I. BIOGRAPHICAL SKETCH

NAME: Robert C. Sharp, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): rsharp07

POSITION TITLE: Postdoctoral Associate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Central Florida, Orlando, FL	B.S.	05/2008	04/2013	Biotechnology
University of Central Florida, Orlando, FL	M.S.	08/2013	12/2014	Biomedical Sciences
University of Central Florida, Orlando, FL	Ph.D.	06/2015	12/2018	Biomedical Sciences
University of Florida, Gainesville, FL	Postdoctoral Associate	01/2019		Pathology and Immunology

A. PERSONAL STATEMENT

My previous experience in biomedical sciences, more particularly in autoimmunity, has helped me begin preparing for my future career as an independent researcher. As a Ph.D. candidate with Dr. Saleh Naser at the University of Central Florida, I investigated both genetic predisposition in the form of single nucleotide polymorphisms (SNPs) along with the presence of an environmental trigger of a bacterial infection in autoimmune patients. More specifically, I examined SNPs found in the T-cell negative regulation genes *protein tyrosine phosphatase non-receptor type 2* and *type 22* (*PTPN2/PTPN22*) along with the bacterial infection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Crohn's disease (CD) and in rheumatoid arthritis (RA) patients. My findings from this study indicated that not only do CD and RA pathogenesis have the same significant SNPs involved in *PTPN2/PTPN22*, but also share the same bacterial infection of MAP. Furthermore, I found that patients with these same significant SNPs had not only higher overall T cell proliferation and a pro-inflammatory phenotype, but also had more of a risk of a MAP bacterial infection. During my time as a Ph.D. candidate, I also developed a new multiplex polymerase chain reaction (PCR) and multi-color fluorescent *in situ* hybridization (*m*-FISH) visualization assays in order to detect multiple pathogens involved in inflammatory bowel diseases. These techniques helped contribute more prove to the fact that the MAP infection is found more readily in CD than other inflammatory bowel diseases, thus being a likely cause of disease. Lastly, as the senior in Dr. Saleh Naser's laboratory, I was the active lab manager and one of the lead workers for a clinical trial from a collaboration with Red Hill Biopharma for the RHB-104 treatment for CD patients. Overall, my previous research has resulted in eight publications (1 first author review, 3 primary first author research papers, 2 co-author research paper, 1 conference paper, and 1 dissertation).

Currently, I am working with Dr. Todd Brusko at the University of Florida, where I am continuing my studies of *PTPN22* and beginning my investigation in other susceptibility genes such as *signal regulatory protein gamma* (*SIRPG*) and c*luster of differentiation* 47 (*CD47*) in type 1 diabetes (T1D). Using the clustered regularly interspaced short palindromic repeats with Cas9 (CRISPR-Cas9) system, we have developed models in $CD8^+$ T cells and natural killer (NK) cells in order to examine the roles of PTPN22, SIRPγ and CD47 in pancreatic beta cell destruction in T1D. Along with this, we have been examining the genotype:phenotype relationship of T cells and NK cells in patients samples with SNPs found in *SIRPG* that are associated with high risk for T1D development.

B. POSITIONS AND HONORS

Positions and Employment

2013 – 2014 M.S. Student, University of Central Florida, Orlando, FL
2013 – 2014 Volunteer Lab Assistant, University of Central Florida, Orlando, FL
2013 – 2016 Teaching Assistant, University of Central Florida, Orlando, FL
2015 – 2018 Ph.D. Student/Candidate, University of Central Florida, Orlando, FL
2019 – Postdoctoral Associate, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

2015 – 2018 Member, American Society of Microbiology
2018 – Member, American Association of Immunologists
2019 – Review Editor, Frontiers in Clinical Microbiology
2019 – Review Editor, COVID-19 Taskforce for Frontiers Journal

Honors

2007 – 2013 Bright Future Scholarship, University of Central Florida, Orlando, FL
2015 – 2017 Burnett School of Biomedical Sciences' Rudy J. Wodzinski Scholarship, University of Central Florida, Orlando, FL
2016 Second Place Speaker Award, Florida Branch American Society for Microbiology Meeting
2018 College of Graduate Studies Presentation Fellowship, University of Central Florida, Orlando, FL
2018 Participants Choice Award, 15th Annual Graduate Research Forum, University of Central Florida, Orlando, FL
2018 Third Place Poster Award, Florida Branch American Society for Microbiology Meeting, Orlando, FL
2020 Outstanding EPIG Project, University of Florida Pathology Department, Gainesville, FL

C. Contributions to Science

***PTPN2/22* and MAP in Rheumatoid Arthritis and Crohn's Disease**

The research done below involves the study of the effects of autoimmune-associated SNPs involved in the T-cell negative regulator genes *PTPN2* and *PTPN22*. Along with these genetic studies, MAP infection was also examined in order to prove the hypothesis that SNPs in immunoregulatory genes along with an environmental trigger can cause autoimmune disorders such as RA and CD. Overall, what was found is that both RA and CD patients not only had higher ratio of risk-variant SNP alleles associated with *PTPN2/22*, but this correlated with higher amounts of MAP infection compared to healthy controls. What was also discovered is that when both risk-variant SNPs and MAP infection were found in the same patient, T-cell proliferation and pro-inflammatory cytokine production was increased, while *PTPN2/22* gene expression was decreased. This phenomenon can potentially lead to an autoimmune response due to the high amounts of immune cell activity and inflammation.

