ABSTRACT

Borrelia burgdorferi (Bb) is an extracellular spirochetal bacterium and the causative agent of Lyme disease (LD). While *in vitro* assessment of Bb-induced robust immune responses suggests they are quite capable of clearing the pathogen, both the innate and adaptive responses are unable to eliminate Bb *in vivo*, resulting in Bb causing persistent infection. Hence, there is a dysregulation of the immune response occurring *in vivo,* which cannot be recapitulated in an *in vitro* system due to the intricacy and complexity of the host tissue milieu. In this study, we utilized intravital microscopy (IVM) along with several transgenic mouse lines, to delineate and identify the innate and adaptive immune dysregulation occurring within skin tissues *in vivo,* which permits this extracellular pathogen to maintain a persistent infection. Regarding adaptive immunity, our studies revealed that, 1) T cells are necessary to promote an optimal Bb-specific antibody response, 2) both B cells and T cells play a role in controlling the kinetics associated with Bb persistence, but offer minimal contribution in controlling Bb persistence long-term, and 3) Bb-specific antibodies are not responsible for the decrease in Bb number after day 8 post-infection. Regarding innate immunity, previous investigation of Bb-elicited innate immune responses identified a rapid and potent interleukin-10 (IL-10) response that affects Bb clearance. We hypothesized that this Bb-elicited IL-10 dysregulates the innate immunity and promotes Bb persistence *in vivo*. An IL-10 reporter (*tiger*) mouse identified macrophages and dendritic cells as the primary producers of IL-10 during active Bb infection in skin. IL-$10^{-/-}$ $LysM^{+}$ mice display significantly higher neutrophil infiltration whereas $TLR2^{-/-}$ $LysM^{+}$ mice had significantly diminished neutrophil infiltration. IL-$10^{-/-}$ $Ia\beta^{+}$ cells display significantly faster activation, whereas $TLR2^{-/-}$ $Ia\beta^{+}$ cells are defective in responding to Bb infection. Hence, Bb-elicited IL-10 diminishes activation of resident immune cells, whereas loss of TLR2 almost completely abolishes innate immune cell activation in response to Bb infection. Lastly, by using the IL-$10^{-/-}$ mouse as a hyperactive and $TLR2^{-/-}$ mouse as a hypoactive immune response model, we investigated the role of Bb velocity in causing persistent infection. Using different Bb chemotactic and motility mutants, Bb persistence analyses revealed that reduction of Bb velocity even by 50%, which is still 20-fold faster than all immune cells in the skin, significantly hampers Bb persistence *in vivo*, perhaps at levels that are unable to maintain the enzootic cycle. Overall this study provides a snapshot of immune dysregulation occurring during Lyme disease and identifies the factors which allow Bb to evade the host immune response.

COLLEGE OF MEDICINE AND LIFE SCIENCES
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DISSERTATION COMMITTEE

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THE UNIVERSITY OF TOLEDO
1872

DISSERTATION PRESENTATION

by

Muhammed Saad Moledina

July 22nd, 2019

Role of Bb-elicited IL-10 in Suppression of Innate Immune Responses within Murine Skin Tissue

Ph.D. in Biomedical Sciences

AWARDS/ LEADERSHIP

2018—Invited for an oral presentation at the 15th International Conference on Lyme Borreliosis and Other Tick-Borne Diseases in Atlanta, GA

2018—Council of Biomedical Graduate Students Graduate Research Forum 2nd place oral presentation

2018—Travel reward by Gordon Research Seminar, 2018 for oral presentation at Biology of Spirochetes, Gordon Research Seminar, Ventura Beach, CA

2017—Fellowship award for best poster (sponsored by NIH), Midwest Microbial Pathogenesis Organizing Committee

2017—Council of Biomedical Graduate Students Graduate Research Forum 1st place poster presentation

2016-2017—Council of Biomedical Graduate Students Risk Manager

2015-2016—Council of Biomedical Graduate Students MMI Representative

2014-2015—Council of Biomedical Graduate Students 1st year Representative

PUBLICATIONS

Hui Xu, **Muhammed Saad A. Moledina**, Syed Sultan, Aaron Yerke, Ki Hwan Moon, Md A. Motaleb & R. Mark Wooten. *CheY1 of Borrelia burgdorferi plays an important role in spirochetal acquisition by naïve ticks.* (in preparation)

Muhammed Saad A. Moledina, Hui Xu, Syed Sultan, Aaron Yerke, Ki Hwan Moon, Md A. Motaleb & R. Mark Wooten. *Intravital confocal microscopy reveals Borrelia burgdorferi CheY2 plays important role in spirochetal motility, dissemination and persistence in murine skin tissue.* (in preparation)

J. P. Lavik, P. Sekar, **Muhammed Saad A. Moledina**, V. Shukla, A. L. Nestor-Kalinoski and R. M. Wooten. *Intravital imaging in murine skin reveals that Borrelia burgdorferi display rapid and constant motility in skin that promotes evasion of host inflammatory responses and persistent infection.* (in preparation)

Muhammed Saad A. Moledina and R. M. Wooten. *Borrelia burgdorferi elicited Interlukin-10 dysregulates neutrophil infiltration and macrophage activity in murine skin.* (in preparation)

Nan Zhang, **Muhammed Saad A. Moledina** and R. M. Wooten. *Production of IL-10, unlike proinflammatory mediators, is independent of macrophage phagocytosis of Borrelia burgdorferi.* (in preparation)

FUTURE PLANS

Saad has accepted a position as a Clinical Research Associate (CRA) at a CRO firm in Cincinnati, OH.

PRESENTED ABSTRACTS

M. Moledina, P. Sekar, J. P. Lavik, Md Motaleb, and R. M. Wooten, "Intravital Microscopy Reveals that Innate, Not Adaptive, Immune Responses are Primarily Responsible for Controlling *Borrelia burgdorferi* During Acute and Persistent Infection" Oral Presentation at 15th International Conference on Lyme Borreliosis and Other Tick-Borne Diseases, Atlanta, GA, 2018

M. Moledina, P. Sekar, S. Sultan, E. Novak, Md Motaleb, and R.M Wooten, "*Borrelia burgdorferi* CheY1, CheY2, And CheY3 Possess Distinct Chemotaxis and/or Virulence Functions During the Natural Enzootic Cycle in Tick and Mouse Reservoirs" Oral Presentation at Graduate Research Forum, University of Toledo, Toledo, OH, 2018

M. Moledina, P. Sekar, J. P. Lavik, Md Motaleb, and R. M. Wooten, "Intravital Microscopy Reveals that Innate, Not Adaptive, Immune Responses are Primarily Responsible for Controlling *Borrelia burgdorferi* During Acute and Persistent Infection" Oral Presentation at Biology of Spirochetes, Gordon Research Seminar, Ventura Beach, CA, 2018

M. Moledina, P. Sekar, J. P. Lavik, Md Motaleb, and R. M. Wooten, "Intravital Microscopy Reveals that Innate, Not Adaptive, Immune Responses are Primarily Responsible for Controlling *Borrelia burgdorferi* During Acute and Persistent Infection" Poster Presentation at Biology of Spirochetes, Gordon Research Conference, Ventura Beach, CA, 2018

M. Moledina, P. Sekar, S. Sultan, E. Novak, Md Motaleb, and R.M Wooten, "*Borrelia burgdorferi* CheY1, CheY2, And CheY3 Possess Distinct Chemotaxis and/or Virulence Functions During the Natural Enzootic Cycle in Tick and Mouse Reservoirs" Poster Presentation at 24th Midwest Microbial Pathogenesis Conference, South Bend, IN, 2017