Final Report for Research Office for General Research Fund Pilot Grant

Award Date: January 2012 (money not released until June 2012 due to Biosafety Committee and IACUC delays)

Award Amount: $10,000

Proposal Title, PI, Department:

Development of a Zebrafish Model for Study of Pathogenesis of Fungal Disease and for Antifungal Drug Discovery; PI Jan Spitsbergen, DVM, Ph.D., Department of Microbiology

Goals and Scholarly Work Performed with Funding

Our goal in this research was to develop reliable and efficient methods to achieve invasive fungal infection in zebrafish following challenge with emerging fungal pathogens of importance in human health. We began our research using *Candida parapsilosis* as suggested by OSU's Biosafety Committee, because it is a BSL-1 agent that was good for optimizing protocols. As reported by other scientists that have tried to achieve successful systemic infection with bacterial pathogens of humans or with *Candida albicans*, zebrafish early life stages with intact innate defense mechanisms are remarkably resistant to infection following bath challenge with *Candida parapsilosis*. Since zebrafish live in water filled with microbes and must withstand the effects of temperature changes on their adaptive immune defenses, their innate defense mechanisms are particularly vigorous compared to those of mammals. We found that both the characteristics of the fungal agent as well as the innate defense mechanisms of the fish were critical to consider in optimizing a successful bath challenge protocol. Our initial attempts to achieve invasive fungal infection in early life stages of zebrafish treated with broad spectrum antibiotics (doxycycline or gentamicin) at the maximal tolerated doses, immunomodulating drugs at the maximal tolerated doses including dexamethasone, cyclophosphamide, plerixifor (inhibitor of CXCR4 which controls leukocyte migration), bortezomib (NFkB-proteosome inhibitor), nodinitib 1 (inhibitor of NOD 1 signaling), or combinations of broad spectrum antibiotics and immunosuppressive drugs were not successful in achieving invasive fungal infection in early life stages of zebrafish using 10^6 or 10^7 colony forming units of yeast culture grown for in YPD medium for 2 days at 28 ℃. Typically for fungal challenges with mammalian species, yeast cultures grown for 2 days are used. Since invasive infections with *Candida parapsilosis* most commonly occur in patients in which a biofilm forms on catheters in blood vessels or urinary tract, or in other implanted devices like artificial heart valves, we reasoned that we might increase the probability of achieving invasive

infection by creating cultures of *Candida parapsilosis* with characteristics resembling biofilm. Therefore, we cultured the organism for 2 weeks in YPD at which time a slime layer had formed on the surface of the culture. More filamentation was evident in the culture when examined using a hemocytometer, although most of the broth culture remained in the yeast form. We successfully achieved invasive fungal infections in zebrafish treated with dexamethasone, dexamethasone plus cyclophosphamide, gentamicin or the mucolytic agent acetylcystine (disrupted the protective mucus layer of the fish) then challenged with 10^6 or 10^7 colony forming units of yeast culture grown for in YPD medium for 2 weeks at 28 ℃ These exciting positive research findings now will allow us to publish this information and to use the preliminary data to apply for funding from NIH, industry or private foundations. These techniques may assist us in development of a zebrafish model to develop better drug treatments for the newly emerging fungal pathogen Exserohilum rostratum which has caused high morbidity and significant mortality in patients injected with contaminated corticosteroid solutions in a nationwide epidemic recognized over the past year.

Expenditure of GFR Funds

We spent the funds on supplies and undergraduate student wages.

External Funding Proposals

Due to the delays in beginning our research resulting from the extended time required for protocol approval by the Biosafety Committee and IACUC and due to the fact that achieving invasive fungal infection by bath exposure of fish was very challenging, we are just now positioned to begin using our preliminary data to leverage more funding. We will apply to NIH, industry and private foundations.