Interaction of *Cryptococcus neoformans* and *Cryptococcus gattii* with the host immune system

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Cryptococcus neoformans and *Cryptococcus gattii* are the etiological agents of cryptococcal meningitis, a fatal disease if left untreated. *C. neoformans* is considered an opportunistic pathogen and causes disease in immunocompromised patients, while *C. gattii* is a primary pathogen that infects healthy individuals. The mechanisms underlying host preference are not yet understood. Infection with *C. neoformans* and *C. gattii* depends on host susceptibility, the virulence of the strain and host-pathogen interactions. The complex interaction of the host with the pathogen eventually decides if the inflammatory response will be more Th1 or Th2 balanced, which will directly influence clinical outcome. Differences between these two species are highlighted in this review, focusing on the differences in complement activation, neutrophil migration, intracellular parasitism and the response to anti-fungal treatment.

Introduction

Cryptococcus neoformans and *Cryptococcus gattii* are the etiological agents of cryptococcal meningitis, a fatal disease if left untreated. Cryptococcal disease is a leading cause of death among HIV-infected individuals (88, 172). Globally over 900 000 cases of cryptococcal meningitis are reported each year, causing over 600 000 deaths by three months after infection. The highest burden was seen in Sub Saharan Africa with approximately 720 000 cases each year (172). *C. neoformans* var. *grubii* is distributed worldwide and is responsible for 99% of cryptococcosis in HIV-infected individuals (157). *C. gattii* infection has been severely underestimated, until the recent outbreak on Vancouver Island that has been responsible for over 200 cases of cryptococcosis and 19 deaths in otherwise healthy humans and in hundreds of animals (74, 94). The epidemic is currently spreading into North America, where it is acknowledged as an emerging fungal disease (49). Both species cause similar disease, but *C. neoformans* is considered an opportunistic pathogen, while *C. gattii* is a putative primary pathogen infecting healthy hosts. *C. gattii* has been classified as a different species due to differences in biochemistry, epidemiology, ecology and genotype. This paper will review the differences in host-pathogen interactions between *C. neoformans* and *C. gattii*, which will decide the outcome of disease.

Taxonomy

Cryptococcus neoformans and *C. gattii* are part of a species complex responsible for cryptococcal disease, a fungal infection that can lead to fatal meningitis. The pathogenic basidiomycete yeast was first isolated from peach juice by Sanfelice in 1894 (194) and was simultaneously isolated from a patient in Germany (23, 24). The yeast was placed in the genus *Saccharomyces*, but in 1901 Vuillemin placed the species in the genus *Cryptococcus* (222). *C. neoformans* was considered a homogenous species until the discovery of four different serotypes, A, B, C and D. Serotype AD, a hybrid genotype between serotype A and D, can be distinguished as a fifth serotype. The serotypes are based on the antigenic properties of the capsule of the pathogen, characterising strains by specific glucuronxylomannan (GXM) structures. The heterogeneity of this species was further strengthened when Kwon-Chung et al. (119) first discovered the teleomorph (or perfect state) of *C. neoformans* named *Filobasidiella neoformans*, by crossing two serotype D strains, or a serotype A and D strain (119). The teleomorph created by crossing serotype B and C strains was found to be

phenotypically different from *F. neoformans* and was named *F. bacillispora* (120). Basidiospores from *F. neoformans* are spherical, oblong, elliptical or cylindrical with finely roughened walls, whereas *F. bacillispora* basidiospores are smoothly walled and bacilliform (26). The hyphae of *F. neoformans* produce hautorial branches, which are not seen in *F. bacillispora*. Kwon-Chung et al. (118) named the anamorph of *F. neoformans*, *C. neoformans* variety *neoformans*, and the anamorph of *F. bacillispora, C. bacillisporus* based on differences in biochemistry. Later *C. bacillisporus* was found to be a synonym for *C. neoformans* var. *gattii*, an atypical strain that was discovered in 1970 (222). In 2002, *C. gattii* was described as a distinct species based upon differences in ecology, epidemiology, pathobiology, biochemistry and genotype (103). *C. neoformans* and *C. gattii* belong to *Filobasidiella* clade of the order Tremellales of the Agaricomycetes (69, 90, 198, 233). Currently the genus *Cryptococcus* exists of at least 70 species that are spread throughout the world. Of these only two species are commonly known agents of cryptococcol meningitis, namely *C. neoformans* and *C. gattii*. However, cases exist that report human disease due to infection by cryptococcal species other than the two major pathogens and it is possible that individuals with compromised cellular immunity are vulnerable to infection by other cryptococcal species (110).

Attempts to subdivide the species in different varieties have been based on molecular and genetic typing using a variety of DNA genetic typing techniques, including polymerase chain reaction (PCR), fingerprinting (37, 206, 224), restriction fragment length polymorphism (RFLP) (209, 225), amplified fragment length polymorphism (AFLP) and multi locus sequence typing (MLST) (20, 41, 151). These different molecular typing techniques have led to the identification of concordant genotypes in *C. neoformans* and *C. gattii* (151). Eight major groups are now identified (see Table 1). Recently, new hybrids have been discovered between genotypic groups as well as between *C. neoformans* and *C. gattii* species (14, 18, 19, 22).

Species/ variety	**Serotype**	**Molecular type[1]**	**Genotype[2]**
C. neoformans var. *grubii*	A	VNI	AFLP1
	A	VNII	AFLP1B
	A	VNB	AFLP1A
AD Hybrid	AD	VNIII	AFLP3
C. neoformans var. *neoformans*	D	VNIV	AFLP2
C. gattii	B/C	VGI	AFLP4
	B/C	VGIII	AFLP5
	B/C	VGIV	AFLP7
	B/C	VGII	AFLP6

Table 1 Schematic overview of molecular type and genotype as found within the *C. neoformans*-*C. gattii* species complex. *C. neoformans* can be clearly divided into *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubbii*, a hybrid population is also present. *C. gattii* can be divided into four genetic groups, although these do not correspond to serotype. [1] (70, 152, 153) [2] (14, 136)

The genome of *C. neoformans* is estimated to be 15-27 Mb, and is larger then the genome of *C. gattii* which is estimated to be 12-18 MB (15). Karyotype analysis showed that

C. gattii has 13 chromosomes as well as a variable chromosome size (15, 138, 231). *C. neoformans* has twelve chromosomes.

DNA sequences of protein coding regions have been compared to reconstruct the evolutionary relationships between the two species. The differences between certain DNA regions can be indicative of the divergence of the two species. The rDNA repeat unit of *C. gattii* and *C. neoformans* appears to be highly conserved among the species and shows 99% homology (67, 69, 81). Fell et al. (69) analyzed the ITS (internal transcribed spacer) region of both species and two genetic identities were seen. Nakamura et al. (162) sequenced a genomic fragment of the CAP59 gene, a gene necessary for capsule formation, for al five serotypes, A, B, C, D and AD. They found 90% similarity between all strains, but the five serotypes could be separated from each other. Serotypes B and C were more closely related then serotype A and D (162). AFLP analyses by Boekhout et al. (14) again showed a clear separation between *C. neoformans* serotypes A, D, AD and *C. gattii* serotypes B and C. Hybrids were found within both clusters, but not between the two species, suggesting that there is no recombination between these species (14). However, Bovers et al.(21) have described the existence of clinical hybrid strains, BD and AC. This suggests that in nature mating does happen between *C. neoformans* and *C. gattii* (18, 21, 22).

