THE GRADUATE COLLEGE OF THE UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Erin Teresa Livingston

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE

GRADUATE COLLEGE

Department of Microbiology and Immunology

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Contribution of *Bacillus* InhA Metalloproteases to Endophthalmitis

COMMITTEE IN CHARGE: Michelle Callegan PhD, Chair; Jimmy Ballard, PhD; Mark Lang, PhD; Anne Pereira, PhD; Michael Elliot, PhD

ABSTRACT: Bacterial endophthalmitis is a devastating infection that can cause blindness following experimental introduction in animal model or accidental introduction as a result of physical trauma in humans into the posterior segment of the eye. Over half of *Bacillus cereus* endophthalmitis cases result in significant loss of useful vision. Often, these eyes have to be enucleated. *B. cereus* produces many virulence factors in the eye that may contribute to retinal damage and robust inflammation. This dissertation focused on *Bacillus* immune inhibitor A (InhA) metalloproteases, which digest extracellular matrix, tight junction proteins, and antimicrobial proteins. We hypothesized that InhAs contribute to *Bacillus* intraocular virulence and inflammation.
We analyzed phenotypes and infectivity of wild-type (WT), InhA1-deficient ($\Delta$inhA1), InhA2-deficient ($\Delta$inhA2), or InhA1, A2, and A3-deficient ($\Delta$inhA1-3) *Bacillus thuringiensis*. *In vitro* analysis of growth, proteolysis, and cytotoxicity were compared between *B. thuringiensis* strains. WT and InhA mutants were similarly cytotoxic, but $\Delta$inhA1-3 had significantly reduced proteolysis. Single InhA mutants had increased growth compared to WT. These results indicate that the InhAs may be important for bacterial growth and proteolysis.

Experimental endophthalmitis was initiated and intraocular *B. thuringiensis*, retinal function loss, and intraocular inflammation, and ocular histology was examined. Eyes infected with single InhA mutants contained greater numbers of bacteria than eyes infected with WT throughout the course of infection, but eyes infected with $\Delta$inhA1-3 cleared the infection. Eyes infected with the triple InhA mutant also had less retinal function loss, inflammation, and ocular tissue damage compared to eyes infected with the WT or single InhA-mutant *B. thuringiensis*. InhA expression of the mutants showed that there may be compensatory expression of the other InhAs in the single InhA mutants. Our data suggests that the InhAs contribute to pathogenesis of *B. thuringiensis* endophthalmitis.

We sought to understand how InhAs contribute to intraocular infection, so the InhAs role in retinal permeability and nutrient acquisition was examined *in vitro*. Retinal cell monolayers treated with the $\Delta$inhA1-3 *B. thuringiensis* were less permeable than WT and single InhA mutants. The permeability differences may be due to cytotoxic affects, since retinal cell monolayers revealed condensed nuclei of $\Delta$inhA1 and $\Delta$inhA2 treated cells. Tight junctions between RPE cells was decreased in cells treated with WT and single InhA mutant supernatants. *Bacillus* InhAs may be more important for bacterial growth since $\Delta$inhA1-3 growth is stunted in environments where nutrients are mainly acquired by breaking down proteins and peptides.

During infection, *Bacillus* must acquire nutrients in the vitreous environment by breaking down large proteins in order to survive and proliferate. These results indicate that, together, the InhA metalloproteases contribute to the severity of infection and inflammation in *Bacillus* endophthalmitis perhaps through mechanisms including bacterial growth. Thus, the InhA metalloproteases may be a therapeutic target in order to reduce the rate of infection of *Bacillus* during endophthalmitis.