusively in pancreatic islet beta cells where it opposes the action of glucokinase, thereby inhibiting glucose-stimulated insulin secretion (GSIS). Consistent with a role for G6PC2 in the regulation of GSIS, data from human genome wide association studies have linked single nucleotide polymorphisms (SNPs) in the *G6PC2* locus to variations in fasting blood glucose (FBG). Molecular studies have shown that *G6PC2* SNPs associated with increased expression are linked to elevated FBG. Because elevated FBG has been associated with both increased cardiovascular-associated mortality and risk for developing type 2 diabetes this suggests that G6PC2 is detrimental to health. We therefore hypothesized that G6PC2 evolved for a different purpose distinct from the regulation of FBG.

In this study we show that glucocorticoids induce human *G6PC2* promoter activity via a canonical glucocorticoid response element (GRE). Glucocorticoids also induce 129SvEv but not C57BL/6J mouse *G6pc2* promoter activity, an observation explained by a SNP that disables the GRE in the C57BL/6J *G6pc2* promoter. Consistent with this observation, glucocorticoids induce *G6pc2* expression in 129SvEv but not C57BL/6J isolated mouse islets *in vitro*. In contrast, glucocorticoids induce *Slc37a4* expression, which encodes the G6P/Pi transporter component of G6Pase, in both 129SvEv and C57BL/6J isolated mouse islets *in vitro*.

In order to determine if the induction of *G6pc2* by glucocorticoids affects metabolism, wild type (WT) and *G6pc2* knockout (KO) mice on the 129SvEv and C57BL/6J genetic backgrounds were fasted either 6 hr or 18 hr, the latter representing a physiological stress that results in weight loss and elevated glucocorticoid levels. Intraperitoneal glucose tolerance tests (IPGTTs) were then performed using 2.0 g/kg glucose to compare glucose tolerance in 6 hr fasted versus 18 hr fasted mice. Little difference in glucose tolerance was observed between C57BL/6J WT and KO mice after 6 or 18 hr fasts, though glucose tolerance was improved in both WT and KO mice after the 18 hr fast due to the presence of enhanced insulin sensitivity. In contrast, in 129SvEv mice, whereas little difference in glucose tolerance was observed between WT mice fasted for either 6 hr or 18 hr, glucose tolerance was improved in 18 hr fasted KO mice versus 6 hr fasted KO mice. We interpret these data to indicate that the induction of *G6pc2* expression in WT 129SvEv mice during the 18 hr fast, with the resulting decrease in GSIS, has been offset by the increase in insulin sensitivity associated with prolonged fasting, leading to no overall change in glucose tolerance. In contrast, glucose tolerance is improved in 18 hr fasted 129SvEv *G6pc2* KO mice because the increase in insulin sensitivity is not offset by an increase in *G6pc2* expression. These data suggest that G6PC2 evolved to modulate GSIS in response to stress.

Androgen Receptor-Dependent Transcriptome Analysis in Pancreatic Beta Cells. Implications for Type 2 Diabetes Risk in Aging Men

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Aging men with testosterone deficiency and men on androgen depletion therapy for prostate cancer have increased risk of developing type 2 diabetes (T2D). Although studies examining this issue have focused on the role of testosterone deficiency as a risk factor for insulin resistance, this approach ignores the role of testosterone deficiency as a potential cause of pancreatic beta cell dysfunction in men. While it has been established that testosterone action is mediated via the androgen receptor (AR), a ligand-activated transcription factor, the role of the AR in beta cell function is unknown. We have generated male mice lacking AR in beta cells ($BARKO^{-/y}$). These mice develop beta cell dysfunction with decreased glucose-stimulated insulin secretion (GSIS) ultimately leading to glucose intolerance. In cultured male pancreatic islets, ligand-activated AR stimulates GSIS via a cAMP-dependent protein kinase A (PKA) pathway. To examine AR-dependent gene expression in male islets, we performed a high throughput whole transcriptome sequencing (RNA-Seq) in islets from male control and $BARKO^{-/y}$ mice. We identified 312 differentially expressed genes (FDR < 0.05). Our analysis of individual transcripts revealed important alterations in beta cell genes involved in cellular bioenergetics and insulin secretion. For example, the expression of Pgc-1-alpha, important to mitochondrial biogenesis, was decreased by 4-fold. Also of interest was the observation that expression of the G-protein coupled receptors Gpr119 and Gpr26, which both signal via cAMP, were upregulated by 2-fold and 5-fold respectively, and that of fibroblast growth factor 21 was upregulated by 5-fold. In addition, the expression of Kcnq1, encoding a voltage-gated potassium channel critical for insulin secretion, was upregulated by 2-fold, and that of Trpc4, mediating the depolarizing currents, was downregulated by 3-fold. Moreover, the expression of hexokinase 2, which regulates glucose metabolism in beta cells, was upregulated by 3-fold. The pathway analysis revealed 23 significantly enriched pathways, such as cytokine-cytokine receptor interaction, Jak-STAT signaling, insulin signaling, MAPK signaling, T2D, and pancreatic secretion. The gene ontology analysis validated the results of the pathway analysis in showing enriched biological processes encompassing hormone secretion, inflammatory response, ion transport, and insulin secretion. In summary, AR is involved in GSIS in vivo and AR-deficient islets show altered expression of genes involved in insulin secretion and inflammation. This study identifies AR as a novel receptor enhancing beta cell function in the male with important implications for the prevention of T2D in aging men.

Insulin-resistance and Decreased Beta-cell Mass in Beta-cell–specific IRS-2 Knockout Mice

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Insulin receptor substrate 2 (IRS-2) is thought to play a pivotal role in β-cell survival and compensatory increases in beta-cell function and mass. To determine the role of IRS-2 in pancreatic β-cells, we generated β-cell–specific IRS-2 knockout mice using he using a Tamoxifen (TMX)-responsive MIP-CreER recombinase. Previous studies have demonstrated that MIP-CreER recombinase does not exhibit Cre-mediated recombination in the brain but specifically in pancreatic β-cells. We therefore investigated whether disruption of IRS-2 specifically in the β-cell is sufficient to induce diabetes.

At 8 weeks of age, mice were injected with TMX or some siblings injected with oil-vehicle as a control. A significant decrease in in IRS-2 expression was confirmed specifically in the β-cells of TMX-treated animals. Throughout the course of the study the β-IRS2-KO mice, maintained on a standard chow diet, had both normal body weight and fasted blood glucose levels relative to control mice. However, 8 weeks following TMX, fasted insulin levels were significantly elevated in β-IRS2-KO mice (2-3 fold; $p \leq 0.05$). Surprisingly, the β-IRS2-KO mice were significantly glucose intolerant and had marked peripheral insulin resistance at the age. At 12 weeks post TMX, β-IRS2-KO mice had less insulin resistance but worsening glucose-intolerance coupled to reduced insulin secretion suggestive of β-cell failure. Analysis of islets in pancreatic sections from these older β-IRS-2 KO mice by both immunofluorescence and immunohistochemistry revealed altered islet architecture and reduced β-cell numbers.

These data confirms the essential role of IRS-2 in the regulation of glucose homeostasis and maintenance of β-cell mass. The outcome of these studies has given us greater insight into the requirement of IRS-2 expression within the β-cell for β-cell survival and preventing the onset of type-2 diabetes.

SESSION 4

Thursday, May 22nd 10:45 – 12:00 pm

Oxidative and Inflammatory Stressing on Islets

Moderator: John Corbett

10:45 – 11:00 am **Ashley R. Burg** - *Superoxide Deficiency Hinders Anti-Viral Responses of Non-Obese Diabetic Macrophages during Diabetogenic Coxsackie B4 Virus Infection*

11:00 – 11:15 am **Katarzyna Broniowska** - *Location, Location, Location! It Is a Key Determinant in the Biological Actions of Free Radicals on Beta Cells*

11:15 – 11:30 am **Ting Zhang** - *FoxO1 Protects Beta-Cell against Oxidative Stress*

11:30 – 11:45 am **Bryndon J. Oleson -** *DNA Damage-Mediated Activation of ATM as a Trigger for Cytokine-Induced Apoptosis*

11:45 – 12:00 pm **Emily K. Anderson-Baucum** - *The Role of Macrophage Deoxyhypusine Synthase in the Progression of Obesity-Associated Metabolic Inflammation*

Superoxide Deficiency Hinders Anti-Viral Responses of Non-Obese Diabetic Macrophages during Diabetogenic Coxsackie B4 Virus Infection

Ashley R. Burg, Lindsey E. Padgett and Hubert M. Tse, PhD

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Type 1 diabetes (T1D) is defined by the autoimmune destruction of pancreatic beta-cells. While the events that initiate the autoimmune attack remain elusive, studies have suggested that pancreatic viral infections may trigger this destructive process. The innate immune anti-viral response can create an inflamed environment, producing reactive oxygen species (ROS) and pro-inflammatory cytokines. We recently demonstrated a critical role for NADPH oxidase (NOX)-derived ROS production in T1D pathogenesis, as superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are highly T1D-resistant. Interestingly, bone marrow-derived macrophages from these mice have reduced viral RNA sensing capacity against the viral dsRNA-mimic, poly(I:C). Therefore, we hypothesize that the absence of NOX-derived ROS will reduce the diabetogenicity of viral infections by dampening innate immune anti-viral responses that contribute to T1D and pancreatic beta-cell destruction. We performed both *in vivo* and *in vitro* viral infection studies with Coxsackie B4 virus (CB4), a hypothesized viral trigger of T1D in both humans and rodents. Following a 6-hour infection with CB4 *in vitro*, NOD.*Ncf1*^{m1J} macrophages displayed significant 1.4- and 2-fold decreases in protein expression of the viral RNA sensors, TLR3 and RIG-I, respectively, compared to NOD macrophages. By 24 hours this resulted in a 2-fold decrease (p<0.0005) in TNF-alpha production, and a dramatic 11-fold decrease (p<0.0001) in IFN-beta release by NOD.*Ncf1*^{m1J} macrophages. This defective response by NOD.*Ncf1*^{m1J} macrophages was recapitulated *in vivo*, as CB4-infected NOD.*Ncf1*^{m1J} mice had a 2.5-fold decrease in the percentages of both TNF-alpha- and IL-1beta-producing macrophages infiltrating the pancreas. These results suggest that depletion of ROS may curtail the viral-induced initiating events in T1D. Future studies will further define this role of ROS in potentiating the innate immune response to diabetogenic viral infections that trigger T1D onset.

Location, Location, Location! It Is a Key Determinant in the Biological Actions of Free Radicals on Beta Cells

Katarzyna Broniowska[1], Bryndon Oleson[1], Jennifer McGraw[1], Aaron Naatz[1], and John Corbett[1]

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Cytokines have been proposed to participate in the development of autoimmune diabetes by impairing beta cell function and inducing beta cell destruction. Nitric oxide, produced following the expression of the inducible nitric oxide synthase (iNOS), mediates the damaging actions of cytokines on function and viability of pancreatic beta cells. In addition to nitric oxide, excessive formation of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, has been shown to cause beta cell damage. The powerful oxidant peroxynitrite, produced from the reaction between nitric oxide and superoxide, has been suggested to be the mediator of beta cell death after exposure to cytokines. In this study the forms of ROS and reactive nitrogen species (RNS) produced in response to cytokine treatment have been determined. Also, we characterized the effects of each reactive species alone, and the effects of the interactions of these reactive species, on the function and viability of beta cells. Cytokine treatment of isolated islets or an insulinoma cell line results in the production of nitric oxide but does not stimulate the formation of peroxynitrite as assessed using the coumarin-7-boronate oxidation. Exposure to the NADPH oxidase activator phorbol 12-myristate 13-acetate (PMA) fails to stimulate superoxide generation that is consistent with the absence of peroxynitrite production by beta cells. In contrast to beta cells, macrophages produce peroxynitrite when stimulated to express iNOS and treated with PMA. When supplied using chemical donors, nitric oxide, superoxide and hydrogen peroxide impair mitochondrial oxidation and cause beta cell death. In contrast, peroxynitrite, supplied using the donor SIN-1, does not impair oxidative metabolism or decrease beta cell viability. Surprisingly, when nitric oxide-treated beta cells are forced to produce superoxide (via treatment with redox cycling agent menadione), mitochondrial oxidative metabolism and beta cell viability are preserved. Under these conditions superoxide interacts with nitric oxide to produce peroxynitrite. These exciting findings suggest that nitric oxide is the toxic species of RNS produced in response to cytokines and that superoxide functions to scavenge this radical and thereby prevents nitric oxide toxicity. The location of superoxide formation is essential for the protection against nitric oxide-dependent toxicity. When superoxide is generated in the extracellular medium (via xanthine oxidase system), peroxynitrite is formed but oxidative metabolism and beta cell viability are not preserved. These findings suggest that the location of radical generation and the site of radical reactions are key determinants in the effects of reactive species on beta cell function and viability. Extrapolating these results to islet inflammation during the development of autoimmune diabetes, these findings would predict that radical generation in beta cells might be a key determinant in impairment of function. The data obtained in this study support our previous reports showing that the damaging actions of macrophage activation on beta cells are mediated by the local generation of IL-1 within pancreatic islets and not reactive species production by these macrophages.

FoxO1 Protects Beta-Cell against Oxidative Stress

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Chronic oxidative stress is thought to be an underlying cause of glucolipotoxicity for beta-cell dysfunction in type 2 diabetes. FoxO1 is a forkhead transcription factor that plays a key role in mediating insulin/IGF-1 signaling in beta-cells. To determine the effect of FoxO1 on beta-cell anti-oxidative function, we generated beta-cell specific FoxO1 transgenic (FoxO1-tg) mice, in which FoxO1 is expressed from the rat insulin II promoter (RIP). We treated FoxO1-tg and control mice (male, n=10/group) with daily intraperitoneal injection of STZ (50 mg/kg) for 5 consecutive days. Such low-dose STZ regimen is known to elicit oxidative stress to beta-cells and induce insulin-deficient diabetes in mice. Prior to STZ administration, there were no significant differences in blood glucose levels between FoxO1-tg mice (112±6 mg/dL) and control littermates (128±9 mg/dL). STZ treatment resulted in severe hyperglycemia, impaired glucose tolerance and insulin deficiency in control mice. In contrast, blood glucose levels and glucose tolerance were maintained in the normal range, correlating with near normal plasma insulin levels in FoxO1-tg mice at 27 days after STZ treatment. No significant differences in body weight were seen between FoxO1-tg and control mice. To gain insight into the underlying mechanism, we showed that FoxO1-tg islets had significantly increased expression of superoxide dismutase (Sod1), catalase (Cat) and glutathione peroxidase (Gpx1), three key functions for defending oxidative stress. We recapitulated this finding in INS-1 cells, in which FoxO1 stimulated beta-cell expression of Sod1 and Cat. These effects correlated with FoxO1 binding to its target sites in the Sod1 and Cat promoters, as determined by ChIP assay. These data suggest that islets with increased FoxO1 activity were bestowed with enhanced anti-oxidative function and this effect protected FoxO1-tg mice from developing diabetes in response to STZ-induced oxidative stress.

DNA Damage-Mediated Activation of ATM as a Trigger for Cytokine-Induced Apoptosis.

Bryndon J. Oleson[1], Katarzyna A. Broniowska[1], Vera L. Tarakanova[2], and John A. Corbett[1]

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Type-1 diabetes mellitus (T1D) is an autoimmune disease characterized by selective destruction of the pancreatic beta-cells. The pro-inflammatory cytokine interleukin-1 (IL-1) is believed to participate in the development of T1D by inhibiting beta-cell function and inducing beta-cell death. The damaging effects of IL-1 on beta-cells are dependent on beta-cell expression of inducible nitric oxide synthase (iNOS) and generation of nitric oxide. This reactive species is responsible for the inhibition of oxidative metabolism and insulin secretion and the induction of DNA damage. The damage caused by short exposures to cytokines (0-24 h) is reversible if further nitric oxide production is prevented using an iNOS inhibitor and islets are allowed time to recover. This reversibility occurs in the presence of the cytokine, indicating that nitric oxide is the mediator of beta-cell damage. Longer exposures to cytokines (36 h and longer) result in irreversible cellular damage and a commitment of beta-cells to death. In this study, we have identified ataxia telangiectasia mutated (ATM) as a key regulator that is responsible for controlling the commitment of beta-cells to death during prolonged cytokine exposure. ATM is a component in the DNA damage response that initiates DNA repair as well as coordinates cell fate decisions following the formation of DNA double-strand breaks (DSBs). Histone H2AX, an ATM substrate that is termed gamma-H2AX when phosphorylated, is a highly sensitive marker of DSBs. We show that treatment of rat islets and INS 832/13 cells with cytokines (IL-1 and IFN-gamma) results in gamma-H2AX formation in a nitric oxide-dependent manner. ATM is the kinase required for H2AX phosphorylation, as ATM inhibition with the selective inhibitor KU-55933 prevents nitric oxide-induced gamma-H2AX formation. Although ATM is classically associated with DNA repair, ATM inhibition has no effect on the repair of IL-1-induced DNA damage in rat islets. In contrast, IL-1-induced gamma-H2AX formation temporally correlates with the induction of apoptosis in rat islets, and ATM inhibition prevents IL-1-stimulated caspase-3 activation in rat islets treated for 36 h with IL-1. Based on our findings, we hypothesize that ATM senses the level of DNA damage in beta-cells during cytokine exposure, and when this damage exceeds the capacity to repair, or more specifically, when DSBs form, ATM is activated and directs the induction of a caspase-dependent apoptotic cascade.

The Role of Macrophage Deoxyhypusine Synthase in the Progression of Obesity-Associated Metabolic Inflammation

Emily K. Anderson-Baucum[1], Masayuki Hatanaka[2], David L. Morris[2], Carmella Evans-Molina[1], and Raghavendra G. Mirmira[1,2]

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The worldwide prevalence of obesity and associated metabolic disorders, such as insulin resistance and type 2 diabetes, has increased dramatically. Research conducted in the past decade has demonstrated that immune cells, including pro-inflammatory "M1" polarized macrophages, accumulate in the pancreatic islets, adipose tissue, liver, and muscle of obese humans and rodents. Inflammatory cytokines secreted from macrophages residing in these metabolic tissues contribute to the development of beta cell dysfunction and peripheral insulin resistance. Eukaryotic translation initiation factor 5A (eIF5A) is an RNA binding protein that promotes translational elongation of specific mRNAs. Interestingly, eIF5A is the only protein known to contain the unique polyamine-derived amino acid, hypusine (hydroxyl-putrescine lysine). Deoxyhypusine synthase (DHS) is the rate-limiting enzyme catalyzing this activating post-translational modification. Recently, our laboratory has shown that DHS and hypusinated eIF5A ($eIF5A^{Hyp}$) promote the translation of mRNAs encoding pro-inflammatory proteins in pancreatic beta cells, thus promoting beta cell dysfunction. However, the role of DHS and $eIF5A^{Hyp}$ in the generation of macrophage-mediated inflammation during obesity remains essentially unknown and unexplored. ***We hypothesized that activation of DHS in macrophages residing in metabolic tissues would promote the translation of inflammatory mRNAs during obesity, resulting in metabolic inflammation, islet dysfunction, and insulin resistance.*** We show that *in vitro* M1 macrophage polarization (10 ng/mL LPS and 50 ng/mL IFN-gamma) increases protein levels of both DHS and $eIF5A^{Hyp}$ ($p<0.05$ compared to control). Puromycin labeling studies and polyribosome profiling demonstrate that M1 polarization promotes the DHS-dependent translation of mRNAs encoding inducible nitric oxide synthase (iNOS) and TNF-α converting enzyme (TACE). In addition, DHS and $eIF5A^{Hyp}$ protein levels were significantly increased in the adipose tissue, but not liver or muscle, of mice fed a 60% high fat diet for 16 weeks. This increase in DHS and $eIF5A^{Hyp}$ protein levels was accentuated in the macrophage-enriched stromal vascular fraction isolated from the adipose tissue of obese mice. Taken together, these data indicate that DHS and $eIF5A^{Hyp}$ promote the translation of inflammatory transcripts in macrophages residing in metabolic tissues.