1. **Sharp RC**, Abdulrahim M, Naser ES, Naser SA. Genetic variations of *PTPN2* and *PTPN22*: role in the pathogenesis of type 1 diabetes and Crohn's disease. *Front Cell Infect Microbiol*. 2015;5:95. doi: 10.3389/fcimb.2015.00095. eCollection 2015. Review. PubMed PMID: 26734582; PubMed Central PMCID: PMC4689782

2. **Sharp RC**, Beg SA, Naser SA. Polymorphisms in *Protein Tyrosine Phosphatase Non-receptor type 2* and *22* (*PTPN2/22*) are linked to hyper-proliferative T-cells and susceptibility to *Mycobacteria* in rheumatoid arthritis. *Front Cell Infect Microbiol*. 2018;8:11. doi: 10.3389/fcimb.2018.00011. eCollection 2018. PubMed PMID: 29423382; PubMed Central PMCID: PMC5788942

3. **Sharp RC**, Beg SA, Naser SA. Role of *PTPN2/22* polymorphisms in pathophysiology of Crohn's disease. *World J Gastroenterol*. 2018 Feb 14;24(6):657-670. doi: 10.3748/wjg.v24.i6.657. PubMed PMID: 29456405; PubMed Central PMCID: PMC5807669

4. **Sharp RC**, Beg SA, Naser SA. Pathophysiology of rheumatoid arthritis is associated with polymorphisms in *Protein Tyrosine Phosphatase Non-receptor type 2* and *22* (*PTPN2/22*) and susceptibility to *Mycobacteria*. *J Immunol*. 2018 May 200;166.12

5. **Sharp RC**. Role of single nucleotide polymorphisms (SNPs) in *PTPN2/22* and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in rheumatoid arthritis and Crohn's disease. *Electronic Theses and Dissertations*. 2018 Dec 6225.

Effects of anti-TNFα on Tuberculosis Infection in Crohn's Disease Patients

This article is a meta-analysis that looks into *Mycobacterium tuberculosis* (TB) and other *Mycobacterium* (MAP for example) infections when CD patients are on anti-TNFα medications. The data we found in this study was that out of twenty-three studies examined, TNFα inhibitors were associated with higher *Mycobacterium*

infection. This brings into question if these *Mycobacterium* infections (specifically MAP infection) could cause and/or exacerbate symptoms of CD patients.

1. Cao BL, Qasem A, **Sharp RC**, Abdelli LS, Naser SA. Systematic review and meta-analysis on the association of tuberculosis in Crohn's disease patients treated with tumor necrosis factor-α inhibitors (anti-TNFα). *World J Gastroenterol*. 2018 Jul 7;24(25):2764-2775. doi: 10.3748/wjg.v24.i25.2764. PubMed PMID:29991880; PubMed Central PMCID: PMC6034143.

Development of a Multiplex PCR and Multi-Color Fluorescent *in situ* Hybridization (*m*-FISH) for Detection of Multiple Pathogens Involved in Inflammatory Bowel Disease

In order to develop an efficient method in detecting multiple pathogens involved in IBD, we developed a multiplex PCR using multiple primers and an *m*-FISH protocol that utilized the same multiplex primers. The pathogens we examined were: *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Klebsiella pneumoniae*, and adherent-invasive *Escherichia coli* strain LF82. What was found in this study is that by both multiplex PCR and *m*-FISH, MAP was found only in the CD patient tissue samples than the ulcerative colitis (UC) patient tissue samples. This study provides more evidence that MAP is associated with CD and not UC.

1. **Sharp RC**, Naser ES, Alcedo KP, Qasem A, Abdelli LS, Naser SA. Development of multiplex PCR and multi-color fluorescent *in situ* hybridization (m-FISH) coupled protocol for detection and imaging of multi-pathogens involved in inflammatory bowel disease. *Gut Pathog*. 2018; 10:51. doi: 10.1186/s13099-018-0278-1. PubMed PMID: 30534203; PubMed Central PMCID: PMC6280354.

