Project title: Engineering metabolic pathways in innate immune cells for T1D tolerance and beta cell regeneration

Project supervisor(s):
Ramon Klein Geltink, PhD, UBC Dept of Pathology & Laboratory Medicine, BC Children's Hospital Research Institute*

Bruce Verchere, PhD, UBC Depts of Surgery and Pathology & Laboratory Medicine, BC Children's Hospital Research Institute

Key words associated with the project (up to 10):
type 1 diabetes, islet, macrophages, dendritic cells, tolerance, immunometabolism, gene therapy, regenerative medicine.

Short- and long-term goals of the project:

Short-term goals: To develop nanomedicine approaches for engineering metabolic pathways in innate immune cells towards a pro-regenerative, anti-inflammatory phenotype
Long-term goals: To develop new gene therapy approaches for inducing tolerance in autoimmune diabetes, towards preventing or reversing type 1 diabetes.

Project description, including relevance to stem cells and regenerative medicine

Initiation of type 1 diabetes requires antigen presentation by innate immune cells (dendritic cells and macrophages) in the pancreas, in the context of a pro-inflammatory environment. Mounting evidence points to islet macrophages as potential therapeutic targets: deletion of macrophages or skewing islet macrophages to an anti-inflammatory, reparative phenotype prevents autoimmune diabetes in NOD mice. Whether islet innate immune cells are pro-inflammatory (M1-like) or reparative (M2-like) is mechanistically tied to the cellular metabolic pathways used by these cells: M1-like macrophages use glucose metabolism primarily, whereas M2-like reparative macrophages, which are not only anti-inflammatory but also produce pro-regenerative factors, use mitochondrial metabolism, highlighting the potential of metabolic engineering for therapeutic purpose. We have developed lipid nanoparticle formulations that enable specific targeting of macrophages, and have the potential to deliver gene therapies to enhance or knockdown expression of key metabolic genes, or deliver pathway-specific inhibitors to therapeutically alter macrophage phenotypes by genetically or transiently manipulating metabolic pathways.

The fellow will use nanomedicine approaches to engineer metabolic pathways in innate immune cells to induce an anti-inflammatory, pro-regenerative phenotype in islet macrophages, with the goal of developing new therapeutic approaches for preventing or reversing T1D by inducing tolerance while promoting beta cell regeneration. Initial studies will be in the NOD mouse model of T1D, prior to and following disease onset. For translation to humans, a first step will be to use human embryonic stem cell-derived macrophages (recently established in collaboration with the Levings lab) to test how therapeutic manipulation of metabolic pathways ex vivo in human macrophages impacts phenotype, with the goal of moving towards humanized mouse models of type 1 diabetes and islet and stem cell derived insulin-producing cell transplants. A co-supervision model will ensure the fellow gains access to the expertise of Dr. Klein Geltink, an early career investigator and expert in immune cell metabolic pathways, and Dr. Verchere, an expert in islet macrophages, modelling type 1 diabetes and cell transplants in mice, and nanomedicine therapies

Project title: Optimizing stem-cell derived beta cell maturation by harnessing metabolism

Project supervisor(s):
Dan Luciani, Associate Professor UBC Dept of Surgery and Scientist BCCHR*
Francis Lynn, Associate Professor UBC Dept of Surgery and Scientist BCCHR

Key words associated with the project (up to 10):
Stem cells; insulin producing cells; metabolism; maturation; glucose-responsiveness

Short- and long-term goals of the project:

- **Short-term goals:**

1) Understand metabolic changes that occur during stem cell-derived beta cell maturation
2) Determine whether those metabolic changes influence stem cell-derived beta cell maturation

- **Long-term goals:**

1) Develop metabolism-based approaches to improve stem cell-derived beta cell maturation
2) Genetically alter stem cell-derived beta cells so that they are metabolically optimized

Project description, including relevance to stem cells and regenerative medicine:
Stem cells can be efficiently differentiated to insulin producing cells that could be used as a regenerative therapy for Type 1 diabetes. The stem cell-derived insulin producing cells (SCß-cells) that are currently produced do not become glucose responsive for many weeks following the initial production of insulin but respond to other metabolic substrates. The overarching aim of this fellowship will be to improve the transition from differentiation to complete functional maturation of SCß-cells. As beta cell function is intricately linked to metabolism, we hypothesize that the metabolic pathways that are active in immature cells prevent them from responding to glucose as do human ß-cells.