SESSION 5

Thursday, May 22nd 1:30 – 2:45 pm

Potential Means of Fixing Type-1 Diabetes

Moderator: Les Satin

1:30 – 1:45 pm **Zunaira Z. Chaudhry** - *The Proinsulin/C-peptide Ratio and HSP90: Potential Biomarkers for Early Detection of Type 1 Diabetes*

1:45 – 2:00 pm **J. Stewart New** - *Modulation of Autoimmune Diabetes By Innate-Like B Cell Derived Antibodies Against N-Acetyl Glucosamine*

2:00 – 2:15 pm **Robert N. Bone** - *Fluoroketone-based Inhibition of the Ca2+-independent PhospholipaseA2beta in Neonates Reduces Incidence of Type 1 Diabetes*

2:15 – 2:30 pm **Lindsey E. Padgett** - *NADPH Oxidase-Deficient Macrophages Display a Dampened M1 Macrophage Phenotype in Type 1 Diabetes*

2.30 – 2.45 pm **Stephen Harrington** - *Allogeneic Islet Transplantation by Encapsulation in Bio-inspired Hydrogel: the Importance of Scaffold Geometry*

The Proinsulin/C-peptide Ratio and HSP90: Potential Biomarkers for Early Detection of Type 1 Diabetes

Zunaira Z. Chaudhry[1], Renecia A. Watkins[2], Craig Beam[8], Lynette Guindon[3], Jennifer Terrell[2], Raghavendra G. Mirmira[1,2,4,5,6], Janice Blum[3], Linda DiMeglio[2], Carmella Evans-Molina[1,4,5,6,7], and the Type 1 Diabetes TrialNet Study Group

[1]Departments of Medicine, [2]Pediatrics, [3]Microbiology and Immunology, [4]Cellular & Integrative Physiology, [5]Biochemistry & Molecular Biology, and the [6]Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN; [7]Richard L. Roudebush VA Medical Center, Indianapolis, IN; and [8]Western Michigan University School of Medicine, Kalamazoo, MI

While the etiology of Type 1 diabetes (T1D) has been classically attributed to autoimmune-mediated beta cell destruction, recent studies suggest that stress pathways, such as endoplasmic reticulum (ER) and/or oxidative stress, are triggered early within the beta cell during T1D evolution and may act to initiate and/or accelerate autoimmune-mediated beta cell destruction. Because immunomodulatory therapies administered at T1D onset have the greatest benefit in those with the highest residual levels of C-peptide secretion, we hypothesized that strategies aimed at detecting activation of these intrinsic beta cell stress pathways may allow for the earlier clinical identification of T1D and more effectively timed therapeutic interventions. Increased serum proinsulin relative to levels of the mature and fully processed insulin molecule (assessed by measuring C-peptide) is indicative of alterations in protein folding and dysfunction at the level of the beta cell ER and is commonly used as a marker of beta cell dysfunction in Type 2 diabetes, while heat shock protein 90 (HSP90) is a protein chaperone upregulated in the beta cell during inflammatory stress. The goal of this project was to test the utility of both measures as candidate biomarkers of beta cell stress and evolving preclinical T1D. Proinsulin/C-peptide (PI/C) ratios and HSP90 were first measured in serum samples obtained from 29 pediatric human subjects at the time of T1D onset (avg age 10.6±3.0 yrs; 55% male) and compared to 16 non-diabetic pediatric controls (avg age 10.5±3.0 yr, 50% male). Interestingly, both PI/C and HSP90 were significantly elevated in subjects with newly diagnosed T1D compared to healthy controls (PI/C $p<0.001$; HSP90 $p=0.02$). To determine if elevations in either PI/C or HSP90 preceded the development of clinically apparent T1D, banked samples were obtained from the TrialNet Pathway to Prevention cohort, which is a longitudinal study of non-diabetic first, second, or third degree relatives of individuals with T1D who are positive for at least one beta cell autoantibody. Samples were obtained from T1D progressors 10-14 months prior to disease onset (n=38; 43.5% male; avg age 20.06 ± 2.26 yrs; n=24 $\leq$18 yrs) and compared to age, gender, and BMI-matched nonprogressors who remained normoglycemic (n=38; 43% male; avg age 20.14 ± 2.212; n=24 $\leq$18 yrs) and were followed in the study for a comparable time period. While HSP90 levels were not different between the groups, the PI/C ratio was 1.57-fold higher in T1D progressors compared to nonprogressors ($p=0.012$; 95% CI 1.11-2.23). In addition, there was a significant positive correlation between PI/C and body mass index ($p=0.034$) and a significant negative correlation between the PI/C ratio and age ($p=0.003$). Together, these data suggest that elevations in the serum PI/C ratio may help predict the onset of T1D at a time prior to the development of clinically apparent hyperglycemia. Future studies are needed to validate these findings in larger populations and to assess changes in the PI/C at additional time points prior to the development of T1D.

Modulation Of Autoimmune Diabetes By Innate-Like B Cell Derived Antibodies Against N-Acetyl Glucosamine

J Stewart New, Brian LP Dizon MD,PhD, and John F Kearney PhD

Department of Microbiology, University of Alabama at Birmingham, Birmingham AL

The immunodominant epitope of Group A Streptococcus (GAS) is the cell wall associated N-acetyl-Glucosamine (GlcNAc) of Group A Carbohydrate (GAC). Neonatal GAS exposure has been demonstrated to protect in multiple murine models of Type 1 Diabetes (T1D), and human cases of T1D have been negatively correlated with cases of Scarlet fever in several studies. Through serum-transfer experiments from GAS-immunized to naïve NOD mice, we have found that T1D protection elicited by GAS exposure is conferred by the antibody response to GAC. Anti-GAC antibodies recognize common GlcNAc components of mammalian glycan chains and are consequently highly reactive with epitopes from pancreatic islets of several species, including human. Epitopes recognized by GAC-specific IgM include a subset of, likely immature, insulin-containing granules; this post-translational modification (PTM) is therefore associated with granules containing multiple T1D-related auto antigens. We have found that anti-GAC antibodies are highly reactive with apoptosis-associated beta cell neoepitopes in mice, and with immature human pancreatic glycans, through open source data available to us through the Consortium for Functional Glycomics. Through in vitro and in vivo studies, we have discovered that GlcNAc-modified autoantigens generated during beta cell apoptosis drive lectin-dependent complement pathway initiation, and we find that GlcNAc-specific antibodies modulate activation of the complement pathway on dying beta cells. Opsonization of apoptotic-beta cell neoeptitopes reduces the capacity of dendritic cells to activate diabetogenic T cells with beta cell derived antigen, and protects from T1D development. In our preliminary studies with T1D patients, we have observed that T1D patients possess significantly lower levels of GAS- but not GlcNAc-reactive IgM antibodies, suggesting the involvement of particular natural anti-GlcNAc antibody-producing clonotypes in T1D prevention. Subsets of anti-GlcNAc antibodies, may serve as biomarkers for identifying at risk-patients whose repertoire may lack these idiotype positive antibodies. Furthermore, boosting this idiotype may serve as a therapy to protect at risk patients from developing T1D.

Fluoroketone-based Inhibition of the Ca^{2+}-independent Phospholipase A_2beta in Neonates Reduces Incidence of Type 1 Diabetes.

Robert N. Bone[1,2], Ying Gai[2,3], Victoria Magrioti[4], Hubert M. Tse[2,5], George Kokotos[4], Sasanka Ramanadham[2,3]

Departments of [1]Pathology, [3]Cell, Developmental, & Integrative Biology, [5]Microbiology, and the [2]Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL, USA and [4]Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Athens, Greece.

Type 1 diabetes (T1D) is an autoimmune mediated disease that leads to ablation of insulin producing pancreatic islet beta-cells, however, the mechanisms involved are not fully defined. Our findings that the group VIA Ca^{2+}-independent phospholipase A_2 beta ($iPLA_2$beta)-derived signals contribute to beta-cell apoptosis and that $iPLA_2$beta expression is increased in islets of diabetes-prone non-obese diabetic (NOD) mice prompted us to assess whether $iPLA_2$beta activation contributes to the development of diabetes in the NOD mouse, a widely used model that spontaneously develops T1D. FKGK18, a potent and reversible fluoroketone-based inhibitor of $iPLA_2$beta amenable for *in vivo* usage, was administered to NOD mice and diabetes outcomes were monitored. Our ongoing studies reveal that the tri-weekly administration of FKGK18, beginning at 10 d of age, reduces the incidence of T1D versus vehicle-treated (control) mice. FKGK18-treated mice had reduced insulitis, greater insulin positive beta-cell area, higher circulating insulin levels, better glucose tolerance, and reduced populations of B-cells and $CD4^+$ T-cells in the islet infiltrate compared to vehicle-treated mice. Further, following inhibition of $iPLA_2$beta, but not $iPLA_2$gamma, we see reduced TNFα secretion from FKGK18 treated CD4+ T-cells, suggesting a blunting of the immune response and reduction of $iPLA_2$beta derived inflammatory products. Since T1D development begins early in life in the NOD mouse, with insulitis beginning around 6 wk of age, we next assessed if FKGK18 administration to adults (8 wk of age) would be as effective as neonatal (10 d of age) administration. We find that adult administration of FKGK18 also reduces T1D incidence, however, the neonatal administration group still had the lowest incidence, versus vehicle-treated controls. Interestingly, while the adult administration group did had reduced incidence, the glucose tolerance was similar to control. Taken together, our findings suggest that age-dependent generation of $iPLA_2$beta-derived lipid signals play important roles in processes that lead to development of autoimmune-mediated T1D.

NADPH Oxidase-Deficient Macrophages Display a Dampened M1 Macrophage Phenotype in Type 1 Diabetes

Lindsey E. Padgett, Ashley R. Burg, Weiqi Lei, and Hubert M. Tse, PhD.

Department of Microbiology, Comprehensive Diabetes Center; University of Alabama-Birmingham; Birmingham, AL

Macrophages are indispensible in the pathogenesis of Type 1 diabetes (T1D), an autoimmune disease characterized by the destruction of insulin-secreting pancreatic beta-cells. As one of the first islet-infiltrating cells, macrophages present antigen to diabetogenic T cells and generate reactive oxygen species (ROS) and pro-inflammatory cytokines to directly lyse pancreatic beta-cells. In contrast to the pathogenicity of M1 macrophages, alternatively activated M2 macrophages, which generate immunosuppressive cytokines, protect Non-Obese Diabetic (NOD) mice against spontaneous diabetes and represent a promising cellular therapy in T1D; however, the environmental cues that govern macrophage polarization remain largely unknown. Our laboratory previously demonstrated the importance of NADPH oxidase (NOX)-derived ROS in T1D pathogenesis, as NOD mice deficient in NOX-derived superoxide (*Ncf1m1J*) were protected against spontaneous T1D due to blunted pro-inflammatory cytokine and Type I interferon synthesis with Toll-like receptor (TLR) agonist stimulation. Thus, we hypothesized that ROS was essential for M1 macrophage differentiation; therefore, ROS ablation would induce a dampened M1 and/or elevated M2 phenotype. Analysis of sera from 16-week-old female NOD.*Ncf1m1J* mice revealed a 15-fold reduction in CCL5, a marker of pro-inflammatory M1 macrophages ($p<0.001$), concomitant with 3-fold enhancement in CCL17, an M2 marker, compared to sera from age- and sex-matched NOD mice ($p=0.0036$). The islet-resident macrophage profile of 16-week female NOD.*Ncf1m1J* mice indicated a significant ($p<0.01$) dampening in M1 macrophage transcripts, such as *Cxcl10* (2-fold), *Ccl5* (4-fold), *Tnfa* (8-fold), and *Nos2* (8-fold) concomitant with a significant ($p<0.01$) elevation in M2 macrophage transcripts, including *Ccl17* (3-fold), *Retnla* (5-fold), *Arg1* (1.5-fold), and *Cd206* (2.5-fold). To further define the role of NOX-derived superoxide on macrophage differentiation, NOD.*Rag.Ncf1m1J* mice were transferred with diabetogenic BDC-2.5 CD4 T cells, which destroy beta-cells by recruiting pro-inflammatory M1 macrophages into the pancreas of recipient mice. A 2-fold dampening in pro-inflammatory M1 macrophages at 5 days, concomitant with a 3-fold elevation in anti-inflammatory M2 macrophages was observed in BDC-2.5-transferred NOD.*Rag.Ncf1m1J* mice compared to NOD.*Rag* mice. Interestingly, treatment of NOD.*Rag* and NOD.*Rag.Ncf1m1J* BDC-2.5-transferred recipients with a potent catalytic antioxidant induced a 3- and 2-fold elevation in M2 macrophages within NOD.*Rag* and NOD.Rag.*Ncf1m1J* pancreata, respectively. These results provide evidence that NOX-derived superoxide is essential for a pro-inflammatory M1 macrophage differentiation; thus, targeting macrophage redox status may represent a promising therapy in halting T1D.

Allogeneic Islet Transplantation by Encapsulation in Bio-inspired Hydrogel: the Importance of Scaffold Geometry

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Immunoprotection of islets by hydrogel encapsulation has emerged as the most readily translatable approach to large-scale clinical islet transplantation. This strategy overcomes two critical barriers facing the procedure by eliminating the need for immunosuppressive therapy and creating an unlimited tissue supply via xenotransplantation of porcine islets. However, immunoprotection methods inherently isolate transplanted tissue from their naturally supportive matrix, increase diffusional barriers, and elicit fibrotic responses *in vivo.* Ultimately, the performance of immunoprotected islet transplants is still not clinically relevant.

In this study, we examined a bio-inspired, covalently cross-linked hydrogel based on hyaluronic acid (HA) and collagen both *in vitro* and *in vivo* for its potential to improve outcomes in islet transplantation. First, rat and canine islets encapsulated in the HA hydrogel were assessed for viability and glucose stimulated insulin secretion over several weeks in culture and compared to islets cultured normally in liquid media. While both groups showed similar viability over time, islets encapsulated in the HA hydrogel retained sensitivity to glucose over time whereas free islets did not, indicating that the gel provides important supportive biological cues to the encapsulated tissue.

We also performed allogeneic islet transplantations into the omentum of out-bred diabetic rats to assess biocompatibility as well as the immunoprotective and bioactive effects of the HA gel in vivo. Normoglycemia was restored immediately after transplantation, and has continued indefinitely (80 days at time of submission). In contrast islets alone reversed diabetes but only for 7-10 days after which, the animals became chronically hyperglycemic. Normoglycemia was restored only when the islets in hydrogel scaffold were deposited in a thin film in close proximity to robust vasculature, and not when deposited in a large gel bolus. Our findings indicate that both the geometry and chemical composition of the encapsulating construct are critical factors in designing a successful immunoprotected islet transplant.

SESSION 6

Thursday, May 22nd 3:15 – 4:30 pm

Pathogenesis and Potential Means of Fixing Type-2 Diabetes

Moderator: Colin Nichols

3:15 – 3:30 pm **Mieke Baan** - *FoxM1 Overexpression in Pancreatic Beta Cells Increases Pancreatic Insulin Content and Lowers Plasma Glucose*

3:30 – 3.45 pm **Gu Jing** - *TXNIP induces IAPP expression via miRNA-124a and FOXA2*

3:45 – 4:00 pm **Damien Demozay** - *Ablation of FoxO3a, but not FoxO1, specifically in beta-cells causes beta-cells failure in mice exposed to a high calorie diet*

4:00 – 4:15 pm **Holly A. Cyphert** - *Characterization of Human MafA/B Activity in Pancreatic Beta Cells*

4:15 – 4:30 pm **Johanne H. Ellenbroek** - *Long-term Ketogenic Diet Causes Glucose Intolerance and Reduced Beta and Alpha-Cell Mass but No Weight Loss in Mice*

FoxM1 Overexpression in Pancreatic Beta Cells Increases Pancreatic Insulin Content and Lowers Plasma Glucose.

Mieke Baan[1], Carly Kibbe[1], Justin Bushkofsky[1], Dawn Belt Davis[1,2]

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The factors regulating the expansion of beta cell mass in compensation for obesity-induced insulin resistance are largely unknown. Our prior work has shown that Forkhead box M1 (FoxM1), a transcription factor that regulates key cell cycle genes, is upregulated in non-diabetic obesity and stimulates human beta cell replication in islets *ex vivo*.

To examine if FoxM1 drives beta cell proliferation *in vivo*, we generated mice expressing murine FoxM1 under control of the mouse insulin promoter (MIP-FoxM1). Body weight and growth rates were not different between MIP-FoxM1 and wildtype controls. As anticipated, expression of FoxM1 mRNA was significantly increased in MIP-FoxM1 islets. To assess whether FoxM1 was functional, we evaluated expression of several of its known cell cycle target genes. Preliminary results show a non-significant 1.8 fold increase in expression of cyclin E2 mRNA ($P = 0.10$, n=5). This was not unexpected, as factors driving the marked beta cell proliferation seen in obesity are likely not present in these lean mice. Nonetheless, total pancreatic insulin content was higher in MIP -FoxM1 mice (649±49 vs 415±12 ug insulin/g pancreas, respectively, mean ± SEM. $P = 0.036$, n=5 and 2), suggesting increased beta cell mass. Fasting blood glucose concentrations were lower in MIP-FoxM1 males at 4, 6, and 8 weeks (204.1±9.077 vs 236.1±7.388; 186.6±13.06 vs 229.6±8.320; 210.4±9.676 vs 244.4±9.305, mean ± SEM, respectively. $P < 0.05$, n=9–14). However, there was no evidence of hypoglycemia. A glucose tolerance test was performed at 10 weeks, and showed no difference in glucose ($P = 0.4848$) or insulin ($P = 0.8574$) levels, suggesting that glucose tolerance and glucose stimulated insulin secretion are not different from controls (n=7-14, respectively).

In conclusion, we have generated a model to study the impact of FoxM1 on the regulation of beta cell mass. We show that FoxM1 overexpression plays an important role by increasing pancreatic insulin content and decreasing fasting blood glucose levels in lean mice. We predict that FoxM1 overexpression in obesity will drive beta cell expansion and will protect from diabetes mellitus.

This work is supported by the National Institutes of Health Ruth L. Kirschstein National Research Service Award Institutional Training Grant T32 RR023916 and T32 OD010423 from the National Center for Research Resources.

TXNIP Induces IAPP Expression via miRNA-124a and FOXA2

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Islet amyloid polypeptide (IAPP), also known as amylin, is a regulatory peptide that is co-expressed and co-secreted with insulin by beta-cells. It tends to aggregate into insoluble amyloid fibrils and smaller non-fibrillar oligomers that are toxic and strongly associated with the progressive loss of pancreatic beta-cell mass in type 2 diabetes. This makes IAPP a potential target and critical factor in the development of type 2 diabetes. However, while glucose and PDX1 are known to induce IAPP transcription, the regulation of IAPP expression is still not fully understood. Interestingly, in our human islet microarray analysis, we not only found a marked induction of IAPP, but also a dramatic increase in thioredoxin-interacting protein (TXNIP) expression in response to glucose. We then went on to show that TXNIP also plays a major role in beta-cell apoptosis and beta-cell loss of diabetes and most recently discovered that TXNIP inhibits insulin transcription and regulates beta-cell function through miR-204. This raised the question whether TXNIP might also be involved in the upregulation of IAPP. In fact, using INS-1 cells, primary mouse and human islets, and beta-cell-specific *Txnip* knockout mice, we found that TXNIP induces beta-cell IAPP expression both *in vitro* and *in vivo*. Our studies further revealed that TXNIP regulates IAPP expression by inducing the expression of a specific transcription factor, forkhead boxA2 (FoxA2) and promoting FoxA2 enrichment at the *IAPP* promoter as assessed by real-time RT-PCR, Western blotting and chromatin immunoprecipitation. Moreover, we also determined the mechanism by which TXNIP induces FoxA2 expression and found that a microRNA, miR-124a, is downregulated by TXNIP and directly targets *FoxA2*. Thus, our findings have identified a novel gene regulatory system implicating TXNIP, miR-124a and FoxA2 in the control of IAPP expression and thereby shed new light onto this important pathway.

Ablation of FoxO3a, but not FoxO1, Specifically in beta-cells Causes beta-cells Failure in Mice Exposed to a High Calorie Diet

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The transcription factor, forkhead box class O (FoxO) 3a, plays a predominant role in primary islets for control Insulin Receptor Substrate-2 (IRS-2) expression, unlikeFoxO1. IRS-2 plays a major role in pancreatic beta-cells by promoting cell growth and survival, so we hypothesized that loss of FoxO3a *in vivo*, rather than FoxO1, may cause beta-cell failure. To test this idea, we generated two inducible mouse models, MIP-CreER/FoxO1 knockout (beta-FoxO1 KO) and MIP-Cre-ER/FoxO3a knockout (beta-FoxO3a KO), where FoxO1 or FoxO3 genes respectively were specifically deleted in the pancreatic beta-cells in young (7-8 weeks) mice by tamoxifen treatment. These were compared to littermate, non-tamoxifen oil-treated (vehicle), controls to assess phenotypic changes.

A knockdown of FoxO1 and FoxO3a expression (by ≥70%) in islets in beta-FoxO1 KO and beta-FoxO3a KO mice respectively was confirmed after tamoxifen treatment, and sustained for >20 weeks. We found no difference in fasted blood glucose, insulin levels and insulin tolerance in both KO mice fed a standard diet. However, whereas beta-FoxO1 KO mice displayed normal glucose tolerance, beta-FoxO3a KO mice are slightly glucose intolerant compared to oil-treated control mice. Interestingly, when the mice were fed a high fat/high sucrose (HFHS) diet, beta-FoxO3a KO mice, but not beta-FoxO1 KO mice, exhibited higher fasting insulin levels compared to control mice. This may be related to HFHS fed beta-FoxO3a KO mice having a higher weight gain than beta-FoxO1 KO mice and equivalent HFHS fed-control mice. Yet, beta-FoxO3a KO mice exposed to HFHS diet showed significant glucose intolerance compared to controls, despite elevated basal insulin levels. Moreover, insulin tolerance tests (ITT) suggested severe peripheral insulin resistance in HFHS fed-beta-FoxO3a KO mice, despite this being a ß-cell specific deletion of FoxO3a. The observed hyperinsulinemia in beta-FoxO3a KO mice may lead to insulin-induced insulin resistance in addition to that caused by the high calorie diet. In contrast, beta-FoxO1 KO mice exposed to HFHS diet showed no difference in glucose and insulin tolerance compared to HFHS fed-control mice. Preliminary immunostaining analysis indicated that beta-cell mass was affected in older beta-FoxO3a KO mice, but not in beta-FoxO1 KO mice, fed with HFHS diet relative to respective control animals.

These data showed that loss of FoxO3a, but not FoxO1, in islets beta-cells leads to beta-cell failure especially under a HFHS dietary challenge. Thus, FoxO3 is critical to maintain beta-cell function and survival and could represent a novel therapeutic target in type 2 diabetes.

Characterization of Human MafA/B Activity in Pancreatic Beta Cells

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MafA and MafB are islet-enriched transcription factors expressed during islet cell development. Loss of MafB (*MafB-/-)* impedes beta cell formation during development by reducing expression of genes important to beta cell maturation. Conversely, beta cell specific knockout of MafA (*MafAdeltabeta*) causes defects in glucose responsiveness and islet architecture postnatally. Strikingly, adult mouse islet beta cells only express MafA while both MafA and MafB are produced in human islet beta cells. Although highly related proteins, there appears to be some dissimilarities in their transcriptional regulatory properties. It is currently unclear how MafA and MafB coexpression (i.e. the MafA/B heterodimer) influences human islet beta cell activity. Towards this end, we generated a transgenic mouse line that produces MafB in beta cells along with endogenous MafA (referred to as MafBtg) to simulate conditions in the human islet. MafB is expressed at similar levels relative to endogenous MafA expression in MafBtg beta cells. Misexpression of MafB in islet beta cells did not affect either fasting blood glucose levels or glucose clearance rates at 4- and 8-weeks. Preliminary results show that MafA and MafB can bind to *insulin* control element sequences as a heterodimer in gel shift assays, and MafB is enriched on the *insulin* promoter in MafBtg mouse islets in chromatin immunoprecipitation experiments. Together these results suggest that MafB is functional in the adult beta cell, and its regulatory role is currently being evaluated by gene expression analysis. Strikingly, transgenic expression of MafB did not rescue glucose intolerance or reverse the islet architecture changes of *MafAdeltabeta* mice, suggesting that the MafB homodimer is not functionally equivalent to MafA homodimer in regulating postnatal beta cell function. In addition to the MafBtg model, we are currently evaluating the role of MafB in the human EndoC-(beta)h1 cell line. Preliminary results suggest that the loss of MafB affects genes important in normal beta cell function (i.e. *insulin*, *G6PC2*). These experimental systems provide ways of analyzing the context dependent regulatory properties of MafB in adult beta cells. Because of our limited knowledge of the human adult beta cell, this information could potentially elucidate the molecular mechanisms that maintain normal beta cell function and yield new directions for diabetes treatment and beta cell regeneration protocols.