***TNF Receptor Superfamily 1B* (*TNFRSF1B*) and MAP in Rheumatoid Arthritis**

With the discovery of SNPs found in *PTPN2/22* and MAP infection together being associated with RA pathogenesis, we began searching for other genes involved in immunoregulation that could be also linked to MAP infection and RA development. Previous work done in our lab (Naser, *et al.*, Can J Physiol Pharmacol., 2018) examined the effect of lower blood active osteocalcin levels (a bone biomarker for osteoporosis development) in CD patients and in patients with MAP infections. It was established that lower blood active osteocalcin levels were found in patients with MAP infection more so than without MAP infection, showing evidence that MAP infection in CD patients could cause osteoporosis complications. In this article, we examine both concepts of SNPs in TNF receptor superfamily 1B (*TNFRSF1B*), a major TNF receptor, and MAP infection causing lower amounts of active osteocalcin in RA patients. We established the roles of SNP *TNFRSF1B:rs3397* and MAP infection in causing a dysregulation of active osteocalcin due to lower expression of *TNFRSF1B* leading to higher MAP infection in RA patients.

1. Naser A, Odeh AK, **Sharp RC**, Qasem A, Beg SA, Naser SA. Polymorphisms in *TNF Receptor Superfamily 1B* (*TNFRSF1B:rs3397*) are Linked to Mycobacterium avium paratuberculosis Infection and Osteoporosis in Rheumatoid Arthritis. *Microorganisms*, 2019 Dec 4; 7(12). doi: 10.3390/microorganisms7120646. PubMed PMID: 31817071; PubMed Central PMCID: PMC6955732.

***SIRPG/CD47* Regulation of T cells Involvement in T1D**

T1D risk allele SNPs rs6043409 (A>G; Val>Ala) and rs2281808 (T>C; intron) found in *SIRPG* has been associated with early T1D development. However, there is very little research on SIRPG expression, activation, and structure analysis of the protein and how SNPs in *SIRPG* alter these factors. Along with this, CD47, SIRPG ligand, has been examined thoroughly in cancer pathogenesis, but not in autoimmune diseases such as T1D. Thus, this project focuses on how SIRPG/CD47 is involved in T1D and what the T1D risk allele SNPs associated with *SIRPG* do towards expression/activation of SIRPG on T cells and NK cells.

1. Shapiro MR, Thirawatananond P, Peters L, **Sharp RC**, Ogundare S, Posgai A, Perry DJ, Brusko TM. De-coding genetic risk variants in type 1 diabetes. *Immunol Cell Biol*. 2021 Jan 22; doi: 10.1111/imcb.12438. PubMed PMID: 33483996.

Complete List of Published Work in My Bibliography: https://www.ncbi.nlm.nih.gov/myncbi/16Q3vziljwJstb/bibliography/public/

D. Additional Information: Research Support

Ongoing Research Support

HIRN Emerging Leaders in T1D Award Sharp (PI) 6/1/2021 – 6/1/2022 Human Islet Research Network (HIRN)

The SIRP:CD47 Signaling Pathway in Pancreatic β-Cell Survival

The overall goal of the proposed project is to characterize the functional consequences of signal regulatory protein (SIRP)/CD47 pathway signaling in human immune cells and islet β-cells and to determine the clinical significance of two T1D risk-associated single nucleotide polymorphisms (SNPs) tagged to SIRPG (rs2281808 and rs6043409). We will characterize SIRPα, SIRPγ, and CD47 expression levels on peripheral blood mononuclear cells (PBMCs) and paraffin embedded human pancreatic tissues from individuals with T1D and rare at-risk subjects, who are positive for islet autoantibodies (AAb) but do not have diabetes, as compared to no-diabetes controls. We will then explore the functional impact of SIRP/CD47 signaling on in vitro β-cell killing. To do this, we will generate SIRPγ and CD47 knock out, overexpressing, and SNP-edited CD8+ T cell avatars (expressing an IGRP-reactive T cell receptor), NK cells, and a human β-cell line. Our goal, is to better understand the mechanisms involved in immunoregulation and β-cell survival, and how these are breached in T1D pathogenesis. The SIRP/CD47 pathway represents a new area for investigation alongside other established T1D-associated immunoregulatory pathways (e.g., CD28/CTLA-4, CD226/TIGIT).

Completed Research Support

2908EPIG Sharp (PI) 8/29/2019 – 5/31/2020 University of Florida Pathology Department

Modeling of the SIRPγ and CD47 Pathway in the Pathogenesis of Type 1 Diabetes

This grant focuses on employing our novel CRISPR/Cas9 genome editing capabilities to create several human cell lines (including T-cells, NK cells and β-cells), along with primary human CD8+ T-cells and NK cells, expressing risk and protective variants of SIRPγ and CD47, resulting in isogenic cellular systems to model immune/β-cell interactions *in vitro*. We will create knockout (KO) models of SIRPG and CD47 along with knock in (KI) protective and risk variants of *SIRPG* on an isogenic background to elucidate the mechanistic basis for the risk variants' contribution(s) toward T1D pathogenesis.