La description des projets n'est disponible qu'en anglais

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-term goals of the project: To develop nanomedicine approaches for engineering metabolic pathways in innate immune cells towards a pro-regenerative, anti-inflammatory phenotype
Long-term goals of the project: 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 (T1D) 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 purposes. We have developed lipid nanoparticle (LNP) formulations that enable specific targeting of macrophages and dendritic cells and can be loaded with multiple cargos enabling the potential to deliver tolerizing therapies in an antigen-specific manner. The project will use primary and stem-cell derived macrophages, and the pre-clinical NOD mouse model, to optimize therapeutic approaches that target innate immune cells using LNPs. One potential target is to enhance or knock down expression of key metabolic genes or deliver pathway-specific inhibitors to therapeutically alter macrophage phenotype by

genetically or transiently manipulating metabolic pathways. The fellow will benefit from extensive infrastructure at BC Children's Hospital Research Institute, collegial and supportive team mentorship, and a diverse and rich diabetes research training environment.

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; transcriptional and functional maturation; glucose metabolism; mitochondria; mitochondrial transfer, mitochondrial dynamics.

Short-term goals of the project:

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 of the project:

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 in culture do not become glucose responsive for many weeks following the initial production of insulin, and remain functionally less mature than primary human islets. The overarching aim of this fellowship will be to improve the transition from differentiation to complete functional maturation of SCß-cells in vitro. We hypothesize that the metabolic pathways that are active in immature cells prevent them from responding to glucose and secrete insulin as do human ß-cells, and can be targeted to achieve full functional maturation.

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 insulin secretion. The applicant will also have the opportunity to drive efforts in the Luciani and Lynn Labs aimed at using transfer of OXPHOS-competent mitochondria, as well as manipulations

of mitochondrial dynamics, as innovative approaches for kick-starting SCß-cell maturation

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, and has established several SCß-cell reporter lines that will be available to the project. 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 to target and optimize SCß-cell mitochondrial metabolism, maturation and function.

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-term goals of the project: 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 of the project: 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:
Initiation of type 1 diabetes (T1D) 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 purposes. We have developed lipid nanoparticle (LNP) formulations that enable specific targeting of macrophages and dendritic cells and can be loaded with multiple cargos enabling the potential to deliver tolerizing therapies in an antigen-specific manner. The project will use primary and stem-cell derived macrophages, and the pre-clinical NOD mouse model, to optimize therapeutic approaches that target innate immune cells using LNPs. One potential target is to enhance or knock down expression of key metabolic genes or deliver pathway-specific inhibitors to therapeutically alter macrophage phenotype by genetically or transiently manipulating metabolic pathways. The fellow will benefit from extensive infrastructure at BC Children's Hospital Research Institute, collegial and supportive team mentorship, and a diverse and rich diabetes research training environment.

Project title: Simultaneous single cell secretion and transcriptome profiling of stem cell derived β cells

Project supervisor(s): Hongshen Ma, Professor, School of Biomedical Engineering

Key words associated with the project (up to 10): stem cell derived beta cells, nanotechnology, insulin, glucagon, somatostatin, secretion, single cell transcriptomics

Short- and long-term goals of the project: This project will investigate SCβ heterogeneity by developing a technology to simultaneously profile single cell hormone secretion, cell surface protein expression and single cell transcriptomics. Our approach involves tethering SCβ cells with nanoparticles functionalized with antibodies to capture secreted pancreatic hormones. After glucose stimulation, hormones secreted by SCβ cells are immediately captured on these nanoparticles. The captured hormones (along with cell surface proteins) are then labeled using detection antibodies tagged with oligonucleotide barcodes similar to CITE-seq. Finally, the secreted hormones are readout along with RNA and proteins using 10X Genomics droplet single cell sequencing.