Long-term Ketogenic Diet Causes Glucose Intolerance and Reduced Beta- and Alpha-Cell Mass but No Weight Loss in Mice

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Background: High-fat, low-carbohydrate ketogenic diets (KD) are effective for weight loss and for treatment of refractory epilepsy. Patients on KD switch from a carbohydrate-based to a fat-based energy metabolism, which leads to a permanent state of ketosis. Recently, short-time studies in rodents have shown that, besides their beneficial effect on body weight, KD lead to glucose intolerance and insulin resistance. However, the long-term effects on pancreatic endocrine cells are unknown. In this study we investigate the effects of long-term KD on glucose tolerance and beta- and alpha-cell mass in mice.

Methods: C57BL/6J mice were fed a control or ketogenic diet for 22 weeks. Glucose tolerance tests were performed after 1, 5, 12 and 20 weeks of diet. After 22 weeks of diet an insulin tolerance test was performed and plasma cholesterol, triglycerides, leptin, MCP-1, IL-1β and IL-6 concentrations were determined. The pancreas was taken for histology of beta- and alpha-cell mass and the liver for determination of liver triglyceride content.

Results: Despite an initial weight loss, KD did not result in weight loss after 22 weeks (34.8±1.2 g (KD) vs. 32.2±0.4 g (control) $p<0.05$). Plasma cholesterol, triglycerides, leptin, MCP-1, IL-1β and IL-6 were increased. The liver triglyceride content was over two-fold increased in KD-fed mice (379±41 nmol/mg protein (KD) vs. 159±19 nmol/mg protein (control) $p<0.01$). KD-fed mice became overtly glucose intolerant at 12 weeks (AUC glucose 2909±130 (KD) vs. 1960±60 (control), $p<0.001$). Insulin-dependent glucose uptake was reduced (inverse AUC glucose 281±12 (KD) vs. 393±28 (control), $p<0.01$). Importantly, a reduction in beta-cell mass (1.31±0.16 mg (KD) vs. 1.75±0.09 mg (control) $p<0.05$) was observed in KD-fed mice together with an increased number of smaller islets. Also alpha-cell mass was found to be decreased, resulting in a lower alpha-to-beta cell ratio.

Conclusion: Long-term KD leads to dyslipidemia, a proinflammatory state, signs of hepatic steatosis, glucose intolerance, and a reduction in beta- and alpha-cell mass, but no weight loss. Altogether this indicates that long-term high-fat, low-carbohydrate KD lead to features that are also associated with the metabolic syndrome and an increased risk for type 2 diabetes in humans.

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– Heritage Hall I/II –

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Poster 02 - John Wiersch - *Alpha Cell Ablation Leads to Delayed Beta Cell Development in Embryonic Mice.*

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Complementary Roles for Glucagon Receptor and GLP-1 Receptor Signaling in Insulin-Cell Differentiation

Iljana Gaffar, John Wiersch, Mario Cassara, Krishna Prasadan, Xiangwei Xiao, Ping Guo, Chiyo Shiota, Zewen Song, George Gittes

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Background: The "glucagon family" of peptides includes alternate splice products of the proglucagon peptide, as well as gastric inhibitory peptide (GIP), that can potentially interact in an overlapping fashion with each other's receptors. We have shown previously that glucagon, glucagon -like peptide-1 (GLP-1) and GIP can all potentially act as inducers of insulin-positive differentiation in the developing mouse embryo. While GIP induction of insulin-positive differentiation appears to occur through the GIP receptor, the receptor targets for glucagon and GLP-1 in the induction of insulin-positive differentiation are less clear.

Methods and Results: There are no studies that directly identify the key endogenous receptor that may be mediating the insulin-positive differentiation induced by glucagon or related peptides. Here we show that both glucagon receptor signaling and GLP-1 receptor signaling can mediate insulin-positive differentiation, and ablation of both of these receptor pathways using antisense in embryonic pancreas leads to nearly complete loss of insulin-positive differentiation in vitro. Deletion of only one of the two receptors will still allow normal development of insulin-positive differentiation. We also tested the AR42J cell line, an acinar carcinoma cell line, that when treated with exendin-4 undergoes insulin-positive differentiation. However, by RT-PCR analysis we found that GLP-1 receptor expression was surprisingly absent in AR42J cells, and thus here the glucagon receptor appears to be a singular mediator of GLP-1 or exendin-4 induced insulin-positive differentiation. When glucagon receptor expression was blocked using antisense in AR42J cells, GLP-1 or exendin-4 induced insulin-positive differentiation was blocked.

Conclusion: These data demonstrate a potentially important new role for glucagon family receptor signaling in embryonic insulin-positive differentiation, and suggest complementary roles for GLP-1 receptor and glucagon receptor in an insulinotrophic paracrine signaling pathway. These findings therefore have implications for our understanding of the regulation of beta cell differentiation.

Alpha Cell Ablation Leads to Delayed Beta Cell Development in Embryonic Mice

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Background: Glucagon is the first peptide hormone detectable in appreciable amounts in the embryonic pancreas. Prior work suggests that this early glucagon expression acts in a paracrine fashion to induce the differentiation of other pancreatic endocrine lineages, particularly beta cells. Several studies indicate that glucagon or its related peptides are necessary for early insulin-positive differentiation in the embryonic mouse pancreas.

Methods: We generated a transgenic mouse with a floxed diphtheria toxin A gene driven by the glucagon promoter (Gcg-iDTA). When combined with mice transgenic for neurogenin3-cre or Pdx1-cre, pancreatic glucagon expressing cells are ablated. To determine if glucagon replacement rescues the alpha cell ablation phenotype, we performed intrauterine embryonic injections and delivered alpha-TC cells into the intraperitoneal cavity. The alpha-TC cells provided continuous systemic delivery of glucagon to the developing embryo. Histologic analysis of the pancreas was performed at E12.5, E15.5, and E17.5.

Results: No alpha cells were seen in embryos carrying both the Gcg-iDTA and cre recombinase transgenes. Embryonic alpha cell ablation delayed the appearance of beta cells until E17.5. Intraperitoneal embryonic injection of alpha-TC cells in control mice increased beta cell number up to two-fold over baseline. Similarly, the delayed development of insulin cells seen in these Gcg-iDTA mice was partially rescued by in utero administration of 300 alpha-TC cells intraperitoneally into E12.5 embryos.

Conclusion: Our study further demonstrates the role of glucagon in modulating early endocrine differentiation. Early enhanced expression of glucagon augments the number of beta cells in wild-type mice and partially rescues beta cell differentiation in alpha cell-ablated mice.

Mnx1 as a critical determinant in beta/delta fate selection during endocrine lineage diversification and beta cell differentiation

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Mnx1 (formerly HB9 or Hlxb9) is expressed in early multipotent pancreatic progenitors, becoming restricted to the beta cells in the mature pancreas. Mice lacking Mnx1 have dorsal pancreas agenesis, while ventral pancreas develops normally with significantly reduced numbers of beta cells that are immature. Human with homozygous Mnx1 mutation has recently showed to cause Permanent Neonatal Diabetes Mellitus. In this study, re-examination of Mnx1 expression pattern during pancreas development were undertaken focusing on the main steps of endocrine lineage allocation During endocrine differentiation, Mnx1 protein is not detected in $Ngn3^{+}$ endocrine progenitors, but it is produced in $Pax6^{+}$ endocrine precursors, for the first time placing it at a specific decision point in endocrine ontogeny. Mnx1 therefore has various stage and cell-type-specific roles. We are interested in determining how Mnx1 integrates into the transcriptional hierarchies controlling lineage decisions during endocrine diversification, specifically in the establishment and maintenance of beta-cell fate and functions. Using a floxed conditional knockout Mnx1 allele, we dissected Mnx1 roles in various stages of pancreas development in a more-detailed, cell-type-specific and temporally controlled manner. Deleting Mnx1 in $Ngn3^{+}$ endocrine progenitors ($Mnx1^{Dendo}$) led to mutant pups that were hyperglycemic at birth, and exhibiting dramatically increased delta and reduced beta-cell numbers. Our new model is that Mnx1 is an instructive lineage allocation factor suppressing delta-cell fate and promoting beta-cell specification/commitment. Furthermore, $insulin^{Cre}$-based beta-cell-specific Mnx1($Mnx1^{Dbeta}$) deletion also showed a significant decrease in Mnx1-negative beta cells and massively increased delta-cell numbers, some of which have undergone transdifferentiation from the beta-cells. These data demonstrated that continued Mnx1 production is essential to maintain the beta-cell fate. To my surprise, beta cells that escaped Mnx1 deletion expanded by proliferation to repopulate the islet mass, even resulting in islet hyperplasia in aged mice. The escaper beta cell numbers were increased greatly over normal tissue and showed increased Mnx1 expression and proliferative capacity. They expressed, produced and secreted more insulin. Menin is a tumor suppressor involved in beta-cell proliferation. It was recently shown to interact physically with Mnx1 to inhibit beta-cell proliferation. $Men^{+/-}$ mice develop multiple endocrine neoplasia type I. In our escaper beta-cell population, Menin expression is also reduced. These data suggest that escaper beta-cell population in the $Mnx1^{Dbeta}$ mutants exhibit similar characteristic to beta cells in the MEN1 insulinoma, implicating that Mnx1 may be responsible for the uncontrollable proliferation and insulin secretion in the MEN1 insulinoma cells. This important new information adds to our knowledge of the transcriptional hierarchy and gene regulatory networks that control beta-cell differentiation.

Insm1 Promotes Endocrine Cell Differentiation by Modulating Expression of a Network of Genes That Includes _Neurog3_ and _Ripply3_.

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Insulinoma associated 1 (*Insm1*) plays an important role in regulating the development of cells in the central and peripheral nervous system, the olfactory epithelium and endocrine pancreas. To better define the role of *Insm1* in pancreatic endocrine cell development we generated mice with an *Insm1*^{GFPCre} reporter allele and used it to perform both immunohistochemical analysis and gene expression profiling by RNA-Seq of *Insm1*-expressing and null populations. Endocrine progenitor cells lacking *Insm1* exhibited broad defects in hormone production, cell proliferation and cell migration and expressed higher levels of transcripts associated with less differentiated progenitor cells. Analysis of the function of *Ripply3*, one such *Insm1*-regulated gene, revealed that it negatively regulates proliferation of endocrine cell progenitors. Moreover, cells lacking *Insm1* contained greater amounts of a non-coding *Neurog3* mRNA splice variant, suggesting that *Insm1* is a positive regulator of Neurog3. In addition, progenitor cells that express either high or low levels of *Pdx1*, and thus are biased towards the formation of specific cell lineages, exhibited cell type-specific differences in the genes regulated by *Insm1,* suggesting that this transcription factor may also have cell type-specific functions. Taken together, our findings indicate that in developing pancreatic endocrine cells *Insm1* promotes the transition from a progenitor ductal epithelial state to a committed endocrine cell by repressing genes expressed in less mature progenitor cells and activating genes involved in RNA-splicing, cell migration, controlled cellular proliferation, vasculogenesis, extracellular matrix and hormone secretion.

3D Differentiation of human induced pluripotent stem cells generates functional insulin producing cells in vitro

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Type 1 diabetes (T1D) is caused by the autoimmune-destruction of pancreatic beta cells. This process results in significant loss of the beta cell mass and reduced production of insulin. Lack of insulin, the key regulator of glucose, leads to elevated blood glucose levels, which can lead to the complications of diabetes like blindness, cardiovascular disease, neuropathy and kidney failure. Our goal was to generate insulin producing cells (IPCs) from human induced pluripotent stem cells (iPS) using a platform that mimics physiological conditions. We established a 3D protocol that utilizes Matrigel and Wnt3a. Matrigel was used to create the three dimensional environment to enable cell clustering and Wnt3a was used to maximize the downstream differentiation of iPS cells.

During the differentiation process, the cells initially formed clusters surrounded by single cells. These clusters transitioned to clusters with projections .The change in the morphology of these clusters was corresponding to the different stages of differentiation. The cells were first differentiated to endodermal cells which express CXCR4. Past this stage, the cells transitioned into pancreatic precursor cells that were positive for the transcription factor Pdx1, the master transcription factor for development of the pancreas. At the end of the differentiation procedure, the cells formed three characteristic types of clusters—tight clusters, cyst-like clusters that had a thick edge and cyst like clusters that contained compact clusters at the core. These structures were similar to what is observed during fetal pancreatic development. On characterization of the cells at the end of the differentiation process, the tight cell clusters were positive for insulin, C-peptide, Nkx6.1, Nkx2.2, glucagon and Glut-2. On glucose stimulation, the cells responded by production of insulin. However, as compared to human pancreatic islets, the glucose responses were very low.

We anticipate that when these cells are transplanted, their insulin production will significantly improve as the cells further mature. We also plan on further study of the distinct types of clusters to provide insight on the differentiation process. A better understanding of these cell types will help us better define the developmental stages of the generation of iPS cell-derived IPCs.

ISLET ß-CELL GROWTH, REGENERATION & REPROGRAMMING

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Differentially Activated Macrophages Create Lineage-Specific Microenvironments for Pancreatic Regeneration in Mice

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Although the identification of cells contributing to pancreatic regeneration has been the subject of intense research, the mediators orchestrating this process remain largely unknown. Accumulating data suggest that the influx of macrophages during tissue regeneration not only serves to debride the site of injury, but it is also critical in coordinating the repair response.

Here, we show that the progressive switch of the macrophages to an M2-phenotype facilitates regeneration of the pancreas. Support for this is gleaned, since depletion of M2 macrophages *in vivo*, as well as compromised polarization observed in old mice both result in impaired pancreatic regeneration. In addition, we show *in vivo* and *in vitro* that polarized macrophages provide signals to promote lineage-specific differentiation in an injury-dependent manner. Finally, we demonstrate that macrophages isolated from the autoimmune-prone NOD strain are characterized by a persistent pro-inflammatory cytokine profile and have an inherent inability to provide appropriate signals for beta-cell generation. This functional impairment might concur to failure of beta-cell regeneration in the setting of autoimmunity and provides a basis for novel macrophage-centered therapeutical approaches in Type 1 Diabetes.

A small molecule kinase inhibitor induces insulin gene expression in pancreatic alpha cells

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Both type 1 and type 2 diabetes involve the loss of insulin-expressing pancreatic beta cells from the islets of Langerhans. A potential therapeutic strategy that is being investigated is transdifferentiation of pancreatic alpha cells into beta cells.

We have utilized the mouse Alpha TC1-6 cell line to look for small molecules that might induce insulin expression in pancreatic alpha cells. Alpha TC1 cells were treated with compounds with defined pharmacological activity and insulin gene expression was evaluated by both measuring endogenous mouse Ins2 mRNA levels with qPCR, and using a human insulin gene reporter assay. We have identified a kinase inhibitor, imidazolo-oxindole protein kinase R (PKR) inhibitor C16, which increases Ins2 mRNA levels up to 30-fold in Alpha TC1 cells after 5 days of treatment. The effects of the compound on insulin gene expression were concentration –dependent with an EC50 of 1.5 micromolar. C16 also increased a signal in the human insulin promoter-driven reporter assay in Alpha TC1 cells. Measurement of proinsulin and insulin protein levels with specific ELISA assays demonstrated that treatment with C16 elevated levels of both intracellular and secreted proteins. The increases in insulin mRNA and protein levels produced by C16 treatment were not mediated by inhibition of the compound putative target PKR. Knockdown of PKR expression using gene specific siRNA did not show an increase in Ins2 mRNA levels in Alpha TC1 cells. Moreover, an allosteric inhibitor of PKR, 7-Desacetoxy-6,7-dehydrogedunin, also did not increase insulin mRNA levels. The identity of a kinase involved in the induction of insulin expression in Alpha TC cells is currently under investigation.

Activated FoxM1 enhances insulin secretion and beta cell survival in young mice while increasing beta cell proliferation in aged mice

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FoxM1 is a transcription factor required for beta cell replication during normal postnatal growth, during gestation, and after partial pancreatectomy. Yet, transgenic overexpression of FoxM1 is not sufficient to augment beta cell mass, even after injury, possibly due to posttranslational regulation of FoxM1. To partially bypass this regulation, we derived a transgenic mouse line expressing an inducible, activated form of FoxM1 in beta cells (Beta-FoxM1* mice). While activated FoxM1 did not alter beta cell mass in two-month old mice, two weeks of expression in four-, eight- and twelve-month old mice effected increased beta cell mass, demonstrating rejuvenation of replicative potential. Unexpectedly, Beta-FoxM1* mice displayed enhanced insulin secretion and improved glucose homeostasis at two months, before beta cell mass increase.

To determine if activated FoxM1 can enhance recovery from beta cell injury, Beta-FoxM1* mice were treated with streptozotocin (STZ). These mice exhibited improved glucose homeostasis and higher beta cell mass than *RIP-rtTA mice* when activated FoxM1 was expressed prior to STZ treatment. Contrary to expectations, elevated beta cell mass resulted from decreased beta cell death rather than increased replication. RNA-Seq analysis and subsequent experiments indicate that this enhanced survival is mediated by increased recruitment of T and B cells and by upregulation of pro-survival factors.

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Islet Cell Composition, Proliferation and Mass in the Human Juvenile Pancreas

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Beta-cell mass in non-diabetic humans varies by as much as 3-5 fold and thus, differences in beta-cell mass may influence diabetic susceptibility. The contribution of the early postnatal period to the establishment of adult islet cell mass is poorly understood. To investigate pancreatic islet cell composition, mass and proliferation early in life, we systematically analyzed cross sections from three regions (head, body, tail) of the whole pancreas from ten human juvenile donors (1 day to 10 years of age) by multicolor immunofluorescence, whole slide imaging and an automated cell-counting algorithm (values are mean ± S.D.). Donors were grouped into four age categories 1-5 days (d; n=2), 2-3 months (mo; n=3), 10-20 months (mo; n=2) and 5-10 years (y; n=3). The islet cell density (including alpha-, beta-, and delta-cells) per pancreatic section area decreased with age (9.24±0.75%, 1-5d; 7.12±0.05%, 2-3mo; 4.46±1.28%, 10-20mo; 3.01±0.48 %, 5-10y). Beta-cell mass (0.11±0.01 g, 1-5d; 0.19±0.05 g, 2-3mo; 0.28±0.13 g, 10-20mo; 0.75±0.08 g, 5-10y), alpha-cell mass (0.06±0.03 g, 1-5d; 0.10±0.03 g, 2-3 mo; 0.12±0.04 g, 10-20mo; 0.32±0.02 g, 5-10y), and delta-cell mass (0.10±0.04 g, 1-5d; 0.11±0.03 g, 2-3mo; 0.10±0.05 g, 10-20mo; 0.23±0.05g, 5-10y) increased gradually with age, with the largest gain between 10-20mo and 5-10y. Of the islet cell populations, the beta-cell population (relative to the total alpha-, beta-, delta-cell pool) increased from 41±9% (1-5d) to 57±6% (5-10y), the alpha-cell population increased from 20±5% (1-5d) to 25±2% (5-10y), and the delta-cell population decreased from 38±5% (1-5d) to 17±4% (5-10y). Total pancreatic proliferation, measured by Ki67 labeling index, revealed a decline in overall tissue proliferation with increasing age (2.23±0.26%, 1-5d; 2.28±1.19%, 2-3mo; 1.3±0.87, 10-20mo; 0.48±0.25 %, 5-10y). Although alpha- and beta-cell proliferation was variable between donors, the mean Ki67 labeling index of alpha-cells and beta-cells was higher, with $Ki67^+$ alpha-cells more frequent than $Ki67^+$ beta-cells, in donors $\leq$ 20mo of age. These results indicate that proliferation, islet cell composition and mass are changing during the first decade of life and suggest that postnatal pancreatic development may be a crucial determinant of adult islet cell mass.

Connective Tissue Growth Factor Promotes Beta Cell Proliferation During Pregnancy

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During pregnancy, the developing fetus creates an increased demand for insulin that requires compensatory actions to support maternal health. These adaptations are achieved as a result of beta cell hypertrophy/hyperplasia, improved sensitivity to glucose, and enhanced glucose stimulated insulin secretion within the maternal islets. Failure of these actions to occur results in gestational diabetes mellitus (GDM), a condition that occurs in 3-7% of pregnancies and is characterized by high maternal blood glucose levels and an increased risk of preeclampsia and C-sections.
Studies from our lab have revealed that connective tissue growth factor (Ctgf) is a previously unrecognized player in embryonic beta cell proliferation. During development, Ctgf is expressed in pancreatic ducts, vasculature, and islets, where it promotes proliferation of beta cells. After birth, beta cells slow their proliferation and cease Ctgf expression. Our data indicates that Ctgf is reexpressed in maternal beta cells during pregnancy, and pregnant mice with haploinsufficiency of Ctgf display impaired glucose tolerance, similar to GDM. Immunolabeling with the proliferation marker, Ki67, reveals pregnant Ctgf haploinsufficent mice display a defect in the increase in beta cell proliferation that normally occurs during pregnancy. Conversely, beta cell hypertrophy associated with pregnancy remains unaffected. Treating adult beta cells with recombinant Ctgf in vitro also stimulates beta cell proliferation.

Using mutant Ctgf alleles, we will determine what role Ctgf plays in the increase in beta cell proliferation, improved sensitivity to glucose, and enhanced GSIS that occurs during pregnancy. Furthermore, Ctgf has known effects on the TGF-beta, BMP, and Wnt signaling pathways. As such, we will use reporter mice as well as commercially available assays to determine if Ctgf influences these pathways to mediate glucose clearance during pregnancy. Taken together, our findings will improve our understanding of GDM as well as the maternal adaptations that normally occur during pregnancy.

