Project Summary

During peripheral inoculation in a mouse model of tularemia, *Francisella tularensis* was unexpectedly found in brain homogenates. No prior evidence suggested that *F. tularensis* has a neural tropism, making our observation novel and perhaps highly significant. *F. tularensis* infection triggers an exuberant host inflammatory response defined by excess production of pro-inflammatory cytokines by immune cells, a condition termed "cytokine storm." The cytokine storm is the direct cause of severe clinical symptoms and ultimate death of the host. Human tularemia, caused by *F. tularensis*, is a zoonotic disease transmitted by aerosol particles, direct contact, and via arthropod vectors, particularly ticks. *F. tularensis* is endemic in the wild animal population of the US, especially Texas, and is a potential biological warfare agent. Severe tularemic disease is thought to be due to pulmonary distress caused by side-effects of the excessive cytokines produced by the host immune system. This may be masking an unappreciated effect of the bacteria on the central nervous system.

The *overarching goal* of this project is to define the consequences of neuronal involvement in *F. tularensis* infection. The specific aims of this proposal is to 1) identify the cellular targets of *F. tularensis* in the central nervous system, 2) determine the contribution of microglial-derived cytokine storm to CNS pathology, and 3) define the activation status of immune cell subsets in the brain. Since in the periphery, macrophages are the target cell for *F. tularensis*, it is likely that microglial cells, the resident macrophages of the central nervous system, may harbor *F. tularensis* in the brain. Rapid activation of microglial cells is an important factor in guarding the neural parenchyma against infectious disease, inflammation, and neurodegeneration while maintaining or redressing tissue homeostasis. We hypothesize that *F. tularensis* infection of microglia results in the overproduction of inflammatory cytokines in the brain which damages surrounding neuronal cells and leads to neuronal impairment.

Methods: Through fluorescent confocal microscopy, we will identify the brain region(s) containing fluorescent *F. tularensis* and its cellular targets. Using Laser Microdissection (LMD), we will excise brain regions containing numerous bacteria, regions immediately adjacent to these bacteria, and region far removed from the area containing bacteria. The production of inflammatory cytokines will be analyzed by Milliplex ELISA and real-time RT-PCR to measure protein and mRNA production, respectively. In collaboration with Dr. Astrid Cardona at UTSA, we will characterize the immune cell composition and activation status by multiparametric flow cytometry.

Expected outcome: Our preliminary data suggests that *F. tularensis* is causing an elevated pro-inflammatory response in the brains of infected mice. Through direct intracranial injections, we have found data suggesting *F. tularensis* invades CNS resident resting microglia cells and, perhaps, other as yet undefined cell types. These may be astrocytes, neurons, and/or infiltrating peripheral macrophages. We hypothesize that inflammation due to *F. tularensis* invasion of the brain could potentially be increasing the mortality of the host. The exuberant inflammatory response in the periphery, combined with central nervous system inflammation, may cause overwhelming tissue damage to tissues, including the brain, ultimately leading to death.

Relevance: The presence of *F. tularensis* in the brain represents a novel observation that has expanded our area of research from immunology into the realm of neuroscience. Since the PI is not an expert in neuroscience, we have formed new collaborations with highly respected neuroscientists from our department (Dr. Arshad Khan, UTEP, an expert in neuroanatomy) and other UT system institutions (Dr. Astrid Cardona, UTSA, an expert in neuro-immunology). Dr. Khan has trained my student and guided the anatomical search for infecting bacteria. Dr. Cardona has agreed to assist in the analysis of the immune cells in the brain to determine their response to infection. Data obtained through use of these seed funds will allow us to generate solid preliminary data to support external federal funding from the NIH and NSF to determine the biological and behavioral implications for neural invasion by *F. tularensis*.