Thesis Title Pathogenicity of the Yeast Cells and the Basidiospores of *Cryptococcus neoformans* var. *neoformans*

Name Kanokwan Kittiniyom

Degree Master of Science (Medical Technology)

Thesis Supervisory Committee

Samaniya Sukroongreung, M.D.

Churairatana Nilakul, M.Sc. (Pharm.)

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ABSTRACT

Cryptococcosis ia a life-threatening infection caused by the encapsulated fungus, *Cryptococcus neoformans*. It is generally accepted that the lungs are the primary portal of entry for the desiccated yeast cells. Sexual reproduction by *C. neoformans* occuring in nature is unknown. Certainly, the small particles, dry basidiospores either from diploid or haploid hyphae would make effective infecting particles, and they have been suggested to be a source of infection.

Searching in the literature, only one report demonstrated pathogenicity of basidiospores in which mice were intracraneally or intraveneously injection with basidiospores resulting in cryptococcosis. This study attempted to evaluate whether a yeast cell or a sexual basidiospore of *C. neoformans* var. *neoformans* is able to produce a similar naturally acquired human cryptococcosis in normal and cyclophosphamide (Cy)-treated mice. Two kinds of equipment were designed, a modified micromanipulator for monitoring a single cell isolate and two inhaling chambers, the first for dry powder inoculating and the second for connecting to a nebulizer.

The pathogenic potential via nasal route of yeast cells (*C. neoformans* MT-12.4) and purified basidiospores (*C. neoformans* RV-45980 X *C. neoformans* MT-100.1) have been evaluated in normal and Cy-treated mice by using three inoculating methods: intranasal instillation, nasal spraying and dry powder blowing. Pathogenicity assessment included enumeration of viable cryptococcal cells in the internal organs and non randomized histopathological observations. This experiment demonstrated that both yeast cells and basidiospores of *Cryptococcus neoformans* var. *neoformans* can cause disseminated cryptococcosis in Swiss albino mice via

nasal route but only when the infectious particles are wet. A large amount of the absolutely dry yeast cells used as inoculum is non-virulent to the mice. This is the first demonstration of pathogenesis of the basidiospores of *C. neoformans* var. *neoformans* in mice via the nasal route. Nasal spraying associated with a nebulizer firstly developed is able to support a new method for nasal inoculating in order to a near naturally acquired cryptococcosis. The spraying droplets act as a vehicle of the infectious particles towards either the nasal cavity or the lungs. In the experimental cryptococcosis via the intraperitoneal route, normal mice are less susceptible to the basidiospores than the yeast cells. The severity of the disease depended on the amount of inocula (yeast cells), or it was dose-dependent. The Cy-treated mice were strikingly more susceptible to basidiospores than the normal mice. Though the decrease susceptibility of the Cy-treated mice to the yeast cells cannot be explained, but at least cyclophosphamide do not increase infectivity of yeast cells of *Cryptococcus neoformans*.

More than one colonial type of *C. neoformans* was isolated from the mice with cryptococcosis caused by basidiospores. This evidence rarely occurs in human acquired cryptococcosis. So that, if any, basidiospores are a kind of the infectious agent, they should be the haploid basidiospores.

The modified micromanipulator is worth enough, for it is easy to manipulate, and very cheap. The inhaling chambers and their accessories are also valid but further development is required.

This research suggests that haploid basidiospores and yeast cells are the infectious particles of *Cryptococcus neoformans*. Droplets carrying infectious particles land on the surface of nasal mucosa and/or the lungs. Colonization of the yeast in the upper respiratory tract may be significantly necessary to the encapsulated yeast cell to establish the disease before spreading to its target organs.