Biochemistry

C. gattii and *C. neoformans* can be separated by phenotypic characteristics. In clinical settings light microscopy is an efficient method for rapid identification of infection with *C. neoformans* or *C. gattii*. Fluid samples obtained from patients, usually blood, CFS or urine, are stained with indian ink (94, 105). Cryptococcal cells generally appear as round or oval shaped cells, surrounded by a large polysaccharide capsule. India ink staining does not differentiate between *C. gattii* and *C. neoformans*, but *C. gattii* cells are more often elliptical or tear shaped then those observed for *C. neoformans* (122).

Both species grow as white-cream colored mucoid colonies on Saraboud dextrose agar, a medium that is commonly used in laboratories for the isolation of the yeast. *C. gattii* colonies have a more mucoid and sticky texture compared to colonies of *C. neoformans* grown on the same agar (124). Birdseed agar is used to distinguish *C. neoformans* and *C. gattii* from non-pathogenic cryptococcal species. *C. neoformans* and *C. gattii* produce melanin, a known virulence factor, causing a dark-brown coloration that can distinguish the pathogens from other fungi. The melanin produced by *C. gattii* is less intense compared to *C. neoformans* and is surrounded by a green hue (118). It must be noted that there are other species that also produce melanin, such as *Cryptococcus podzolicus* and *Cryptotrichosporon anacardii* (168, 177).

Biochemical differences between *C. gattii* and *C. neoformans* can more reliably distinguish between the two species. *C. gattii* reacts with cavanine-glycine-bromthymol blue (CGB) agar, turning the medium blue, while *C. neoformans* does not show any reaction. These differences are based upon differences in the ability of both species to utilize glycine as a sole source of nitrogen and carbon and their differences in susceptibility to L-canavanine (154, 180). *C. gattii* is naturally resistant to canavanine by a mechanism that metabolizes canavanine into a non-toxic product (180). The blue coloration is caused by alkalization of the medium, due to the release of ammonium during glycine degradation. *C. gattii* can utilize glycine as a sole source of carbon and nitrogen, *C. neoformans* cannot use this compound, thus suggesting a difference in nitrogen metabolism between *C. gattii* and *C. neoformans*.

Other species-related differences have been observed. *C. gattii* and not *C. neoformans* can use D-proline as the sole source of nitrogen (59, 165). *C. gattii* degrades creatine with creatine deaminase in the presence of ammonium, ammonium inhibits creatine degradation by the same enzyme in *C. neoformans* (154, 180). Bennet et al. (12)showed that the uptake of L-

malic acids is 10 times greater for *C. gattii* than for *C. neoformans* and assimilation of l-malic acid, fumaric acid, succinic acids is greater in *C. gattii* compared to *C. neoformans*. Another way of differentiation is a medium that effectively differentiates B and C serotypes from A and D serotypes on the basis of resistance to low concentrations of cycloheximide and assimilation of glycine (192).

Differences between serotypes have been described between the enzymes glucose-phosphate isomerase, phosphoglucosemutase (190), phenol oxidase (99), and the properties of Cu, Zn superoxide dismutase (87).

These differences have lead to the description of *C. gattii* as a separate species, and can help in identifying the two *Cryptococcus* species. However the wide variety of techniques that can accurately decipher genotypes are more specific then the biochemical differences and can therefore more accurately distinguish between the different varieties present in the *C. neoformans* and *C. gattii* species complex.

Ecology

Cryptococcus neoformans is distributed worldwide and has primarily been found in bird droppings and soil. *C. neoformans* has been isolated from guano of numerous avian species, including duck, parrot, peacock, owl, and caracara (25, 101, 139), but is most commonly isolated from pigeons droppings (10). It is not know if *C. neoformans* originates from the guano and enters the soil or is present in the soil and colonizes droppings. The fungus is more likely isolated from aged, dry pigeon guano and surrounding dust and soil, than it is from fresh, moist droppings. Dry guano will yield more viable cells compared to samples taken from fresh droppings (77, 188), which suggests that dried avian guano offers favourable conditions for the growth of the fungus. *C. neoformans* has less competition for growth and survival on dry excrement compared to moist excrement, where conditions are favourable for other fungi and bacteria (188). The primary niche of *C. neoformans* is most likely the soil itself, although researchers have speculated that the fungus is originally present in bird guano and amplifies when it comes in contact with exposed environment (133).

Birds are usually not affected by the yeast as the high body temperature of birds of 40 ºC does not favour growth (26). There are rare cases of birds that are infected with the fungus, among these are pigeons, parrots and kiwi's (35, 65, 71, 79, 91, 145, 183). This suggests that in spite of high body temperature, birds are not fully protected from the pathogen. If the pathogen does survive within the body of birds, this would explain why only *C. neoformans* is found in bird excrement and not *C. gattii*, which is more sensitive to temperature changes (175). *C. gattii* is know to infect parrots, but infection is usually limited to body surfaces and infection does not lead to disseminated cryptococcosis (145). Disseminated disease is seen in kiwi's, but these animals have a lower body temperature of 38 ºC, which may accommodate the growth of *C. gattii*.

There are rare occasions where *C. neoformans* has been isolated from the bodies of birds (186). Research has shown that the intestinal bacteria flora of pigeons inhibits growth of the pathogen (2). This makes it probable that bird guano enriches the soil and promotes growth of the fungus already present there and that the pigeon is only a carrier of the fungus, not a primary source. The pathogen has been cultured frequently from the beak, paws and feathers of birds (171) that have been contaminated with the fungus due to exposure to guano in their nests and surroundings. Many of the infections by *C. neoformans* of pigeons can be explained by pecking injuries, where infected beaks contacted the skin (145). The food of pigeons or housekept birds has not been reported to be infected by *C. neoformans*. No other primary source has been found that could lead to *C. neoformans* infection in birds, making it more likely that the original reservoir of *C. neoformans* is soil. Birds do play a role as a carrier, as guano attracts and promotes growth of the pathogen and this is transmitted by birds

on body surfaces. They can carry the yeast along long distances and interaction with other animals and humans can lead to infection. Zoonotic transmission from birds to human has been described (126, 204). The lack of *C. gattii* in the excrement of birds can be explained due to the high pH present in bird guano. *C. gattii* is more sensitive to these changes than *C. neoformans* (26).

Bird guano is the most commonly reported source for *C. neoformans*, but this is not the only ecological niche of this pathogen. *C. neoformans* has also been found in numerous fruits and vegetables such as mandarins, guava, melon, cauliflower, and stringbeans (139). The pathogen has also been isolated from decaying wood in the hollows of trees (80, 128, 129, 181, 182).

Cryptococcus gattii was originally believed to occur only in tropical and subtropical climates and was found to associate with *Eucalyptus camaldulensis* by Ellis and Pfeifer (63). Currently *C. gattii* has been associated with over 54 species of trees and the pathogen has been isolated from Australia, the United States, Africa, Europe and Asia (62, 80, 127, 178, 182, 205, 210). The spread of *C. gattii* throughout the world was thought to be due to the export of eucalyptus trees from Australia. However, now many more tree species have been associated with *C. gattii*, many of which are commonly exported for commercial use.