With existing human islet and SCß-cell perifusion, transcriptomic and proteomic data in hand, the successful applicant will be able to delve into differences in metabolism between these cell types and identify factors that potentially reduce glucose responsiveness of SCß-cells. The applicant will then be able to test inhibitors or activators of these pathways, or use metabolic

substrates that circumvent these pathways to determine their roles in SCß-cell maturation and glucose-stimulated insulin secretion. The Lynn and Luciani Labs at BCCHR, along with collaborators at UBC (J Johnson), will support the applicant with expertise, technical assistance and infrastructure. Lynn leads the BCCHR Tissue & Disease Modelling Core for SCß-cell generation and gene editing. Luciani and Johnson co-lead the JDRF CoE Islet Phenotyping Core for functional characterization of human β-cells and SCß-cells by live-cell imaging, perifusion and Seahorse metabolic analysis.

At the end of the first year the applicant will have generated a significant amount of data that could be used to support further fellowship and grant applications and will be well positioned to develop new pharmacological and gene-editing approaches for metabolically optimizing SCß-cell maturation and function.

Project title: Mechanisms by which lipids modulate insulin secretion from human stem cell-derived β cells

Project supervisor(s): Dr James Johnson

Key words associated with the project (up to 10): human islets, lipidomics, insulin secretion, stem cells, lipids, high throughput imaging

Short- and long-term goals of the project: The short-term goal is to investigate the mechanisms of lipid modulation in human stem cell-derived β on insulin secretion response of to specific dietary macronutrient challenge. Lipidomic profiling will determine the direct effects different free fatty acids have on hormonal secretion of beta-cells and their lipidomic profile. The long-term goal is to build better β cells for the treatment of type 1 diabetes.

Project description, including relevance to stem cells and regenerative medicine:

The project will use human embryonic stem cell-derived (hESC) beta cells to determine the mechanisms of lipid modulation in human beta-cell insulin secretion. This project has 3 primary aims:

1) Individual free fatty acids found in circulation of those with and without T1Ds will be given to hESC beta cells and primary human islets at concentrations found in circulation to determine insulin and glucagon responses to lipids.
2) hESC beta cells and human islets will be exposed to free fatty acids over 7 days to determine effects on maturity markers, proliferation, and cell death.
3) Determine the mechanisms of acute lipid-stimulated insulin release and chronic lipotoxicty by combining lipidomics and (phospho)proteomics approaches on hESC beta cells and human islets.

The successful applicant will have the opportunity to work within my ~2500 sqft lab with a highly interactive group of excellent students and fellows, with supervisors that emphasize mentorship above all else.

Project title: Sex differences in beta cell identity during maturation and stresses associated with T1D

Project supervisor(s):
Dr. Elizabeth Rideout, Assistant Professor, Life Sciences Institute, UBC*
Dr. James Johnson, Professor, Life Sciences Institute, UBC

Key words associated with the project (up to 10):
type 1 diabetes, sex differences, beta cell identity, beta cell maturation, endoplasmic reticulum stress, stem-cell differentiation, insulin production, protein translation

Short- and long-term goals of the project:
Insulin production is required to prevent/reverse T1D, but it comes at a cell-autonomous cost of increased endoplasmic reticulum (ER) stress. We found that male and female beta cells differ in expression of identity markers and function during ER stress.

• Our short-term goals are to combine studies in primary human and mouse beta cells to monitor beta cell identity during maturation and stress in each sex.

• Our long-term goals of this project are to leverage knowledge of beta cell identity during maturation and stress in each sex to optimize stem cell differentiation and ensure beta cell identity is maintained.

Project description, including relevance to stem cells and regenerative medicine: Stem cell-based therapies for T1D require the production of large quantities of insulin-producing beta cells, where each cell makes large quantities of insulin. Insulin accounts for roughly half of all protein production in a mature beta-cell. Making and folding insulin is therefore an inherently stressful process for beta cells. Understanding how beta cells acquire and maintain their identity in stressful conditions, which allows them to make and release insulin in response to changes in blood glucose, is a critical task in developing stem cell-based T1D therapies.

Our unpublished data shows significant sex differences in how beta cell adapt to the stress of producing insulin. Specifically, we show that male and female beta cells differ in their response to one type of stress called endoplasmic reticulum (ER). This includes robust differences in the expression of genes related to beta cell identity during ER stress, and differences in how well beta cells maintain insulin production in this context.

