MCW
GRADUATE SCHOOL

Doctoral Dissertation Defense Announcement

"When commensals become pathogens: Using an *Enterococcus* dissemination model to understand mechanisms of opportunistic infection"

Kevin C Jennings

Candidate for Doctor of Philosophy

Microbiology and Immunology

School of Graduate Studies

Medical College of Wisconsin

Committee in Charge:

Nita Salzman, MD, PhD (Mentor)

Christopher Kristich, PhD

Robert Lochhead, PhD

Amy Hudson, PhD

Brian Smith, PhD

Date: Thursday, May 23, 2024

Time: 11:00 AM (CST)

Defense Location: Bolger Auditorium

Zoom: https://mcw-edu.zoom.us/j/94931677583?pwd=ekUrenZDdlZLMjEwZUdyTXRLaTZhQT09

Meeting ID: 949 3167 7583

Passcode: KFg3PY6t

Graduate Studies:

Biochemistry of the Cell (INBS 16202)
Techniques in Molecular and Cellular Biology (INBS 16242)
Molecular and Cellular Biology (INBS 16244)
Mechanisms of Cellular Signaling (INBS 16250)
Classical and Molecular Genetics (INBS 16252)
Ethics and Integrity in Science (BIOE 10222)
Cellular and Molecular Immunology (MIIM 25234)
Cellular Microbiology (MIIM 25236)
Research Ethics Discussion Series (BIOE 10444)
Mucosal Immunology (MIIM 25259)
Seminar (MIIM 25300)
Immunological Tolerance (MIIM 25265)
Advanced Immunology (MIIM 25264)
Doctoral Dissertation (MIIM 25003)

Dissertation

"When commensals become pathogens: Using an *Enterococcus* dissemination model to understand mechanisms of opportunistic infection"

Enterococci are common commensal bacteria that colonize the gastrointestinal tracts of most mammals, including humans. Importantly, these bacteria are one of the leading causes of nosocomial infections. This study examined the role of colonic macrophages in facilitating *Enterococcus faecalis* infections in mice. We determined that depletion of colonic phagocytes resulted in the reduction of *E. faecalis* dissemination to the gut-draining mesenteric lymph nodes. Furthermore, we established that trafficking of monocyte-derived CX3CR1-expressing macrophages contributed to *E. faecalis* dissemination in a manner that was not reliant on CCR7, the conventional receptor involved in lymphatic migration. Finally, we showed that *E. faecalis* mutants with impaired intracellular survival exhibited reduced dissemination, suggesting that *E. faecalis* can exploit host immune cell migration to disseminate systemically and cause disease. Our findings indicate that modulation of macrophage trafficking in the context of antibiotic therapy could serve as a novel approach for preventing or treating opportunistic infections by disseminating enteric pathobionts like *E. faecalis*.

Kevin Jennings
Curriculum Vitae
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Education:

Southeastern Community College
Keokuk, Iowa
2010-2012

Coe College
Cedar Rapids, Iowa
2012-2014

Carroll University
Waukesha, Wisconsin
BS Biochemistry
2014-2016

Medical College of Wisconsin
Wauwatosa, Wisconsin
Microbiology & Immunology
2017-present

Research Experience

Undergraduate Research Projects

Assessing salt tolerance in *Triops newberryi*
Carroll University – Summer 2016
Tested growth, reproduction, survival, and activity of the small crustacean *T. newberryi* in various salt concentrations

Research Grants Awarded/Pending/Submitted

The role of intestinal macrophages in commensal *Enterococcus faecalis* containment and dissemination (NIH F31, August 2020, Impact Score: 20, Percentile: 14.0, Not Funded)

The role of intestinal macrophages in commensal *Enterococcus faecalis* containment and dissemination (NIH F31 resubmission, November 2020, Not Funded)

The role of intestinal macrophages in commensal Enterococcus faecalis containment and dissemination (American Heart Association Predoctoral Fellowship, March 2021, Not Funded)

Understanding the role of mononuclear phagocytes in *Enterococcus faecalis* dissemination

(Center for Immunology Growth and Research in Immunology Training (GRIT) Award, February 21, 2022, **Funded**)

Effects of microbial composition on *E. faecalis* dissemination (Center for Microbiome Research Pilot Award, October 2022, **Funded**)

