GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

MEDICAL COLLEGE OF WISCONSIN

PROGRAM FOR THE FINAL PUBLIC EXAMINATION
FOR THE DOCTOR OF PHILOSOPHY
OF

MONICA ARIEL THOMAS

WEDNESDAY, MARCH 21, 2018

11:00 AM

MICROBIOLOGY AND IMMUNOLOGY CONFERENCE ROOM

BASIC SCIENCE BUILDING 276

COMMITTEE IN CHARGE

BRIAN VOLKMAN, Ph.D.

BLAKE HILL, Ph.D.

MICHAEL DWINELL, Ph.D.

MATTHEW SCAGLIONE, Ph.D.

ALEXANDER ARNOLD, Ph.D.

DISSERTATION

Structural and Functional Analyses on the Human Chemokine CCL28 with Implications for its Role in Homeostasis and Disease

ABSTRACT

Chemokines, or *chemo*tactic cyto*kines*, are a family of small, secreted proteins that orchestrate cell migration through interactions with cell-surface G protein-coupled receptors (GPCRs). Though chemokines and chemokine receptors (CKRs) are most well studied in the context of the immune system, the chemokine network is known to play important roles in a variety of homeostatic (e.g., embryonic development, wound healing) and pathologic (e.g., cancer, asthma, HIV) processes. With ~50 known chemokine ligands and only ~20 known CKRs, there exists a high degree of promiscuity in the chemokine system (i.e., one chemokine can interact with one or multiple CKRs and vice versa). Interestingly, specificity is maintained and every chemokine does not interact with every CKR. Decades of structural studies have revealed important motifs mediating diverse aspects of chemokine function, and yet the molecular determinants of chemokine-CKR selectivity, specificity, and promiscuity are yet to be fully elucidated. As every chemokine and chemokine-CKR pair is involved in a unique subset of physiologic and pathologic processes, a deeper structural understanding of each specific player in the chemokine network will aid in the development of specific, targeted therapies for a large number of human diseases.

In this dissertation work, I sought to understand the structural basis for the homeostatic and pathologic functions of the human chemokine CCL28, a protein with dual roles in homeostatic mucosal immunity. Also known as mucosae-associated epithelial chemokine (MEC), CCL28 is constitutively expressed in diverse mucosal tissues and acts as both a chemoattractant and antimicrobial agent at mucosal surfaces. CCL28 interacts with two CKRs, CCR10 and CCR3, and dysregulation of the CCL28-CCR10-CCR3 axis has been implicated in a number of pathologic conditions, most notably post-viral asthma. As such, CCL28, CCR10, and CCR3 have been identified as attractive targets for the development of novel asthma therapeutics. However, prior to beginning this dissertation work, structural knowledge of CCL28 and CCL28-CKR interactions was sparse, complicating efforts to design CCL28-CCR10-CCR3 axis-specific inhibitors.

To this end, we began our efforts to structurally understand homeostatic and pathologic functions of CCL28 by establishing a role for the tertiary fold of CCL28 in the pathogenesis of post-viral asthma (**Chapter 3**). In collaboration with Dr. Mitchell Grayson's Laboratory, we identified that human recombinant CCL28 is necessary and sufficient for the development of an asthma phenotype in a mouse model of post-viral asthma. Moreover, we found that the tertiary fold of CCL28 is absolutely necessary for its *in vitro* chemotactic activity and its ability to drive asthma pathology *in vivo*. Given the significance of the fold of CCL28 for its pathologic activity, we used nuclear magnetic resonance spectroscopy (NMR) to experimentally determine the solution structure of human recombinant CCL28 (**Chapter 4**). Intriguingly, the structure revealed unique features not seen in other chemokines, including an extended C-terminal tail and an additional disulfide bond. We investigated the structural and functional significance of these unique structural features and identified a novel structural role for the additional disulfide bond in CCL28. Moreover, in collaboration with Dr. Anna Huppler's laboratory, we found that CCL28 experiences a pH- and salt-dependent structural lability that may be important for its antifungal activity.

Chemokines interact with their cognate CKRs in a two-step, two-site manner, whereby CKR activation occurs upon insertion of the flexible N-terminal domain of the chemokine into the orthosteric pocket of the CKR (i.e., site 2). As such, the composition and length of chemokine N-termini are known to have a major role in CKR activation. Interestingly, the length of CCL28's N-

terminal domain remains somewhat ambiguous, with different sources utilizing different N-terminal versions of CCL28. To attempt to disambiguate the N-terminal length of CCL28, we generated a series of N-terminal truncation variants and tested them for their ability to activate both CCR10 and CCR3 (**Chapter 5**). Surprisingly, we found that CCR10 and CCR3 displayed unique activation profiles for each of the N-terminal variants tested. Further, we utilized computational modeling techniques and molecular dynamics simulations to identify key residues that may be mediating the observed CCL28-mediated activation differences between CCR10 and CCR3. Importantly, we show this method can be modified to analyze other chemokine-CKR site 2 interactions *in silico* and ultimately further our molecular understanding of chemokine-CKR selectivity, specificity, and promiscuity.