Betatrophin (Angptl8) Expression in Zebrafish and Impact on Beta Cell Numbers

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Increasing beta-cell number can potentially restore glycemic control in diabetic patients. Several candidate molecules have been identified that may increase beta-cell genesis thereby leading to an increased beta-cell mass. One such molecule is Angptl8, also called “Betatrophin”, which was found to be upregulated in the liver by insulin resistance. We have found that contrary to reports that the gene is exclusive to mammals, *angptl8* is also present in zebrafish. Similar to mice, *angptl8* is expressed in the liver of adult and larval zebrafish and expression is increased under conditions of overnutrition and in conditions of insulin resistance. Overexpression of either human or zebrafish Angptl8 in the liver of zebrafish larvae results in an increased number of beta-cells in unfed larvae at 6 dpf. We are investigating whether overexpression of Angptl8 in combination with overnutrition has a cooperative effect leading to a further increase in the beta-cell numbers. We have also generated loss of function mutations in *angptl8* using CRISPR/Cas genome editing. We are investigating if absence of *angptl8* function affects either the basal number of beta-cells or the compensatory beta-cell genesis following 8 hours of overnutrition. Angptl8 has been suggested to function through Angptl3 resulting in alterations in lipid metabolism. Zebrafish *angptl3* is expressed in the same tissues as *angptl8* although the expression does not appear to be altered by overnutrition or insulin resistance. We are investigating if gain or loss of Angptl8 function through the transgenic or knockout approaches results in alterations in lipid metabolism in the zebrafish.

Pax4 Induced Alpha-to-beta Cell Phenotypic Change

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Pancreatic beta-cell replacement is a promising approach for the treatment of Type 1 Diabetes. Reports showing beta-cells can be formed by transdifferentiation of other cells suggest this may be a therapeutic alternative to restore beta-cell mass. Pax4 is a transcription factor that plays an important role in the development of pancreatic beta-cells. Mice deficient for Pax4 fail to form beta-cells but develop excess alpha-cells, suggesting that Pax4 controls alpha - and beta-cell fates. This study was designed to explore whether Pax4 can induce alpha-cells to transdifferentiate into beta-cells. To accomplish that, we first developed an adenoviral vector encoding CMV promoter driven Pax4, namely Ad5.Pax4. Then we examined the gene expression and the functionality of Pax4 in an alpha-cell line, alphaTC1-9 cells. Both western blotting and immunohistochemistry assays showed efficient Pax4 expression in alphaTC1-9 cells following Ad5.Pax4 treatment. More importantly, we found Ad5.Pax4 treatment induced insulin expression and greatly reduced glucagon expression in these cells, suggesting Pax4 has the potential to convert alpha-cells into beta-cells. Furthermore, we found insulin secretion was stimulated by high concentration of glucose, suggesting the Pax4-treated alphaTC1-9 cells have functional glucose-stimulated insulin secretion (GSIS). To further confirm the identity of these cells, we examined a panel of transcription factors essential for alpha- and beta-cell development. Although very few cells showed positive staining for beta-cell specific transcription factors in our immunofluorescence assays, our quantitative PCR (qPCR) assay detected significant increase for Pdx1, Nkx6.1, Ngn3 and MafA compared to uninfected and Ad5.GFP-treated alphaTC1-9 cells. In contrast, expression of alpha-cell specific transcription factors such as MafB and Arx did not change significantly. In summary, our study provided evidence that forced Pax4 expression could convert alpha-cells into functional beta-cells, and this could provide a new avenue to beta-cell replacement therapy.

The Outcome of Pdx1/Ngn3/MafA Reprogramming of Acinar Cells Varies Greatly Depending on Specific Experimental Conditions

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It has been previously reported that adenoviral-mediated expression of *Ngn3/Pdx1*/*MafA* (3TF) in pancreatic acinar cells result in new insulin-expressing beta cells. To explore both the transcriptional and epigenetic mechanisms involved, we developed mice that express 3TF in combination with the fluorescent reporter protein mCherry in a tetracycline-inducible, acinar cell-specific manner. Pancreata of *Rag*$^{+/+}$ mice that expressed 3TF for 7 or 28 days undergo profound changes in both their gene expression and morphology, taking on a histological appearance similar to that of pancreatic metaplasia. At 7 days, amylase (*Amy*) immunostaining is profoundly reduced, and is absent at 28 days. Conversely, at these same time points both chromogranin A (*Chg A*), a neuroendocrine marker, and ghrelin (*Ghrl*), an endocrine hormone, are induced in approximately 100% and 60% of mCherry expressing cells, respectively. However, despite high-level and sustain expression of 3TF (based on RNA-seq at 1 and 7 days) cells that co-stained for both mCherry and insulin were not observed. Instead, we observed a massive infiltration of CD45^{+} cells that peaked at 7 days and was still present at 28 days. Since the reprogramming outcome using transgene-mediated 3TF expression in *Rag*$^{+/+}$ mice differed so markedly from the use of adenoviral-mediated expression in *Rag*$^{-/-}$ mice, we inserted the same 3TF cassette into a recombinant adenovirus and used it to infect the pancreas of *Rag*$^{-/-}$ mice. Under these conditions, we also observed scattered insulin^{+} cells. Thus, experimental differences associated with the use of a recombinant adenovirus or transgene, the *Rag* genotype of the mice, or factor expression levels, dramatically affect the outcome of acinar reprogramming. Current experiments seek to identify the priniciple variable and associated mechanisms.

Signals Derived From The Islet Microenvironment Modulated By Vascular Endothelial Growth Factor-A Promote Beta Cell Proliferation

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Reduced pancreatic beta cell mass is a hallmark of diabetes, which makes the ability to increase or restore beta cell mass a major therapeutic goal. However, factors that effectively stimulate beta cell proliferation have not been identified. While testing the hypothesis that increased endothelial cell signaling would increase beta cell mass using a model of inducible vascular endothelial growth factor-A (VEGF-A) overexpression in beta cells (beta-VEGF-A mouse), we found that increased VEGF-A leads to reduced, not increased, beta cell mass. Surprisingly, withdrawal of the VEGF-A stimulus is followed by robust beta cell proliferation, leading to islet regeneration, normalization of beta cell mass, and reestablishment of the intra-islet capillary network.

Using islet and bone marrow transplantation approaches we found that beta cell proliferation is dependent on the local microenvironment of endothelial cells, beta cells, and bone marrow-derived macrophages recruited to the islets upon VEGF-A induction. Blocking macrophage recruitment by partial bone marrow ablation greatly reduces beta cell proliferation, indicating that infiltration of these macrophages is required for the beta cell proliferative response in regenerating islets. Using a genetic model of macrophage-specific knockdown in beta-VEGF-A mice we can effectively reduce macrophage recruitment to islets, and are testing whether this similarly reduces beta cell proliferation.

Transcriptome analysis of whole islets, and FACS-sorted endothelial cells and macrophage populations shows that macrophages recruited to beta-VEGF-A islets express phenotypic M2 markers, and some M1 markers, along with high levels of MMPs associated with tissue restoration and remodeling, suggesting that these macrophages have a unique regenerative phenotype. This data also shows that intra-islet endothelial cells produce several modulators of cell proliferation and may secrete factors directing macrophage phenotype activation.

Based on this data we propose a new paradigm for beta cell regeneration where beta cell self-renewal is mediated by coordinated interactions between macrophages recruited to the site of beta cell injury and intra-islet endothelial cells.

ISLET TRANSCRIPTING & TRANSCRIPTION FACTORS

Poster 15 - Junqin Chen - *Thioredoxin-Interacting Protein Self-Induction: Role of ChREBP and AMP-Kinase.*

Poster 16 - Jason M. Spaeth - *FoxP1/2/4 Transcription Factors Are Required For Endocrine Alpha- and Beta-cell Proliferation and Function*

Poster 17 - David Scoville - *Identification of New Co-regulatory Partners of the MafA Transcription Factor*

Thioredoxin-Interacting Protein Self-Induction: Role of ChREBP and AMP-Kinase

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Thioredoxin-interacting protein (TXNIP) has emerged as a regulator of key cellular processes involved in diabetes development and plays a critical role in pancreatic beta cell biology and glucose toxicity. We have previously found that high glucose increases TXNIP expression and elevated TXNIP induces beta- cell apoptosis; whereas TXNIP-deficiency protects against beta-cell apoptosis, and prevents type 1 and type 2 diabetes. We further reported that glucose-induced TXNIP expression is mediated by the carbohydrate response element-binding protein (ChREBP). Surprisingly, using our unique rat INS-1 beta cell line overexpressing human TXNIP as well as primary mouse islets and quantitative real-time RT-PCR, luciferase reporter studies and chromatin immunoprecipitation, we have now discovered that TXNIP stimulates its own expression by increasing ChREBP binding to the carbohydrate response element in its promoter. We further found that TXNIP reduced phosphorylation and increased nuclear translocation of ChREBP, key processes conferring ChREBP-mediated transcriptional activation of target genes. In contrast, AMP-activated protein kinase (AMPK) has been reported to promote phosphorylation and inhibition of ChREBP, suggesting that AMPK inhibition might play a role in the observed TXNIP effects on ChREBP. Indeed, we now found that AICAR (5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside), an AMPK activator, blunted the TXNIP-conferred self-induction. Moreover, TXNIP overexpression in INS-1 beta cells decreased AMPK phosphorylation/activation, suggesting that TXNIP-induced TXNIP expression was mediated by inhibition of AMPK and the resulting decrease in ChREBP phosphorylation, increase in nuclear ChREBP translocation and enhanced TXNIP transactivation. Taken together, we have discovered a novel pathway that sheds new light onto the vicious cycle of beta cell glucose toxicity, increased TXNIP, and diabetes progression.

FoxP1/2/4 Transcription Factors Are Required For Endocrine Alpha- and Beta-cell Proliferation and Function.

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Members of the Forkhead Box (Fox) superfamily of transcription factors are critical for pancreatic islet formation and function. For example, FoxA2 is a 'pioneer' factor that specifies early pancreas cell fates, activates *Pdx1*, and is required to maintain euglycemia in neonatal mice (1,2). In addition, FoxM1 controls several cell cycle regulators and augments beta-cell mass under conditions of metabolic stress (e.g. pregnancy and pancreatic injury) (3,4). In contrast, the function of the FoxP family of regulators (FoxP1-4), are largely unknown in the pancreas, but well-studied in other tissues including the lung and heart (5-7). Our preliminary data from an *in vitro* DNA-binding screen suggested that FoxP1, FoxP2 and FoxP4 could potentially regulate promoters of islet-enriched genes, including *MafA* and *Pdx1*. As a consequence, we set out to define the expression and roles of these proteins in the endocrine pancreas. Immunofluorescence analysis indicated that FoxP1, FoxP2 and FoxP4 are broadly expressed in the developing pancreas, and appear to be expressed in all major cell types of the adult pancreas. Previous unpublished studies tested for physiological impacts after Cre-mediated inactivation of *FoxP2* alone or *FoxP1;FoxP4*. However, there was no effect on islet development or function, suggesting functional redundancy. Thus, we generated an islet-specific deletion of *FoxP1*, *FoxP2* and *FoxP4* by crossing floxed *FoxP* allele mice with *Pax6-Cre* transgenic mice (*FoxP1/2/4 cKO*). Mutant mice were born in expected ratios, clear glucose normally, but develop transient postnatal hypoglycemia, which is resolved by 8W of age. Immunohistochemical analyses demonstrated that *FoxP1/2/4 cKO* mice have an overt loss of alpha cells, due (in part) to reduced proliferation. The proliferative impact is likely mediated through by significant reductions in *cyclin A2*, *cyclin B1* and *cyclin D1/D2* mRNA levels by P10. Furthermore, *FoxP1/2/4 cKO* mice exhibit significantly reduced fasting plasma insulin and glucagon levels at 4W, which is resolved by 8W suggesting that both alpha- and beta-cell function is delayed. This is likely through a reduction in the expression of maturity markers in alpha- (MafB and Arx) and beta-cells (MafA, Nkx6.1, Pdx1), which we will monitor immunohistochemically and by gene expression analysis from isolated islets. This study establishes that the FoxP1/2/4 transcription factors are required for postnatal endocrine alpha- and beta-cell proliferation and function.

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Identification of New Co-regulatory Partners of the MafA Transcription Factor

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Several transcription factors are essential for the development, differentiation, and/or function of the pancreatic islet cells. Of the known transcription factors important for islet beta-cell differentiation and function, MafA and MafB play prominent yet distinct roles. Although they have identical promoter binding properties, MafB is uniquely involved during development in the early maturation of the beta-cell, while MafA is only essential to beta-cell activity postnatally. We are interested in determining how MafA mediates transcriptional activation, and whether these unique coregulatory interactions distinguish the adult beta-cell functions of MafA from the developmental roles of MafB. Toward this end, we have used an 'in cell' cross-linking, co-immunoprecipitation, and mass spectrometry strategy to isolate MafA binding partners from the betaTC-3 cell line. Several potentially interesting MafA interacting proteins have been identified, including the entire MLL2/3 complex (i.e. MLL2, MLL3, Rbbp5, Ash2l, Wdr5, dpy30, Utx, Paxip1, PA1, and NCoA6). MLL2/3 are two of six mammalian MLL complexes capable of both for H3K4 mono- and trimethylation, both strong epigenetic marks of activation. Interestingly, the MLL2/3 complex appears to be unable to interact with certain other islet enriched transcription factors, such as Isl1 and HNF1α. It was, however, able to interact with MafB in the recently established human beta-cell line, EndoC-betaH1. This suggests that MafA-MLL2/3 plays a vital transcriptional stimulatory role in islet β-cells by catalyzing H3K4 mono- and/or trimethylation.

We are currently taking both *in vitro* and *in vivo* approaches to determine the significance of the MLL2/3 complex in driving MafA *trans*-activation in mice. The focus of these experiments has been on NCoA6, a unique protein of the MLL2/3 complex that is necessary for promoter recruitment. Results will be presented illustrating how siRNA-directed knockdown of MafA or NCoA6 affects the epigenetic state and expression level of MafA activated genes. In addition, we are currently examining how islet beta-cell function is impacted upon removal of NCoA6 from floxed *NCoA6* mice with *insulin* promoter-driven Cre. The resulting beta-cell specific knockout of *NCoA6* (termed $NCoA6^{deltabeta}$) produces an impaired glucose tolerance phenotype analogous to the beta-cell specific deletion of *MafA* (i.e. $MafA^{deltabeta}$). However, only a subset of known MafA target genes is downregulated in $NCoA6^{deltabeta}$ mice, suggesting only partial overlap between targets of MafA and the MLL2/3 complex. We are examining in further detail the similarity of the $NCoA6^{deltabeta}$ and $MafA^{deltabeta}$ mice, focusing specifically on the consequence to gene expression and epigenetic architecture of MafA-regulated genes.

METABOLISM, SECONDARY SIGNALS & SECRETION

Poster 18 - Mark Perelis - *Clock Silencing in Adulthood Impairs Rhythmic Insulin Release and Reprograms Protein Secretion Transcription Networks.*

Poster 19 - Anton E. Ludvik - *A possible role of HKDC1 in maternal glucose homeostasis.*

Poster 20 - Medha Priyadarshini - *Potential Role of FFAR3 in Pancreatic Beta Cell Function.*

Poster 21 - Xiaodong Zhu - *Microtubules Negatively Regulate Insulin Secretion In Pancreatic Beta Cells.*

Poster 22 - Scott A. Soleimanpour - *The diabetes susceptibility gene Clec16a regulates mitophagy.*

Poster 23 - Latha Ramalingam - *Doc2b serves as a scaffolding platform for concurrent binding of multiple Munc18 proteins.*

Clock Silencing in Adulthood Impairs Rhythmic Insulin Release and Reprograms Protein Secretion Transcription Networks

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The molecular clock is encoded by a transcription-translation feedback loop in brain and peripheral tissues that maintains physiologic constancy through transcriptional programming. Studies in liver and pancreas show that clock transcription factors vary in abundance across the light-dark cycle and generate temporal windows in the transcription of metabolic gene networks, raising the possibility that clock control of transcription may impact physiologic homeostasis. Indeed, pancreatic clock deregulation throughout development and adulthood causes hypoinsulinemic diabetes, although the cellular and genomic basis for this pathology has not been defined. Here we show that wild-type mice display pronounced circadian oscillations of insulin release in response to both glucose and KCl and that selective clock ablation within beta cells during adulthood causes hypoinsulinemia and impaired glucose tolerance. Beta cell failure occurs when clock function is abrogated in adulthood due to impaired nutrient-stimulated insulin exocytosis, while oxygen consumption is intact, indicating a defect downstream of metabolism. RNA sequencing further reveals that clock transcription factors regulate a repertoire of beta cell genes involved in vesicle trafficking, docking, and exocytosis. Collectively, these findings demonstrate that the circadian clock produces rhythmic cycles of glucose -stimulated insulin secretion due to programming of islet transcription networks involved in peptide hormone trafficking and release into the circulation.

A Possible Role of HKDC1 in Maternal Glucose Homeostasis

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Pregnancy is accompanied by a diverse range of metabolic adaptations that balance both maternal and fetal nutrition. In particular, alterations in glucose metabolism are necessary to ensure sufficient fuel for the developing fetus. Gestational diabetes mellitus (GDM) occurs when women fail to maintain normal glucose homeostasis during pregnancy. A recent genome-wide association study using the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study cohort identified a novel and highly significant ($p < 10^{-20}$) association between a putative hexokinase, HKDC1, and 2-hr glucose levels during an oral glucose tolerance test (OGTT) at ~28 weeks of pregnancy. As this gene is previously uncharacterized, we first explored its expression pattern in human RNA libraries; observing wide tissue distribution including the kidney, colon, and small intestine. As many of the genes associated with diabetes have a role in pancreatic beta cells, we further examined if HKDC1 is expressed in human islets. HKDC1 is, in fact, expressed in human islets, suggesting a role in beta -cell function. Regarding its biological function, we hypothesize that it is a novel hexokinase, as it shares significant sequence homology to hexokinases I-III. To test this, bacterially synthesized and purified human HKDC1 protein has demonstrated robust, high affinity hexokinase activity *in vitro*, confirming that it is a previously unknown hexokinase. To explore the role of HKDC1 genetically, we have established a HKDC1 gene trap mouse model, which results in a truncated nonfunctional HKDC1 allele. Homozygous animals have proven to be embryonic lethal. Studies in heterozygous animals between the ages of 4 and 28 weeks have demonstrated normal development, weight gain, and fasting blood glucose levels. At 28 weeks of age, however, female animals have impaired glucose tolerance as compared to controls. Further phenotyping studies including studies during pregnancy are on-going in our laboratory. Taken together, we have begun to explore a novel genetic link between HKDC1 and glycemic control during pregnancy, and thus far have observed it is a novel hexokinase that has an *in vivo* role in glucose homeostasis.

Potential Role of FFAR3 in Pancreatic Beta Cell Function

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Insulin secretion and synthesis are tightly regulated to maintain glucose homeostasis. Short chain fatty acids (SCFAs), the byproducts of gut microbial fermentation of complex carbohydrates, have been recently recognized as novel players in aspects of metabolic regulation including glucose homeostasis. Of importance, these molecules are known to activate free fatty acid receptor 2 and 3 (FFAR2 and -3) and consequently, this activation has been shown to modulate adiposity, energy expenditure, glucose control and inflammation. Interestingly, both receptors have been shown to be expressed in pancreatic beta cells; however, their role in pancreatic beta cells remains uninvestigated. In this report, we focus on FFAR3. First of all, we explored and observed that FFAR3 has altered expression in islets in various insulin resistant states suggesting it may have a potential role in adapting to the stress of insulin resistance in pancreatic beta cells. To further investigate a potential role of FFAR3, global knockout of FFAR3 ($FFAR3^{-/-}$) mice were generated. Using isolated islets from age-matched $FFAR3^{-/-}$ and $FFAR3^{+/+}$ mice, we have observed that (a) $FFAR3^{-/-}$ islets secrete more insulin in a glucose-dependent manner as compared to islets from their wildtype counterparts ($FFAR3^{+/+}$ mice), and (b) that endogenous ligands of FFAR3, the SCFAs (in particular propionate, butyrate, and beta-hydroxybutyrate) modulate their ability to secrete insulin in response to glucose. To help understand these results, transcriptome analyses by RNA-sequencing of $FFAR3^{-/-}$ and $FFAR3^{+/+}$ islets observed 4166 significantly modified genes. Noteworthy changes include multiple genes downregulated in $FFAR3^{-/-}$ islets that are involved in aspects of glucose homeostasis and insulin secretion (e.g., Nr4a2, Pax6, Foxo3, Pfkm, Neurod1, Mafa, GPR119, Glp1r, Isl-1, and Irs-2). Interestingly, the glycolytic enzymes, hexokinase 1 and 2 (HK1 and HK2), showed increased expression in $FFAR3^{-/-}$ islets, and we suggest that this may contribute to the altered islet-glucose sensing in $FFAR3^{-/-}$ islets. Interestingly, various inflammatory and oxidative stress pathways were also found to be upregulated in $FFAR3^{-/-}$ islets. Taken together, our data suggest that FFAR3 has a role in glucose stimulated insulin secretion, and subsequent studies are underway to explore this mechanism in greater detail. Future studies are set to more rigorously define the role of FFAR3 by *in vivo* approaches

Microtubules Negatively Regulate Insulin Secretion In Pancreatic Beta Cells

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Glucose-stimulated insulin secretion (GSIS) from pancreatic beta cells maintains glucose homeostasis. A hypothesis that insulin secretion is promoted by microtubule-dependent transport, which delivers insulin granules to exocytic sites, has been a matter of debate. In contrast to this common notion, we report that microtubules (MTs) regulate insulin secretion by restraining excessive delivery of insulin granules to the plasma membrane. Using high- and super-resolution microscopy on intact islets, combined with GSIS detection, we found that MTs in functional beta cells emerge from the Golgi complex and form a meshwork-like network that anchors insulin granules. Importantly, the density of this network is significantly higher in dysfunctional beta cells of diabetic mice compared to normal mice. Furthermore, we show that MT depolymerization facilitates delivery of insulin granules to the cell membrane and dramatically enhances GSIS, suggesting that MTs withhold granules away from secretion sites and that glucose induces dynamic MT turnover for GSIS to occur. Our results demonstrate that MTs act as a cellular “rheostat” for precisely metered insulin secretion, which is tuned according to physiological needs by modulating MT density and dynamics.