Harnessing spatial transcriptomics to study localized immune responses to disseminating *Enterococcus faecalis* (10x Genomics-Mellows Center Visium CytAssist Grant, June 2023, Not Funded)

Abstracts and Presentations

Meetings and Conferences Attended

Molecular Genetics of Bacteria and Phages Meeting (Madison, Wisconsin August 5-9, 2019)
Poster Presentation

> Growth Phase and Osmotic Stress Drive Subcellular Localization of *Enterococcus faecalis* OGIRF_11271, a Conserved Protein Involved in Cell Envelope Integrity

Center for Immunology Scientific Retreat (Milwaukee, Wisconsin March 12, 2020)
Poster Presentation

> Mononuclear phagocytes respond to ceftriaxone-induced *Enterococcus faecalis* dissemination

Center for Immunology Scientific Retreat (Wauwatosa, Wisconsin June 9, 2022)
Oral Presentation

> CCR2-dependent migration of CX3CR1-hi antigen presenting cells facilitates *Enterococcus faecalis* dissemination

International Cytokine and Interferon Society Meeting (Big Island, Hawaii September 20-23, 2022)
Poster Presentation

> CCR2-dependent migration of CX3CR1-hi antigen presenting cells facilitates *Enterococcus faecalis* dissemination

Midwest Microbial Pathogenesis Conference (Madison, Wisconsin September 30-October 1)
Poster Presentation

> CCR2-dependent migration of CX3CR1-hi antigen presenting cells facilitates *Enterococcus faecalis* dissemination

Center for Immunology Scientific Retreat (Wauwatosa, Wisconsin June 7, 2023)
Poster Presentation

> Colonic CX3CR1-hi macrophages facilitate *Enterococcus* dissemination through CCR2-dependent migration to the colon-draining mesenteric lymph node

Institution or Department Presentations

Research in Progress (Medical College of Wisconsin, April 19, 2019)
Oral Presentation

Determining cellular localization of OG1RF_11271 and OG1RF_11272 in *Enterococcus faecalis*

Research Day (Medical College of Wisconsin, September 19, 2019)
Poster Presentation

Growth Phase and Osmotic Stress Drive Subcellular Localization of *Enterococcus faecalis* OGIRF_11271, a Conserved Protein Involved in Cell Envelope Integrity

MCW Graduate School Annual Poster Session (Medical College of Wisconsin, November 7, 2019)
Poster Presentation

Growth Phase and Osmotic Stress Drive Subcellular Localization of *Enterococcus faecalis* OGIRF_11271, a Conserved Protein Involved in Cell Envelope Integrity

Research in Progress (Medical College of Wisconsin, February 21, 2020)
Oral Presentation

The role of mononuclear phagocytes during *Enterococcus faecalis* dissemination

MCW Graduate School Annual Poster Session (Medical College of Wisconsin, February 5, 2021)
Poster Presentation

Ceftriaxone-mediated macrophage depletion – A novel mechanism for *Enterococcus* dissemination?

Graduate Student Association Symposium (Medical College of Wisconsin, March 18, 2021)
Oral Presentation

Mononuclear phagocytes respond to ceftriaxone-induced *Enterococcus faecalis* dissemination

Research in Progress (Medical College of Wisconsin, March 26, 2021)
Oral Presentation

The role of intestinal macrophages in commensal *Enterococcus faecalis* containment and dissemination

Immunology Research Roundtable (Medical College of Wisconsin, November 5, 2021)
Oral Presentation

Understanding the role of intestinal mononuclear phagocytes in *Enterococcus faecalis* dissemination

Research in Progress (Medical College of Wisconsin, February 18, 2022)

Oral Presentation

CCR2-dependent migration of CX3CR1-hi mononuclear phagocytes facilitates *Enterococcus faecalis* dissemination

Research Day (Medical College of Wisconsin, October 27, 2022)
Poster Presentation

CCR2-dependent migration of CX3CR1-hi mononuclear phagocytes facilitates *Enterococcus faecalis* dissemination

Immunology Research Roundtable (Medical College of Wisconsin, February 10, 2023)
Oral Presentation

Transcriptomic heterogeneity of mesenteric lymph node antigen presenting cells

Research in Progress (Medical College of Wisconsin, February 24, 2023)
Oral Presentation

