ABSTRACT

Borrelia burgdorferi is a spirochetal bacterium that causes Lyme disease, which is the most prevalent tick-borne disease in North American and Eurasia. Notable, this pathogen displays unique endoflagellar motility to efficiently disseminate through dense host tissues, and eventually persist within target tissues to cause inflammation-associated disease. Unfortunately, the importance of different *B. burgdorferi* virulence mechanisms, particularly those involved in bacterial motility/chemotaxis, cannot be accurately assessed *in vitro* since the complexity of the host tissues cannot be appropriately replicated for this obligate parasite. Hence, our initial goal was to develop intravital microscopy techniques that allow direct observation of fluorescent *B. burgdorferi* within the intact ear skin of living mice, including distinguishing specific motility parameters and *B. burgdorferi*-interactions with host immune cells. Our analyses determined that *B. burgdorferi* injected into murine skin undergo massive proliferation between Days 3-5, followed by a rapid decrease to a stable low level by Day 12; these levels are maintained for >2 years. *B. burgdorferi* move continuously within skin tissues, primarily in a back and forth motion that is interspaced with directed runs, and achieve average velocities of ≥200μm/min throughout most of the infection, which is ≥40x faster than any observed immune cell in those tissues. Neutrophils were observed to migrate into infected tissues within 2-6h post-infection, but these numbers decreased between 48-72h even though the *B. burgdorferi* numbers continued to increase. Thus, we were unable to distinguish whether the decrease in *B. burgdorferi* numbers observed after Day 5 post-infection was due to clearance by the initial inflammatory responses versus the activities mediated by *B. burgdorferi*-specific antibodies that reach significant levels around 5-7 days post-infection. However, our intravital techniques appear to provide an improved and excellent model for accurately dissecting *B. burgdorferi* virulence mechanisms *in vivo*.

We then focused on determining the relative importance of spirochetal motility and chemotaxis for evasion of immune clearance using *B. burgdorferi* mutants that lacked either MotB (a motor protein required in flagellar rotation; Δ*motB*) and CheY3 (a chemotaxis response regulator; Δ*cheY3*), respectively. *In vitro*, the Δ*motB* mutant is non-motile whereas the Δ*cheY3* mutant is motile but unable to reverse direction. Δ*motB B. burgdorferi* did not possess the characteristic spirochetal shape as motor rotation was necessary for forming the endoflagellar ribbon, as shown by cryo-ET. Even *in vivo*, Δ*motB* was non-motile and was cleared from all tissues by ≤96h post-injection. *In vivo*, Δ*cheY3 B. burgdorferi* retained the spirochetal shape but not the motility pattern of wild-type (WT) *B. burgdorferi*; the mutants displayed rapid velocities, but were unable to reverse directions and were cleared from all tissues by Day 5 post-injection. Interestingly, Δ*motB*-infection elicited very few *B. burgdorferi*-specific antibodies, whereas Δ*cheY3* elicited similar *B. burgdorferi*-specific antibody levels as WT through 28-days post-injection, but did not increase further compared to WT *B. burgdorferi* infection. Notably, the complexity of antigens recognized by Δ*cheY3*-elicited antibodies was less than WT, but contained antibodies against many proteins expressed by WT *B. burgdorferi in vivo*. These findings suggest that both *motB* and *cheY3* are essential for *B. burgdorferi* persistence, and that Δ*cheY3 B. burgdorferi* may serve as a vaccine to protect against Lyme disease.

DISSERTATION COMMITTEE

R Mark Wooten, Ph.D., Major Advisor

Robert Blumenthal, Ph.D.

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The University of Toledo College of Medicine and Life Sciences

Infection, Immunity & Transplantation (IIT) Track

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THE UNIVERSITY OF TOLEDO
1872

DISSERTATION PRESENTATION

Padmapriya Sekar

April 30, 2015

The effects of key motility and chemotaxis genes for *Borrelia burgdorferi* dissemination and evasion of immune clearance in murine tissues

Ph.D. in
Biomedical Sciences

PUBLICATIONS

Sultan SZ, Sekar P, Zhao X, Manne A, Liu J, Wooten RM, Motaleb MA Motor rotation is essential for the formation of the periplasmic flagellar ribbon, cellular morphology, and *Borrelia burgdorferi* persistence within *Ixodes* tick and murine hosts. Infect Immun. 2015 May; 83(5):1765-77

J. P. Lavik,* P. Sekar,* 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; * denotes co-first author)

Sekar, P., E. A. Novak, Md A. Motaleb, and R. M. Wooten. Loss of *cheY3* in *Borrelia burgdorferi* leads to loss of chemotaxis, inability to reverse direction, and early immune clearance from murine tissues. (in preparation)

Future Plans

Priya has accepted a post-doctoral position working with Dr. Wooten studying the effectiveness of different *B. burgdorferi* motility and chemotaxis mutants to work as attenuated vaccines against Lyme disease.