Project description, including relevance to stem cells and regenerative medicine: Islet transplantation from cadaveric donors combined with immunotherapy is a clinically effective treatment for type 1 diabetes (T1D). However, there are insufficient cadaveric donors to meet the demand for all T1D patients. Furthermore, the life span of transplanted islets is limited, with <20% of patients maintain insulin independence after 5 years. Stem cell derived β (SCβ) cells provide a potentially limitless supply of insulin-producing cells for transplantation. However, current stem cell differentiation protocols produce cells with limited functional maturity, which exhibit glucose stimulated insulin secretion (GSIS) at ~1/3 the level of donor islets.

It is currently unclear whether this GSIS deficiency is a result of SCβ cells being homogenously immature, or if SCβ cells are heterogeneous groups at different stages of maturity. If the latter, it may be possible to screen for the functionally mature phenotype to determine their underlying molecular drivers in order to guide cell engineering efforts toward generating more functionally mature cells. To investigate the heterogeneity of SCβ cells, we will develop a new technology to simultaneously profile hormone secretion, gene expression, and protein expression at the single cell level.

Our approach involves tethering SCβ cells with nanoparticles functionalized with antibodies to capture secreted pancreatic hormones including insulin, glucagon, and somatostatin. After glucose stimulation, hormones secreted by SCβ cells are immediately captured on these nanoparticles. Using an approach similar to CITE-seq, hormones captured on nanoparticles, along with cell surface proteins, are labeled using detection antibodies tagged with oligonucleotide barcodes. Finally, the secreted hormones are readout simultaneously as single cell RNA and proteins using single cell sequencing.

The postdoctoral associate will:

- Develop the nanoparticle technology for capturing secreted hormones from SCβ cells
- Work with the UBC antibody, flow cytometry, and sequencing cores to develop detection antibodies tagged with oligonucleotides for single cell sequencing
- Collaborate with Prof. Francis Lynn's group to profile hormone secretion heterogeneity in SCβ cells and human islet cells
- Develop data analytics pipelines for single cell multi-omic (secretion, transcriptome, cell surface protein) analysis of SCβ cells
- Collaborate with Prof. Francis Lynn's group to identify and test markers associated with highly functional SCβ cells

Project title: Developing a human stem cell-derived model of type 1 diabetes

Project supervisor(s): Megan Levings

Key words associated with the project (up to 10): stem cells, islets, antigen presentation, T cells, autoimmunity, human

Short term goals: generate stem cell-derived insulin-producing cells, antigen presenting cells and T cells from a new HLA-A2+DR4+DQ8+ induced pluripotent stem cell line. T cell and chimeric antigen receptor engineering will be used to generate islet antigen specific T cells such that this three cell system can be used to model autoimmunity. Regulatory T cells can be added to the system to study tolerogenic cell therapy.

Longer term goals: use this multiple stem-cell-derived, autologous cell system to create a new humanized mouse model where new pro-tolerogenic therapies can be tested.

Project description: multiple promising tolerogenic therapies are in development for type 1 diabetes (T1D), but a major limiting factor is the lack of tractable in vitro and in vivo models to study and manipulate autoimmunity. This project aims to overcome this limitation by leveraging existing methods to produce insulin-producing cells, antigen presenting cells and T cells from a stem cell line with a T1D-relevant haplotype (A2+DR4+DQ8+). Specifically, during the first year the candidate would learn how to generate these multiple cell types and validate their phenotype and function. Genetic engineering of stem cell-derived CD8+ T cells will be used to introduce autoantigen specific T cell receptors and the ability of islets to stimulate these cells either directly or indirectly via antigen presenting cells will be tested. Once established, this model will be used to study effects of tolerogenic strategies including regulatory T cell therapy and lipid nanoparticle therapy, both in vitro and in vivo.