Collectively, this dissertation work provides evidence that the unique structural elements of CCL28 play important roles in its homeostatic and pathologic functions, and provides a basis for future drug discovery efforts targeting the CCL28-CCR10-CCR3 axis. More broadly, this dissertation work suggests that subtle nuances of chemokine structure influence the multiple functional roles chemokines play in the human body. Importantly, this work also establishes a computational approach for the investigation of chemokine-CKR interactions, a useful method for the generation of testable hypotheses regarding the molecular basis of chemokine promiscuity and specificity.

GRADUATE STUDIES

Advanced Protein Chemistry
Biomolecular NMR: Structure and Molecular Recognition
Contemporary X-ray Crystallography
Special Topics in Biochemistry: Oxidative Stress Signaling in Cancer
Ethics & Integrity in Science
Research Ethics Discussion Series
Readings and Research
Seminar

CURRICULUM VITAE

Monica A. Thomas

EDUCATION

Ph.D.	**Medical College of Wisconsin** Milwaukee, WI *Biochemistry*	**2018** (Anticipated)
M.D.	**Medical College of Wisconsin** Milwaukee, WI *Medical Scientist Training Program*	**2020** (Anticipated)
B.S.	**University of Utah** Salt Lake City, UT *Chemistry, Cum laude*	**2012**

RESEARCH EXPERIENCE

Graduate Research

Dissertation: Structural and Functional Analyses on CCL28 with Implications for its Role in Homeostasis and Disease

Advisor: Dr. Brian Volkman
July 2014 – present

My dissertation work focuses primarily on the structural and functional characterization of CCL28, a chemokine known to play critical roles in homeostatic mucosal immunity and also known to be intimately involved in the pathogenesis of asthma. Utilizing nuclear magnetic resonance spectroscopy and other biophysical methods, I investigate how the structure and dynamic properties of CCL28 influence its ability to not only bind its cognate G-protein coupled receptors and ultimately drive asthma, but also how these features influence the ability of CCL28 to act as a broad-spectrum antimicrobial agent. By understanding the structural intricacies of CCL28, and by understanding CCL28-GPCR interactions on a molecular level, we can work toward designing specific, targeted therapeutics that can one day be used in the clinic to treat disease.

Undergraduate Research

Using Protein Engineering to Design a Protein Capsid-based Small Molecule Delivery System

Dr. Kenneth Woycechowsky
University of Utah
June 2011 - May 2012

Engineering a Flow Chamber for the Microscopic Analysis of Real-time Enveloped Virus Budding

Dr. Saveez Saffarian
University of Utah
September 2010 – September 2011

PUBLICATIONS

1. **Thomas MA**, He J, Peterson FC, Huppler AR, Volkman BF. The Solution Structure of CCL28 Reveals Structural Lability that May Enhance Antifungal Efficacy. *In preparation.*

2. Fox JC, **Thomas MA**, Larsen O, Nakayama T, Dishman AF, Yoshie O, Rosenkilde MM, Volkman BF. Structure-Function Guided Modeling of Chemokine-GPCR Specificity for the Chemokine XCL1 and its Receptor XCR1. *In Preparation.*

3. **Thomas MA***, Kleist AB*, Volkman BF. Decoding the Chemotactic Signal. **Submitted.**

4. **Thomas MA***, Buelow BJ*, Nevins AM, Jones SE, Peterson FC, Gundry RL, Grayson MH, Volkman BF. Structure-Function Analysis of CCL28 in the Development of Post-viral Asthma. *J Biol Chem.* 2015;290(7):4528-4536. doi:101074/jbc.M114.627786.