The Diabetes Susceptibility Gene *Clec16a* Regulates Mitophagy

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ABSTRACT

Clec16a has been identified as a disease susceptibility gene for type 1 diabetes, multiple sclerosis and adrenal dysfunction, but its function is unknown. Utilizing high throughput analysis of protein interaction partners found during immunoprecipitation and mass spectrometry, we report that Clec16a participates in a multi-subunit protein complex to regulate expression of Parkin, a master regulator of mitophagy. Islets from mice with pancreas-specific deletion of Clec16a have abnormal mitochondria with reduced oxygen consumption and ATP concentration, both of which are required for normal beta cell function. Indeed, pancreatic Clec16a is required for normal glucose-stimulated insulin release. Furthermore, we find that Clec16a is a membrane-associated endosomal protein which regulates pathways downstream of Parkin and its targets, including autophagosomal flux and ER homeostasis. Moreover, patients harboring a diabetogenic SNP in the Clec16a gene have reduced islet Clec16a expression and reduced insulin secretion. Thus, Clec16a controls beta cell function and prevents diabetes by controlling mitophagy. This novel pathway could be targeted for prevention and control of diabetes and may extend to the pathogenesis of other Clec16a and Parkin

Doc2b serves as a scaffolding platform for concurrent binding of multiple Munc18 proteins

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Glucose-stimulated insulin secretion (GSIS) from pancreatic islet beta cells requires SNARE proteins to mediate insulin granule exocytosis. Exocytosis is facilitated by SNARE protein assembly into docking and fusion complexes, and this assembly process further requires regulatory proteins such as Munc18c, Munc18-1 and Doc2b. Knockout mouse models of each of these regulatory proteins support the concept that each is required in the process of GSIS and SNARE complex assembly. Interestingly, while Munc18-1 is required for first phase GSIS, Munc18c is only needed for second phase GSIS, yet Doc2b is required for both phases. While Munc18-1 and Munc18c are well-known as transient high-affinity binding factors for their cognate t-SNAREs Syntaxin 1A and Syntaxin 4, respectively, the molecular role for Doc2b in the context of SNARE and Munc18 protein regulation in GSIS at the molecular level is unclear. Because Doc2b has been shown to bind to Munc18-1 via Doc2b's C2A domain, and the Munc18c isoform via Doc2b's C2B domain, we hypothesized that Doc2b may function as a molecular scaffold for these Munc18 isoforms in the beta cell, permitting their independent and transient associations with the Doc2b scaffold. Indeed, macromolecular heterotrimeric complexes of Munc18c-Doc2b-Munc18-1 were detected by reciprocal co-immunoprecipitations from MIN6 beta cell lysates. Co-immunoprecipitations using recombinantly expressed and purified Munc18 and Doc2b proteins indicated that Munc18c and Munc18-1 do not directly associate, and can be co-immunoprecipitated only upon addition of Doc2b to binding reactions. In a second and antibody-free approach, GST-Munc18-1 and GST-Munc18c interaction assays confirmed that Doc2b was required for the heterotrimeric interaction. Competition-based GST-Doc2b interaction assays further showed that Munc18-1 and Munc18c do not compete for occupancy on Doc2b, supporting the concept that they bind to independent domains and in a similar stoichiometric ratio to the Doc2b scaffold. Given that very recent evidence from our group shows that Doc2b enrichment in the islet beta cell promotes highly efficient insulin exocytosis and whole body glucose homeostasis, further studies of the stimulatory mechanisms modulating Munc18c-Doc2b-Munc18-1 interactions in insulin-secreting beta cells will be pursued toward developing Doc2b as a target for diabetes mitigation and prevention.

ISLET ION CHANNELS

Poster 24 - Eric J. Glynn - *Chronic Glucose Exposure Alters Glucose-Sensitivity in Pancreatic Islets via K(ATP) Channel Regulation in Beta-Cells.*

Poster 25 - Nicholas Vierra - *The C-Terminus of TALK-1 is a Charge-Sensitive Module Critical for Regulation of Channel Activity.*

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Chronic Glucose Exposure Alters Glucose-Sensitivity in Pancreatic Islets via K(ATP) Channel Regulation in Beta-Cells

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Introduction and Objectives: Islet beta-cells respond to chronic changes in the glucose environment in numerous ways; however, functional adaptations in response to chronic glucose are poorly understood. Here we show the effect of chronic glucose exposure on the glucose-sensitivity of islets. Our goals were to (1) characterize how different concentrations of glucose chronically affect islet function, (2) identify the role of insulin feedback on glucose-sensitivity, and (3) test whether changes in glucose-sensitivity were due to changes in trafficking of K(ATP) channels to the plasma membrane.

Methods: Islets were cultured for 18 hrs in media containing 2.8-16.7 mM glucose. The glucose induced calcium responses of islets from each pretreatment group were assessed to obtain relative shifts in islet glucose sensitivity. Patch-clamp analysis was used to evaluate K(ATP) channel conductance in beta-cells of islets in the pretreatment groups most affected.

Results: Islets pretreated with <5mM glucose had reduced glucose-sensitivity while pretreatment with >5mM glucose predominantly enhanced glucose sensitivity. Co-culture with diazoxide enhanced glucose sensitivity, while co-culture with tolbutamide reduced glucose-sensitivity. Inclusion of the AMPK activator, AICAR, partially reversed the effect of high glucose and diazoxide. Patch-clamp analysis revealed a correlation between the number of K(ATP) channels in the plasma membrane of beta-cells and the respective change in glucose-sensitivity resulting from pretreatment. Reducing the number of K(ATP) channels in the membrane would be expected to cause a left-shift in glucose-sensitivity of calcium oscillations.

Conclusions: Islets adapt to chronic glucose by altering the number of K(ATP) channels in the plasma membrane. This decreases the threshold of glucose required to stimulate calcium oscillations and insulin secretion. We propose that glucose and insulin may have opposing actions on K(ATP) trafficking. These results reveal a novel adaptive mechanism that is relevant to *in vivo* glucose homeostasis. Supported by R01DK46409 (L.S.S.).

The C-Terminus of TALK-1 is a Charge-Sensitive Module Critical for Regulation of Channel Activity

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Insulin secretion from human beta-cells is dependent on Ca^{2+} entry through voltage dependent Ca^{2+} channels (VDCCs). K^+ channels modulate the beta-cell membrane potential, which influences VDCC activity and hormone secretion. The most abundant K^+ channel of the human pancreatic islet is the two-pore domain K^+ (K2P) channel TALK-1, however, its physiological function is unknown. Furthermore, a non-synonymous coding sequence polymorphism in the C-terminal tail (*Ct*) of TALK-1 has been associated with an increased risk for developing type II diabetes. To identify mechanisms that regulate beta-cell TALK-1 channel activity, we utilized a Tl^+-sensitive fluorescent screen to assess if TALK-1 is regulated by specific kinases. Cells expressing TALK-1 channels were monitored for changes in Tl^+ flux in response to co-expression with a constitutively active kinase (192 myristoylated kinases in total were assessed), which revealed 9 kinases that modify Tl^+ flux through TALK-1 channels. Interestingly, three phosphatidylinositol phosphate kinases (PIKs) including PIK3CG, PI4K2B, and PIKFYVE increased TALK-1 channel activity. To determine if modulation of TALK-1 channels via PIKs is due to build-up of membrane phosphoinositides, we depleted membrane PIP_2 with a rapamycin-recruitable phosphatase and found that TALK-1 channel activity was reduced. As positively charged residues in the *Ct* of other K^+ channels have been shown to mediate PIP_2 sensitivity, we assessed if PIP_2 influences TALK-1 channel activity via charged residues in the *Ct*. We found that neutralization of the *Ct* residues R269 and K274 to alanine significantly reduced PIP_2 activation of TALK-1 channels. Finally, we examined islets from Talk-$1^{-/-}$ mice to assess how TALK-1 channel activity modulates Ca^{2+} homeostasis. We found that glucose and tolbutamide stimulation resulted in 1.8 ± 0.1 fold greater Ca^{2+} entry into Talk$1^{-/-}$ islets compared with control islets. Furthermore, increasing islet PIP_2 levels by inhibiting the 5'-inositol phosphatase SHIP2 and the 3'-inositol phosphatase PTEN resulted in greater inhibition of Ca^{2+} entry in control islets (46 ± 3%) compared with Talk$1^{-/-}$ islets (28 ± 2%). Thus, these results uncover a PIP_2 regulatory mechanism for activation of beta-cell TALK-1 channels, which involves a charged *Ct*. Importantly, these data also indicate that TALK-1 channels limit beta-cell Ca^{2+} entry in response to depolarization and PIP_2 elevation. Therefore, these results predict an important role for TALK-1 channels during secretagogue induced beta-cell Ca^{2+} influx and insulin secretion.

In the Absence of ZnT7, Deletion of ZnT8 Impairs Glucose Tolerance

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Zinc homeostasis is maintained in the body by two types of proteins, metallothioneins and zinc transporters. Zinc transporters are divided into two families, Zip and ZnT. The ZnT family of zinc transporters is responsible for reducing cytoplasmic zinc concentration by causing an efflux of zinc ions out of the cell or influx of zinc into intracellular vesicles.

Zinc transporter 8 (ZnT8), a member of the ZnT family, is encoded by the *SLC30A8* gene and is highly expressed in adult islet beta cells, localizing to insulin secretory vesicles. It is hypothesized that ZnT8-mediated transportation of zinc ions into insulin secretory vesicles aids in insulin processing and crystallization.

Data from multiple human genome wide association studies (GWAS) have shown that a non-synonymous single nucleotide polymorphism (SNP) in the *SLC30A8* gene is associated with increased susceptibility to type 2 diabetes (T2D). To explore this connection, several groups have generated and studied mice in which the S*lc30a8* gene has been deleted globally or specifically in beta cells. The phenotypes observed have been highly variable, although mild. For example, we have previously reported that global deletion of *Slc30a8* on a pure C57BL/6J background markedly decreases the content of free zinc in isolated islets, which is consistent with other reports, but has no significant impact on glucose-stimulated insulin secretion (GSIS) in isolated islets *in vitro* or glucose tolerance *in vivo*. Most recently, it has been shown that, in humans, *SLC30A8* haploinsufficiency protects against T2D. We hypothesized that the human haploinsufficiency and GWAS data could be reconciled with the KO mouse data if the non-synonymous *SLC30A8* SNP that is associated with increased susceptibility to T2D were acting in a dominant negative fashion to inhibit the activity of another ZnT in beta cells.

Previously published data led us to further hypothesize that ZnT7, encoded by the *Slc30a7* gene, might functionally compensate for the absence of ZnT8 and be the target of dominant negative inhibition by the ZnT8 SNP variant. Thus, it has been shown in rat insulinoma cells (RIN5) that over-expression of ZnT7 enhances GSIS. We therefore generated and characterized double knockout (KO) mice lacking both ZnT7 and 8. Intraperitoneal glucose tolerance tests using 2 g/kg glucose show that glucose tolerance is impaired in the absence of ZnT7 but not ZnT8, and further impaired by the combined absence of ZnT7 and 8, consistent with the hypothesis that ZnT7 can compensate for the absence of ZnT8. However, during oral glucose tolerance tests using 2 g/kg glucose, while glucose tolerance is impaired in the absence of ZnT7, it is not further impaired by the absence of ZnT8. We hypothesize that in the absence of ZnT7, incretins, gut peptide hormones secreted in response to an oral glucose load that enhance GSIS, are able to compensate for the absence of ZnT8. Current studies are examining the impact of combined deletion of ZnT7 and 8 on GSIS *in vivo* and *in vitro* and whether the T2D-associated ZnT8 SNP affects dimer formation between ZnT7 and 8.

Beta Cell SERCA2b Protein Stability is Regulated Via NO- and AMPK-Dependent Pathways Under Inflammatory Diabetic Conditions

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The sarco-endoplasmic reticulum Ca^{2+} ATPase 2b (SERCA2b) pump maintains a steep calcium concentration gradient between the beta cell cytosol and ER lumen and acts as the primary gatekeeper of endoplasmic reticulum (ER) Ca^{2+} homeostasis. Our previous work has revealed markedly diminished beta cell SERCA2b mRNA and protein expression in human and rodent models of Type 1 and Type 2 diabetes mellitus. Meanwhile, SERCA2b haploinsufficient mice and mice with a beta cell specific deletion of SERCA2b exhibit defective glucose homeostasis and altered ex vivo islet glucose stimulated insulin secretion. Here, we sought to define the transcriptional and translational regulation of SERCA2b under diabetic conditions. To begin to address this goal, INS-1 832/13 cells and mouse islets were treated with 5 ng/ml IL-1beta + 25 mM glucose (IL-1beta + HG) to mimic the pro-inflammatory milieu of diabetes, and cycloheximide and actinomycin D were employed in time-course studies. Under basal conditions, SERCA2b protein half-life (t1/2) was 24 hr, while the mRNA t1/2 was 6 hr. IL-1beta + HG treatment led to iNOS/nitric oxide (NO) induction, activation of cleaved caspase-3, and reduced SERCA2b protein t1/2 to 16hr; however the mRNA t1/2 was unchanged. Concurrent treatment with the iNOS inhibitor, L-NMMA, prevented these changes, indicating that altered SERCA2b expression is dependent upon NO activation. Because SERCA2b pump activity is highly energy dependent, we hypothesized that SERCA2b protein stability and hence activity under inflammatory conditions may also be regulated through AMP-activated protein kinase (AMPK)-regulated signalling pathways. Prolonged pharmacologic activation of AMPK with 2 mM AICAR decreased SERCA2b protein t1/2. Conversely, the AMPK inhibitor, compound C (10 uM) prevented both IL-1beta and NO donor-dependent loss of SERCA2b. Compound C also prevented cleaved caspase-3 activation and restored cell viability. Taken together, these data suggest that SERCA2b loss under inflammatory diabetic conditions occurs through NO- and AMPK-dependent mechanisms. We also illustrate a novel pathway through which AMPK limits beta cell energy expenditure through restraint of proteins with Ca^{2+} ATPase activity. Future studies will define the specific pathways responsible for SERCA2b degradation and test the effects of acute AMPK activation and changes in energy status on beta cell calcium flux

Disruption of Ca^{2+}-Induced Ca^{2+} Release and Glucose-Stimulated ERK1/2 Activation in INS-1 Cells Expressing the $Ca_v1.2$ II-III Loop

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L-type calcium channels play important roles in glucose-stimulated insulin secretion and pancreatic beta cell proliferation; however, the individual roles of $Ca_v1.2$ and $Ca_v1.3$, the L-type channels expressed in beta cells, have not been fully elucidated. One of the most divergent regions of the $Ca_v1.2$ and $Ca_v1.3$ primary structures is the II-III intracellular loop, a region important for protein-protein interactions. Using sucrose density gradient fractionation, we found that expression of the II-III loop of $Ca_v1.2$ in INS-1 cells ($Ca_v1.2$/II-III cells) displaces endogenous $Ca_v1.2$ channels from lipid raft domains. Since lipid rafts are thought to serve as intracellular signaling platforms, we investigated calcium signaling, insulin secretion, electrical activity, ERK activation and proliferation in $Ca_v1.2$/II-III cells. Both calcium transients and insulin secretion stimulated by tolbutamide (200 μM) in INS-1 cells and $Ca_v1.2$/II-III cells were blocked by nicardipine (2 μM) but thapsigargin (1 μM) blocked these events in INS-1 cells only, suggesting that calcium flux through L-type calcium channels but not release from intracellular stores is intact in $Ca_v1.2$/II-III cells. Using single-cell calcium imaging of Fura2-AM-loaded cells and a genetically-encoded ER-targeted FRET-based Ca^{2+} sensor (D1ER), we determined that Ca^{2+}-induced Ca^{2+} release (CICR) is disrupted in $Ca_v1.2$/II-III cells. Further, we observed that $Ca_v1.2$/II-III cells have an increased frequency of action potentials in response to glucose (18 mM) compared to INS-1 cells. Apamin (1 μM) increased action potential frequency in INS-1 cells to a level not different from $Ca_v1.2$/II-III cells in the absence of apamin, but did not affect action potential frequency in $Ca_v1.2$/II-III cells, suggesting that SK channels are not efficiently activated in $Ca_v1.2$/II-III cells. In addition, ERK1/2 phosphorylation stimulated with glucose (18 mM) or glucose + GLP-1 (50 nM), was significantly slowed in $Ca_v1.2$/II-III cells compared to INS-1 cells. ERK1/2 phosphorylation in $Ca_v1.2$/II-III cells was completely blocked by nicardipine but insensitive to ryanodine (20 μM), suggesting that CICR does not contribute to ERK1/2 activation in $Ca_v1.2$/II-III cells as in INS-1 cells. Finally, we found that glucose + GLP-1 stimulated proliferation of INS-1 cells but not $Ca_v1.2$/II-III cells, as assessed using a [^{3}H]-thymidine incorporation assay. We conclude that $Ca_v1.2$ plays a role in CICR, activation of SK channels, and glucose + GLP-1 stimulated proliferation in INS-1 cells.

Exploring Beta-Cell Excitotoxicity as a Cause for Type 2 Diabetes

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Intracellular calcium is a vital secondary messenger for excitable cells including cardiac and skeletal muscle, neurons, and endocrine cells. Transient spikes in the intracellular calcium concentration ($[Ca^{2+}]_i$) trigger muscle contraction, neurotransmitter release, or hormone secretion, respectively. However, the over-stimulation of excitable cells can lead to chronically increased $[Ca^{2+}]_i$ which may then cause cellular dysfunction or death. The deleterious effects of chronic hyperstimulation on neurons has been termed excitotoxicity. We hypothesize that a sustained elevation of $[Ca^{2+}]_i$ due to overstimulation by a hyperglycemic environment leads to beta-cell excitotoxicity.

Previously we have shown that the genetic deletion of *Abcc8*, a key component of ATP-sensitive potassium channels, causes a sustained elevation in basal $[Ca^{2+}]_i$. *Abcc8*$^{-/-}$ mice are born with islets that exhibit normal cytoarchitecture. However, within a month they begin to develop markedly disrupted islets, with alpha-, delta-, and PP-cells localized throughout the core instead of predominately in the mantle. To better understand the progression of this phenotype we have measured changes in islet morphology beginning at 4 weeks of age and extending to at least 20 weeks. We have observed decreased insulin$^+$ area per islet, decreased distance of alpha-cells from the islet center, and increased prevalence of "empty" islet area in aging *Abcc8*$^{-/-}$ mice. Additionally, we see a trend toward impaired glucose tolerance in *Abcc8*$^{-/-}$ compared to *Abcc8*$^{+/+}$ mice.

Since the mechanisms whereby a sustained increase in $[Ca^{2+}]_i$ cause beta-cell dysfunction are not understood we are in the process of generating RNA-seq datasets from *Abcc8*$^{-/-}$ beta-cells to determine changes in gene expression that occur in response to hyperstimulation. Additionally, we are making a novel, allelic series of mice that will enable us to perturb specific protein mediators known to affected an increase in $[Ca^{2+}]_i$. We will use these mice to explore the importance of specific Ca^{2+} signaling pathways in causing beta-cell dysfunction and death.

ISLET STRESS & SURVIVAL

Poster 30 - Guanlan Xu - *TXNIP-Induced microRNA-204 Inhibits Beta Cell Function.*

Poster 31 - Stephen Filios - *miR-200b Increases Beta Cell Apoptosis and is Induced by TXNIP*

Poster 32 - Leena Haataja - *Successful and Unsuccessful Folding in the Oxidation of Proinsulin*

Poster 33 - Daleep Arora - *Identification of small molecules that protect pancreatic beta cells against ER stress induced cell death.*

Poster 34 - Lance Thielen - *Oxidative Stress Cannot Mimic TXNIP-Induced microRNA Expression in INS-1 Beta-Cells.*

Poster 35 - Carly Kibbe - *Cholecystokinin Protects Pancreatic Beta Cells From Stress-Induced Apoptosis.*

Poster 36 - Wataru Yamamoto - *SERCA2b Overexpression Improves Beta Cell Survival Under Inflammatory and ER Stress Conditions.*

Poster 37 - Justin Bushkofsky - *Tcf19 is a Novel Islet Factor Involved in Proliferation and Atf6 Mediated Endoplasmic Reticulum Stress.*

TXNIP-Induced microRNA-204 Inhibits Beta Cell Function

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Loss of functional pancreatic beta cell mass is a common characteristic of type 1 and type 2 diabetes. Thioredoxin-interacting protein (TXNIP) plays a critical role in this process. We previously showed that TXNIP is increased in diabetic islets, whereas the genetic deficiency or pharmacological inhibition of TXNIP protects against diabetes by preventing beta cell loss. Our gene expression profiling study demonstrated that 95% of the genes altered by TXNIP overexpression were downregulated even though TXNIP is not known to act as a transcriptional repressor, suggesting that TXNIP might confer its effects via miRNAs. In the present study, we performed miRNA microarray assays comparing TXNIP-overexpressing and LacZ-expressing INS-1 beta cells. By setting 1.6 fold as a threshold, we found that five miRNAs (miR-139-5p, 193, 204, 200c, 141) were upregulated in response to TXNIP overexpression, and confirmed this by real-time RT-PCR. Interestingly, diabetic B6-obese and BTBR-obese mouse islets, in which TXNIP was significantly increased, also showed significantly higher expression of these miRNAs. In contrast, miRNA levels were significantly lower in TXNIP-deficient mouse islets as compared to controls. When studying the potential function of these miRNAs, we found that only miR-204 overexpression significantly inhibited insulin expression. However, miR-204 did so at the transcriptional level rather than targeting insulin directly. Using 3'UTR reporter assays, real-time RT-PCR, immunoblotting and chromatin immunoprecipitation studies, as well as primary human islets we demonstrated that miR-204 instead targets and downregulates the expression of the insulin transcription factor MAFA, which in turn inhibits insulin transcription and results in reduced beta cell insulin content. Taken together, we found that TXNIP and diabetes induce the expression of distinct miRNAs, which in turn control important beta cell functions, such as insulin transcription. The present study thereby sheds new light on the role of miRNAs in TXNIP signaling and diabetes and their effects on beta cell biology.

miR-200b Increases Beta Cell Apoptosis and is Induced by TXNIP

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Beta cell death is a prominent feature of both type 1 and type 2 diabetes and we have found that Thioredoxin Interacting Protein (TXNIP), plays a critical role in this process. Recently, we also discovered that TXNIP regulates beta cell microRNAs (miRs) and a miR microarray revealed an increase in members of the miR-200 family.

