Investigating the role of altered immune cell metabolism and inflammation in type 2 diabetes

Abstract/Project Summary

Type 2 diabetes (T2D) is a worldwide epidemic, affecting the Native Hawaiian population and economy in Hawai‘i. Inflammation associated with obesity is a major risk factor in T2D and often precedes T2D, but the role of altered immune cell metabolism and inflammation in T2D is unknown. Thus, there is a need for research of clinical biospecimens obtained from individuals with T2D to characterize and understand the metabolic and inflammatory mechanisms contributing to T2D as a metabolic disease.

The overall goal of this proposal is to study the role of altered immune cell metabolism and inflammation in T2D by (1) characterizing and comparing immune cell metabolism and (2) phenotyping the inflammatory immune cell subset populations in individuals with T2D and age/gender matched controls. We hypothesize that (1) T2D alters immune cell metabolic function leading to a chronic proinflammatory state, and (2) associates with an increased composition of inflammatory immune cells.

First, to characterize and compare immune cell metabolism in individuals with T2D, we will compare the metabolic profiles of T2D and healthy control peripheral blood mononuclear
cells (PBMCs) by measuring glycolysis and mitochondrial respiration rates on the Agilent Seahorse XFe96 Analyzer. Second, to phenotype and quantify the immune cell subset populations in individuals with T2D, we will use a BD LRS Fortessa flow cytometer capable of detecting fluorochrome-conjugated antibodies specific for markers of particular immune cell subsets.

We expect to show that (1) T2D alters the metabolic function of immune cells by decreasing glycolysis and increasing oxidative phosphorylation in PBMCs, and (2) inflammatory T cell subsets will be increased in T2D compared to controls. This project will fill a gap in knowledge on the role of altered immune cell metabolism and identify which cell types may be disease relevant. Moreover, this novel information may be relevant for improving the treatment and management of type 2 diabetes.

**Background and Significance/ Compelling Case**

Type 2 Diabetes (T2D) is a metabolic disease characterized by defects in insulin secretion and sensitivity resulting in high blood glucose levels (Shu, Benoist & Mathis, 2012). Approximately 13.1% of the adult population have diabetes in Hawai‘i, and 41.5% of them are prediabetic (Dall et al., 2014). In addition, diabetes is the fourth leading cause of death in Native Hawaiians (King et al., 2012). Diabetes has not only affected the health of Hawai‘i, but also the economy. Annually, diabetes and prediabetes cost an estimated $1.5 billion in Hawai‘i (Dall et al., 2014). To provide insight in stopping the diabetes epidemic in Hawai‘i, clinical research needs to characterize and understand the mechanisms behind T2D as a metabolic disease.

Increasing evidence supports the role of obesity in inflammation caused by the immune system (Donath & Shoelson, 2011). Proinflammatory conditions onset by obesity contributes to
insulin resistance and dysfunction (Kalupahana, Moustaid-Moussa & Claycombe, 2012). Contrasting, improved insulin sensitivity was shown in obese individuals who have experienced a decrease of inflammatory biomarkers such as C-reactive protein, TNF-α and IL-6 accompanying their weight loss (Kopp et al., 2003). Therefore, characterizing the role of the immune cells responsible for regulating immune inflammation is important in understanding T2D.

Studies have shown that energy metabolism has a role in immune cell programming (MacIver, Michalek & Rathmell, 2012). Evidence shows that immune cell metabolism is a key regulator of T cell function and fate, and that changes in immune cell metabolism can enhance or suppress specific T cell functions (MacIver, Michalek & Rathmell, 2012). T cells are lymphocytes that are produced by the thymus gland. Once activated, T cells can participate in the body’s immune response by recognizing and attacking antigens. T cell activation causes a shift in metabolism to protect against pathogens and orchestrate action of other immune cells (Nishimura et al., 2009). T cell suppression fails to allow the response to cytokine signals and differentiation into distinct helper T cell subsets, leading to excessive inflammatory responses, a characteristic that we see in T2D (Jameson, 2002). However, whether metabolic mechanisms in specific T cell immune cell populations are altered in T2D remains unclear.
Figure 1. Obesity results in inflammation and changes in immune system cells in adipose tissue. (Figure from Sue et al., 2012)

Specific immune cell types including lymphocytes have been linked to inflammatory conditions in T2D (Figure 1). Obese adipose tissue has been shown to activate CD8+ effector T cells specifically to promote the recruitment and activation of macrophages that produce proinflammatory cytokines (Nishimura et al., 2009). Additionally, roles for adipocyte-specific CD4+ T cells and regulatory T cells (Tregs) have been implicated in controlling both local macrophage recruitment and systemic insulin resistance (Winer et al., 2009; Feuerer et al., 2009). Yet, few studies have investigated whether there is an alteration in the composition of specific subsets of lymphocytes in T2D. This study will focus specifically on T cell subsets in order to
understand the immune system’s role in the inflammation seen with individuals with T2D.