SELECT PRESENTATIONS

Padmapriya Sekar, John-Paul Lavik, Md A. Motaleb, R. Mark Wooten. The effects of different motility and chemotaxis genes for *Borrelia burgdorferi* dissemination in murine tissues. 2012. 2011-12 Graduate Research Forum, Toledo, OH (Poster)

Padmapriya Sekar, John-Paul Lavik, Md A. Motaleb, R. Mark Wooten. The impact of *Borrelia burgdorferi* infection on behavior of immune cells of murine skin tissue. 2013. 2012-13 Graduate Research Forum, Toledo, OH (Oral)

Padmapriya Sekar, John-Paul Lavik, Md A. Motaleb, R. Mark Wooten. Intravital assessment of immune evasion mechanisms employed by *Borrelia burgdorferi* in murine skin tissue. 2013. 20th Midwest Microbial Pathogenesis Conference, Columbus, OH (Poster)

Padmapriya Sekar, John-Paul Lavik, Md A. Motaleb, R. Mark Wooten. Assessment of cellular immune responses to acute and persistent *Borrelia burgdorferi* infection in skin. 2014. 2014 Biology of Spirochetes Gordon Research Seminar, Ventura Beach, CA (Oral) - Travel award

Padmapriya Sekar, John-Paul Lavik, Md A. Motaleb, R. Mark Wooten. Assessment of cellular immune responses to acute and persistent *Borrelia burgdorferi* infection in skin. 2014. 2014 Biology of Spirochetes Gordon Research Conference, Ventura Beach, CA (Poster)

Padmapriya Sekar, John-Paul Lavik, Md A. Motaleb, R. Mark Wooten. The effects of key motility and chemotaxis genes for *Borrelia burgdorferi* dissemination and evasion of immune clearance in murine tissues. 2014. 2013-14 Graduate Research Forum, Toledo, OH (Oral)

Padmapriya Sekar, Elizabeth A Novak, Md A. Motaleb, R. Mark Wooten. Intravital imaging reveals that the chemotaxis gene *cheY3* affects *B. burgdorferi* motility patterns, dissemination, and persistence within murine tissues. 2014. 21th Midwest Microbial Pathogenesis Conference, Chicago, OH (Poster)

Padmapriya Sekar, Elizabeth A Novak, Md A. Motaleb, R. Mark Wooten. Loss of chemotaxis gene Δ*cheY3* in *Borrelia burgdorferi* leads to early immune clearance but does elicits a strong antibody response. 2015. American Association of Immunologists Conference, New Orleans, LAA (Poster)

ABSTRACTS IN JOURNALS

Sekar, P., E. A. Novak, Md A. Motaleb, R. M. Wooten. Intravital assessment of a *Borrelia burgdorferi cheY3* mutant in mouse skin tissues reveals critical spirochetal properties for motility, dissemination, and persistence in the development of Lyme disease. 2015. Bacterial Locomotion and Signal Transduction XIII, Tucson, AZ. (Oral presentation)

Sekar, P., E. A. Novak, Md A. Motaleb, R. M. Wooten. Loss of chemotaxis gene Δ*cheY3* in *Borrelia burgdorferi* leads to early immune clearance but does elicit a strong antibody response. 2015. American Association of Immunologists Conference, New Orleans, LA (Poster)

AWARD

Finalist for oral presentation awards at the 2012-13 Graduate Research Forum, The University of Toledo, Toledo, OH (2013)

Invited speaker at the 2014 Biology of Spirochetes Gordon Research Seminar, Ventura, CA (2014)

Awarded American Heart Association Predoctoral Fellowship (2014)

Awarded UT Health Science Campus Retirees Award (2014)

Awarded Outstanding Graduate Student Award, Ph.D. in Biomedical Sciences Program (2015)