Rachael L. Hardison  
PhD Candidate

“*Haemophilus* pathogenesis during otitis media: Influence of nutritional immunity on bacterial persistence and intracellular lifestyles”

October 24, 2018  
James L035  
9:00am
VITA

1990 .............................................. Born – Salem, VA

2012 .................................. BS in Biology, Xavier University

2014 – present ................................. Graduate Research Associate,
The Ohio State University

COMMITTEE MEMBERS

Kevin Mason, PhD, Co-advisor

Sheryl Justice, PhD, Co-advisor

Stephanie Seveau, PhD, Chair

Jennifer Edwards, PhD

John Gunn, PhD
Nontypeable *Haemophilus influenzae* (NTHI) is a gram-negative opportunistic pathogen and major causative agent of otitis media (OM) and other diseases of the upper and lower respiratory tract. As a commensal, NTHI resides in the human nasopharynx, an environment with sufficient levels of nutrients to allow colonization. Permissive risk factors, such as a preceding viral infection, can allow NTHI to migrate up the Eustachian tube and into the sterile middle ear, an environment that is initially depleted of nutrients and prone to a robust and rapid inflammatory response. Nutritional immunity occurs when the host tightly sequesters essential nutrients, such as heme-iron, in sterile areas like the middle ear. As NTHI is auxotrophic for heme-iron, the ability for NTHI to sense, respond and adapt to shifts in heme-iron availability is important for NTHI survival during the transition from a commensal to an opportunistic pathogen and in the progression of OM. We have previously described that transient heme-iron restriction of NTHI promotes diverse phenotypes, including changes in biofilm formation, increased survival of NTHI and altered disease severity in a preclinical model of OM, and the transition to an intracellular lifestyle for NTHI. Specifically, transient heme-iron restriction promotes intracellular bacterial community (IBC) formation by NTHI in epithelial cells in vitro. However, the host and bacterial responses to nutrient restriction that impact intracellular fate and persistence of NTHI in chronic OM are unknown.

This work demonstrates a role for transient heme-iron restriction in inducing persistence through adaptation to two relatively underexplored NTHI lifestyles: long-term stationary phase persistence, and survival in the host cell as a cytosolic pathogen. In this study, we gained mechanistic insight into NTHI persistence through the use of an in vitro model of long-term stationary phase growth. Transient heme-iron restriction potentiates extended survival of NTHI for weeks in vitro. We hypothesized that transient heme-iron restriction induced genetic
alterations that contribute to persistence of NTHI. By performing whole genome sequencing of NTHI isolates from long-term culture, we identified microevolution of NTHI in response to transient nutrient limitation through mutation of icc in two independent in vitro experiments. Mutation in icc results in decreased 3',5'-cyclic adenosine monophosphate phosphodiesterase activity that is associated with increased competence for NTHI and survival of NTHI within middle ear mucosal tissue in IBCs that persist during chronic OM.

This study provides the first evidence for the role of NTHI adaptation to nutrient limitation in promoting the formation of IBCs in vitro and in vivo in a preclinical model of OM. We hypothesized that transient heme-iron restriction of NTHI resulted in altered uptake into epithelial cells leading to escape or evasion of the endolysosomal pathway. Using colocalization studies and pharmacological inhibition of endocytosis, we demonstrated a role for entry by macropinocytosis and subsequent escape or evasion of the endolysosomal pathway in NTHI IBC formation in epithelial cells. Further, inhibition of macropinocytosis altered the intracellular fate of transiently restricted NTHI for degradation in the endolysosomal pathway. Blocking macropinocytosis reduced the number of IBCs in cultured middle ear epithelial cells, providing evidence for the feasibility of this approach to reduce OM persistence. Collectively, the results from these studies reveal that transient heme-iron restriction induces genetic and phenotypic changes that promote NTHI competence, a transition to persistence in a long-term stationary phase state, and a novel lifestyle as a cytosolic pathogen in IBCs leading to new mechanisms for survival during disease progression. The new data generated herein will direct future studies toward investigation of a role for NTHI IBCs in driving recurrent OM.
Hardison RL, Justice SS and Mason KM. (2018). Taking a Detour: Bacterial trafficking patterns of NTHI in host epithelial cells promote persistence. 2018 Hayes Graduate Research Forum. The Ohio State University. Columbus, OH. Oral Presentation

Hardison RL, Justice SS and Mason KM (2017). Taking a Detour: Bacterial trafficking patterns of NTHI in host epithelial cells promote persistence. 2017 Biomedical Sciences Graduate Program Retreat. The Ohio State University. Columbus, OH. Oral Presentation.


Hardison RL, O’Bryan M, Justice SS, and Mason KM. (2015). Heme-iron starvation enhances stationary-phase persistence of
nontypeable Haemophilus influenzae. 2015 Midwest Microbial Pathogenesis Conference. Indianapolis, IN. *Poster Presentation.*

RECENT PUBLICATIONS


AWARDS AND HONORS

Ohio State University Infectious Disease Institute T32 Fellowship, 2017-2018

Midwest Microbial Pathogenesis Conference Trainee Fellowship, 2017

Nationwide Children’s Hospital Infectious Disease Consortium Research Grant, 2017 and 2014

Outstanding Oral Presentation Award, Nationwide Children’s Hospital Infectious Disease Consortium, 2017

Research Institute Trainee Association Graduate Fellowship, Nationwide Children’s Hospital, 2015-2016

Second Place Poster Award, OSU Center for Microbial Interface Biology Symposium, Columbus, OH, 2016

First Place Poster Award, 2015 Midwest Microbial Pathogenesis Conference, Indianapolis, IN, 2015

Second Place Oral Presentation Award, 18th International Symposium on Recent Advances in Otitis Media, National Harbor, MD, 2015

Career Development Grant, The Ohio State University Council of Graduate Students, 2015

FUTURE PLANS

I will be continuing my research training as a postdoctoral scientist in the Edwards Lab in the Center for Microbial Pathogenesis at The Research Institute at Nationwide Children’s Hospital. My long term career goal is to pursue a career in industry or technology commercialization.