**Figure 2.** A component diagram depicting the T cell’s hypothesized involvement in Type 2 Diabetes.

Understanding the role of immune cells in the context of T2D may contribute significantly in the effort to treat the diabetes epidemic in Hawai‘i. The immune system has been well linked to type 1 diabetes (Pino, Kruger & Bortell, 2011), but this link has not yet been well defined in T2D. Additionally, the number of T2D studies focusing on Native Hawaiian and Pacific Islander (NHPI) populations do not reflect the high incidence rates in NHPI nor the cost it has on Hawaii’s economy. In order to fulfill the needs of the NHPI population and the economy in Hawai‘i, there is a need to fulfill the gap in knowledge by understanding the role of altered immune cell metabolism and populations in T2D.

In this study, we will aim to characterize and compare immune cell metabolism and phenotype inflammatory immune cell subset populations in individuals with T2D. We hypothesize that **T2D alters immune cell metabolic function leading to a chronic**
proinflammatory state, and associates with an increased composition of inflammatory immune cells.

Experimental Design and Methods

**Aim 1: To characterize and compare immune cell metabolism in individuals with T2D.**

**Rationale:** Studies highlight a potential role for altered immune cell metabolism in the pathogenesis of T2D. Changes in metabolism dictate immune cell activation. T cells and dendritic cells both depend on oxidative phosphorylation for energy generation, but switch to glycolysis post-activation (Raval & Nikolajczyk, 2013). In macrophages, the metabolic switch from oxidative phosphorylation to glycolysis is necessary in secretion of proinflammatory cytokines (Tannahill et al., 2013). Yet, few studies of T2D have examined metabolism of inflammatory immune cells such as T cells.

**Hypothesis 1:** T2D alters the metabolic function of immune cells by decreasing glycolysis and increasing oxidative phosphorylation in PBMCs.

**Methods:** The metabolic profiles of participants diagnosed with T2D and age/gender matched healthy control peripheral blood mononuclear cells (PBMCs) will be assessed using the Agilent Seahorse XFe96 Analyzer (Smolina et al., 2017). Glycolysis and mitochondrial respiration rates will be measured using the Glycolytic Rate Assay Kit and the Mito Stress Test. In the Glycolytic Rate Assay Kit, Proton Efflux Rates (PER) are measured following mitochondrial inhibition to reflect basal glycolysis and compensatory glycolysis. In the Mito Stress Test, Oxygen Consumption Rates (OCR) are measured throughout a series of compound additions targeting the
Electron Transport Chain (ETC) to reflect basal respiration, ATP production, proton leak, maximal respiration, spare respiratory capacity, and nonmitochondrial respiration.

Figure 3. Graph illustrating the data output of the Seahorse XF Glycolytic Assay and Mito Stress Test (Agilent Technologies).

**Aim 2: To phenotype and quantify inflammatory immune cell subsets in individuals with T2D compared to controls.**

**Rationale:** Inflammation has been linked to metabolic conditions including T2D. Studies suggest inflammatory cytokines are a risk in developing T2D (Spranger et al., 2003). Specifically, elevated levels of serum interleukin-1 receptor antagonist precede the onset of T2D six years prior to diagnosis (Carstensen et al., 2010).