In the present study, we demonstrate that TXNIP positively regulates beta cell levels of the miR-200 family members, miR-200a, miR-200b, miR-200c, miR-141, and miR-429. These results were found in INS-1 beta cells constitutively overexpressing TXNIP and in primary mouse islets, and show that TXNIP deficient HcB-19 mice have decreased levels of miR-200 family members as measured by qPCR. In contrast, islets of obese diabetic B6 ob/ob mice, which have increased TXNIP levels, also exhibit an up-regulation in their expression of miR-200 family members.
Furthermore, we determined that overexpression of miRNA-200 family members, and in particular miR-200b, caused apoptosis in INS-1 beta cells as assessed by Bax/Bcl2 mRNA ratio, cleaved caspase-3 protein levels, and terminal deoxynucleotidyl transferase dUTP nick end labeling. We also investigated which downstream targets of miR-200 may cause an increase in beta cell death. We found that one potential target, Zinc finger E-box binding homeobox 1 (Zeb1) mRNA was decreased in response to overexpression of members of the miR-200 family in INS-1 beta cells. Similar results were also found at the protein level, as measured by Western blot. We also discovered that knocking down Zeb1 using small interfering RNA induced apoptosis as assessed by an increase in cleaved caspase-3 demonstrating that Zeb1 down-regulation can mimic the effects of miR-200 on beta cell apoptosis.

Taken together, the results of this study indicate that TXNIP causes an increase in the beta cell expression of miR-200 family members, that these miRs increase beta cell apoptosis, and that this may be mediated through the targeting of Zeb1.

Successful and Unsuccessful Folding in the Oxidation of Proinsulin

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We have previously reported that in the syndrome of Mutant INS-gene-induced Diabetes of Youth (MIDY, derived from heterozygous proinsulin mutations), misfolded mutant proinsulin engages in abnormal intramolecular disulfide isomers. During normal folding to the native state, proinsulin makes three evolutionary conserved disulfide bonds, two “interchain ”: Cys(B7)-Cys(A7) and Cys (B19)-Cys(A20) and one “intrachain”: Cys(A6)-Cys(A11). In this study, we have investigated the hypothesis that there is a cooperativity in newly-synthesized proinsulin folding in the endoplasmic reticulum of pancreatic beta cells and a favored sequence of disulfide bond formation. To test this hypothesis, we have created a novel series of proinsulin mutants bearing only two natural Cys partners from which only one native disulfide bond of proinsulin can form (“keep mutants”). In addition, we have examined the threat posed by unpaired Cys residues as an intramolecular perturbant to the formation of other native disulfide pairings. Finally, we have introduced MIDY mutations that neither add or lose a Cys residue. Specifically we concentrated on keep-B19/A20 proinsulin to study B19/A20 disulfide bond formation by nonreducing SDS-PAGE. We find that B19/ A20 disulfide bond is able to form independently of any other disulfide pairs, whereas Cys(B7)-Cys (A7) is not formed in the absence of pre-existing Cys(B19)-Cys(A20) disulfide bond. Moreover, we identified two single Cys residues capable of perturbing proinsulin B19/A20 disulfide bond formation. Finally, we show that several MIDY mutations directly inhibit B19/A20 disulfide bond oxidation. Our results highlight the importance of the proinsulin folding pathway for proper insulin production, and identify discrete molecular events in intramolecular proinsulin maturation that can go wrong, leading to ER stress and insulin deficiency.

Identification of small molecules that protect pancreatic beta cells against ER stress-induced cell death

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Pancreatic bet cell dysfunction and loss is a pathological component of type 2 diabetes (T2D). Endoplasmic reticulum (ER) stress plays an important role in causing the decline in beta cell function and mass in T2D. We developed a novel phenotypic beta cell-based assay to identify small molecule capable of protecting beta cells against ER stress-induced cell death. Mouse beta cell line βTC6 cells were treated with ER stressor Tunicamycin (Tm) to induce ER stress and cell death and a collection of 17,600 compounds were screened for those that increased beta cell survival assayed by cellular ATP levels. Approximately 80 compounds were identified that showed beta cell protective effect. A number of selected hit compounds were also able to promote additional beta cell lines including MIN6 and INS-1 and human primary beta cells against various chemical and pathophysiological ER stressors such as Thapsigargin and palmitate. These compounds also restored the glucose stimulated insulin secretion (GSIS) in the presence of Tm. Finally, we found that these compounds protect beta cell survival by resolving ER stress and that the combination of compounds with distinct mechanisms of action achieved a greater protective effect against ER stress-induced cell death than singly. Identification of small molecules that can prevent ER stress-induced beta cell death could provide a new avenue for the treatment of diabetes.

Oxidative Stress Cannot Mimic TXNIP-Induced microRNA Expression in INS-1 Beta-Cells

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The death of pancreatic beta-cells via apoptosis represents a critical factor in the pathogenesis of diabetes. Thioredoxin-interacting protein (TXNIP), whose gene is most significantly upregulated in response to glucose in human islets, is a ubiquitously expressed redox protein that has been shown to promote apoptosis in beta-cells. TXNIP is also upregulated in diabetes and induces oxidative stress, which is believed to be one of the main mechanisms by which TXNIP causes its detrimental effects on beta-cells. Recently, we discovered that TXNIP also regulates the expression of microRNAs and a microRNA microarray analysis revealed the following five microRNAs (miR-139-5p, miR-193, miR-204, miR-200c, and miR-141) to be the most significantly upregulated in response to TNXIP overexpression in INS-1 beta-cells. The aim of the present studies was therefore to determine whether this observed increase in microRNA expression was also due to TXNIP-induced oxidative stress. To address this question, we subjected INS-1 beta-cells to various H_2O_2 and staurosporine concentrations (7-15uM H_2O_2 and 1-100nM staurosporine) and treatment durations (2-24 hours) to induce oxidative stress. RNA was harvested from the treated cells and quantitative RT-PCR was performed, testing for alterations in expression of these five microRNAs. However, our results revealed no significant change in microRNA expression in response to oxidative stress, indicating that oxidative stress was not able to mimic the TXNIP effects. Taken together, our results suggest that TXNIP is inducing microRNA expression by mechanisms other than induction of oxidative stress and thereby provide additional support for the notion tha*-t TXNIP has beta-cell effects beyond its role as a redox regulator.

Cholecystokinin Protects Pancreatic Beta Cells From Stress-Induced Apoptosis

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Beta cell apoptosis is a key defect in diabetes, but to date there are no effective therapies that target this defect. Understanding the mechanisms involved in beta cell apoptosis could lead to the development of new therapies to preserve beta cell mass and prevent or reverse diabetes. Cholecystokinin (CCK) is a gut hormone released in response to nutrient intake. Interestingly, pancreatic islets also produce CCK. We have previously shown that CCK is highly upregulated in islets of obese (ob/ob) mice. CCK deficient ob/ob mice have reduced beta cell mass and hyperglycemia due to increased beta cell apoptosis. Furthermore, isolated islets from CCK deficient ob/ob mice are more susceptible to endoplasmic reticulum (ER) stress-induced apoptosis following thapsigargin treatment. These results suggest CCK is necessary to protect beta cells from apoptosis under stress conditions. Therefore, we set out to test the hypothesis that CCK overexpression can protect beta cells from stress-induced apoptosis. To study the effect of CCK overexpression in vivo we generated transgenic mice with CCK expression driven by the mouse insulin promoter (MIP-CCK). When left unstressed, lean MIP-CCK animals displayed normal plasma glucose and insulin levels in addition to normal glucose-stimulated insulin secretion compared to control animals. These results suggest beta cell-specific overexpression of CCK does not affect islet function or glucose metabolism in healthy animals. CCK administration has been shown to induce pancreatitis in mouse models. Pancreatic sections from aged animals were stained with hematoxylin and eosin and levels of inflammation and fibrosis were scored. There was no significant difference in severity score between MIP-CCK and control animals, suggesting that beta cell-specific overexpression of CCK does not result in pancreatitis. Streptozotocin (STZ) was administered to MIP-CCK animals to study the effect of CCK overexpression under stress conditions. MIP-CCK mice remain euglycemic 30 days after STZ treatment ($n=7$-9, $p<0.05$). STZ-treated MIP-CCK mice also display a 2-fold reduction in beta cell apoptosis ($n=5$, $p<0.05$). These results suggest CCK overexpression protects against STZ-induced diabetes through decreased beta cell apoptosis. The MIP-CCK mice were also crossed with Akita mice, a model of ER stress-induced diabetes due to beta cell apoptosis. Akita heterozygous males that overexpress CCK are less hyperglycemic than controls at 22 weeks of age (1053+/-23 mg/dL vs. 649+/-84 mg/dL, $n=3$, $p=0.0078$). In summary, beta cell-specific expression of CCK protects mice from stress-induced apoptosis and ameliorates STZ- and ER stress-induced diabetes. Therefore, in addition to its beneficial endocrine effects on insulin secretion and satiety, local CCK production in the beta cell appears to protect against beta cell apoptosis. CCK directed therapies could have multiple positive effects that would lead to improved diabetes control.

SERCA2b Overexpression Improves Beta Cell Survival Under Inflammatory and ER Stress Conditions

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The maintenance of intracellular Ca^{2+} homeostasis in the beta cell plays a vital role in insulin secretion and endoplasmic reticulum function. Accumulating evidence suggests that reductions in the ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) may play a primary causative role in triggering beta cell ER stress. Cytosolic and ER Ca^{2+} levels are closely regulated by the activity of the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump. SERCA2b is the most abundant isoform expressed in pancreatic islets, and our previous work has demonstrated significantly decreased beta cell SERCA2b expression in human and rodent models of Type 1 and Type 2 diabetes mellitus. We therefore hypothesized that overexpression of SERCA2b under conditions of diabetic and ER stress may improve ER calcium homeostasis and beta cell viability. To test this hypothesis, rat insulinoma (INS-1 832/13) cells were transduced with a SERCA2b adenoviral construct or a LacZ construct as a control and then treated with 25 mM glucose combined with 5 ng/mL of the pro-inflammatory cytokine IL-1beta (HG + IL-1beta) to mimic the pro-inflammatory milieu of diabetes or with 10 uM tunicamycin to induce the unfolded protein response in time-course experiments. HG + IL-1beta led to decreased SERCA2b protein expression and increased expression of cleaved caspase-3, while tunicamycin led to increased cleaved caspase-3 expression independent of changes in SERCA2b levels. SERCA2b adenoviral overexpression was able to prevent HG + IL-1beta as well as tunicamycin-induced upregulation of cleaved caspase-3 and led to restoration of cell viability under both conditions. These results were confirmed by a caspase3/7 activity assay, which revealed a significant a reduction in caspase3/7 activity in SERCA2b overexpressed INS-1 cells following HG + IL-1beta and tunicamycin treatment. Effects on INS-1 cell proliferation under basal and stressed conditions were also assessed by measuring thymidine incorporation. Proliferation was significantly decreased with HG + IL-1beta treatment, while SERCA2b overexpression was able to partially abrogate this effect. These data suggest a critical role for SERCA2b and the regulation of ER Ca^{2+} homeostasis in the maintenance of beta cell survival and response to stress. Work is ongoing to correlate alterations in SERCA2b expression with changes in cytosolic and $[Ca^{2+}]_{ER}$ levels using Fura 2-AM imaging and ER-targeted and genetically encoded calcium indicators coupled with fluorescent lifetime imaging (FLIM).

Tcf19 is a Novel Islet Factor Involved in Proliferation and Atf6 Mediated Endoplasmic Reticulum Stress

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Transcription factor 19 (Tcf19) is a putative transcription factor with a genetic association to type 1 diabetes. Tcf19's role in the pathogenesis of type 1 diabetes remains unclear. Tcf19 is expressed in both human and rodent islets and increases in non-diabetic obesity. Efforts to prevent apoptosis and promote proliferation hold promise as therapeutic interventions for preserving beta cell mass and preventing diabetes. We have recently shown that Tcf19 plays a dual role in beta cell proliferation and apoptosis.

Tcf19 expression correlates with beta cell expansion in obese mice, supporting Tcf19's role as a transcriptional regulator of beta-cell proliferation. Tcf19 knockdown in INS-1 cells caused a 45% decrease in proliferation, and flow cytometry revealed this decrease was due to a lack of progression past the G1/S-phase transition. Tcf19 knockdown decreased expression of cyclins important for the G1/S-phase transition (*CcnA1*, *CcnA2*, *CcnE1* and *CcnE2*). With growth arrest, Tcf19 expression also decreases. Across conditions, *Tcf19* expression is highly correlated with that of the proliferative gene, *Ki67*.

Beta cells have a large secretory capacity, leaving them susceptible to endoplasmic reticulum (ER) stress. If the ER stress is unresolved, beta cell death can result. When we knockdown Tcf19 in INS-1 cells, we see an increase in expression of pro-apoptotic genes and a decrease in ER homeostasis genes. We hypothesized that Tcf19 is a stress activated transcription factor that would then activate expression of other genes to maintain ER homeostasis. In fact, *Tcf19* expression increases 4.8-fold after 8hr exposure ($p<0.0001$) to thapsigargin. Three key ER sensors work together to relieve ER stress by halting translation (PERK) and activating chaperones and other genes involved in ER homeostasis (Atf6 and IRE1). A pharmacologic inhibitor of Atf6 significantly blunted the increase in *Tcf19* expression under ER stress ($p<0.0001$), while inhibitors of PERK and IRE1 had no effect. This suggests that stress activation of Tcf19 is ATF6 mediated. Additionally, *Tcf19* and *Atf6* gene expression are highly correlated under various treatment conditions ($R^2=0.86$, $p<0.0001$). Tcf19 knockdown in INS-1 causes a 30% decrease in *Atf6* expression ($p=0.002$) and preliminary results also show a decrease in *Atf6* expression in islets from Tcf19 knockdown mice. Chromatin immunoprecipitation after thapsigargin treatment shows low-level occupancy of Tcf19 on the unfolded protein response element of the Atf6 promoter ($p=0.07$). Together, this suggests that Tcf19 also acts as a direct regulator of Atf6 expression. Tcf19 also has occupancy at its own promoter under ER stress conditions ($p=0.04$), suggesting autoregulation.

Tcf19 plays a critical role in both cell cycle progression and ER homeostasis. Tcf19 is necessary for progressing through the G1/S-phase of the cell cycle and is an important regulator of the ER stress response. Tcf19 is regulated by Atf6 and also appears to directly regulate Atf6 expression. We propose that Tcf19 may mediate the ability of beta cells to maintain adequate insulin production in the face of increased insulin demand.

ALPHA-CELLS & GLUCAGON SECRETION

Poster 38 - David Jacobson - *Two-Pore-Domain TASK-1 Potassium Channels Modulate Pancreatic Islet Glucagon Secretion.*

Poster 39 - E. Danielle Dean - *Hepatic-Derived Factor Stimulates Human Alpha Cell Proliferation.*

Poster 40 - Camille Allard - *Estrogen Suppresses Glucagon Secretion In Diabetic Mice And Cultured Islets.*

Poster 41 - Mingyu Li - *Modeling alpha cell hyperplasia to search for the liver-derived inducer in zebrafish*.

Two-Pore-Domain TASK-1 Potassium Channels Modulate Pancreatic Islet Glucagon Secretion

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Glucose regulation of pancreatic alpha-cell Ca^{2+} entry through voltage-dependent Ca^{2+} channels is essential for normal glucagon secretion and becomes defective during the pathogenesis of diabetes mellitus. The two-pore-domain potassium (K2P) channel, TASK-1, is an important modulator of membrane voltage and Ca^{2+} entry, however, its role in alpha-cells has not been determined. Therefore, we addressed how TASK-1 channels modulate alpha-cell electrical activity, calcium entry and glucagon secretion. We find that TASK-1 channels are expressed in human and rodent alpha-cells. Furthermore, alpha-cell K2P currents are blocked by the specific and potent TASK-1 channel inhibitor A1899. Alpha-cell K2P currents are also significantly reduced following ablation of mouse alpha-cell TASK-1 channels. Inhibition of TASK-1 channels with A1899 causes membrane potential depolarization in both the human and mouse alpha-cells, which results in increased Ca^{2+} influx. Furthermore, ablation of alpha-cell TASK-1 channels increases alpha-cell electrical excitability under elevated glucose (11 mM) conditions. Although TASK-1 inhibition with A1899 augments alpha-cell Ca^{2+} influx under both low (1 mM) and high (14 mM) glucose conditions, treatment of mouse and human islets with A1899 only increases glucagon secretion under elevated (14 mM) glucose conditions. Therefore, these data suggest an important role for TASK-1 channels in limiting alpha-cell excitability and glucagon secretion during glucose stimulation

Hepatic-Derived Factor Stimulates Human Alpha Cell Proliferation

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Intervention to decrease glucagon action could be a useful therapeutic approach to treat type 2 diabetes. However, interruption of glucagon action by several mechanisms (glucagon receptor (Gcgr) knockout, antibodies, and antagonists) results in hyperglucagonemia and alpha cell hyperplasia. Recently, we demonstrated that interruption of glucagon signaling in the liver stimulates alpha cell proliferation and this occurs independent of the pancreatic environment using an islet transplantation model. To test whether human alpha cells also respond to this stimulus, we transplanted human adult islets under the kidney capsule of NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ mice (NSG). After a two week period of engraftment, mice were treated with a 10mg/kg Gcgr monoclonal antibody (Gcgr mAb) once a week for two weeks. Gcgr mAb treatment reduced fasting blood glucose levels (PBS - 109.3±3.7 mg/dl, Gcgr mAb - 79.0±2.1mg/dl, n=49-50, p<0.0001). Fasting and glucose/arginine-stimulated serum glucagon and GLP-1 levels increased in Gcgr mAb-treated mice (fasting glucagon: PBS - 113±11pg/ml, Gcgr mAb - 1075±88pg/ml, n=37-42, p<0.0001; stimulated glucagon: PBS - 270±29pg/ml, Gcgr mAb - 3327±381pg/ml, n=42-43, p<0.0001; fasting GLP-1: PBS - <41pg/ml, Gcgr mAb - 82±11pg/ml, n=10; stimulated GLP-1: PBS - <41pg/ml, Gcgr mAb - 290±24pg/ml, n=10). Gcgr mAb treatment increased alpha cell proliferation in the NSG mouse pancreas 14-fold (PBS - 0.96±0.4%, Gcgr mAb - 13.7±0.6%, n=3, p<0.0001). In addition, alpha cell proliferation in the human donor islet grafts increased in 3 of 5 donors (Donor 1: PBS - 0.09%, Gcgr mAb - 2.42%; Donor 2: PBS - 2.29%, Gcgr mAb 2.85%; Donor 3: PBS - 0.09%, Gcgr mAb - 1.37%; Donor 4: PBS - 0.04 ±%, Gcgr mAb - 0.65%; Donor 5: PBS - 1.48%, Gcgr mAb - 1.19%). These data support the hypothesis that a circulating factor stimulates human alpha cell proliferation. To test if human or mouse alpha cells respond to this signal in vitro, we cultured islets in media containing serum from Gcgr global knockout ($Gcgr^{-/-}$) mice for 72 hours. $Gcgr^{-/-}$ serum treatment increased alpha cell proliferation in cultured human islets (Donor A: media alone - 0.10%, media + $Gcgr^{+/+}$ serum - 0.69%, media + $Gcgr^{-/-}$ serum - 1.20%; Donor B: media - .69%, media + $Gcgr^{+/+}$ serum - 3.32%, media + $Gcgr^{-/-}$ serum - 3.46%) similarly to increases observed in mouse islets (mouse: media - 1.02±0.23%***, media + $Gcgr^{+/+}$ serum - 2.41±0.49%**, media + $Gcgr^{-/-}$ serum - 7.46±0.87%; n=4-11, **p<0.01, ***p<0.001 vs media + $Gcgr^{-/-}$ serum-treated). These data indicate that an unidentified, liver-derived factor secreted in response to a loss of hepatic glucagon signaling stimulates mouse and human alpha cell proliferation.

Estrogen Suppresses Glucagon Secretion In Diabetic Mice And Cultured Islets

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A large body of evidence implicates hyperglucagonemia as instrumental in the maintenance of hyperglycemia in both type 1 (T1D) and type 2 diabetes (T2D). Thus, suppressing hyperglucagonemia is a major therapeutic strategy for controlling hyperglycemia in diabetes. During chronic insulin deficiency and hyperglycemia, insulin suppression of glucagon secretion is lost. Thus, restoring insulin's ability to suppress glucagon secretion would represent a novel therapeutic approach to hyperglucagonemia. Our lab showed that estrogen (E2) treatment improves pancreatic islet transplantation (PIT) in diabetic mice. In these mice, E2 treatment acutely suppresses hyperglycemia after PIT which is associated with a pronounced suppression of hyperglucagonemia. We show that the estrogen receptor alpha (ER alpha) is expressed in mouse and human alpha-cells and ER alpha needs to be activated in alpha-cells of the recipient diabetic mouse for E2 to suppress hyperglycemia following PIT. Further, E2 treatment suppresses glucagon secretion from cultured mouse islets. To test whether E2 synergizes with insulin to suppress hyperglucagonemia in the insulin-deficient diabetic mouse alpha-cells we used male mice rendered diabetic with streptozotocin. Treatment with insulin and E2 produced a synergistic suppression of hyperglycemia compared to insulin or E2 alone. However, in these mice E2 showed no effect on hyperglucagonemia either alone or combined with insulin. In summary, E2 reduces glucagon secretion in diabetic mice transplanted with islets as well as in cultured islets. However, E2 does not suppress hyperglucagonemia in diabetic mice solely treated with insulin. Taken together, these findings suggest that E2 requires the presence of islets to suppress glucagon secretion. Studies are ongoing to determine the identity of the islet factor(s) that synergize with E2 to suppress hyperglucagonemia.