The overarching goal of this project is to determine how beta cell identity contributes to insulin production during maturation and ER stress in each sex. In doing so, we will gain a more accurate understanding of how to make better functioning and more resilient beta cells from stem cells. The fellow will lead project this project in close collaboration with Dr. Rideout, an expert in metabolism and sex-based analysis and Dr. Johnson, an expert in insulin production, beta cell biology, and stem-cell derived beta-7 cells (Johnson). The fellow will train in a world-class research environment with close connections between a large diabetes community.

In Year 1 of this project, the fellow will:

• Study beta cell identity during maturation and stress in primary mouse and human islets of both sexes.

• Integrate sex-based analysis and stem cell biology to make new discoveries about beta cell identity.

In future years, the fellow will:

• Optimize iPSC differentiation protocols based on the data acquired in Year 1.

• Present their work at an international conference.

Project title:
ProIAPP-derived peptides as biomarkers of human stem cell derived b-cell maturity and transplant function

Project supervisor(s):
Bruce Verchere, PhD, UBC Depts of Surgery and Pathology & Laboratory Medicine, BC Children's Hospital Research Institute

Key words associated with the project (up to 10): diabetes, b-cell function, prohormones, proinsulin, proIAPP, stem cells, transplants

Short- and long-term goals of the project:

Short-term goals: To identify and validate biomarkers of islet and stem cell derived b-cell transplant function and to refine assays for their measurement in pre-clinical models and humans.
Long-term goals: To assess graft function of stem-cell derived b-cell transplants in T1D recipients through measurement of prohormone biomarkers.

Project description, including relevance to stem cells and regenerative medicine:
The b-cell peptides insulin and islet amyloid polypeptide (IAPP) are first produced as larger precursor propeptides, proinsulin and proIAPP. The proposed sponsor's lab has shown that forms of incompletely processed proIAPP (as for proinsulin) are disproportionately elevated in the blood of persons with T1D (Courtade et al *J Clin Endo Metab* 2017), as well as in recipients of islet transplants (Chen et al *Am J Transpl* 2022). A paradigm is emerging that elevated prohormone levels are biomarkers that reflect b-cell dysfunction in T1D (Rodrigues-Calvo *Diabetes* 2021) and may predict graft failure. Proper processing of proinsulin and proIAPP to their mature and active forms is a property of healthy, differentiated b-cells, and likely occurs in later stages in the differentiation of human embryonic stem cells into b-cells (SCb-cells) for transplantation. The overall hypothesis of the proposed fellowship is that measurement of proinsulin and proIAPP derived peptides will enable in vivo assessment of SCb-cell maturity and function, and may predict graft outcomes.

The fellow will refine approaches for measurement of b-cell prohormones in human plasma and those generated by SCb-cells at different stages of differentiation using proteomic approaches, including mass spectrometry, protein biochemistry, and ELISAs. Assessment of the prohormone

processing machinery at different stages of human SCb-cell differentiation will be made in vitro and in vivo using these assays as well as immunohistochemical approaches. Once measurement approaches are optimized, assessment of prohormone forms will be made in blood of pre-clinical mouse models of human SCb-cell transplantation in diabetes, and in SCb-cell clinical trials, where available. The fellowship promises exposure to proteomics and protein biochemistry, mouse models of T1D and transplantation, stem cell differentiation, and clinical studies. The project will include ppportunities for collaboration with other JDRF-CoE labs in stem cell differentiation and b-cell function assessment.

Project title: Assessment of differentiated immune-evasive stem cells for the treatment of diabetes

Project supervisor(s):
David Thompson*, University of British Columbia
Timothy Kieffer, University of British Columbia

Key words associated with the project (up to 10):
Stem cells, diabetes, plasma, assays, immunohistochemistry, histology, clinical trial, rodents, implants

Short- and long-term goals of the project:
Short-term goals:
1) conduct various hormone and immune marker assays on blood samples periodically collected from patients
2) perform histology and immunohistochemistry on devices retrieved from patients
Long-term goals:
1) conduct cell implants in animal models of diabetes
2) assess glucose homeostasis and graft performance in animal models
3) perform histology and immunohistochemistry on devices retrieved from animal models