Botes et al. (16) have shown growth and survival of *C. neoformans* var. *grubii* on woody debris. It has been proposed that tree hollows containing dead wood debris are the primary ecological niche of *C. neoformans* and *C. gattii* as both species have been found here, individually or simultaneously present (129). *C. neoformans* and *C. gattii* are capable of producing the enzyme laccase, which is implicated in the degradation of lignin by wood-rotting fungi. It is not known if *Cryptococcus* species are capable of degrading wood by itself or if interaction with other microbes is necessary.

Epidemiology

The divergence of *C. gattii* and *C. neoformans* as two distinct species is likely due to the different ecological niche that each species occupies. There is little evidence of transmission of the pathogen between individuals and this has led researchers to believe that the main route of infection is by contact with environmental sources as well as routes of zoonotic transmission. This makes the natural habitat and geographical distribution of the fungus important in understanding and studying the epidemiology of cryptococcol meningitis.

C. neoformans var. *neoformans,* like *C. neoformans var. grubbii,* is distributed world wide, but is more frequently found in Europe (55, 121, 217, 227). *C. gattii* has high prevalence in geographically restricted regions and has originally been associated with tropical and subtropical areas and is endemic in Australia, Malaysia (216), Venezuela (28), Papua New Guinea (40, 199), South Africa (161), Brazil, Mexico (28) and Southern California (26). *C. gattii* has been associated with an outbreak among humans and animals in Vancouver Island, British Columbia, Canada (112, 144). Between 2002 and 2005 the incidence of *C. gattii* infection was 36 cases/million/year, a much higher number then the prevalence in endemic areas of Australia of 0.94 cases/million/year (112). This increased virulence in Vancouver Island has been associated with a recent recombination event. The outbreak is presently spreading to the pacific coast of North America (43, 211).

Cryptococcus gattii cases have also been reported in other temperate/Mediterranean climates, such as those in Europe (38, 66, 152, 226). Most isolates are serotype B. *C. gattii* infects predominantly immunocompetent individuals (33, 157, 207), although reports of *C. gattii* serotype C suggest a correlation between HIV-infected patients and infection with *C. gattii* genotype VGIV in certain regions of sub-Saharan Africa (135).

Geographical distribution and cryptococcosis incidence has been correlated with the flowering season of the *E. camaldulensis* in Australia (64). However, the flowering season of

the eucalyptus tree coincides with the rainy season in Australia and a study in Brazil has shown that occurrence of *C. gattii* is favoured in rainy months (77). This suggests that the rainy season, not the flowering season, may influence the incidence of *C. gattii*.

Infection and virulence factors

It is widely accepted that the port d'entrée of infection for the etiological agent of cryptococcosis is the lung (26). Due to ciliary function of the epithelium and airway turbulence in the lungs particles larger then 5 μm cannot enter the alveoli. Capsulated yeast cells are 4 to 20 μm in size, making it unlikely that these particles can enter the alveoli and cause infection (26, 133). It is thought that the infectious particles are basidiospores or desiccated yeast cells. Yeast cells that are deprived of nutrients and moist can lose their capsule, reducing their size to 5 μm and less (163). Aerisoled particles smaller than 5 μm have been isolated from the air and above bird excrements (163, 187). However, these cells display poor viability (189, 230) and basiodiospores have been shown to be more virulent in a mouse model (214). Basidiospores are approximately 2 μm in size and arise as a result of sexual reproduction between cells, or as the product of mono-karyotic fruiting between cells of the same mating type. These infectious particles have been isolated from the environment (63). As most environmental strains are type MATα, it is believed that most infectious particles are produced through mono-karyotic fruiting.

Cryptococcus neoformans and *C. gattii* have several virulence factors in common that set them apart from other fungi. *C. neoformans* and *C. gattii* are the only fungi in the order Tremellales that are able to grow at high temperatures between 37-39 ºC. This enables the yeast to survive in the mammalian body, temperature sensitive strains are no longer virulent (175). The main virulence factor of C. *neoformans* is the polysaccharide capsule. The capsule consist of 88% GXM and galactoxylomannan (GalXM) and some mannoproteins (235). These capsule proteins are shedded and can be found in the body fluid of infected patients (158). The capsule protects the pathogen against phagocytosis by inhibiting phagocytosis in the absence of opsonins (113, 143). Mutant strains lacking a capsule are avirulent in mouse models and capsular pathogens are not as easily phagocytosed by phagocytic cells as acapsular strains (30, 73). Furthermore the capsule can have strong immunomodulatory effects and promotes survival within the host (235). Shedded polysaccharide can have immunomodulatory effects and high levels of capsule polysaccharide antigen in the cerebral spinal fluid (CSF) can change the osmolarity, leading to increased intra cranial pressure, headaches and visual disturbance (46). The capsule is not strictly needed for virulence as acapsular strains can be virulent, but only in severely immunocompromised mouse models (193).

Melanin is another important factor for virulence. Melanin is produced by laccase in the presence of catecholamines such as dopamine, present in the brain (26). *C. neoformans* and *C. gattii* recovered from brain tissue of infected patients have been show to be melanised (166). Melanin protects the pathogen from ultraviolet radiation and it can protect the fungus from toxic free radicals produced by the host (143). Mutant strains that are not capable of producing melanin have been shown to be less virulent (27) and less susceptible to antifungal drugs (221). Melanised cells are more resistant to phagocytosis and cell death caused by phagocytic effector cells (96). However there are several non-pathogenic *Cryptococcus* species that also produce melanin (26), although these species do not prosper at 37 ˚C.

Urease catalyses the hydrolysis of urea to ammonia and carbamate. In certain bacteria, such as *Helicobacter pylori*, urease has proven to be a significant virulence factor (26). Cox et al. (39) disrupted the urease gene in *C. neoformans* and showed that mice injected with the mutant strains had a prolonged survival time. A similar study by Osterholzer et al. (170) showed a 100-fold increase in fungal burden after two weeks in mice infected with a urease

producing strain compared to infection with a non-urease producing mutant strain. Furthermore, it was found that mice infected with an urease producing strains displayed a potent, non-protective, Th2 response and promoted the accumulation of immature dendritic cells (170) Urease negative strains lead to much lower fungal loads in the brain, indicating that urease has an important role in dissemination to the brain. Urease promotes accumulation in organs with close capillary beds, such as the brain. This suggests that urease promotes central nervous system (CNS) invasion by enhancing yeast sequestration within microcapillary beds during hematogenous spread, thereby facilitating blood-to-brain invasion by *C. neoformans* (169). Urease production is significantly higher in *C. neoformans* strains, compared to *C. gattii* strains (125, 218), although exceptions exist as not al *C. neoformans* strains produce urease (26).

More research is necessary to compare the different clinical manifestations of *C. neoformans* and *C. gattii* in healthy individuals, to asses whether the decreased urease production observed in *C. gattii* results in decreased predilection for the CNS and, secondly if *C. neoformans* infections leads to a more Th2 balanced immune response compared to *C. gattii* infection. The interaction between virulence factors and host immunity is complicated and is most likely influenced by many factors.

