2013 CFID Undergraduate Summer Research Award Proposal

***Aspergillus fumigotus:* harnessine sporulation to treat disease**.

Applicant: Mohamad Rabbani, McGill University
Supervisor: Don Sheppard MD

Background

The mold *Aspergillus fumigatus* spreads through dispersion of its asexual spores, called conidia. When inhaled, these conidia are efficiently eliminated by innate immune defenses. But, in immunocompromised patients, conidia escape killing to germinate into hyphae which invade host cells and proliferate in blood vessels, often disseminating to other organs. lnvasive aspergillosis (lA) occurs in up to 15% of Canadian patients with acute leukemia or undergoing allogenic stem cell transplantation, and the mortality rate of IA remains greater than 50%, despite the use of new antifungal agents. Novel approaches to modify the natural history of this illness are urgently needed.

A *fumigatus* is an opportunistic pathogen with relatively low intrinsic virulence. As a result the growth rate of the organism plays a crucial role in its ability to cause disease, and strains with reductions in hyphal growth rate exhibit reduced or no virulence. Efficient hyphal growth is favored by the fact that during invasive aspergillosis there is no conidiation allowing the organism to direct its energy towards vegetative growth. Conversely, work from our group and others has demonstrated that during conidiatian, vegetative growth is inhibited, as the organism commits its resources to the generation of new conidia. We therefore hypothesize that forcing the induction of conidiation of *A. fumigatus* during IA will result in impairment of vegetative growth, leading to a reduction of the fitness of the fungus, and impairing virulence. If this hypothesis is confirmed, developing molecules that activate conidiation will be explored as a new therapeutic tool.

Control of conidiation in *Aspergilli* has been well studied in the non-pathogenic model A. *nidulans* [1, 2]. In *A. nidulans,* conidiation is activated by a key development transcription factor *br/A* [1, 3] which acts as a master switch that is required for controlling the expression of the genes involved in conidiation. We have identified the *br/A* orthologue in A *fumigatus,* and through gene disruption studies and whole organism genome profiling we have confirmed that this gene controls conidiation and directly regulates vegetative growth in *A. fumigatus* [4]. We will exploit the role of this master switch of conidiation to test our hypothesis that suppression of conidiation is required for virulence in IA.

ExperimentalApproach:

To test the ability of forced conidiation to suppress virulence, we will construct a mutant strain of A *fumigatus* in which a copy of the *br/A* gene will be placed under the control of a tetracycline responsive promoter. In this system, *br/A* expression is induced at high level only in the presence of tetracycline. This will allow the forced expression of *br/A* and the induction of conidiation *in vitro* or *in vivo* simply through exposure to tetracycline, an agent with no activity against *A. fumigatus.*

Methods: The *Br/A* ORF will be amplified by PCR and cloned following an inducible promoter under control of the tetracycline operator. *A. fumigatus* protoplasts will be co-transformed with the plasmid bearing this construct and the plasmid bearing the operator activator. Recombinants will be selected for their resistance to the appropriate markers and for forced expression of *br/A* in tetracycline enriched growth medium. The *in vitro* phenotype of the selected transformants will be studied, especially for growth defect and morphological changes. Strains exhibiting reduced growth in response to tetracycline will then be tested for virulence in an inhalational mouse model of invasive aspergillosis developed by the Sheppard lab. Quantification of the fungal burden and inflammatory mediator production as well as histopathological observations will be performed at appropriate time points.

Anticipated ResuIts

We do not anticipate any difficulties with strain construction, phenotypic analysis or the virulence assays as these techniques are all in routine use in the Sheppard lab [5, 6]. We predict that forced expression of *br/A* will result in canidiation and a reduction in vegetative growth *in vitro.* Further, we anticipate this reduced growth will translate to improved survival in our animal model. It is also possible that forced conidiation will result in the production of viable conidia within mouse lungs, and that this could enhance infection *in vivo.* However since spores are less resistant to immune mediated killing than hyphae, we hypothesize the production of spores will not enhance but rather attenuate virulence.

Significance and Future Directions

This study outlines a novel approach *to* manipulating the fungal life cycle in order to attenuate virulence. If these pilot experiments provide promising results they will form the basis for a novel approach to the treatment of invasive aspergillosis. Small molecules that fungi use to stimulate conidiation have already been described, and these compounds will then form the basis for further studies aimed at developing a novel therapeutic agent. Importantly, this type of therapy could easily be combined with classic antifungal agents to improve outcomes during this important infection.

References

1. Yu, J. H., J.-H. Mah, and J. A. Seo. Growth and developmcntal control in the modcl and pathogenic Aspergilli. Eukaryotic Cell 2006; 5 (10):1577-1584
2. Mah, J.·H. and J. H. Yu. Upstream and downstream regulation of asexual development of Aspergillus fumigates. Eukaryotic Cell 2006; 5 (10):1585-1595
3. Aguitte, J. Spatial and temporal controls of the Aspergillus *Br/A* developmental regulatory gene. Mol Microbiol 1992;8(2):211-8
4. Twumasi-Boatong K., et al. Transcriptional profiling identifies a role for *Br/A* in nitrogen depletion and for StuA in the regulation of secondary metabolite clusters in Aspergillus fumigates. Eukaryotic Cell 2009;8(1):104·115.
5. Gravclat, F. N., D. S. Askew, and D.C. Sheppard. Targeted gene deletion in Aspergillus fumigates using the hygromycin resistance split marker approach. Methods Mol Biol 2012;14S:119-30
6. Sheppard, D.C., et al. Standardization of an experimental murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2006;50(10):3501-3