**Hypothesis 2:** Inflammatory T cell subsets will be increased in T2D compared to controls.

**Methods:** PBMCs of individuals with and without T2D will be thawed and stained with AARD to sort live and dead cells and with fluorochrome conjugated antibodies specific for markers of senescent, naïve, central memory, intermediate, and effector CD4$^+$ and CD8$^+$ subsets (CD3, CD4, CD8, CCR7, CD28, CD45RA, TIGIT, PD-1, TIM-3). The stained cells will then be phenotyped using flow cytometry on the BD Bioscience LSR Fortessa. The flow cytometer will extract the
cellular heterogeneous sample, mix it with saline solution, then lead the cell suspension into a narrow channel into a single file line, allowing each cell to individually pass an interrogation point and through a laser beam. The laser beam will scatter light from each cell in multiple directions allowing two detectors to measure side and forward scattered light in voltage pulse which is proportional to a cell’s granularity and size respectively. Additionally, the flow cytometer will detect and quantify emitted light from excited fluorescent molecules, the fluorochrome conjugated antibodies. A computer converts the data into a histogram to be analyzed, identifying the specific immune cell subsets in T2D and in controls.

**Expected Results:** We expect to show that: (1) T2D alters the metabolic function of immune cells by decreasing glycolysis and increasing oxidative phosphorylation in PBMCs, and (2) inflammatory T cell subsets will be increased in T2D compared to controls.
Figure 5. Schematic illustrating the workflow of this proposed study (pluriSelect, Agilent Technologies).

**Timetable**

A proposed timeline for the planned studies is illustrated in the table below and the workflow illustrating the use of biospecimens is shown in Figure 5.

<table>
<thead>
<tr>
<th>OBJECTIVES</th>
<th>Fall 2018</th>
<th>Spring 2019</th>
<th>Summer 2019</th>
<th>Fall 2019</th>
<th>Spring 2020</th>
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<tbody>
<tr>
<td><strong>Specific Aim 1-</strong> To characterize and compare immune cell metabolism in individuals with T2D.</td>
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<td>1.1: Learn cell culture maintenance and the protocol for thawing whole PBMCs</td>
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<td>1.2: Receive training on the Agilent Seahorse XFe96 Analyzer</td>
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<td>1.3: Run titration experiments</td>
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<td>1.4: Run Assay</td>
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### Specific Aim 2 - To phenotype the inflammatory immune cell subset populations in individuals with T2D and age/gender matched controls.

- **2.1: Receive training on the Flow Cytometer**
- **2.2: Immunophenotype**
- **2.3: Analyze Data**

### Finishing Thesis/Presentation

- **3.1: Write Thesis**
- **3.2: Complete extra experiments, as needed**
- **3.3: Create and present poster**

### Resources and Materials

My Principal Investigator, Dr. Michael Corley and his Cell and Molecular Biology graduate student, Alina Pang will be supervising all experiments. Dr. Corley’s laboratory is a total of 400 square feet (sq. ft.) in the Biosciences Building (BSB) of the John A. Burns School of Medicine (JABSOM) Kaka’ako campus, giving access to the shared space as follows:

**Corley Lab**
• 3 lab benches to accommodate 6 people (315 sq. ft.)
• Imaging and GelDoc station room (85 sq. ft.)

Common/shared space

• Tissue culture room (110 sq. ft.)
• Cold room (122 sq. ft.)
• Equipment room (660 sq. ft.)
• Decontamination/autoclave room (114 sq. ft.)
• Chemical fume hood room (110 sq. ft.)
• Staff break room (207 sq. ft.)
• Two conference/meeting rooms (327 sq. ft.; 224 sq. ft.)
• Copy/Fax Supply room (142 sq. ft.)

Dr. Corley’s lab is part of an NIH funded Diabetes Center at the JABSOM and have access privileges to Core resources that include the BD Bioscience LSR Fortessa Flow Cytometer and Agilent Seahorse XFE96 Analyzer. The PBMC samples from ZenBio and all materials used in this project will be funded by the Center for Biomedical Research Excellence (COBRE) for Diabetes.

Documentation of Training

My laboratory safety training and dates of completion are illustrated in the table below.

See appendix for copies of certificates.