Both *C. neoformans* and *C. gattii* are capable of phenotypic switching. The cryptococcal cells can switch from a smooth colony to a more virulent mucoid colony. The pathogen changes the polysaccharide capsule and the cell wall, in order to escape the immune system and adapt to the host environment (105). For example only smooth colonies could be grown from brain homogenates of infected mice, presumable because the smooth variant is better equipped to cross the blood-brain barrier (105). Mucoid colonies on the other hand have a thicker layer of capsule causing enhanced intracellular survival in the lungs (105). Smooth and mucoid colonies elicit different immune responses leading to differences in virulence. Guerrero and Fries (82) found that interleukin 10 levels are higher in the alveoli of mice infected with smooth colonies compared to mucoid colonies. Furthermore Guerrero and Jain (83) indicate that mucoid, but not smooth colony infection in mice is associated with the emergence of Th17 cells and higher levels of interleukin 17 in lung tissue. They show that there is a difference in macrophage activation between smooth and mucoid colonies of C. *neoformans*. Infection with mucoid colony cells leads to activation of macrophages that play a significant role in maintaining damage promoting inflammation in the lung (83).

Jain et al. (104) review the differences between phenotypic switching in *C. neoformans* and *C. gattii*. *C. gattii* is primarily found in mucoid colony morphology, where *C. neoformans* displays smooth colony morphology. Cells from mucoid colonies tend to be more resistant to phagocytoses and increase intracellular survival of the pathogen (104). 2008.

Pathogenesis

Fungal infection by *Cryptococcus* starts in the lung, where a primary pulmonary lymph node complex is formed. The disease process can occur in two ways, as symptomatic disease or asymptomatic disease, where the yeast dies or remains latent until reactivation occurs due to an event that weakens the immune system. It is believed that the normal response of an immunocompetent host is in most cases effective in containing the fungus (26). There are relatively few cases of cryptococcosis, although there is a high risk of exposure and studies have shown that there is a high frequency of infection as most children carry antibodies against *C. neoformans* (200). Studies have shown that antibodies against the GXM of *C. neoformans* are present in a majority of children above 2 years of age, with and without clinical symptoms (1, 76). This suggests that infection is quite common, but is usually cleared by the immune system or remains dormant.

Dormant infection of *C. neoformans* was investigated by Garcia-Hermoso et al (75). They analyzed clinical isolates of French patients using RAPD patterns and found that the strains from nine African patients that had moved from Africa to France more than 10 years ago, differed from those seen in 17 patients that originally came from France. The patients who had lived in Africa were infected prior to moving to Europe, meaning that they were infected long before clinical manifestation of infection (75). Other cases have been published were patients have been exposed to infection with *C. neoformans* and *C. gattii* long before the onset of disease (17, 58, 85). A Dutch tourist was diagnosed with infection of *C. gattii*, the strain was identical to the highly virulent strain found in the Vancouver island outbreak in British Colombia (85). The patient had visited Vancouver island a year before and had just undergone her first corticosteroid treatment for systemic lupus erythematosus. As the Vancouver island outbreak strains have not been found anywhere outside America, it is reasonable to believe that the patient was infected during her visit to Canada and the infection remained dormant until corticosteroid therapy. These results suggest caution for patients treated with corticosteroid or other immune suppressive drugs in areas endemic for *C. gattii*. Acute infection with either species of *Cryptococcus* can occur when immunocompromised patients are exposed to large numbers of cryptococcal cells (167).

When infection is symptomatic it can cause pulmonary symptoms, or the fungus can disseminate to other sites of the body. Clinical symptoms of cryptococcosis disease can vary greatly due to the diversity in host response to the infection dependent on the immunological status and the virulence of the fungal strain involved. Clinical symptoms have been noted in many tissues such as the skin, eye, genitourinary tract, bones and joints, muscle, heart, gastrointestinal tract, breast, lymph nodes, thyroid and the adrenal gland (26). The most common symptoms however are seen in the lungs, as the primary site of infection, and the CNS (157). The yeast has a high predilection for the CNS, the exact mechanisms for this tropism are however not yet known. The fungus can multiply and survive under the hypoxic conditions in the brain and this preference may be due to the less aggressive immune system behind the blood brain barrier (26). Melanogenesis may play a role in this process as the neurotransmitters in the brain can serve as substrates for the production of melanin, a know virulence factor for *C. neoformans* (133). The brain is not the only organ that produces these factors, suggesting that there may be other mechanisms at work as well. Possibly there are receptors on neuronal cells that can specifically bind to the yeast cell. It has indeed been shown that *C. neoformans* can bind to the endothelial cells of brain capillary vessels as well as lung and glial cells (31, 148). The capsule seems to have a part in the adherence to glial monolayers as non-encapsulated yeasts show stronger adherence compared to encapsulated yeasts (149). Another factor that has been indicated in the CNS tropism of the yeast is the mating type. The alpha mating locus has been indicated as a important virulence factor (123). In congenic strains of *C. neoformans* var. *grubii* MATα strains show a stronger predilection to the CNS during co-infection with both MATa and MATα strains (164). Furthermore the promoter for α pheromone is induced during the proliverative stage of CSN infection, indicating the MATα locus as a factor in tropism (45).

Clinical manifestations

An intense granulatomous condition has been associated with the infection of immunocompetent hosts, as granuloma`s are considered the natural immune response to the pathogen. Clinical symptoms associated with cryptococcal infections of immunocompetent hosts are higher frequencies of cerebral involvement, hydrocephalus, papilledema, neurological sequelea and pulmonary disease, than seen in immunocomprimised hosts (207). Brain and lung cryptococcoma's are also more frequent in immunocompetent hosts, cryptococcoma's are granuloma`s associated with *Cryptococcus* infection and are absent in

immunocomprimised hosts due to the lack of a proper immune response (33). Furthermore, immonocompetent hosts tend to have a longer duration of disease and are more likely to need surgery compared to immunocomprimised hosts (207).

Immunocomprimised patients show a response with an absence of the typical granuloma formation, extensive capillary involvement, dissemination and minimal involvement of lymphocytes (203). There is also more extensive pulmonary disease seen in immunocompromised hosts then in otherwise healthy patients as well as a higher occurrence of meningitis (33, 207). These features are mostly explained due to the lack of a proper CD4+ T-cell response as reconstitution of CD4+ numbers by HAART therapy in AIDS patients can cause a change from massive capillary involvement in the disease to granoloma-like formations (26, 203).

Only few studies have focused on the differences in clinical outcome due to cryptococcal varieties, as most healthy patients are infected with *C. gattii* serotype B and most immunocomprimised patients are infected by *C. neoformans* var. *grubii*, serotype A. It is difficult to correlate strain variety to clinical symptoms, without looking at immune status as relatively few healthy individual are infected by *C. neoformans*, and equally few immunocompromised patients are infected by *C. gattii*. However, there are studies in Australia that have looked at healthy individuals infected with either *C. neoformans* or *C. gattii*. Australia is endemic for both cryptococcal species, making this comparison possible, however the group of *C. gattii* infected hosts tends to be overrepresented.

Cryptococcus gattii infection has been correlated with the occurrence of lung and brain cryptococcoma's, long disease combined with CNS infection, mass lesions in the brain, obstructive hydrocephalus, single or multiple mass lesions on a chest röntgenogram and a longer period of hospitalization compared to *C. neoformans* (33, 106, 156). The latter could be an effect of the occurrence of brain cryptococcoma, which tend to lead to more severe neurological infliction.