** Authors contributed equally to work*

ABSTRACTS AND PRESENTATIONS

1. **Thomas MA,** Wedemeyer MJ, Volkman BF. Understanding Chemokine Promiscuity at G protein-coupled receptors. *Keystone Symposia. GPCR Structure and Function: Taking GPCR Drug Development to the Next Level.* 2018.
2. **Thomas MA,** Peterson FC, Volkman BF. A Structural Approach to Understanding Promiscuity and Specificity in the Chemokine Network. *Graduate School Research Poster Session, Medical College of Wisconsin.* 2017.
3. **Thomas MA,** Peterson FC, Volkman BF. A Structural Approach to Detangling the Chemokine Network. *Graduate Student Symposium, Medical College of Wisconsin.* 2017.
4. **Thomas MA**, Peterson FC, Volkman BF. Investigating the Structure and Dynamics of the Chemokine CCL28. *Chicago Area NMR Discussion Group.* 2016.
5. **Thomas MA,** Peterson FC, Huppler AR, Grayson MH, Volkman BF. A structural basis for the dual immune function of the human chemokine CCL28. *National MD/PhD Student Conference.* 2016.
6. **Thomas MA,** Peterson FC, Huppler AR, Grayson MH, Volkman BF. Structural Investigations on the Pathologic Function of CCL28. *Gordon Research Conference, Chemotactic Cytokines.* 2016.
7. **Thomas MA,** Peterson FC, Huppler AR, Grayson MH, Volkman BF. Structural Investigations on the Pathologic Function of CCL28. *Gordon Research Seminar, Chemotactic Cytokines.* **Invited talk.** 2016.

8. **Thomas MA**, Peterson FC, Volkman BF. Using Structural and Dynamic NMR Analyses to Dissect the Pathologic Function of a Novel Chemokine. *Biophys J.* 2016;110(3):221a. doi:10.1016/j.bpj.2015.11.1223.
9. **Thomas MA**, Buelow BJ, Peterson FC, Grayson MH, and BF Volkman. Structure of the Human Chemokine CCL28 Provides Insight into its Role in Post-Viral Asthma. *Medical College of Wisconsin Annual Graduate School Poster Session*. 2015.
10. **Thomas MA**, Lilavivat S, Chen H, Thomas G, and K Woycechowsky. Engineered Hexahistadine-Tags as Molecular Switches for Reversible Protein Self-Assembly. *National Conferences on Undergraduate Research*. 2012.

WORKSHOPS AND MEETINGS

2018	Keystone Symposia, GPCR Structure and Function: Taking GPCR Drug Development to the Next Level, Santa Fe, NM *Poster Presentation*
2016	Rosetta Workshop, Vanderbilt University, Nashville, TN *Protein modeling and docking workshop*
2016	Chicago Area NMR Discussion Group (CANMRDG), Milwaukee, WI *Poster presentation*
2016	National MD/PhD Student Conference, Keystone, CO *Poster presentation*
2016	Gordon Research Seminar & Conference, Chemotactic Cytokines, Girona, Spain *Invited talk and poster presentation*
2016	Visiting scholar, University of California, San Francisco *Lab of Dr. Brian Shoichet, March 28 – April 15* *In silico docking using DOCK*
2016	Biophysical Society 60th Annual Meeting, Los Angeles, CA *Poster presentation*
2015	Chicago Area NMR Discussion Group (CANMRDG), Chicago, IL
2015	UIUC/Pittsburgh Supercomputing Center, Pittsburgh, PA *Computational biophysics workshop*
2014	National Magnetic Resonance Facility at Madison, Madison, WI *Advanced structure refinement workshop*

ACADEMIC AND PROFESSIONAL HONORS

2017	NIH F30 Predoctoral Fellowship Award

2010-2012	Dean's list, University of Utah
2011	Myriad Woman in Science Scholarship, University of Utah
2011	Outstanding Sophomore in the Department of Physics, University of Utah
2011	Mack Thomas Rozelle Scholarship, University of Utah
2009-2010	Dean's list, Southern Utah University

PROFESSIONAL EXPERIENCE

2017-2018	GSA Symposium Committee Medical College of Wisconsin
2016-present	Association for Biomolecular and Computational Simulation (ABACUS) Medical College of Wisconsin *Co-President, Co-Founder*
2014-2015	USMLE Step 1 Tutor Medical College of Wisconsin
2012	Undergraduate Teaching Assistant, Biochemistry University of Utah
2011	Undergraduate Teaching Assistant, Organic Chemistry University of Utah

VOLUNTEER EXPERIENCE

2016-2017	Science Fair Mentor, Wauwatosa STEM Elementary School
2015	Science Fair Judge, Wauwatosa STEM Elementary School
2012-2015	Saturday Clinic for the Uninsured
2012-2014	Partnership for Urban Medical Education and Advancement

PROFESSIONAL SOCIETY MEMBERSHIPS

2016-present	Biophysical Society
2014-present	Federation of Clinical Immunology Societies
2014-present	American Chemical Society