Lab and cores: this project harnesses expertise across the centre, with opportunities to work with Levings, Verchere and Lynn to learn stem cell-based differentiation protocols. The T cell differentiation work will include collaborations with Zandstra. The candidate will gain diverse expertise across a variety of experimental systems including stem cell differentiation, genetic engineering, immunotherapy and tolerance, and animal models. As a future step, the project will work with the animal modelling core to transfer various cell types in vivo to create a new human-cell based model of T1D. In addition to training programs through the Centre and BC Children's Hospital Research Institute, the

candidate will be affiliated with the School of Biomedical Engineering which has robust career development opportunities.

Knowledge translation: the candidate will have the opportunity to present their work at multiple local opportunities, and to attend at least one international conference per year. Through Centre activities they will engage with people with lived experience partners. The candidate's work will be published in well-recognized journals.

Project title: Mechanosensitive Immune Cell-Based Therapy for Anti-Inflammatory response in Type-1 Diabetes

Project supervisor(s): Dr. Peter Zandstra, Professor, School of Biomedical Engineering

Key words associated with the project (up to 10): Stem cell differentiation, Immune cells, Mechanotransduction, Mechanosensitive receptors, Extracellular matrix, Inflammation, Mechanobiology, Anti-Inflammatory cytokines, Synthetic Biology, Type 1 diabetes.

Short-term goals of the project:

- Design synthetic receptors with adjustable tension settings to create customizable mechanosignaling pathways, where both force detection and response mechanisms can be tailored to specific needs.
- Employ a chemically defined protocol to differentiate human pluripotent stem cells (hPSCs) to mature T cells expressing tension-tuned surface receptors.
- Test the ability of T cells to secrete anti-inflammatory cytokines in response to changing external matrix stiffness and tensile forces using synthetic substrates and specialized DNA based tension sensors.

Long-term goals of the project:

- Perform preclinical studies with mechanosensitive T cells in experimental Type 1 Diabetes mouse models and test their ability to reduce inflammation in vivo.

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

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the immune system's attack on insulin-producing beta cells causing inflammation in pancreatic tissues. The Inflamed pancreatic tissues are mechanically softer than healthy tissues and create an inhospitable environment for the remanent insulin-producing beta cells. The proposed research aims to develop mechanosensitive immune cells from stem cells to detect mechanical changes within the tissues and respond by releasing anti-inflammatory cytokines, potentially reducing inflammation. This innovative approach holds promise for improving T1D treatment.

In Year 1 of this project, the fellow will:

- Design tension-tuned synthetic notch receptors with customize mechanosensitive circuits by exchanging the ligand binding domain inputs with antibody-based domain to detect various ECM proteins, such as laminin found abundantly in inflamed lesions. Simultaneously, modify output genetic expression by substituting the intracellular transcription domain and incorporating specific downstream effector target genes for anti-inflammatory cytokines like Interleukin-10.

- Generate mature T cells expressing tension-tuned surface receptors from human pluripotent stem cells using chemically defined protocols optimized by Zandstra lab.

- Evaluate cells expressing mechanosensitive receptors for their capacity to produce specific mechanical responses to varying external matrix stiffness and tensile forces using synthetic substrates with variable stiffness and DNA-based tension gauge tethers (TGT). Validate downstream gene expression through the inclusion of fluorescent reporters introduced during circuit design.

In following years, the fellow will:

- Evaluate the therapeutic potential of mechanosensitive T cells in preclinical models of T1D by assessing their ability to deliver anti-inflammatory cytokines and reduce inflammation in the target tissues in vivo.

A fellow with substantial expertise in mechanobiology will lead this project under the supervision of Prof. Zandstra, an expert in stem cell bioengineering. The Zandstra laboratory boasts extensive experience encompassing microscopy, micropatterning, single-cell gene expression analysis, synthetic biology, and immune cell development protocols. The fellow will have access to in-house facilities, including lentiviral transduction-cell culture, flow cytometry, animal housing, and genotyping. Additional fellow will benefit from in lab collaborations with experts in synthetic biology and sequencing which will enrich the project's technical depth. The fellow will disseminate the findings of this project via peer-reviewed publications and presentations at scientific conferences.