Immune response

The majority of the human population is able to clear or contain an infection with the causative agent of cryptococcosis. Clearance or containment of *C. neoformans* in immunocompetent people should therefore be relatively effective. After inhalation of the pathogen natural cellular defence mechanisms are activated, involving neutrophils and other phagocytic cells (84). These cells recruit a cell-mediated immune response involving T-cells, the main mediators of the clearance of the pathogen. A Th1-asociated immunune response has shown to be protective against cryptococcal infection, while a Th2-associated immune response is not protective (11, 93, 95). Mice lacking CD4+ T cells show an increased susceptibility to infection with *C. neoformans,* if compared to immunocompetent mice (29). This coincides with the fact that humans with immune deficiencies such as AIDS are also highly susceptible to the disease. Adoptive transfer of T-cells could confer immunity to *C. neoformans*, providing more evidence for the prominent role of this cell population (78, 132). CD4+ T-cells display a protective Th1 response involving cytokines such as TNFα and IFNγ. Neutrophils, eosinophils, Th1-associated classical activated macrophages and DC's are attracted to the site of infection. These cells contribute to the formation of giant cells and granuloma`s. Granuloma`s can contain the pathogen and are thought to be the primary immune response to infection in immunocompetent patients (26, 92, 203). CD8+ T-cells also appear crucial to a proper immune response, lack of these cells diminishes influx of phagocytotic cells and CD4+ T-cells. CD8+ T-cells secrete mainly IFNγ and IL-2, possibly attracting more CD4+ T-cells to the site of infection. The exact interactions between CD4+ and CD8+ T-cells are not clear (92, 97, 98). The balance between a protective Th1 and a non-

protective Th2 response, consisting of cytokines such as Il-4, Il-5, Il-10, is thought to be influenced by NKT cells and Gamma delta T-cells as well as B-cells (108).

Antibody mediated immunity has a role in the defence against cryptococcosis, although the role of antibodies is not as clear or as crucial as cell mediated immunity. Several studies have shown that B-cell deficient mice show no differences upon infection with *C. neoformans* (26). Antibodies against capsular polysaccharides and cryptococcal proteins are found in the serum of patients. The absence of antibodies correlates with a poorer prognosis, while the presence of antibodies is associated with enhanced recovery in patients presenting with meningitis (47). Antibody treatment promotes the formation of giant cells *in vivo* and mice treated with antibodies show a more intense granulomatous response, suggesting the presence of antibody enhances host resistance (195, 201). Furthermore, the presence of B-cells in SCID mice leads to an enhanced resistance and a lowered fungal burden in tissue (3). Patients with defects in antibody mediated cell immunity, such as a XID mutation or hyper IgM syndrome, are more susceptible to cryptococcal infection then immunocompetent patients, providing a role for B-cells and antibody mediated immunity in cryptococcosis (102, 107, 146, 212, 213, 215). Reports are, however, contradicting as antibodies have been found that are protective, prolong survival and decrease the organ burden, but disease enhancing and non-protective antibodies are also observed ((26, 179). B-cells and antibodies also have a role in directly modulating the immune response (185).

A study by Speed et al. (208) compared the antibody levels in serum of immunocompetent individuals who were infected by *C. gattii* or *C. neoformans*. The serum showed IgG levels that persisted for a long time and IgA levels that declined after two weeks. Patients infected with *C. gattii* had a higher prevalence of IgA and showed higher IgA antibody levels. It is possible that *C. gattii* is more immunogenic, but it is also conceivable that the onset of disease in *C. gattii* is more rapid and that the IgA antibodies do not have enough time to decline (208). The increased prevalence of cryptococcoma`s seen in patients infected with *C. gattii* could be explained by a more effective antibody mediated immune response.

Increased risk of cryptococcosis has been associated with immune status, sex and age. Children rarely develop cryptococcosis (26, 200), and disease occurs between 20-50 years old in HIV-infected people, while immunocompetent people ≥45 years of age have an increased risk of infection (86). Many studies have indicated that male sex is a risk factor for cryptococcosis (47, 55, 86), however this is not reflected in all studies (32).

In Australia an increased risk for cryptococcosis was found among aboriginal residents (26, 106). Racial susceptibility of aboriginal heritage in Australia is most likely due to geographic isolation of certain groups that coincide with increased exposure to the pathogen, such as seen in aboriginals that live in rural areas (33). Urban living aboriginals do not have an increased risk to *C. gattii*, as they do not have a greater risk of exposure. A decreased risk for *C. neoformans* infection was found for Africans (56), suggesting that genetic susceptibility to the antigen may play a role. Major histocompatibility complex (MHC)-dependent susceptibility to *C. neoformans* infection in mice has been demonstrated, suggesting the importance of MHC-genes in host resistance (147, 220).

Host immunity is a well-established risk factor most prominent in the occurrence of cryptococcosis among HIV-infected individuals. Other diseases that affect the immune status of the host have been related to an increased risk of cryptococcosis, such as lymphoproliferative disorders, cirrhosis and hypogammaglobulinemia (26). Most prevelant risk factors besides HIV are recipients of organ transplant and patients undergoing corticosteroid therapy (86).

Intracellular parasitism

Cryptococcus neoformans and *C. gattii* are considered facultative intracellular pathogens, as they can survive intracellularly *in vivo* (9, 196) and *in vitro*. The important role of alveolar macrophages for a proper immune response has already been reported (202). *C. neoformans* is protected from phagocytocis by macrophages *in vitro* in the absence of opsonins (68, 115, 116). However in the presence of opsonins, the pathogen is readily phagocytosed (68).

The pathogen can survive and proliferate within infected cells. Once ingested by the macrophage, the phagosome becomes permeable and provides the pathogen with access to the nutrients in the cytoplasm. The pathogen then replicates by budding and an accumulation of polysaccharide-containing vesicles is observed. Intracellular replication eventually leads to rupture of the host cell, releasing the pathogen into the extracellular milieu (5, 219). A novel mechanism has been observed by which the pathogen can be expulsed from the macrophage by extrusion of the phagosome, the pathogen and the macrophage will both survive (6, 140) This could be a way for the pathogen to survive without killing the host cells and recruiting an immune response. Lee et al. (130) show that *C. neoformans* survives in microglia and replicates in spacious phagocytes, but when ingested by close fitting macrophages it doesn't replicate and is extruded. This process could be mediated by the polysaccharide capsule as this diffuses within the phagosome in spacious phagocytes, while it remains compact and homogeneous in close fitting phagocytes (130). In a study by Alverez et al. (6) *C. neoformans* var. *grubii*, *C. neoformans* var. *neoformans* and *C. gattii* were all analysed for phagosomal extrusion and survival of the host cells. This was observed for all strains, but was found to be more prevalent in *C. gattii*. Whether this is associated with clinical outcome should be investigated further (6).

Another option via which the pathogen can escape the immune system is by spreading from cell to cell. Macrophages fuse and the pathogen enters a previously uninfected macrophage without entering the extracellular environment (7, 141). Large vacuoles remain in the empty macrophages, which are most likely lethal (7).

