

# The Triple Threat of Cryptococcosis: It's the Body Site, the Strain, and/or the Host

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**ABSTRACT** Cryptococcosis is the leading invasive fungal infection in the world today. Over the past century, the causative agents, *Cryptococcus neoformans* and *Cryptococcus gattii*, have risen from the status of medical curiosities to common but life-threatening central nervous system pathogens. In an elegant experimental pathobiology study of these two organisms carried out by Ngamskulrungraj et al., there are three matters that merit further discussion. First is the question of whether there is a variable specific pathobiology for each yeast strain. Does it make biological and clinical sense to designate *C. neoformans* and *C. gattii* as two separate species? Second is the matter of how the organisms differ pathologically at the site of infection. Finally, there is the possibility that the human immune system responds differently to each species. Although no single study can provide definitive mechanistic answers to the important questions, this experimental pathology study and its discussion clearly frame the issues to be dissected.

In their study, “The primary target organ of *Cryptococcus gattii* is different from that of *Cryptococcus neoformans* in a murine model,” Ngamskulrungraj et al. (1) found that a *C. gattii* strain grew well in the murine lung but was inefficient at leaving the lung and producing disease in the brain. A *C. neoformans* strain, on the other hand, easily left its original site of infection and, with its known cerebral tropism, caused severe disease in the brains of mice. Mechanistically, the causes of these differences remain uncertain. This study clearly emphasizes that there are differences between these cryptococcal species despite their phylogenetic and phenotypic relatedness. Are the differences between *C. neoformans* and *C. gattii* significant, and how do these differences relate to the site of infection each favors and the human host immune responses they elicit?

As to the question of their differences, from a clinical standpoint, anything *C. neoformans* can do, *C. gattii* can do also. Their respective diseases overlap. However, the perception of many investigators and clinicians is that *C. gattii* infects primarily immunocompetent hosts whereas *C. neoformans* causes disease primarily in immunocompromised patients. Furthermore, *C. gattii* tends to produce larger granulomas or cryptococcomas in the lung and brain than does *C. neoformans*. Clinical differences between the two species' disease production are created by complex interactions influenced by the differences in ecology, epidemiology, biology of the strain, and host factors (2). The pathobiology of the two species reveals more differences. For example, the genomes of the two species show distinct sequence differences (3, 4) and molecular pathogenesis studies have revealed functional differences between similar genes in the two species. Disruption of the trehalose synthase gene (*tpsI*) in *C. neoformans* and *C. gattii*, for instance, creates null mutants that survive high mammalian temperatures and are avirulent in mammals, but a  $\Delta$ *tpsI* mutant of *C. gattii* had poor capsule and melanin formation, whereas a  $\Delta$ *tpsI* mutant of *C. neoformans* had no change in these prominent virulence factors (5). Hence, the genetic networks are not exactly the same.

Of course, the total virulence composites of individual strains of both *C. neoformans* and *C. gattii* are variable. In fact, *C. neoformans* strains and even *C. gattii* strains that are very closely related can have widely different transcriptional circuits and phenotypic

differences in virulence when carefully analyzed in controlled murine models of infection (6). Therefore, microevolution is clearly present in these two species, and even recently, recombinant strains within a species, such as the *C. gattii* Vancouver (genotype VGII) outbreak strains, have evolved into new ecological sites and changed their virulence composites (7). It is an important concept for all who study these yeasts to remember that the plasticity of the cryptococcal genome and its epigenetic factors can be measured on a real-time basis within strains and species. This adaptability helps to explain how they survive stresses. We are just beginning to see how this genetic variation or plasticity among and within cryptococcal strains occurs (8, 9) and the role that microevolution of disease plays in drug resistance (10, 11). Thus, it would be naive for us to completely disregard the basic biology of the two different species, but the differences between *C. neoformans* and *C. gattii* strains are moving targets. As this study demonstrates, the differences in virulence between species and strains are measurable but the stability of these differences and their relevance to clinical disease are less certain (12). At present, a clinician managing an individual case of cryptococcosis probably does not need to know the identity of the infecting species and the Infectious Diseases Society of America guidelines for cryptococcosis treatment are the same, regardless (13).

