Effect of *Francisella* Pathogenicity Island Protein PdpD on IL-1β expression Levels in Host Macrophage Cells

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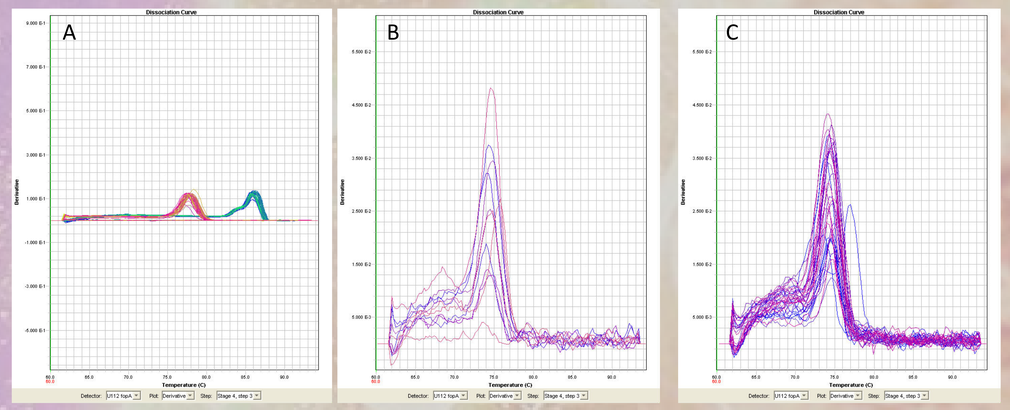
Introduction

Francisella tularensis is a gram negative, intracellular bacterial pathogen responsible for the disease tularemia. It has been classified by the United States government as a Class A Biological Agent of Bioterrorism/Biowarfare. *F. tularensis* can evade the initial immune response by phagasomizing in host macrophage cells (Broms, et al. 2010). A phagasome is a vacoule formed around an invading substance. *F. tularensis* breaks free from the phagasome and replicates inside the host cell. Detection of the bacteria triggers the inflamasome which triggers the activation of cytokines such as IL-1β. A cytokine is a protein used to signal the immune system.

The Francisella Pathogenicity Island (FPI) is a segment of the *Francisella* genome that contains the coding genes involved in the pathogenicity of *F. tularensis* (Chong and Celli, 2010). In previous studies, when the FPI transcriptional regulator *mglA* was knocked out, immune response levels were lower than infection with the wild strain. This suggests that without the FPI, *F. tularensis* does not stimulate wild-type levels of cytokine secretion. The FPI protein PdpD was knocked out and found to be non-essential to cellular growth or reproduction. However when this protein is knocked out, the difference in secretion of the cytokine TNF-α between the PdpD knockout strain and the wild-type is greater than in the other knock out strains. This project will continue to focus on how *Francisella tularensis* impacts the immune response including the possible role of PdpD function.

Figure 1. Dissociation Curves for qt-PCR



(A): A depiction of an ideal dissociation curve with two sets of samples (TNF-α and β-actin).
(B): Dissociation curve for the IL-1β negative controls.
(C): Dissociation curve for the IL-1β sample set.
(D): Dissociation curve for the β-actin negative controls.
(E): Dissociation curve for the β-actin sample set.

Results

The dissociation curve is a graph showing the point at which a double-stranded DNA molecule dissociates into single-stranded DNA. A dissociation curve is unique to each DNA molecule. Ideally, the peak of the curves for each line should be in a tight grouping, as in Figure (1). Also, the dissociation curves for the negative controls should be a tight band of relatively flat lines across the graph. However, the results were less than ideal; Figure (2) and Figure (3) (the negative controls)have clear dissociation curve peaks deviating from the flat band. In figures (4) and (5), there are multiple peaks for one sample set; these results suggest contamination. Due to contamination, all results are invalid.

Discussion

The results were invalid due to contamination. Because the qt-PCR results shown clearly exhibit contamination in all samples and controls, contamination maybe due to outside DNA contaminating all of the samples. In the most recent qt-PCR, however, the β-actin negative controls came back without any contamination, suggesting that the IL-1β primers are contaminated. Both contamination scenarios are likely, to resolve the contamination issues buying new primers and taking more care in the procedure will likely suffice.

Conclusions

Data is inconclusive due to contamination.

Materials and Methods

- Macrophage cells (J774A.1) were infected with *Francisella tularensis novocida.* (donated by Dr. Francis Nano, University of Victoria, Victoria, British Columbia, Canada)
- RNA from the macrophage cells was extracted at 2, 5, 12, and 24 hours post-infection using TRIzol reagent (Invitrogen, Carlsbad CA) as per manufacturer instructions.
- The RNA was reverse transcribed into cDNA using reverse-transcription-polymerase-chain-reaction (rt-PCR) later used in qt-PCR.
- The cDNA from the rt-PCR was used as the template for quantitative-polymerase-chain-reaction (qt-PCR) in order to quantify thethe expression levels of IL1-β. The primers used targeted the IL-1β gene and the β-actin gene.
- The results from the qt-PCR was then analyzed using the $2^{-\Delta\Delta C_T}$ method. The $2^{-\Delta\Delta C_T}$ method represents data as a fold change in expression that normalizes (scales) to an internal reference and compairs to a control.

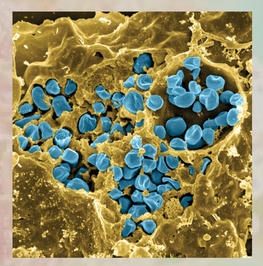


Figure 2. Scanning electron micrograph image of a macrophage cell infected by *Francisella tularensis.*

Image from - *http://www.pnas.org/content/103/39.cover-expansion*

Works Cited

Background photo for poster - http://napervillethyroiddoctor.blogspot.com/2011/05/dr-hagmeyer-naperville-ils-thyroid.html

Photo of *F. tularensis* in Figure 2.- http://www.pnas.org/content/103/39.cover-expansion

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Chong, A; Celli, J. (2010) The *Francisella* intracellular life cycle: toward molecular mechanisms of intracellular survival and proliferation. Frontiers in Microbiology, 1:138

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