The fungal outbreak on Vancouver Island has been going on since 1999 and is now spreading to the mainland (43, 211). The Vancouver Island outbreak is caused by a rare genotype VFII/AFLP6, but similar strains have been found in other parts of the world, suggesting that a recent event in the Vancouver Island area may be responsible for the increased virulence seen in this strain (111, 112). To investigate the cause of hyper virulence among the VIO strains compared to other VGII/AFLP6 strains found in other parts of the world, Ma et al. (142) found a significant correlation between intracellular parasitism of the VIO strain and other strains of the same genotype. The observed increased proliferation rate found in murine macrophages as well as human macrophages, was not due to over expression of any know virulence factors such as described earlier, but was found to be associated with an increase in mitochondrial gene regulation and a change in mitochondrial morphology. A tubular mitochondrial morphology was correlated with increased proliferation rates. Tubular mitochondria are thought to be the result of mitochondrial fusion, a protective response that allows rapid intracellular growth, thus enhancing virulence.

Ma et al. (142) found a strong correlation between the intracellular proliferation rate and mean survival time of mice. This further indicates the importance of intracellular parasitism on the virulence of *Cryptococcus*. This correlation was found in both species *C. neoformans* and *C. gattii*. In fact, *C. neoformans* strains had a high proliferation rate compared to non-VIO *C. gattii* strains. Unfortunately, mitochondrial gene expression and changes in mitochondrial morphology were not investigated in *C. neoformans* strains.

Complement activation

Complement factors play an important role in the natural cellular immune response to infection by *Cryptococcus*. The polysaccharide capsule activates compliment via the

alternative pathway. Complement factor C3b/iC3b binds to the capsule and acts as a potent opsonin for phagocytotic cells. The classical pathway is activated by antibodies and acts primarily through activation of the alternative pathway as demonstrated by Diamond et al. (48). The lectin binding pathway, the alternative pathway as well as the classical pathway are all blocked in mice that are deficient in late complement factors. Mershon et al. (150) have shown that these mice have a lower rate of survival compared to mice lacking Factor B. Mice lacking Factor B are unable to activate the alternative pathway, but can still activate the lectin binding pathway (150). This suggests that the alternative pathway is not the only pathway involved in protection against *Cryptoccoccus*. *C. neoformans* secretes mannoproteins that can be recognized by Mannose Binding Lectin and activate the lectin binding complement pathway (131). The efficiency of the opsonin capacity depends not only on activation of the alternative complement pathway by the capsule, but also on the presence of cytokines that upregulate the efficiency of complement mediated phagocytosis by phagocytic cells and the availability of phagocytic cells with complement receptors. Genetically deficient mice for the fifth component of complement, C5, an important factor in the activation of the classical, as well as the alternative pathway, are more susceptible to infection by *C. neoformans* than healthy mice as are guinea pigs depleted of late complement factors involved in the alternative pathway (48, 57, 184). Complement factor is important in clearance of fungi from extraneuronal sites, but not from the CNS. Once the disease disseminates to the brain complement factor could not lower fungal burden. In human spinal fluid no opsonization has been observed, suggesting low complement activity in the central nervous system, possibly contributing to the predilection of *C. neoformans* and *C. gattii* for the CNS (48).

Early reports by Washburn et al. (228) state that *C. neoformans* serotypes A and D bind three times more C3b molecules per surface unit compared to *C. gattii* serotypes B and C. C3b is bound to the capsule of cryptococci which mainly exists of GXM, with minor contribution of GalXM and MP. GXM consists of a mannose main chain and each serotype differs in the degree of xylose, glucuronic acid and O-acetyl substitution. Washburn at el. ascribed the differences in binding of C3b to *C. neoformans* and *C. gattii* to these differences in side chain substitution. The active C3 thioester group can efficiently bind to mannose present in the GXM core. Possibly the addition of side chain substitutions can cause hindrance and complicate the binding of C3b. Serotype B and C have more extensive xylose substitution, possibly causing the difference seen in binding kinetics. Later reports by Young et al. (234), however, show that there is no difference among serotypes in the maximum amount of C3 fragments that can bind to the capsule, but, rather a difference was noted in the rate of accumulation of C3 onto the capsule. *C. neoformans* serotypes A and D have a higher rate of accumulation then *C. gattii* serotypes B and C. The result by Washburn et al.(228) is due to a shorter measurement, not allowing maximum deposition of C3 unto the capsule of serotypes B and C. Possibly the extensive xylose substitutons of serotypes B and C on GXM slow the accumulation process of C3 fragments (191). Sahu et al. (191) demonstrate that the attachment efficiency of C3b strongly depends on the xylose content. They found that serotype A and D, with respectively one and two xylose residues branching out, bind C3b slower and to a lesser extent if compared to serotypes B and C, with respectively three and four xylose residues. This contradicts previous findings, where serotypes B and C bind C3b slower in less or equal amounts.

Kozel et al. (114) used *C. neoformans* serotype D mutant strains to determine the effect of differences in O-acytelation and single xylose side chains on the mannose backbone of GXM. Xylose negative mutants could bind C3b and showed a more rapid accumulation when compared to xylose positive mutants. This indicates that the binding of C3 fragments is not dependent on xylose residues as suggested in earlier reports by Sahu et al. (191). Possibly, the xylose substitutions increase the efficiency with which Factor H can interact with Factor I

on the cryptococcal capsule, as these two factors are important for the conversion of C3b in iC3b, which is abundantly present on the capsule. The slower rate of accumulation however does not influence the efficiency of phagocytosis by neutrophils.

O-acytelation had little or no effect on complement activation and is not required for inhibition of neutrophils. O-acytelation did have an effect on the binding of antibodies to the capsule. It seems to influence the ability of antibodies to crosslink at the surface of the capsule, without directly inhibiting the binding of antibody to the capsule. The xylose substitution did have an impact on the clearance of GXM from the spleen, as xylose negative GXM appeared to accumulate in the spleen. O-acetyl negative GXM was cleared faster from the serum and showed lower levels in the liver when compared to O-acetyl positive GXM.

These results do not explain the observation that xylose-negative strains were avirulent compared to xylose-positive strains, where o-acetyl negative strains showed increased virulence (114, 116, 117, 191). These observations suggest that the GMX structure influences the virulence of the pathogen in different ways that are not yet understood.

Neutrophil migration

C. neoformans mainly infects people with a defect in their immune system, thus given this opportunistic pathogen a clear pass to infect the lungs and disseminate to the CNS and other body sites. *C. gattii* however mainly infects immunocompetent people and to do this it must be able to surpass the immune defences of the body. Culture filtrate of *C. gattii* has been proposed as a novel anti-inflammatory compound due to its immunosuppressive abilities (155). This suggest that there must be differences in the interaction of *C. gattii* and *C. neoformans* with the host defence system. It has been reported that neutrophil migration and function is impaired in infection with *C. gattii*. Dong and Murphy (52) show that *C. neoformans* serotype A stimulates neutrophil migration *in vitro* and *in vivo*. Mice were injected with gelatinous sponges that contained culture filtrate antigens from *C. neoformans* serotype A or *C. gattii* serotype B or C. After six hours neutrophils, monocytes and lymphocytes were harvested after treatment with antigen from *C. neoformans*. This was not the case for *C. gattii* serotypes B and C, where more lymphocytes were found, but less neutrophils. This was in agreement with the finding that *C. gattii* serotype B and C inhibited *in vitro* migration of neutrophils to known chemo-attractants and inhibited random movement of neutrophils. Inhibition of neutrophil migration into sites of infection happens at different stages of migration. The GXM capsule polysaccharide has direct chemoattracting activity that can affect the ability of neutrophils to respond to other signals (51). Furthermore, interference with chemokinesis and downregulation of chemokine receptors by the capsule can interfere with a proper response of leukocytes to chemoattractants (36, 134, 159). GXM can also affect the attachment of leukocytes to the endothelium and the migration towards the site of inflammation. GXM has been shown to inhibit the adhesion of neutrophils to activated endothelial cells, by affecting both neutrophils and endothelial cells. GXM induces L-selectin shedding on leukocytes (50, 53, 54), thus inhibiting extravasation. Rolling on the endothelium is inhibited by GXM, probably through interference with E-selecing binding (60, 61).