Modeling alpha cell hyperplasia to search for the liver-derived inducer in zebrafish

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Inhibition of glucagon (Gcg) signaling or action is a potential treatment for type 2 diabetes. However, several approaches that interrupt glucagon signaling lead to hyperglucagonemia and alpha cell hyperplasia. For example, mice in which the glucagon receptor (Gcgr) is inactivated develop alpha cell hyperplasia, but the responsible signal is not known. Recent work from our group using an islet transplantation model in these mice indicates that the signal is a circulating factor of liver origin. To understand the molecular mechanism of the alpha cell hyperplasia and to identify the responsible factor, we are working to create a zebrafish model in which Gcgr signaling has been interrupted. The genetically and chemically tractable zebrafish, which provides a robust discovery platform, has two glucagon receptor genes (*gcgra* and *gcgrb*) in its genome. Sequence, phylogenetic, and synteny analyses suggest that these are co-orthologs of the human *GCGR*. Similar to its mammalian counterparts, *gcgra* and *gcgrb* are mainly expressed in liver. We inactivated the zebrafish *gcgra* and *gcgrb* using TALEN (Transcription activator-like effector nuclease) first individually and then both genes and assessed the number of glucagon cells by crossing with an alpha-cell reporter line, *Tg (gcga:GFP)*. The number of alpha cells in *gcgra-/-* (27.7 ± 1.5) and *gcgrb-/-* (23.3 ± 0.7) were greater than in wild-type fish (20.6 ± 0.5) (P<0.01) at 7 days postfertilization (dpf), and the increase was more conspicuous in juveniles (45 dpf). Interestingly, *gcgra-/-; gcgrb-/-* double mutants had a more pronounced increase in alpha cell number (32.9 ± 0.8) at 7 dpf, and exhibited an increased rate of alpha-cell proliferation. Moreover, the free glucose is lower in *gcgra-/-* (295.0 ± 16.0), *gcgrb-/-* (308.8 ± 28.4.0), and *gcgra-/-; gcgrb-/-* (275.1 ± 36.9) fish compared with wild-type fish (395.9 ± 8.3) (P<0.05), similar to the blood glucose changes in mice with Gcgr inactivation. These results indicate a highly conserved, compensatory alpha cell response in zebrafish. The robust alpha cell hyperplasia in *gcgra-/-; gcgrb-/-* larvae provides a platform to screen for chemical and genetic suppressors of the compensatory response, and ultimately to identify the stimulus for alpha cell hyperplasia and its signaling mechanism.

TYPE-1 DIABETES PATHOGENESIS & TREATMENT

Poster 42 - Joseph G Daft - *Antimicrobial Peptide and Mucin Expression Differ Between the Diabetic NOD Mouse and Non-Diabetic NOR Mouse.*

Poster 43 - Zachary R. Shaheen - *mTORC1 Regulates Macrophage Activation during Infection with the Diabetogenic Encephalomyocarditis Virus.*

Poster 44 - Yong Wang - *Preconditioning with Diazoxide Reduces Ischemic Injury to Non-Human Primate Islets.*

Poster 45 - Angela M. Henschel - *Biobreeding (BB) Rat Islets Exhibit Underlying Dysfunction that Is Ameliorated by a Hydrolyzed Casein Diet.*

Poster 46 - Ivan Restrepo-Angulo - *Effect of IL-12/23(p40) Inhibition on Diabetes Outcomes in Non-Obese Diabetic Mice*

Poster 47 - Emily Sims - *Beta Cell Derived miR-21 as an Intrinsic Protective Response and Biomarker in Type 1 Diabetes Mellitus.*

Antimicrobial Peptide and Mucin Expression Differ Between the Diabetic NOD mouse and Non-Diabetic NOR Mouse

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Type 1 Diabetes (T1D) is defined as the selective immune destruction of insulin producing beta cells within the islet. Alterations in the intestinal microbiota, increased intestinal permeability, and an aberrant immune system are thought to play key roles in the development of T1D. Recent studies have shown that mice that develop T1D have a different microbiota compared to mice that do not develop T1D. However why mice that are on the same diet in the same facilities have different microbiota is unknown. We hypothesize that altered antimicrobial peptide (AMP) and mucin production early in life determines ones microbiota, protecting some, but not others from T1D.
Non-obese diabetic (NOD) female mice were compared to age and sex matched Non-obese diabetic resistant (NOR) mice. AMP and mucin gene expression was measured in the ileum and colon of the two strains and protein expression was examined by immunohistochemistry. At 2 weeks of age there is a significant increase in the AMP, defcr-4, in the ileum of NOD mice compared to NOR mice. In addition a decrease in the mucins, Muc 1, 2, and 3 was measured in the colons of NOD mice compared to NOR mice. This correlates with alterations that we have measured in Bacteroidetes and Firmicutes between the two strains of mice. We postulate that these differences in AMP and mucin expression between the two strains leads to alterations in microbiota, which leads to differences in disease outcome.

mTORC1 Regulates Macrophage Activation During Infection with the Diabetogenic Encephalomyocarditis Virus

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Environmental factors, such as viral infections, are proposed to initiate pancreatic beta-cell damage and confer risk for autoimmune diabetes pathogenesis. Encephalomyocarditis virus (EMCV) is a positive single-stranded RNA picornavirus that induces diabetes development in genetically susceptible mice. EMCV-induced diabetes is associated with the activation of macrophages and the expression of pro-inflammatory mediators such as interleukin-1 (IL-1beta), tumor necrosis factor (TNF-alpha), and nitric oxide (a product of inducible nitric oxide synthase – iNOS). We have recently identified the chemokine receptor CCR5 as a required signaling receptor for EMCV-stimulated inflammatory gene induction (iNOS, IL-1beta, and COX-2) in macrophages. Macrophage responses to EMCV infection are further dependent on the activation status of phosphatidylinositide 3-kinase (PI3K). In this study, the mechanisms by which CCR5-PI3K signaling regulates macrophage inflammatory expression during EMCV infection were examined. Pharmacological inhibition of the PI3K substrate Akt prevents EMCV-stimulated expression of iNOS and COX-2. However, Akt inhibition does not prevent EMCV-stimulated COX-2 and iNOS mRNA accumulation nor does it affect EMCV-stimulated signaling pathways known to be required for COX-2 and iNOS gene expression. In contrast, Akt regulates translation of iNOS and COX-2 in macrophages via the mammalian target of rapamycin (mTORC1). Pharmacological inhibition of PI3K, Akt, or mTORC1 each attenuates EMCV-stimulated phosphorylation of the mTORC1 targets 4EBP1 and p70S6K. Furthermore, EMCV-stimulated Akt and p70S6K phosphorylation requires CCR5. CCR5-dependent mTORC1 activation may be protective for macrophages during virus infection. In support of this hypothesis, rapamycin (a chemical inhibitor to mTORC1) attenuates EMCV-stimulated COX-2 and iNOS expression and enhances virus replication in macrophages. The mechanism by which a CCR5 -PI3K-Akt-mTORC1 signaling pathway modifies cellular susceptibility to virus infection is currently being examined.

Preconditioning with Diazoxide Reduces Ischemic Injury to Non-Human Primate Islets

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Background: Diazoxide (DZ) is a ATP-dependent K^+ channel activator clinically used to treat hyperinsulinemic hypoglycemia. Previously, we demonstrated that DZ protect rodent islets against ischemia reperfusion (IR) injury. Here, we investigate whether DZ supplementation to University of Wisconsin solution (UW) during pancreas procurement and preservation has similar cytoprotection in non-human primate (NHP) model. **Methods**: Cynomolgus monkey pancreata were flushed with UW or UW + DZ during pancreas procurement and preserved for 8 hrs prior to isolation. Islet yield, *in vitro,* and *in vivo* function were evaluated. **Results**: (i) Significantly higher islet yields were observed in UW + DZ group than in UW alone (57,887 vs. 23,574 IEq/pancreas; $p = 0.021$. and 5,396 vs. 1,646 IEq/gram, $p = 0.019$). (ii) The DZ treated islets had significantly higher insulin content (59.22 vs. 40.35 ng/islet) and higher insulin positive cells (96.15% vs. 82.74%). (iii) The DZ treated islets had significantly less apoptotic cells per islet (1.64% vs. 9.85%). (iv) The DZ treated islets had improved *in vitro* islet function assessed by glucose-induced intracellular calcium response. Additionally, DZ treated islets had better *in vivo* graft function with higher cure rate (88.9%) than the UW alone (62.5%) ($p < 0.05$). The mice transplanted with islets from the UW+DZ group also reversed diabetes within a significantly lower number of days than the UW group (6.8 days ± 1.5 vs. 14.1 days ± 3.5 respectively; $p < 0.05$) with better average blood glucose levels. **Conclusion**: This study confirmed previous observations in rodent, now showing that DZ provides a significant cellular protection on NHP islets against IR injury. Therefore, this study warrants exploration of DZ in human clinical application

Biobreeding (BB) Rat Islets Exhibit Underlying Dysfunction That is Ameliorated by a Hydrolyzed Casein Diet

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Type 1 Diabetes (T1D) is increasing in incidence worldwide. Both genetic and environmental factors contribute to one's susceptibility. Many environmental factors have been implicated in T1D etiology including viral infection, intestinal microbiome, and diet. Notably, placing high-risk HLA infants on a casein hydrolysate formula has been associated with a decreased risk of sero-conversion to autoantibody positivity and a large-scale trial of this diet is ongoing (TRIGR: Trial to Reduce IDDM in the Genetically at Risk). Other groups have shown that spontaneously diabetic Biobreeding (BB) DR*lyp/lyp* rats fed a low antigen, hydrolyzed casein (HC) diet exhibited reduced disease penetrance, improved intestinal epithelium integrity, altered gut microbiome and an immune shift in the Th1/Th2 balance. How HC diet may act at the level of the islet has not been defined. DR*lyp/lyp* rats possess the high-risk MHC-II, *RT1u/u* (*Iddm1*), and a mutation in *Gimap5* (*Iddm2*), which results in T-cell lymphopenia, affecting in particular the regulatory T-cell compartment. Congenic DR+/+ littermates are wild-type for *Gimap5* and are not lymphopenic or spontaneously diabetic; however, diabetes can be induced in young DR+/+ rats by viral stimuli. Fischer rats (F344) possessing *Iddm1* and/or *Iddm2* remain disease free indicating that they lack additional susceptibility loci possessed by BB rats. We have discovered that unlike other rat strains, BB rat beta-cells show evidence of duress by expressing the chemokine eotaxin independent of insulitis and T1D onset. More recent studies have included global gene expression profiling of BB and F344 islets where both DR*lyp/lyp* and DR+/+ islets exhibit high expression of chemokines and other genes consistent with a response to bacterial antigen exposure. Our goal here was to define transcriptional and functional differences in islets of BB rats fed HC versus a normal plant protein diet. Consistent with previous studies, DR*lyp/lyp* rats fed HC diet from weaning were either protected from diabetes or showed a significant delay compared to the normal diet ($p<0.001$). HC fed DR+/+ and DR+/- rats exhibited a lower pro-insulin:C-peptide ratio by 6 weeks of age compared to normal diet-fed BB rats suggesting reduced beta-cell stress. Global gene expression analysis of islets of d50 DR+/+ rats fed HC versus normal diet showed reduced expression of many genes related to NFkB activation and TLR ligation including *Cxcl10*, *Ptgs2*, *Ccl2*, *Mip1a* and *CD40* (absolute fold change > 3.0, RP-FDR<10%). Furthermore, many of the genes differentially regulated in islets of d50 DR+/+ rats fed HC were previously identified in a pairwise comparison of the islet transcriptomes of d50 F344-*Iddm1* versus d50 DR+/+ rats, suggesting that HC diet altered the islet expression profile of DR+/+ rats to more closely resemble that of the nonsusceptible F344-*Iddm1* strain (p-value of overlap<1e-6). These data support the hypothesis that DR+/+ rats possess an underlying islet-level dysfunction that is improved by feeding a low antigen diet.

Effect of IL-12/23(p40) Inhibition on Diabetes Outcomes in Non-Obese Diabetic Mice

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INTRODUCTION: The pathophysiology of type 1 diabetes (T1D) involves, in part, the immune-mediated destruction of beta cells by T cells. Both CD8+ and CD4+ T cells contribute to disease pathogenesis. Interleukin-12 (IL-12) and -23 (IL-23) are pro-inflammatory cytokines produced by antigen presenting cells (APCs), and contribute to the formation of CD4+ T helper subtypes, Th1 and Th17, respectively. Both cytokines contain a 40 kDa subunit (p40) that differentially pairs with either a 35 kDa peptide (p35, for IL-12) or with a 19 kD peptide (p19, for IL-23). Early studies in NOD mice documenting the role Th1 cells in T1D relied upon the manipulating the common p40 subunit (before IL-23 was identified as an overlapping cytokine). Recently, a human monoclonal antibody (mAb) against IL-12/23(p40) (ustekinumab) has been approved for use in psoriatic arthritis and plaque psoriasis. To determine if use of this drug might be beneficial in humans with T1D, we obtained a murine form of this mAb and tested its potential to prevent or reverse diabetes in an Institutional Animal Care and Use Committee (IACUC) approved NOD mice.

METHODS: Anti-IL-12/23(p40), anti-IL-23(p19), anti-IL-17, and control IgG (Centocor, Philadelphia, PA) were administered to pre-diabetic NOD mice weekly by intraperitoneal injection, and diabetes incidence (defined as a blood glucose >300 mg/dl on at least two separate occasions), insulitis, cytokine levels, and peripheral circulating CD4+CD25+Foxp3+ T cells (Tregs) were monitored. In a second study, diabetic NOD mice received anti-IL12/23(p40), anti-IL-17, or IgG, by weekly intraperitoneal injection and were followed for 4 weeks for diabetes reversal.

RESULTS: Eight week-old pre-diabetic NOD mice that were administered mAbs anti-IL12/23(p40), anti-IL-23(p19), and anti-IL-17 showed no differences in diabetes incidence or changes in pro-inflammatory cytokine levels. Interestingly, the anti-IL23(p19) group showed a slight, but non-significant delay in diabetes incidence ($p=0.06$ by log-rank test). Notwithstanding the lack of effect on diabetes outcome, all of these groups showed a trend for an increase in the percentage of T regulatory cells (Tregs) in the blood. Diabetic animals receiving mAbs showed no reversal in diabetes. Because of the slight delay observed in diabetes incidence with the anti-IL-23(p19) mAb, we asked if treatment beginning at an earlier age (6 weeks) would alter outcomes. 6 week-old mice administered anti-IL-23(p19) group showed a significant delay in the rate of diabetes incidence ($p=0.03$ by log-rank test), accompanied by a decrease in the severity of insulitis compared to controls ($p<0.01$ by t test), but without differences in Treg percentages.

CONCLUSION: Our study suggests that treatment with murine anti-IL12/23 mAb (commercially marketed as ustekinumab for humans) does not prevent or reverse diabetes in NOD mice. Because delay in diabetes incidence was observed with anti-IL23(p19), we suggest a possible role for this cytokine (or Th17 cells) in the pathogenesis of T1D in NOD mice. Development of humanized mAb against IL-23(p19) might be justified for prevention of human T1D.

Beta Cell Derived miR-21 as an Intrinsic Protective Response and Biomarker in Type 1 Diabetes Mellitus

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Although Type 1 Diabetes Mellitus (T1DM) has traditionally been defined as autoimmune destruction of the pancreatic beta cell, recent data suggest the intrinsic beta cell response to inflammatory and metabolic stress plays a major role in the determination of disease initiation and progress. Thorough exploration of this emerging paradigm not only allows for an increased understanding of T1DM pathophysiology, but also presents an opportunity for alternative treatment strategies and earlier disease detection through identification of activated beta cell stress pathways. Altered microRNA (miRNA) expression has been demonstrated in the beta cell and serum in diabetes, suggesting a role for these small, non-coding RNAs in diabetes pathophysiology. miRNA 21 (miR-21) expression augments cell survival in multiple tissues, and cytokine-induced activation of NF-KB signaling induces miR-21 expression. The tumor suppressor, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), has been identified as a target of miR-21 in other tissues, but the relationship between miR-21 and PTEN within the beta cell remains unexplored. Because PTEN acts to decrease β cell survival through augmentation of cell cycle regulators and inhibition of the PI3K/AKT pathway, targeted reduction of *PTEN* translation could contribute to a pro-survival effect of increased beta cell miR-21 expression. ***We hypothesized that cytokine-induced transcription of miR-21 in the beta cell acts as a compensatory mechanism to repress translation of PTEN in an attempt to limit beta cell death.*** Furthermore, we predicted that beta cell derived miR-21 in the serum may serve as a marker of inflammatory stress within the islet, such as that seen in developing T1DM.

To test this hypothesis, INS-1 beta cells were treated with a cytokine mix of IL-1beta, TNF-alpha, and IFN-gamma for 6, 24, and 48 hours to mimic the early inflammatory milieu of T1DM. RNA isolation, reverse transcription, and qRT-PCR were performed to analyze miR-21 expression, and immunoblots were performed to quantitate PTEN levels. Beta cell expression of miR-21 was increased by 6 hours of cytokine treatment, and peaked with a 2-fold increase at 24 hours. Beta cell PTEN protein levels were decreased in parallel by 30% at 24 and 48 hours. Expression of miR-21 in media of treated cells was increased by 6 hours of cytokine exposure, and reached significance with a nearly 5-fold increase after 24 hours of treatment. This effect was magnified in exosomes isolated from media, where miR-21 levels were almost 20-fold higher than controls. These results suggest an association between increased production and release of miR-21 and decreased PTEN protein levels in the beta cell in this *in vitro* model of early T1DM and suggest the potential for miR-21 as a serum biomarker or treatment modality for early T1DM. Future studies will evaluate direct relationships between miR-21 and PTEN in the beta cell using miR-21 mimics/inhibitors and luciferase assays and verify elevations of miR-21 in serum samples from mice and humans with developing diabetes.

TYPE-2 DIABETES PATHOGENESIS

Poster 48 - Brandon B. Boland - *The Cell Biology of the Adaptive Plasticity for Insulin Production in Pancreatic Beta-Cells from Obese Diabetic Mice.*

Poster 49 - Miles H. Fuller - *Genetic Deletion of the Short Chain Fatty Acid Receptor, FFAR2, leads to Impaired Gestational Glucose Homeostasis.*

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The Cell Biology of the Adaptive Plasticity for Insulin Production in Pancreatic Beta-Cells from Obese Diabetic Mice

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RATIONALE: The pathophysiology of obesity-linked beta-cell dysfunction in Type 2 Diabetes Mellitus (T2DM) is characterized by insulin secretory dysfunction, loss of beta-cell mass, and a presumed inadequate production of insulin to meet metabolic demand. Here, we used the *db/db* mouse model, to better examine the beta-cell biology of compensatory changes in insulin production in obese T2DM.

OBJECTIVE: To quantify and better characterize the altered beta-cell biology of insulin production in obese T2DM. Recent findings in isolated *db/db* murine islets demonstrated an unexpected marked upregulation of insulin production, to such a degree that altered the whole cell biology of an 'obese beta-cell', characterized by a sizeable expansion of the rough endoplasmic reticulum (RER) and Golgi apparatus correlating with massive degranulation. This could be reversed close to normal beta-cell biology in matter of hours by exposure to normoglycemic media – revealing a rapid adaptive plasticity for insulin production in response to glucose. Here, quantitative electron microscopy (EM) and immunofluorescent analyses of beta-cells from murine C57BL/6J WT versus *db/db* islets was conducted to better assess dynamic changes in beta-cell biology of insulin production in obese T2DM.

FINDINGS: Quantified EM analysis of freshly isolated islets from WT versus *db/db* indicated *db/db* beta-cells had a marked reduction in mature insulin granules compared to WT beta-cells (by ≥80%; $p < 0.001$), whereas immature insulin granule content was increased (≥3 fold; $p < 0.001$). There was a significant expansion of both the RER (2 fold; $p < 0.001$) and Golgi (2 fold; $p < 0.001$) in *db/db* beta-cells compared to WT, which underlies the marked increase in proinsulin biosynthesis in *db/db* beta-cells. Mitochondrial size was also significantly increased in *db/db* beta-cells (~2 fold; $p < 0.001$) as was the average area of the cell occupied by mitochondria ($p \leq 0.02$). Immunofluorescent analysis of pancreatic sections from *db/db* mice compared to WT mice revealed a loss of co-localization between markers of *cis*-Golgi (GMP130) and *trans*-Golgi (TGN38). Although the protein expression of TGN38 did not change in WT versus *db/db* islets, it was markedly dispersed in *db/db* beta-cells indicating a large specific expansion of the *trans*-Golgi network. Likewise, the tight localization of proinsulin to a *trans*-Golgi compartment in WT ß-cells was also dispersed in many *db/db* beta-cells, again consistent with expansion of the *trans*-Golgi network. Finally, there was also a marked increase in the presence of rarely seen multilaminar bodies (MLB) in *db/db* beta-cells indicative of increased membrane recycling concurrent with a dynamic RER and Golgi expansion. A (first ever) 3D EM tomographic reconstruction of a *db/db* beta-cell MLB revealed three membrane bi-layer compartments of differing structures emanating from a localized MLB center. This also emphasizes the dynamic nature of the adaptive plasticity of the whole cell biology in an obese beta-cell in a valiant effort to intensify insulin production for the increased metabolic demand.

CONCLUSION: Quantitative morphological assessment of *db/db* beta-cells revealed a pronounced increase in the cell biology of insulin production. This further indicates that beta-cells in obese T2DM are working hard to maximally produce insulin to meet the metabolic demand, even in the face of insulin resistance.

Genetic Deletion of the Short Chain Fatty Acid Receptor, FFAR2, leads to Impaired Gestational Glucose Homeostasis

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Dynamic changes in maternal islet function occur throughout pregnancy to compensate for the changes in insulin resistance. These changes help to maintain metabolic stability in the mother and are linked to the programming of fetal energetics. Previously, using pregnancy as a model system of insulin resistance, we identified the expression of the Free Fatty Acid Receptor-2 (FFAR2) transcript to be induced in mouse islets. Therefore, we hypothesized that FFAR2 plays an important role in the regulation of glucose homeostasis during pregnancy. As shown here, we have now determined that FFAR2 is differentially expressed in multiple peripheral tissues throughout gestation and in the post-partum period. Further exploring, mouse models with FFAR2 genetically ablated was then utilized to elucidate the role of this receptor in glucose homeostasis during pregnancy. Results show that the FFAR2$^{-/-}$ mice exhibit impaired glucose tolerance during pregnancy due in part to hypoinsulinemia. Additionally, FFAR2$^{-/-}$ mice had diminished beta cell expansion during pregnancy, compared to the female WT littermate, from incomplete beta cell proliferation. Interestingly, serum levels of the short chain fatty acid (SCFA) acetate, the endogenous ligand for FFAR2, were elevated in both FFAR2$^{-/-}$ and WT littermate mice during pregnancy as compared to prior to pregnancy. Together, these results identify signaling through FFAR2 as a component of gestational glucose homeostasis and provide mechanistic insight into the linkage between this class of nutrients, short chain fatty acids and maternal-fetal glycemic control.