Project description

The candidate will have the opportunity to participate in a world's first clinical trial with stem cells engineered to be immune-evasive as a source of insulin replacement.
• See ClinicalTrials.gov Identifier: NCT05210530 for more information about the trial.
• See Ramzy et al. Cell Stem Cell 2021 Dec 2;28(12):2047-2061 PMID: 34861146 for results from previous trial and example of the types of analyses performed.
• The candidate will have the opportunity to determine the appropriate sample collection and analyses to test novel hypotheses.
• The candidate will have the opportunity to conduct parallel studies in animal models to assess the immune-evasiveness of the cells, and ability to reverse diabetes following implant in different locations, including encapsulated and non-encapsulated formats.
Background: Transplant of islets obtained from organ donors is a highly effective treatment, with some remaining insulin-independent more than ten years. However, this therapy is severely restricted by limited supply of donor organs. ViaCyte is developing a pancreatic progenitor product from stem cells that represents a renewable source of

cells, and an implantable device to contain the cells. Our clinical testing of this potential product with low doses of cells indicates the procedure is safe and the device supports both the maturation and function of the implanted cells. In a patient implanted with a higher dose of cells, peak insulin use was reduced >70% and blood glucose levels were maintained in the healthy range >90% of the time. The ViaCyte stem cells have now been genetically engineered to escape detection by the immune system. As a world's first, September 2022 we will embark on clinical trials in patients with type 1 diabetes to examine the safety and immune-evasiveness of these cells, and subsequently, with larger doses, whether this approach can restore normal control of blood glucose levels and eliminate insulin injections, in the absence of chronic immunosuppression. Our team will apply unique and rigorous assessments of graft function. If successful, this clinical trial may lead to the development of a product that can cure millions of patients with diabetes putting an end to insulin injections.

Project title: Building better beta-cells from stem cells via non-cell autonomous YAP signaling

Project supervisor(s):

Primary supervisor: Dr. Janel Kopp*, Assistant Professor, Life Sciences Institute, UBC

Secondary supervisor: Dr. James Johnson, Professor, Life Sciences Institute, UBC

Key words associated with the project (up to 10):

type 1 diabetes, beta cell maturation, stem-cell differentiation and propagation, insulin production, recapitulating pancreatic development

Short- and long-term goals of the project:

Differentiation of insulin-producing beta-cells from pluripotent stem cells is quite efficient up until the pancreatic progenitor cell stage. We recently found that selectively inducing expansion of pancreatic progenitor cells using mosaic YAP1 activity in mice promoted the differentiation of neighboring cells into endocrine cells.

Our short-term goals are to determine whether selectively activating YAP1 in a subset of human embryonic stem cell-derived pancreatic progenitor cells expands those progenitor cells, while promoting normal neighboring progenitor cells to differentiate into endocrine cell. **Our long-term goals** are to identify the non-cell autonomous mechanisms induced by YAP1 activity in progenitor cells driving these events.

Project description, including relevance to stem cells and regenerative medicine:

The current stem cell-based therapy being tested for T1D utilizes near faithful recapitulation of developmental biology to create pancreatic progenitor cells capable of differentiating into insulin-producing beta cells in vivo. Further differentiation of the progenitor cells in the dish for the creation of insulin producing beta cells to transplant is still limited due to our incomplete understanding of the processes needed for progenitor cells to accurately differentiate into mature functioning beta cells. One difference between current efforts to differentiate cells ex vivo and normal in vivo differentiation is the absence of a proliferative expansion at the pancreatic progenitor stage. We recently found that mosaic activation of Yap1 in mouse pancreatic progenitor cells resulted in expansion of the Yap1-expressing progenitor cells and

differentiation of non-Yap1 activated cells into acinar and endocrine cells. This suggested that proliferation of some progenitor cells is coordinated with differentiation of other progenitor cells in a non-cell autonomous manner. The overarching focus of this project is to examine whether similar results can be obtained when this manipulation is performed in the human embryonic stem cell differentiation platform ex vivo and what impact this has on the differentiation and maturation of endocrine cells. In doing so, we will gain a more accurate understanding of how to make better functioning beta-cells from stem cells. The fellow will lead project this project in close collaboration with Dr. Kopp, an expert in pancreatic development and endocrine differentiation. They will also be co-supervised by Dr. Johnson, an expert in insulin production, beta cell biology, and stem-cell derived beta-cells. The fellow will train in a world-class research environment.

In Year 1 of this project, the fellow will:

- Create a doxycycline inducible active Yap1 allele to temporally and mosaically activate Yap1 expression in human embryonic stem cells.
- Assess the effect of mosaic Yap1 activation on endocrine differentiation from human embryonic cells cultures.

In future years, the fellow will:

- Identify the molecules responsible for non-cell autonomous effect of YAP1 activation on endocrine cell differentiation.