Dong and Murphy (52) suggested that due to inhibition of neutrophil migration to the lungs *C. gattii* infection could not be contained in an early stage, possibly providing an explanation why *C. neoformans* is cleared in immunocompetent hosts unlike *C. gattii*. The capsular polysaccharide GXM was found responsible for the chemotactic event in *C. neoformans* serotype A, and it is possible that the structural differences in GXM that categorise the serotypes, are responsible for the different effects of *C. gattii* and *C. neoformans* on neutrophil migration (52). Later research by Wright et al. (232) did not report a difference in neutrophil infiltration in the lungs of mice infected with either *C. neoformans* or *C. gattii*. They observed an inhibition of neutrophil function at the site of infection, which

could result in the survival of extracellular yeast, leading to the formation of cryptococcoma's, which are seen more commonly with *C. gattii* infection. Furthermore, they showed differences in the metabolite concentrations secreted by both species. *C. neoformans* has higher concentrations of metabolites, most prominently ethanol and acetate. This is reflected in a lower pH in cryptococcal supernatant from *C. neoformans*. Two compounds were found exclusively in *C. gattii* infection, namely acetoin and dihydroxyacetome. These metabolites are thought to be part of a stress response (232). A more recent study observed lower levels of neutrophil infiltration in the lungs of mice infected with *C. gattii* together with a reduced production of inflammatory cytokines, mainly IFNγ and TNFα (34). Cheng et al. (34) speculated that the early neutrophil response is impaired due to inhibition in neutrophil migration. Because the early neutrophil response plays an important role in the induction of cell mediated immunity, only a weak Th1 response is mounted (34). A weak Th1 response has been associated with a reduced survival time (8). *C. gattii* inhibits proper migration of neutrophils and impairs neutrophil function leading to a weaker inflammatory response. This could be a factor in the mechanisms responsible for the preference of *C. gattii* for immunocompetent host.

In short, immunogenic differences exist between *C. neoformans* and *C. gattii*, but the underlying mechanism of these differences are not completely understood yet.

Therapy

Pulmonary cryptococcosis can be controlled by the host immune response, but once the yeast has disseminated to the brain, infection is uniformly lethal. Reports from Zimbabwe indicate that the median survival from diagnosis to death is 14 days in untreated HIV-related disease and cryptococcal meningitis, and only 22% of the patients survive for longer then 30 days without treatment (89). Treatment of fungal pathogens is difficult, due to the many similarities between the cellular machinery of fungi and humans. Amphotericin B (AMB) is the main treatment of choice and often complemented with flucytosine (229). Azoles, such as fluconazole and itraconazole, are also frequently used in the management of cryptococcosis. Fluconazole effectively penetrates the CSF and can suppress infection in AIDS patients. Fluconazole is often used for longer courses of treatment, such as in the case for patients with AIDS for whom treatment is considered indefinite (176). Resistance to fluconazole has been reported, especially in parts of Africa were fluconazole is often used as primairy therapy for cryptococcosis. In a study in Cape Town, South Africa, Bicanic et al. (13) described 32 cases of relapse out of 27 patients that originally presented with cryptococcol meningitis and were treated with fluconazole as initial treatment. Sixty seven percent of the culture-positive relapses were associated with isolates that had reduced susceptibility to fluconazole (13, 174). An increased resistance to fluconazole has been associated with *C. gattii* (109, 174). Sixty clinical isolates form AIDS patients were tested for resistance to AMB, itraconazole and fluconazole. None were resistant to AMB or itraconazole, one out of 56 *C. neoformans* strains was found resistant to fluconazole, while 3 out 4 *C. gattii* strains were resistant to this drug (174). Drug resistance is not a problem for first line therapy with AMB and flucytosine. AMB resistance strains have been detected (44), but in general resistance to AMB is rare (197, 223). New drug development has led to the discovery of new azoles such as voriconazol, posonazol, albaconazol, that are safer and more active against *Cryptococcus* (72, 160). No resistance has been found for these drugs, but resistance should be monitored when these drugs will be used by a broader population (160). MIC (minimum inhibitory concentration) values for several azoles, especially fluconazole, were found to be higher in *C. gattii* strains then in *C. neoformans* strains (44, 174). Patients infected with *C. gattii* do not respond as well to treatment as patients infected with *C. neoformans* ((173, 207). This difference could be explained due to the lower susceptibility to antifungal treatment seen for strains of *C. gattii*.

Iqbal et al. (100) investigated the susceptibility of the strains involved in the ongoing *C. gattii* outbreak on Vancouver Island and found a significant correlation between subtype and MIC values. VGI and VGIII had comparatively low MIC values, where VGII was found to be associated with high MIC values. Studies investigating the association of *in vitro* susceptibility and clinical outcome with treatment are contradictory and more research is needed to confirm if lower MIC values comply with good clinical outcome (4, 42).

Conclusion

Infection with *C. neoformans* and *C. gattii* is dependent on host susceptibility, the virulence of the strain and host-pathogen interactions. The complex interaction of the host with the pathogen eventually decides if the inflammatory response will be more Th1- or Th2 balanced, which will directly influence clinical outcome. Many factors play a part here and since the genome of *C. neoformans* and *C. gattii* are now available, more virulence factors are discovered (137). This is important for a better understanding of the pathogenesis of both species, that will lead to the discovery of effective prevention and treatment strategies needed to control the ongoing spread of *C. gattii* in Canada and the USA, as well as the containment of *C. neoformans* in countries were antiretroviral therapy is not readily available.

