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The Thistle QA CEU No is: **MT-2015/009**.

Each attendee should claim **THREE** CEU points for completing this Quality Control Journal Club exercise, and retain a copy of the relevant Thistle QA Participation Certificate as proof of registration on a Thistle QA EQA.

MICROBIOLOGY LEGEND

CYCLE 37 ORGANISM 6

Cryptococcus neoformans

Cryptococcus neoformans is an encapsulated yeast that can live in both plants and animals. Its teleomorph (the sexual reproductive stage, typically a fruiting body) is *Filobasidiella neoformans*, a filamentous fungus belonging to the class Tremellomycetes.

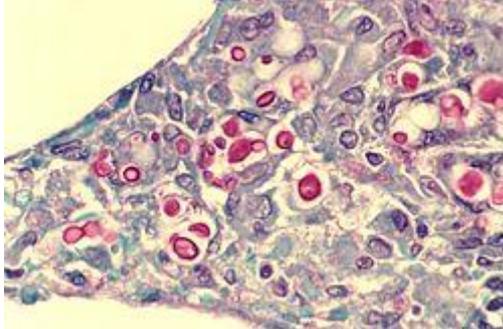
Classification

Cryptococcus neoformans is composed of two varieties (v.): *C. neoformans* v. *neoformans* and *C. n.* v. *grubii*. A third variety, *C. n.* v. *gattii*, is now considered a distinct species, *Cryptococcus gattii*. *C. n.* v. *grubii* and *C. n.* v. *neoformans* have a worldwide distribution and are often found in soil contaminated by bird excrement.

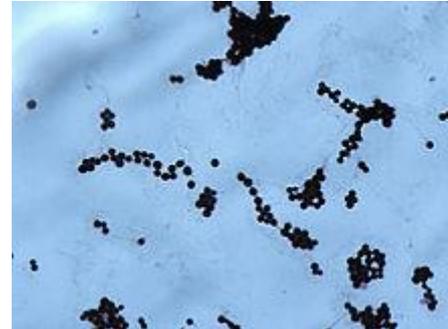
Characteristics

C. neoformans grows as a yeast (unicellular) and replicates by budding. It makes hyphae during mating, and eventually creates basidiospores at the end of the hyphae before producing spores. Under host-relevant conditions, including low glucose, serum, 5% carbon dioxide, and low iron, among others, the cells produce a characteristic polysaccharide capsule. The recognition of *C. neoformans* in Gram-stained smears of purulent exudates may be hampered by the presence of the large gelatinous capsule which apparently prevents definitive staining of the yeast-like cells. In such stained preparations, it may appear either as round cells with Gram-positive granular inclusions impressed upon a pale lavender cytoplasmic background or as Gram-negative lipid bodies.

When grown as a yeast, *C. neoformans* has a prominent capsule composed mostly of polysaccharides. Under the microscope, the India ink stain is used for easy visualization of the capsule in cerebral spinal fluid. The particles of ink pigment do not enter the capsule that surrounds the spherical yeast cell, resulting in a zone of clearance or "halo" around the cells. This allows for quick and easy identification of *C. neoformans*. Unusual morphological forms are rarely seen. For identification in tissue, mucicarmine stain provides specific staining of polysaccharide cell wall in *C. neoformans*. Cryptococcal antigen from cerebrospinal fluid is thought to be the best test for diagnosis of cryptococcal meningitis in terms of sensitivity, though it might be unreliable in HIV-positive patients.



C. neoformans seen in the lung of a patient with AIDS: The inner capsule of the organism stains red in this photomicrograph.



C. neoformans stained by Gram stain

Pathology

Infection with *C. neoformans* is termed cryptococcosis. Most infections with *C. neoformans* occur in the lungs. However, fungal meningitis and encephalitis, especially as a secondary infection for AIDS patients, are often caused by *C. neoformans*, making it a particularly dangerous fungus. Infections with this fungus are rare in those with fully functioning immune systems. So, *C. neoformans* is sometimes referred to as an opportunistic fungus. It is a facultative intracellular pathogen.

In human infection, *C. neoformans* is spread by inhalation of aerosolized basidiospores, and can disseminate to the central nervous system, where it can cause meningoencephalitis. In the lungs, *C. neoformans* cells are phagocytosed by alveolar macrophages. Macrophages produce oxidative and nitrosative agents, creating a hostile environment, to kill invading pathogens. However, some *C. neoformans* cells can survive intracellularly in macrophages. Intracellular survival appears to be the basis for latency, disseminated disease, and resistance to eradication by antifungal agents. One mechanism by which *C. neoformans* survives the hostile intracellular environment of the macrophage involves upregulation of expression of genes involved in responses to oxidative stress. Traversal of the blood–brain barrier by *C. neoformans* plays a key role in meningitis pathogenesis. However, precise mechanisms by which it passes the blood-brain barrier are still unknown.

Meiosis (sexual reproduction), another possible survival factor for intracellular *C. neoformans*. The vast majority of environmental and clinical isolates of *C. neoformans* are mating type a. Filaments of mating type a have haploid nuclei ordinarily, but these can undergo a process of diploidization (perhaps by endoduplication or stimulated nuclear fusion) to form diploid cells termed blastospores. The diploid nuclei of blastospores are able to undergo meiosis, including recombination, to form haploid basidiospores that can then be dispersed. This process is referred to as monokaryotic fruiting. Required for this process is a gene designated *dmc1*, a conserved homologue of genes *recA* in bacteria, and *rad51* in eukaryotes. *Dmc1* mediates homologous chromosome pairing during meiosis and repair of double-strand breaks in DNA. One benefit of meiosis in *C. neoformans* could be to promote DNA repair in the DNA-damaging environment caused by the oxidative and nitrosative agents produced in macrophages. Thus, *C. neoformans* can undergo a meiotic process, monokaryotic fruiting, that may promote recombinational repair in the oxidative, DNA-damaging environment of the host macrophage, and this may contribute to its virulence.

Treatment

Cryptococcosis that does not affect the central nervous system can be treated with fluconazole alone.

Cryptococcal meningitis should be treated for two weeks with intrathecal amphotericin B and oral or intravenous flucytosine. Intravenous amphotericin B may be used in patients who do not tolerate amphotericin B. In Africa, oral

fluconazole is often used. However, this does not result in cure, because it merely suppresses the fungus and does not kill it; viable fungus can continue to be grown from cerebrospinal fluid of patients not having taken fluconazole for many months.

References

1. http://en.wikipedia.org/wiki/Cryptococcus_neoformans

Questions

1. Discuss the morphological characteristics of *Cryptococcus neoformans*.
 2. Discuss the role of *Cryptococcus neoformans* in disease.
 3. Discuss the lab diagnosis of *Cryptococcus neoformans*.
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