

ABSTRACT

Burkholderia pseudomallei is an encapsulated Gram-negative pathogen which resides and thrives in the soils of tropical and subtropical regions, where it causes the disease melioidosis. Melioidosis is a severe febrile illness which can rapidly lead to fatal septicemia if not properly treated. Accurate diagnosis is often delayed due to the wide variety of signs and symptoms of this disease. Furthermore, the extensive repertoire of antibiotic resistance mechanisms employed by *B. pseudomallei* further impede an appropriate response to this infection. Due to the ubiquitous nature of this bacterium in endemic soils, the ease at which it may be grown, and the lethality of the ensuing disease, *B. pseudomallei* is believed to have a great risk for deliberate misuse with the potential to cause mass casualties. As such, it has been classified a Tier 1 Select Agent by the CDC as well as the USDA. There is great interest in the development of vaccines to prevent *B. pseudomallei* infection, as no FDA-approved vaccine currently exists to protect against melioidosis.

The complement system is an ancient surveillance system which works to maintain homeostasis within the body. As a component of the innate immune response, the complement system plays an important role in pathogen clearance. Out of necessity, many pathogens have evolved complement evasion mechanisms (e.g., secretion of proteases, expression of complement regulator-binding proteins) to survive within the host and successfully cause disease. Importantly, there is great interest in the identification of proteins involved in these immune evasion strategies, as these effectors are recognized as potential therapeutic targets.

Previous studies indicate that evasion of the complement system is critical for successful *B. pseudomallei* infection and persistence within the host. Indeed, we demonstrate here that *B. pseudomallei* resists complement-mediated direct killing in normal human serum as well as binding by complement C3 opsonins using select agent-exempt attenuated strains. The goal of this study was to understand the mechanism by which this complement resistance was occurring. We hypothesized that recruitment of complement negative regulator Factor H to the *B. pseudomallei* surface is responsible for the observed complement resistance. In this study, we demonstrate that *B. pseudomallei* binds functional Factor H, indicating that this interaction may offer an immune evasion strategy for this pathogen. Indeed, when *B. pseudomallei* interaction with Factor H was prevented, we observed a significant growth defect in serum, indicating that Factor H-binding significantly aids in *B. pseudomallei* survival in serum. Comprehensive *in silico* and *in vitro* screening for putative bacterial receptors for Factor H identified several proteins of interest. Recombinant expression of these proteins was evaluated for the ability to confer Factor H-binding to *E. coli*. These studies demonstrated that expression of *B. pseudomallei* Protein 1 corresponds to the ability to bind Factor H. Bioinformatic analysis revealed that Protein 1 is highly conserved across numerous *Burkholderia* pathogens, indicating that a therapeutic targeting this surface-exposed protein may offer cross-protection against other diseases. We conclude this study with an *in silico* prediction of Protein 1 residues that may be surface-exposed. These findings provide valuable insight into a novel immune evasion mechanism used by *Burkholderia pseudomallei* and identify a potential therapeutic target which may offer protection against numerous important *Burkholderia* pathogens.



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DISSERTATION
PRESENTATION

by

Irum Syed

April 28th, 2022

**Identification and
Characterization of a
Burkholderia pseudomallei
Factor H-Binding Protein**

Ph.D. in Biomedical
Sciences

PRESENTED ABSTRACTS

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Ferreira VP, Huntley JF, Wooten RM. Identification of Factor H-Binding Proteins Expressed by *Burkholderia pseudomallei*. Graduate Research Forum. Toledo, OH. Oral presentation. March 25, 2021.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Ferreira VP, Huntley JF, Wooten RM. Factor H-Binding by *Burkholderia pseudomallei* Outer Membrane Proteins. Graduate Research Forum. Toledo, OH. Oral presentation. March 16-18, 2020.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Ferreira VP, Huntley JF, Wooten RM. Identification of Factor H-Binding Protein(s) Expressed by *Burkholderia pseudomallei*. 9th World Melioidosis Congress. Hanoi, Vietnam. Poster presentation. October 16-17, 2019.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Ferreira VP, Huntley JF, Wooten RM. Identifying *Burkholderia* Protein(s) Involved in Factor H-Binding. Midwest Microbial Pathogenesis Conference. Toledo, OH. Poster presentation. September 21, 2019.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Ferreira VP, Huntley JF, Wooten RM. The Identification of a Factor H-Binding Protein in *Burkholderia pseudomallei*. Graduate Research Forum. Toledo, OH. Poster presentation. March 21, 2019.

PRESENTED ABSTRACTS cont.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Wooten RM. The Identification of a Factor H-Binding Protein in *Burkholderia pseudomallei*. Midwest Microbial Pathogenesis Conference. Iowa City, IA. Poster presentation. September 29, 2018.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Wooten RM. The Identification of a Factor H-Binding Protein in *Burkholderia pseudomallei*. Graduate Research Forum. Toledo, OH. Poster presentation. March 15, 2018.

Syed I, Nejedlik LS, Lambert CL, Woodman ME, Mulye M, Wooten RM. The Identification of a Factor H-Binding Protein in *Burkholderia pseudomallei*. ASM: Biothreats. Baltimore, MD. Poster presentation. February 13, 2018.

SUBMITTED/AWARDED GRANTS

Awarded

Kohler International Grant
University of Toledo
Spring/Summer 2019

Submitted

Predoctoral Fellowship
American Heart Association
Fall 2019

FUTURE PLANS

Irum will continue her work on *Burkholderia pseudomallei* in the Wooten lab.

PUBLICATIONS

Syed I, Nejedlik L, Lambert C, Morgan K, Ferreira VP, and Wooten RM. *Burkholderia pseudomallei* Binds Host Complement Factor H as an Immune Evasion Strategy (2022). *Front Cell Infect Microbiol.* (*under review*)

Syed I, Wooten RM. Interactions Between Pathogenic *Burkholderia* and the Complement System: a review of potential immune evasion mechanisms (2021). *Front Cell Infect Microbiol.* doi: 10.3389/fcimb.2021.701362

Syed, I. “*Accurately identifying Melioidosis can be problematic*”. Toledo Blade. January 6, 2020.

LEADERSHIP ROLES

Advanced Leadership Academy Graduate
Toledo, OH. Spring 2020.

Interactive Career Panel Moderator
Midwest Microbial Pathogenesis Conference
Toledo, OH. September 21, 2019.

Bacteriology Laboratory Teaching Assistant
Toledo, OH. Fall 2018.