CCR2-dependent migration of CX3CR1-hi mononuclear phagocytes facilitates *Enterococcus faecalis* dissemination from the intestinal tract

Bibliography

Papers

Jennings, Kevin, Johnson, Kaitlin, Hayward, Michael, Kristich, Christopher, Salzman, Nita. "CCR2-dependent migration of CX3CR1-hi antigen presenting cells facilitates *Enterococcus faecalis* dissemination from the intestinal tract". *Mucosal Immunology* – Submitted 3/17/23, Rejected

Jennings, Kevin, Johnson, Kaitlin, Hayward, Michael, Kristich, Christopher, Salzman, Nita. "CCR2-dependent CX3CR1+ colonic macrophages promote *Enterococcus faecalis* dissemination". *Infection and Immunity* – Submitted 1/5/24, **Accepted, Selected for IAI "Spotlight"**, a feature in the journal that highlights research articles of significant interest from the current issue.

Mentoring

Mentoring activity

First Year IDP Student Coach – Guided a new student in study strategies, test taking skills, and grasping course material (Leah Martinez, 2019-2020, ~weekly/monthly)

Rotation Student Mentor – Worked to optimize macrophage culturing and infections with Enterococcus faecalis. Performed various survival assays and incorporated fluorescent imaging strategies (Matt Mortenson, Nov-Dec 2019, daily)

Clinical & Translational Science Institute 500 Stars Program, Student Mentor – Established foundational principles of laboratory research. Developed a small

project that focused on changes in intestinal cytokines/receptors during ceftriaxone treatment. Training emphasized critical and common laboratory techniques, including RNA isolation, cDNA synthesis, and qPCR. (Miranda Lyles, June-September 2021, daily)

Rotation Student Mentor – Tissue dissection, qPCR, flow cytometry and standard microbiological techniques used to establish mechanisms of mononuclear phagocyte migration and the corresponding impact on *E. faecalis* dissemination. (Ashley Bauer, November-December 2021)

Students Understanding Principles of Research Education through Medicine, Engineering, and Science (SUPREMES) High School Student Mentor – Tissue dissection, bacterial cell culture, pouring plates, standard pipette techniques, statistical analysis, basics in immunology "mini-course" online teaching. (Khadijah Dhoondia, January-May 2022)

Clinical & Translational Science Institute 500 Stars Program, Student Mentor – Tissue dissection, cell preparation/staining, flow cytometry. Small project to characterize MLN APC populations via flow cytometry. Assessed the impact of APC depletion on MLN APC abundance and surface marker expression. (Khadijah Dhoondia, July-August 2022)

Rotation Student Mentor - Tissue dissection, bacterial cell culture, pouring plates, standard pipette techniques, flow cytometry, statistical analysis. Continued previous projects involving E. faecalis mutant dissemination and observing intracellular bacteria in APCs via "ImageStream" flow cytometry. (Dilini Kumarasinghe, September-November 2022)

Memberships in Professional Societies

American Chemical Society, member (2016, 2017)
American Association for the Advancement of Science, member (2018-present)
American Heart Association, member (2019-2021)
International Cytokine and Interferon Society, member (2022)
American Society for Microbiology, member (2023-present)

Leadership and Service

Committee service

Enhancing Scholarly Communication Committee, Member (2017-present), Vice President (2018)
Diversity, Equity, and Inclusion in Science Committee, Member (2020-present)

Graduate School Service Activities

Graduate School Interview Day Volunteer (2017, 2018, 2019, 2020, 2021, 2022)
Graduate School Welcome Day Volunteer (2018, 2019, 2020)
Big Sib Volunteer (2020)
Peer Mentor (2020)

Community Service Activities
WSTEM Fair, Student Mentor, helped 4th and 5th grade students design, prepare, and present their science fair projects, taught the purpose of a scientific question, hypothesis, controls, and data analysis (January-April 2020)

Honors and Awards

MCW Travel Award (8/28/2019)

Outstanding Presentation and Poster Award (Research Day, 10/1/2019)

Center for Immunology Travel Award (10/4/2022)

Infection and Immunity "**Spotlight**" Article - a feature in the journal that highlights research articles of significant interest from the current issue (4/19/2024)