The Progression of Intra-Islet GLP-1 Expression Differs in Mouse Models of Type 1 versus Type 2 Diabetes

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The incretin Glucagon-like Peptide (GLP-1) has been reported to elicit a number of positive effects associated with improved glucose homeostasis, including improved insulin sensitivity and increase in glucose-induced insulin secretion by beta cells, as well as enhancement of beta cell survival and proliferation. GLP-1 shares with glucagon the same precursor molecule proglucagon, but arises from a distinct posttranslational process. The common belief is, in intestinal L-cells, proglucagon is cleaved by prohormone convertase 1/3 (PC1/3) to give rise to GLP-1, while in pancreatic islets, proglucagon is processed by PC2 to produce glucagon. However, recently it has been shown that GLP-1 is also produced in pancreatic islet cells in response to varying stimuli, including glucose concentration, cytokines, and even insulin levels, which is in agreement with our own observations. In order to understand the nature and impact of intra-islet GLP-1 production, we investigated whether GLP-1 production in pancreatic islets is altered in the course of diabetes development. Both type 1 (NOD/ShiLtJ mouse) and type 2 (db/db mouse) diabetes models were employed. Mice were monitored closely and sacrificed at different stages according to age, in db/db mice, or blood glucose level, in NOD mice. The pancreas of each mouse was then used for immunohistochemical (IHC) assessment of pancreatic islet GLP-1 and PC1/3 expression. Using an average of 20 to 30 islets per group, islets cells positive for GLP-1, glucagon, or co-positive cells were then quantified based on associated nuclei. Our data indicate that the number of GLP-1-expressing islet cells increased with the development of diabetes in db/db mice, in accordance with the upregulation of PC1/3 expression in these cells. Interestingly, intra-islet GLP-1 expression was, however, not significantly changed during the development of type 1 diabetes in NOD mice. These data suggest that there are clear differences in intra-islet GLP-1 production during the development of type 1 and 2 diabetes. These differences may have an impact on the clinical and pathophysiological processes of these diseases and may be a target for therapeutic approaches.

PAK1 Knockout Mice Harbor an Increased Susceptibility to Diet-Induced Diabetes

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Type 2 diabetes (T2D) results from defects in insulin secretion from the pancreatic islet beta cell and in insulin action/signaling at the peripheral tissues. Recently, p21-activated kinase (PAK1) has come to the forefront as a vital regulator of both these processes, as well as preserving beta cell mass. The human PAK1 gene is in the T2D susceptibility locus on Chromosome 11, and modeling this, whole-body PAK1 knockout ($PAK1^{-/-}$ KO) mice are glucose intolerant, are insulin resistant, and have impaired glucose-stimulated insulin secretion. Relatedly, human islets from T2D cadaveric donors exhibit an 80% reduction in PAK1 protein abundance, as do obese and diabetic rodent models. However, whether PAK1 protein loss is related to diabetogenic stimuli, and/or whether reduction of PAK1 rendered enhanced susceptibility to progression to frank T2D, has remained unexplored. Toward this, we exposed cadaveric human islets to either pro-inflammatory cytokines or glucolipotoxic culture conditions for 72 h and found a significant loss in PAK1 protein, demonstrating that PAK1 protein is susceptible to loss under diabetogenic stress. Moreover, whole-body $PAK1^{-/-}$ KO mice challenged with a palmitate-based high-fat (HF) diet (Western Diet, 42% kcal from fat) for as little as 10 weeks rapidly developed severe glucose intolerance and fasting hyperglycemia, as compared with wild-type littermate mice fed the same diet. Consistent with defects in insulin secretion, the HF-Fed $PAK1^{-/-}$ KO mice showed a 50% reduction in peak serum insulin content, compared to that of HF-Fed wild-type mice. Accordingly, while HF-Fed wild-type mice showed a compensatory 23% increase in beta cell area, HF-Fed $PAK1^{-/-}$ KO mice succumbed to a 42% decrease in beta cell area, a loss which placed their beta cell mass below that of LF-Fed and Chow-Fed wild-type mice. These findings suggest that the high-fat diet undermined the coping mechanism to maintain functional beta cell mass in the $PAK1^{-/-}$ KO mice, exposing their underlying susceptibility to dysglycemia/diabetes. Overall, these data suggest that PAK1 deficiency can produce a pre-diabetic phenotype with clear susceptibility to dietary phenomena. Further studies of the mechanisms modulating PAK1 abundance in insulin-secreting beta cells will be required to prevent and remediate this point of susceptibility to diabetes development and progression.

SR-135, a Peroxynitrite Decomposing Catalyst, Preserves Beta-Cell Function and Prevents Apoptosis in High Fat Diet-Fed B6D2F1 Mice

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Type 2 diabetes is reaching epidemic proportions as obesity has become a global problem. Nutrient overload associated with obesity causes insulin resistance and beta-cell defects, the hallmark of type 2 diabetes. Molecular mechanisms underlying obesity-induced insulin resistance and beta-cell defects, however, are not fully understood. Recent evidence indicates that peroxinitrite ($OONO^-$) plays a pivotal role in the pathogenesis of diabetes and diabetic complications. Increased peroxynitrite formation has been observed in obese rodents (1,2) and diabetic patients (3,4). Peroxynitrite is a powerful oxidizing and nitrating species, causing DNA damage, lipid peroxidation, and post-translational modification of proteins (5,6). Peroxynitrite-mediated nitration of tyrosine residues of the key insulin signaling proteins have been shown to interfere with the insulin signaling (7-9). Chemical catalysts that destroy peroxynitrite, therefore, may have therapeutic value for treating type 2 diabetes. To this end, SR-135, an analogue of biscyclohexano-fused Mn(III) complexes of bis(hydroxyphenyl)dipyrromethenes, was synthesized and validated for its peroxynitrite decomposing catalyst activity. To assess its anti-diabetic effects, B6D2F1 mice (the F1 hybrids of C57BL/6 and DBA/2) were fed with a high fat-diet (HFD) to induce diabetes. Mice on a HFD gained weight at a faster rate and showed significantly higher fasting blood glucose levels as compared to mice on a lean-diet. Intra-peritoneal injection of SR-135 (5 mg/kg) every other day for two weeks significantly reduced fasting blood glucose levels (204.9±8.3 vs. 154.3±6.8) of HFD-fed mice. Intra-peritoneal glucose tolerance test (IPGTT) also indicated that SR-135 significantly enhanced obesity-induced glucose tolerance as compared to vehicle or the control drug SRB. Islets isolated from mice treated with SR-135 showed significantly enhanced glucose-stimulated insulin secretion and elevated insulin content. Moreover, SR-135 treatment also preserved islet architecture, lowered islet size, reduced tyrosine nitration, and prevented apoptosis. These results suggest that a peroxynitrite decomposing catalyst preserves beta-cell function under nutrient overload.

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NOVEL ISLET METHODOLOGIES

Poster 53 - Ping Guo - *Adeno-associated virus-mediated cre-lox targeting of the adult mouse pancreas through intra-pancreatic ductal injections.*

Poster 54 - James J. McGarrigle - *Isolation and Analysis of Ngn3/CD133+ Endocrine Precursor Cells From Human Exocrine Tissue.*

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Poster 61 - Tal Murthy - *Development of Novel Broad Range, Highly Sensitive, Small Volume Chemiluminescent Assays for Beta Cell Profiling.*

Adeno-associated virus-mediated cre-lox targeting of the adult mouse pancreas through intra -pancreatic ductal injections

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The cre recombinase system has been very useful for conditional gene targeting. However, in mice, the requirement for genetic alterations makes for a very cumbersome, time-consuming, and labor-intensive system. Here we describe a very efficient, pancreas-specific cre-lox expression system, using adeno-associated virus (AAV) delivery into wild-type mice. In this proof-of-principle experiment, we used a dual virus system, with one virus carrying a glucagon promoter driving cre recombinase, and a second virus carrying a CMV-loxP-GFP-loxP-TmRed reporter construct. We initially observed the rapid onset of broad GFP expression throughout the pancreas when the CMV-loxP-GFP-loxP-TmRed reporter virus alone was infused into the pancreatic duct through a laparotomy. When we instead co-infused the CMV-loxP-GFP-loxP-TmRed reporter virus with an AAV-Glu-cre virus through the pancreatic duct, we saw α-cell specific TmRed expression, with 98% of α-cells in the pancreas labeled. The broad pancreatic expression of the CMV-loxP-GFP-loxP-TmRed reporter prior to cre recombination, and the specific tagging of α-cells following glucagon-cre virus infusion suggest an efficient and enhanced ability to target the pancreas and specific pancreatic cell types for studies of function and regeneration.

Isolation and Analysis of Ngn3/CD133+ Endocrine Precursor Cells From Human Exocrine Tissue

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Type I Diabetes Mellitus (T1DM) is a metabolic disease caused by the autoimmune destruction of insulin-producing beta cells in human pancreatic islets. Human islet transplantation is now seen as a viable treatment against T1DM, however the procedure is limited due to the lack of availability of pancreata from human donors. At present the vast majority of donor pancreas tissue, mainly comprised of exocrine tissue, is discarded following the isolation of islet cells from the organ. During pancreatic development, cells that commit to an endocrine cell fate begin to express the basic Helix-Loop-Helix (bHLH) transcription factor Neurogenin 3 (Ngn3), which is the key factor that triggers their differentiation into all islet cell types, including the insulin producing beta-cells. Furthermore it has been shown that Ngn3 endocrine progenitor cells in the human pancreas co-express CD133, a transmembrane glycoprotein. Exploiting the fact that exocrine and endocrine pancreatic tissue have a common embryonic origin, we set about to isolate CD133+ progenitor cells, isolated from exocrine tissue discarded during human pancreatic islet isolations, which are also known to express Ngn3. Using Magnetic Cell Sorting, CD133+ cells were isolated and cultured in a short term suspension culture. These individual cells were shown to aggregate into sphere like structures, known as pancospheres. Histological analysis of the pancospheres indicated the presence of Pdx1, a transcription factor necessary for pancreatic development and beta-cell maturation, as well as the protein chromogranin A. Furthermore *in vivo* analysis of these pancospheres in nude mice indicated an alteration in blood glucose levels during intraperitoneal glucose tolerance test (IPGTT). Together these data indicate that endocrine precursor cells can be isolated from “waste” exocrine tissue following human pancreatic islet isolation and that such cells, when cultured, can develop into pancospheres that can express proteins necessary for pancreatic development and beta-cell maturation. Further in-depth genetic, proteomic and functional analysis of these pancopheres will have to be performed to determine their exact significance to the field of diabetic research.

Development of A Microfluidic-based Array for High-Resolution and High-Content Imaging of Islets

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BACKGROUND: In order to facilitate *in vitro* study of pancreatic islets, a microfluidic chip was designed, fabricated and verified to trap, immobilize, and orient individual islet in an array format to allow high-resolution and high-content imaging in order to study physiology and pathophysiology of islets.

METHODS: (1) A microfluidic array was designed based on hydrodynamic principle and fabricated based on photo lithography technique. (2) Fluid flow simulation performed using COMSOL 4.4 to verify design principle and minimal shear stress on islet cells. (3) A thin PDMS membrane (100μm) was integrated as an oxygen controller to generate various oxygen patterns. (4) Islets were loaded and stimulated using gravity based fluid flow.

RESULTS: (1) Flow simulation verified the design principle, showing maximum velocity in trapping sites. (2) The device is capable of trapping up to 200 islets individually in an array with 90% efficiency. (3) The oxygen controller can provide fast oxygen microenvironment (less than 60 s) and generate uniform oxygen concentrations and profiles. (4) The device allowed multiparametric High-Resolution and High-Content Imaging of islet cellular and subcellular metabolic activity and ion signaling such as mitochondrial energetics, ROS levels, calcium influx, and redox activity.

CONCLUSION: We designed, developed, and validated a novel microfluidic platform that allows trapping individual islets with high efficiency. Device also allowed for co-culturing islets and hypoxia studies, showing an improvement over conventional hypoxia chambers. The device allowed high-resolution imaging of islet using fluorescence and confocal microscopy and high-content multiparametric imaging of key insulin stimulator-secretion coupling factors. This work demonstrates the feasibility of array-based cellomics analysis.

Validation of LC-MS Metabolomics Method in Whole Islets and Application to Studies of Gluco/Lipotoxicity

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Glucose and fatty acid flux into the beta-cell is increased as a result of insulin resistance, which leads to an increased demand for insulin secretion. This causes strain on the cells that can eventually lead to the beta-cell dysfunction and/or death that results in Type 2 diabetes. The mechanism for this beta-cell failure is not fully understood, although exposure to high levels of glucose and/or lipids is thought to play a major role. Many cellular pathways are potentially affected during gluco/lipotoxicity; metabolomics provides a powerful tool for investigating alterations in these pathways. A desirable metabolomics method would be able to quantify a wide range and amount of metabolites with good reproducibility. Accordingly, liquid chromatography-time-of-flight mass spectrometry is an ideal technique because of its high sensitivity and selectivity.

Previous metabolomics studies have typically been conducted with clonal beta-cells due to their ease of access and specificity for beta-cell metabolism. However, clonal cells are derived from a cancer line and as such may favor pathways involved in cell growth, which could result in different metabolics than would be seen in a native cell population. For this reason, it is important to corroborate data obtained from clonal cells with data derived from native islets. However, there are some challenges in conducting metabolomics studies on whole islets- islets are not as easily obtained or manipulated as clonal cells and they vary widely in size. Because beta-cells within an islet may be exposed to varying concentrations of stimulants, the metabolic profile is more variable than in dissociated cells, leading to poor reproducibility of results. One way to combat this is to increase the number of islets per sample.

We have developed a sample preparation method for the reproducible measurement of ~70 metabolites within whole islets through optimization of islet number, extraction method, and extraction solvent. Islets were incubated in 2.8 mM or 16.7 mM glucose prior to metabolism quenching and metabolite extraction. Analyses were performed using a hydrophilic interaction liquid chromatography/anion exchange (HILIC/AEX) column or a reverse phase column coupled to a TOF-MS to measure polar metabolites and lipids, respectively. A series of 10 stable isotope-labeled metabolites were spiked into the samples to allow for quantification and to normalize data from run to run. We observed increased levels of glycolysis and TCA cycle components and increased cellular energetics in response to glucose stimulation, all of which have previously been reported in beta-cells. This suggests that our method is able to reliably detect changes in intracellular metabolites. Future work involves time course measurements of metabolites in islets exposed to glucose and/or fatty acids.

Development and Optimization of a Microfluidic-based Multi-Parametric Assay for Evaluating Human Islet function prior to Transplantation

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Islet transplantation is a promising therapy for T1DM. *In vivo* animal transplantation has predictive correlation. However, these experiments are time consuming. We developed a microfluidic multiparametric assay to determine islet functionality prior to transplant. A total of 8 human islet isolations were tested. Islet preparations were stressed by pelleting for 30 min and 120 min to create a hypoxic setting. Standard *in vivo* experiments, viability and glucose static incubation tests were completed simultaneously. (1) There was no significant difference in the glucose stimulation indices of the 3 groups or in the viability (2) intracellular calcium concentration of the control was similar to the 30 min hypoxic group ($p>0.05$), while the control or 30 min group had higher calcium levels when compared to 120 min group ($p<0.05$). (3) There were no significant differences between mitochondrial potential changes among the 3 groups, consistent with the standard viability assay. (4) *In vivo* nude mice transplant data showed that 46.42% in the control and 43.33% in the 30 min hypoxic group reversed diabetes (≤ 220 mg/dL glucose). No reversal of diabetes with islets exposed to 120 min of hypoxia. (5) Mitochondrial potential indices demonstrated similarity between the control group and 30 min hypoxic group ($p>0.05$) while mitochondrial indices of control group and 120 min showed a significant difference ($p<0.05$). (6) The islets that had good calcium and mitochondrial potential responses to glucose had more insulin secretion than 30 min or 120 min hypoxic groups ($p<0.05$). The microfluidic-based assay can provide real-time, parametric, and functional analysis without islet dissociation and be superior to currently available standards with potential application for islet transplantation.

Multi-donor Reaggregated 3D Islets for Drug Testing

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Introduction: There is a gradual increase in the number of people affected with diabetes all over the world. With the complications associated with the use of diabetic drugs like GLP-agonists, there is definitely a need to discover new drugs. 3D clusters of human islet cells would provide an optimal drug screening assay. However, there is great variability in the insulin response of islets from different donors, hence the drug testing needs to be repeated several times in order to get an average drug response. In order to overcome this barrier, we created multi donor reaggregated islets by mixing islet cells from different donors. For this study we compared the glucose dose response curve of multi donor Kanslets with the average of the glucose dose response curve of the single donor Kanslets when reaggregated using micromolds.

Methods: We created multi- donor Kanslets from two different donors. Native islets from two different donors were dispersed and cryopreserved as single cells and then were later thawed to create single donor reaggregates from cells of respective donors as well as multi-donor reaggregates from both the donors in equal proportion. Single donor islet clusters from the two donors as well the multi-donor reaggregates were challenged with different doses of glucose (1.4, 2.8, 5.6, 11.2, 16.8, 22.4, 28, 33.6 and 22.4mM+ potassium chloride) and insulin secretion measured.

Results: We were successfully able to reaggregate islets cells from two different donors to form the multi-donor islet clusters. Single donor and multi-donor clusters were highly viable. While the glucose sensitivity of one donor was poor, with little insulin secreted even at high glucose concentrations, the response the second donor appeared normal. From the multi-donor clusters, the glucose-stimulated insulin release was approximately in the middle of the glucose response curves of the two donors. A bioequivalence test for the glucose dose response curve for multi-donors and averaged single donor reaggregates suggested that both the groups were bioequivalent. Upper and lower bounds of 95% confidence interval for the difference between the two groups (-10%, 0.55%) was well within the equivalence interval (-20%, +25%).

Conclusion: It is possible to make highly viable multi-donor reaggregated islets and their glucose response curve fell in the middle of the glucose response curve of the two individual donors. Continued experiments will compare insulin secretion as well as drug response curve to other insulin secretogauges like glybenclamide and inhibitors like somatostatin.

High Content Screening And Analysis Of Beta Cell Function

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The ß-cells of the Islets of Langerhans of the pancreas synthesize and secrete insulin in synchrony with food consumption and elevation of blood nutrient concentrations. A sufficient number of properly functioning ß-cells is a critical determinant of normal blood glucose levels and prevention of diabetes. The spectrum of potential therapeutic mechanisms directed toward ß-cells includes 1) stimulation of insulin secretion, 2) reduction of cellular stress and dysfunction, 3) stimulation of proliferation or differentiation, and 4) inhibition of cell death.

Using the Cellomics Array Scan VTI and the more recent CellInsight NXT Platform has provided an opportunity to examine b-cell function at both the individual cell level (INS1832/3) and in the context of the whole islet (Rat primary islets). Both instruments provide a multi-parametric analysis of cell viability and function in a single assay. For example integrated measurements of ß-cell number, viability, death, death signaling, DNA damage, oxidative stress, and proliferation can all be assayed at the same time. Oxidative stress is induced using tert-butyl hydroperoxide, menadione and or high glucose. Endoplasmic reticulum stress can be induced using tunicamycin and thapsigargin. Challenges to the b-cells/islets in the presence and absence of compound can simulate the *in vivo* environment in a diabetic patient. This assay platform can be used for drug discovery target validation, compound testing, and drug discovery flow-scheme support. This capability will promote development of novel agents that delay diabetes progression by sparing or expanding the population of insulin-secreting islet ß-cells.

Automated Quantification of Pancreatic Beta Cell Mass

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Beta cell mass is a parameter commonly measured in studies of islet biology and diabetes. However, the rigorous quantification of pancreatic beta cell mass using conventional histological methods is a time-consuming process. Rapidly evolving virtual slide technology with high-resolution slide scanners and newly developed image analysis tools has a potential to transform beta cell mass measurement. To test the effectiveness and accuracy of this new approach, we assessed pancreata from normal C57Bl/6J mice, and from mouse models of beta cell ablation (streptozotocin-treated mice) and beta cell hyperplasia (leptin-deficient mice) using a standardized systematic sampling of pancreatic specimens. Our data indicate that automated analysis of virtual pancreatic slides is highly reliable and yields results consistent with those obtained by conventional morphometric analysis. This new methodology will allow investigators to dramatically reduce the time required for beta cell mass measurement by automating high-resolution image capture and analysis of entire pancreatic sections.

Development of Novel Broad Range, Highly Sensitive, Small Volume Chemiluminescent Assays for Beta Cell Profiling.

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Quantification of insulin, C-peptide, intact and total proinsulin is vital in furthering diabetes research. Many commercial human ELISAs require extra sample dilution steps to ensure concentrations fall within a limited standard curve range. This results in increased sample preparation time as well as potential re-dilution and re-testing of samples. Furthermore, sensitivity is limited when using standard colorimetric detection methods in ELISA, whereas chemiluminescence platforms offer vast improvements. That being said, many of the improvements in sensitivity and dynamic range by luminescence are limited due to many being read on expensive flash chemiluminescence readers or using proprietary instrumentation.

A new set of STELLUX chemiluminescent assays have been developed at ALPCO to address these needs specifically for beta cell profiling in human samples. The STELLUX insulin, C-peptide, intact proinsulin and total proinsulin ELISAs offer a broad range, high sensitivity, and utilize small sample volumes. These assays were designed for the end user to have the capability of running multiple sample types, disease states, and treatment states in one assay without having to worry if the sample is at the correct dilution. All of these assays are highly characterized for accuracy, precision, sensitivity and specificity. Additionally, each production lot contains a complete validation package. In this poster we present the performance and the experimental data that supports the detection of the 4 analytes in serum, plasma, and tissue culture supernatants. Differences in beta cell outputs are seen when evaluating serum from normal, Type 1 Diabetic, and Type 2 Diabetic fasted or fed samples. All samples were detected without performing upfront dilutions and use a standard glow chemiluminescence plate reader that is available in most laboratories.

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