<table>
<thead>
<tr>
<th>Training Certificate</th>
<th>Date of Completion</th>
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<tr>
<td>General Biosafety Principles and Practices</td>
<td>December 4, 2017</td>
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<tr>
<td>Laboratory Safety Training</td>
<td>December 4, 2017</td>
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Role of Researcher

I started volunteering at Dr. Michael Corley’s lab in November of 2017 after completing the appropriate training. I began by helping with qPCR, cell culture maintenance, gel electrophoresis, cell processing, and metabolic assays on the Agilent Seahorse. Dr. Corley’s lab is funded as a project of an NIH Diabetes Center grant, studying the immune system’s role in diabetes. I will be responsible for completing a Specific Aim of Dr. Corley’s funded study. Specifically, I will be thawing blood samples and collecting metabolic and flow cytometry data. Moreover, I will be responsible for organizing data and ensuring that samples are maintained.
References


the Human Immunology Project. *Nature reviews Immunology, 12*(3), 191-200.
http://doi.org/10.1038/nri3158

http://doi.org/10.1038/nm.1964


http://doi.org/10.1016/j.smim.2012.12.001


Appendix

Certificate of Training
In
General Biosafety Principles and Practices

Ashley Lee

The above University of Hawaii employee has completed online refresher training for Biosafety Principles and Practices and has shown through examination the comprehension and understanding of:

- Review Biosafety Program
- Containment
- Biological Safety Levels (BSL)
- Personal Protective Equipment (PPE)
- Decontamination and Disinfection
- Dealing with Biological Spills
- Biological Waste Management
- Risk Assessment
- Incident Response
- Records and Documentation

December 4, 2017
Date of Training

[Signature]
Biosafety Compliance Program

The certificate expires one year from the date of training.
LABORATORY SAFETY TRAINING

Ashley Lee

This certificate verifies that the holder has been instructed in General Lab Safety, a general awareness training which includes elements of: General Lab Hazard Awareness; Safe Lab Practices; Labeling and Signage; Personal Protective Equipment; Kaka’ako Campus Safety and Security Policies; the UH Chemical Hygiene Plan; Standard Operating Procedures; Safety Data Sheets; Chemical Management; Engineering Controls, including Chemical Fume Hoods; Spill Response; Emergency Preparedness; Incident Reporting.

Janet Medei, Kaka’ako EHSO Supervisor
Instructor

December 4, 2017
Date of Completion

University of Hawai’i • Kaka’ako Environmental Health & Safety Office
651 Hilo Street, Biosciences Building 112 • Honolulu, Hawaii 96813
http://www.hawaii.edu/shs/kakaako

HAZARDOUS WASTE GENERATOR TRAINING

Ashley Lee

This certificate is awarded upon completion of the UH JABSOM Kaka’ako Hazardous Waste Generator Online training as required by the UH Manoa Environmental Health and Safety Office. This certificate verifies that the holder has been instructed on the basis of procedures for Hazardous Waste Generator safety and compliance with University, C&C, State, and Federal regulations. This certificate should be kept for record.

Janet Medei, Kaka’ako EHSO Supervisor
Instructor

December 4, 2017
Date of Completion

University of Hawai’i • JABSOM Kaka’ako Environmental Health & Safety Office
651 Hilo Street, Biosciences Building 112 • Honolulu, Hawaii 96813
Certificate of Completion
For the Course
Understanding and Using the Biological Safety Cabinet

Ashley Lee

The above University of Hawaii employee has completed the informational course on Understanding and Using the Biological Safety Cabinet and has shown through examination the comprehension and understanding of:

- How the Biosafety Cabinet (BSC) works
- HEPA filters - what they are and how they work
- How BSC's protect the person, product and environment.
- How Fume hoods and LFCB's differ from BSC's.
- 3 Classes of BSC's
- Working safely and effectively in a BSC
- Spills and emergencies in the BSC
- Care and maintenance of the BSC
- Annual certification requirements
- NSF 49 - Class II BSC's standards

December 4, 2017
Date of Training

Stephen E. Case
Biosafety Compliance Program

The certificate has NO expiration but refresher training is recommended.

Certificate of Training
In
Bloodborne Pathogens and Safe Sharps Use
(OSHA) 29 CFR 1910.1030, (HIOSH) HAR§12-205.1

Ashley Lee

The above University of Hawaii employee has completed online annual refresher training for Bloodborne Pathogens and Safe Sharps Use and has shown through examination the comprehension and understanding of:

- OSHA standard 1910.1030
- Epi and symptoms of selected BBP Modes of transmission
- Site-specific Exposure Control Plan
- Use of engineering, work practices and PPE
- Proper waste management
- Hep B vac. and exposure control methods
- Hazard recognition
- Question and answer sessions

December 4, 2017
Date of Training

Stephen E. Case
Biosafety Compliance Program

The certificate expires one year from the date of training.