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References

1. **Abadi, J., and L. Pirofski.** 1999. Antibodies reactive with the cryptococcal capsular polysaccharide glucuronoxylomannan are present in sera from children with and without human immunodeficiency virus infection. J. Infect. Dis. **180:**915-919. doi: 10.1086/314953.
2. **Abou-Gabal, M., and M. Atia.** 1978. Study of the role of pigeons in the dissemination of *Cryptococcus neoformans* in nature. Sabouraudia. **16:**63-68.
3. **Aguirre, K. M., and L. L. Johnson.** 1997. A role for B cells in resistance to *Cryptococcus neoformans* in mice. Infect. Immun. **65:**525-530.
4. **Aller, A. I., E. Martin-Mazuelos, F. Lozano, J. Gomez-Mateos, L. Steele-Moore, W. J. Holloway, M. J. Gutierrez, F. J. Recio, and A. Espinel-Ingroff.** 2000. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. Antimicrob. Agents Chemother. **44:**1544-1548.
5. **Alvarez, M., T. Burn, Y. Luo, L. A. Pirofski, and A. Casadevall.** 2009. The outcome of *Cryptococcus neoformans* intracellular pathogenesis in human monocytes. BMC Microbiol. **9:**51. doi: 10.1186/1471-2180-9-51.
6. **Alvarez, M., and A. Casadevall.** 2006. Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. Curr. Biol. **16:**2161-2165. doi: 10.1016/j.cub.2006.09.061.
7. **Alvarez, M., and A. Casadevall.** 2007. Cell-to-cell spread and massive vacuole formation after *Cryptococcus neoformans* infection of murine macrophages. BMC Immunol. **8:**16. doi: 10.1186/1471-2172-8-16.
8. **Aratani, Y., F. Kura, H. Watanabe, H. Akagawa, Y. Takano, A. Ishida-Okawara, K. Suzuki, N. Maeda, and H. Koyama.** 2006. Contribution of the myeloperoxidase-dependent oxidative system to host defence against *Cryptococcus neoformans*. J. Med. Microbiol. **55:**1291-1299. doi: 10.1099/jmm.0.46620-0.
9. **Baker, R. D., and R. K. Haugen.** 1955. Tissue changes and tissue diagnosis in cryptococcosis; a study of 26 cases. Am. J. Clin. Pathol. **25:**14-24.
10. **Barreto de Oliveira, M. T., T. Boekhout, B. Theelen, F. Hagen, F. A. Baroni, M. S. Lazera, K. B. Lengeler, J. Heitman, I. N. Rivera, and C. R. Paula.** 2004. *Cryptococcus neoformans* shows a remarkable genotypic diversity in Brazil. J. Clin. Microbiol. **42:**1356-1359.
11. **Beenhouwer, D. O., S. Shapiro, M. Feldmesser, A. Casadevall, and M. D. Scharff.** 2001. Both Th1 and Th2 cytokines affect the ability of monoclonal antibodies to protect mice against *Cryptococcus neoformans*. Infect. Immun. **69:**6445-6455. doi: 10.1128/IAI.69.10.6445-6455.2001.
12. **Bennett, J. E., K. J. Kwon-Chung, and T. S. Theodore.** 1978. Biochemical differences between serotypes of *Cryptococcus neoformans*. Sabouraudia. **16:**167-174.
13. **Bicanic, T., T. Harrison, A. Niepieklo, N. Dyakopu, and G. Meintjes.** 2006. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. Clin. Infect. Dis. **43:**1069-1073. doi: 10.1086/507895.
14. **Boekhout, T., B. Theelen, M. Diaz, J. W. Fell, W. C. Hop, E. C. Abeln, F. Dromer, and W. Meyer.** 2001. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. Microbiology. **147:**891-907.
15. **Boekhout, T., A. van Belkum, A. C. Leenders, H. A. Verbrugh, P. Mukamurangwa, D. Swinne, and W. A. Scheffers.** 1997. Molecular typing of *Cryptococcus neoformans*: taxonomic and epidemiological aspects. Int. J. Syst. Bacteriol. **47:**432-442.

16. **Botes, A., T. Boekhout, F. Hagen, H. Vismer, J. Swart, and A. Botha.** 2009. Growth and mating of *Cryptococcus neoformans* var. *grubii* on woody debris. Microb. Ecol. **57:**757-765. doi: 10.1007/s00248-008-9452-1.
17. **Bottone, E. J., P. A. Kirschner, and I. F. Salkin.** 1986. Isolation of highly encapsulated *Cryptococcus neoformans* serotype B from a patient in New York City. J. Clin. Microbiol. **23:**186-188.
18. **Bovers, M., F. Hagen, E. Kuramae, H. Hoogveld, F. Dromer, G. St-Germain, and T. Boekhout.** 2007. Promiscuous mating of *Cryptococcus neoformans* and *Cryptococcus gattii*: discovery of a novel AB hybrid. PhD Thesis: Chapter 2. .
19. **Bovers, M., F. Hagen, and T. Boekhout.** 2008. Diversity of the *Cryptococcus neoformans-Cryptococcus gattii* species complex. Rev. Iberoam. Micol. **25:**S4-12.
20. **Bovers, M., F. Hagen, E. E. Kuramae, and T. Boekhout.** 2008. Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. Fungal Genet. Biol. **45:**400-421. doi: 10.1016/j.fgb.2007.12.004.
21. **Bovers, M., F. Hagen, E. E. Kuramae, M. R. Diaz, L. Spanjaard, F. Dromer, H. L. Hoogveld, and T. Boekhout.** 2006. Unique hybrids between the fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*. FEMS Yeast Res. **6:**599-607. doi: 10.1111/j.1567-1364.2006.00082.x.
22. **Bovers, M., F. Hagen, E. E. Kuramae, H. L. Hoogveld, F. Dromer, G. St-Germain, and T. Boekhout.** 2008. AIDS patient death caused by novel *Cryptococcus neoformans* x C. gattii hybrid. Emerg. Infect. Dis. **14:**1105-1108.
23. **Buschke, A.** 1895. Über eine durch Coccidien Hervergerufene Krankheitdes menschen. Dtsch. Med. Wochenschr. 21:14.
24. **Busse, O.** 1894. Über parasitäre zelleinschlüsse und ihre züchtung. Zentralbl. Bakteriol. **16:**180.
25. **Caicedo, L. D., M. I. Alvarez, M. Delgado, and A. Cardenas.** 1999. *Cryptococcus neoformans* in bird excreta in the city zoo of Cali, Colombia. Mycopathologia. **147:**121-124.
26. **Casadevall A, and Perfect JR.** 1998. *Cryptococcus neoformans*. ASM Press.
27. **Casadevall, A., A. L. Rosas, and J. D. Nosanchuk.** 2000. Melanin and virulence in *Cryptococcus neoformans*. Curr. Opin. Microbiol. **3:**354-358.
28. **Castanon-Olivares, L. R., R. Arreguin-Espinosa, G. Ruiz-Palacios y Santos, and R. Lopez-Martinez.** 2000. Frequency of Cryptococcus species and varieties in Mexico and their comparison with some Latin American countries. Rev. Latinoam. Microbiol. **42:**35-40.
29. **Cauley, L. K., and J. W. Murphy.** 1979. Response of congenitally athymic (nude) and phenotypically normal mice to *Cryptococcus neoformans* infection. Infect. Immun. **23:**644-651.
30. **Chang, Y. C., and K. J. Kwon-Chung.** 1994. Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. Mol. Cell. Biol. **14:**4912-4919.
31. **Chang, Y. C., M. F. Stins, M. J. McCaffery, G. F. Miller, D. R. Pare, T. Dam, M. Paul-Satyaseela, K. S. Kim, and K. J. Kwon-Chung.** 2004. Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier. Infect. Immun. **72:**4985-4995. doi: 10.1128/IAI.72.9.4985-4995.2004.
32. **Chen, J., A. Varma, M. R. Diaz, A. P. Litvintseva, K. K. Wollenberg, and K. J. Kwon-Chung.** 2008. *Cryptococcus neoformans* strains and infection in apparently immunocompetent patients, China. Emerg. Infect. Dis. **14:**755-762.
33. **Chen, S., T. Sorrell, G. Nimmo, B. Speed, B. Currie, D. Ellis, D. