Abstract

Candida species are the number one cause of mortality from a fungus-associated disease and the fourth leading cause of hospital acquired bloodstream infections in the United States (1). Candida albicans, a dimorphic pathogen which alternates between yeast and filament forms, is the most common pathogen in this group (1). Better understanding of interaction between C. albicans and the immune system may lead to improved methods of detecting and treating invasive candidiasis. Preliminary research has linked the dynamic physical structure of C. albicans with its ability to cause systemic disease and has indicated that immune cells may move yeast from one tissue to another. I propose to use a transparent zebrafish model to test the hypothesis that inflammatory responses are associated with the infection spread. I will use both quantitative assessment of inflammatory cytokine levels and high resolution imaging of C. albicans as it spreads and causes systemic disease.

Project Description

Candida albicans, the most prevalent member of the Candida species, is a common commensal fungus which becomes pathogenic in the immunocompromised. Candida species present a burden to public health by increasing the cost and the length of hospitalization and by raising mortality (1). Better understanding of interaction between C. albicans and the immune system is crucial to developing improved methods of detecting and treating invasive candidiasis. Candida albicans is a dimorphic microbe which normally alternates between yeast and hyphae forms during the course of an infection; these two morphologies are key to understanding the pathogenesis of the fungus. In my preliminary research I have worked with Brittany Seman using yeast-locked and hyphae-locked mutant strains to examine how each morphology, or the combination thereof, could be linked to Candida's ability to spread from one tissue to another to cause systemic disease. Of particular interest, we find many yeast cells inside phagocytic immune cells; this suggests that immune cells may move yeast from one tissue to another. With this fellowship I will expand upon this knowledge by turning to examine the other side of the equation – the host immune response. When an invader such as Candida is detected, the host acts swiftly with the innate immune system, the “blunt force” that comes before the more tailored adaptive response. Specialized, professional immune cells such as neutrophils or macrophages are attracted to the initial site of infection and several physiological changes begin to occur; these cells can influence each other and themselves through the
release of small proteins called cytokines (3). Cytokines, particularly IL-1β and TNFalpha, can effect powerful cell signal cascades to recruit immune cells to the site of infection where they might pick yeast up and transport them away. They also induce a pro-inflammatory response that loosens barriers to movement and can allow pathogens to pass through blood vessels (5). We hypothesize that these inflammatory responses are associated with the spread of infection. I plan to examine the relationship between morphology, dissemination, and the inflammatory response through a multifaceted approach using zebrafish models and drug-repressible strains of Candida albicans that overexpress genes for either hyphal growth (Ptet-UME6) or yeast growth (PtetNRG1). I have designed experiments that build on my previous work imaging infections, while adding quantitative PCR to measure expression of inflammatory cytokines during different stages of infection. Newly acquired transgenic zebrafish express green fluorescent protein (GFP) when the proinflammatory cytokine TNFalpha is produced (4) have the potential to act as a profound tool, amplifying the possibilities of this research. Part One: Visualizing Active Dissemination Similar to my preliminary work, I will microinject groups of approximately 25 wildtype zebrafish at 48 hours post fertilization with 10-20 fluorescent Candida cells into the yolk of the fish and screen via epifluorescence microscopy to ensure correct cell inoculum. In some experiments, I will use a single strain of Candida: mutants forming either only yeast (Ptet-NRG1) or only hyphae (PtetUME6) or a wild type (Ptet-T21) able to form both, and in some groups I will use a coinfection of two strains. I have previously taken images of infection progression and measured levels of dissemination and mortality at specific time points (18, 24, and 44 hours post infection). I will continue to collect this data but will also add time-lapse confocal microscopy, setting up a confocal microscope to record images at regular intervals over the course of an infection to more accurately determine when and what is occurring during dissemination. Part Two: Quantifying Components at Play My next step in elucidating factors influencing dissemination is quantification of proinflammatory gene expression with PCR. To do this, I will infect much larger groups of 150 fish in the same manner as in Part One. I will still use a confocal microscope to categorize subgroups of fish with disseminated and non-disseminated infections at specific time points. Instead of measuring mortality as in Part One, however, I will collect 10-20 zebrafish from each group for each time point and extract RNA, make cDNA, and perform qPCR to measure inflammatory cytokine expression (e.g. IL1-b, IL-6, or TNFalpha). I will compare expression between fish infected with different C. albicans strains exhibiting different morphologies; and also examine differences between disseminated and nondisseminated infection. Additionally, if time allows, I hope to perform experiments similar to

**Budget Justification**

CUGR Request: Stipend: $2,070 Supplies: $430 Travel: $500 Total: $3,000 Justification for Student Stipend: This grant will provide partial salary for Ms. Moore, 248 hours of work at $8.35/hour for a total of $2,070. This will cover 6 weeks and one day of full-time work. The remainder of her salary for the rest of the summer will be covered on a current extramural grant. Ms. Moore will carry out the majority of the proposed experiments. These experiments include performing time-course experiments of zebrafish-Candida albicans interactions, microscopy, quantitative PCR and analysis of the resulting data. Justification for General Supplies: This grant will provide money for purchase of general supplies, including fungal growth media, plastics,
syringes, needles, injection equipment, PCR supplies etc. in the amount of $430. The remainder of the supplies necessary will be covered by a current extramural grant. Justification for Travel:

This grant will provide money for Ms. Moore to travel with the PI (Wheeler) to the bi-annual North Atlantic Zebrafish Research Symposium in Dalhousie, Nova Scotia in June 2015. The $500 budgeted will cover part of the expected registration, housing and travel fees. Travel is estimated at $200, registration is $60, food is estimated at $150 over three days, and lodging is estimated at $120 for two nights. The budgeted amount is expected to only partially cover expenses, and the remainder will be covered by a current extramural grant. Itemized Budget:

- Total Budget Requested: $500
- Stipend Amount Budget: $200
- Materials and Supplies Budget: $150
- Travel Budget: $60
- MISC