The second principle so elegantly described by this work is the importance of the site of infection. Since its first description in 1914, clinicians have known that *Cryptococcus* has a unique predisposition to invade the human central nervous system, and today it is responsible for an estimated one million cases of cryptococcal meningitis per year worldwide (14), making it a leading cause of meningitis. How it survives and migrates to sites of infection continues to fascinate and perplex pathobiologists, but molecular pathogenesis studies are beginning to provide insights into

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the sugar-coated yeast's *modus operandi*. Our group has shown that even yeast survival in the relatively benign cerebrospinal fluid is controlled by multiple genes, including *Ena1*, *Rub1*, and *Pik1* (15). Furthermore, the stressors provided by its immediate environment require it to utilize different pathways of metabolism. For instance, its use of fundamental processes of carbon and energy metabolism, glycolysis and gluconeogenesis, vary in their importance depending on whether it resides in the lung or the brain (16). Ngamskulrungrroj et al. observed differences in survival rates in blood between *C. neoformans* and *C. gattii*.

These yeasts must be able to live and grow within the harsh, inhospitable host environment as temperature, nutrition, and other innate factors work to eradicate it. Our clinical epidemiology studies have suggested that the two species prefer different sites of infection: *C. neoformans* more commonly causes disease in the central nervous system, and *C. gattii* more frequently produces pulmonary infections (17, 18). This predilection seems to be supported by the controlled study by Ngamskulrungrroj et al. However, it needs to be carefully emphasized that *C. gattii* can do anything that *C. neoformans* can do and they can both do it in the same populations and at the same infectious site. It would be wrong to categorize one species as a pulmonary pathogen and the other as a central nervous system pathogen or one species as the immunosuppressant organism and the other as the immunocompetent organism. Pathobiology is simply not that simple.

Finally, there is the question of how each species, *C. gattii* or *C. neoformans*, interacts with the human immune system, a question researchers have been addressing for the last 30 years (19). In fact, there has been great progress. On a simple basis, we understand the importance of cell-mediated host immunity through our clinical observations of risk groups such as AIDS patients, transplant recipients, and patients undergoing corticosteroid and anti-tumor necrosis factor antibody treatments and basic scientific studies that are validated by elegant and detailed immunological studies of both cell-mediated and humoral immunity. However, as this study clearly illustrates, our understanding remains imprecise despite these foundation studies and insights. The study by Ngamskulrungrroj et al. suggests that *C. gattii* produces an immune response that is different from and more protective against infection than that produced by *C. neoformans* (1). On the other hand, a recent study with a different set of mice and strains found that *C. neoformans* appeared to elicit a more robust and protective immune response than *C. gattii* (20). Determination of the details of how strains and, specifically, species, with their structurally different protective capsules and their ability to even produce different metabolites that directly impact immune cell responses, present themselves to the host (21) requires further studies for better insights. However, findings in this report challenge us to obtain a greater understanding of the complex interactions between hosts and yeasts at the site of infection.

After over a century of studying cryptococcosis as a fungal disease in humans, we have outstanding molecular tools, robust animal models, and basic understandings of pathology from both the yeast and host perspectives. However, we still do not have all the answers. From its humble beginning as an isolate obtained by Francesco Sanfelice in peach juice in 1894 and its link to a single case of human disease a year later, this yeast has evolved into a major human pathogen which kills over half a million individuals per year (14). Work to understand and control cryptococcosis

must continue. The sugar-coated yeast, whether it is *C. neoformans* or *C. gattii*, is flexible, complex, and deadly and deserves our respect and attention. To emphasize its mystery, perhaps rather than *Cryptococcus*, this “cryptic” yeast should be referred to as “Cryptic-coccus.” Many of its disease features are still hidden from us.

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