ABSTRACT

Francisella tularensis is the causative agent of the lethal disease tularemia. Despite decades of research, little is understood about why *F. tularensis* is so virulent. Bacterial outer membrane proteins (OMPs) are involved in various virulence processes, including protein secretion, host cell attachment, and intracellular survival. Many pathogenic bacteria require metals for intracellular survival and OMPs often play important roles in metal binding and uptake. Previous studies identified three *F. tularensis* OMPs that play roles in iron acquisition. We have identified two new proteins, FTT0267 (named *fmvA*, for *Francisella* metal and virulence) and FTT0602c (*fmvB*), which are homologs of those iron acquisition genes and demonstrated that both are upregulated during mouse infections. Based on sequence homology and *in vivo* upregulation, we hypothesized that FmvA and FmvB are OMPs involved in metal acquisition and virulence. Despite sequence homology to previously-characterized iron-acquisition genes, FmvA and FmvB do not appear to be involved iron uptake, as neither *fmvA* nor *fmvB* were upregulated in iron-limiting media and neither Δ*fmvA* nor Δ*fmvB* exhibited growth defects in iron limitation. However, among other metals examined in this study, magnesium-limitation significantly induced *fmvB* expression, Δ*fmvB* was found to express significantly higher levels of lipopolysaccharide (LPS) in magnesium-limiting medium, and increased numbers of surface protrusions were observed on Δ*fmvB* bacteria in magnesium-limiting medium, compared with wild-type *F. tularensis* grown in magnesium-limiting medium. RNA sequencing analysis of Δ*fmvB* revealed the potential mechanism for increased LPS expression, as LPS synthesis genes *kdtA* and *wbtA* were significantly upregulated in Δ*fmvB*, compared with wild-type *F. tularensis*. To provide further evidence for the potential role of FmvB in magnesium uptake, we demonstrated that FmvB was outer membrane-localized. Finally, both Δ*fmvA* and Δ*fmvB* were found to be significantly attenuated in mice and cytokine analyses revealed that Δ*fmvB*-infected mice produced lower levels of various cytokines, including GM-CSF, IL-3, and IL-10, compared with mice infected with wild-type *F. tularensis*. Taken together, these studies have characterized two previously-unstudied *F. tularensis* proteins, have shown that both play roles in *F. tularensis* virulence, and provide new insights into the importance of magnesium for intracellular pathogens.

DISSERTATION COMMITTEE

Jason F. Huntley, Ph.D., Major Advisor

Robert Blumenthal, Ph.D.

William Maltese, Ph.D.

Kevin Pan, M.D., Ph.D.

R. Mark Wooten, Ph.D.

Dayanidhi Raman, Ph.D., Graduate School Representative

The University of Toledo College of Medicine

Medical Microbiology & Immunology (MMI) Track

Department of Medical Microbiology & Immunology

THE UNIVERSITY OF TOLEDO
1872

DISSERTATION PRESENTATION

Xiaojun (Wilma) Wu

April 22, 2016

Identification of two novel *in vivo*-upregulated *Francisella tularensis* proteins involved in metal acquisition and virulence

Ph.D. in
Biomedical Sciences

PUBLICATIONS

Wu X, Ren G, and Huntley JF. Generating Isogenic Deletions (Knockouts) in *Francisella tularensis*, a Highly-infectious and Fastidious Gram-negative Bacterium, Bio-protocol, 2015, 5(12): e1500.

Wu X, Ren G, Gunning W, Weaver D, Kalinoski A, Khuder S, and Huntley JF. FmvB: A *Francisella tularensis* magnesium-responsive outer membrane protein that plays a role in virulence (manuscript under review at PLoS One).

Future Plans

Currently applying for post-doctoral fellowship positions in microbiology and immunology in the Midwest.

ABSTRACTS & PRESENTATIONS

Xiaojun Wu, Guoping Ren, and Jason F. Huntley. Identification of Two Novel *In Vivo* Up-regulated *Francisella tularensis* Proteins Which are Involved in Iron Acquisition. Ohio Branch of the American Society for Microbiology, Ashland, OH, April 2013.

Xiaojun Wu, Guoping Ren, and Jason F. Huntley. Identification of Two Novel *In Vivo* Up-regulated *Francisella tularensis* Proteins Which are Involved in Iron Acquisition. Midwest Microbial Pathogenesis Conference, Columbus, OH, August 2013.

Xiaojun Wu, Guoping Ren, and Jason F. Huntley. Identification of Two Novel *In Vivo* Up-regulated *Francisella tularensis* Proteins Which are Involved in Iron Acquisition. Ohio Branch of the American Society for Microbiology, Columbus, OH, April 2014.

Xiaojun Wu, Guoping Ren, and Jason F. Huntley. Identification of Two Novel *In Vivo* Up-regulated *Francisella tularensis* Proteins Which are Involved in Iron Acquisition. Midwest Microbial Pathogenesis Conference, Chicago, IL, September 2014.

Xiaojun Wu, Guoping Ren, and Jason F. Huntley. Identification of Two Novel *In Vivo* Up-regulated *Francisella tularensis* Proteins Involved in Metal Acquisition and Virulence. FASEB Molecular Pathogenesis: Mechanisms of Infectious Disease, Keystone, CO, July 2015.

Xiaojun Wu, Guoping Ren, and Jason F. Huntley. Identification of Two Novel *In Vivo* Up-regulated *Francisella tularensis* Proteins Involved in Metal Acquisition and Virulence. Midwest Microbial Pathogenesis Conference, Indianapolis, IN, August 2015.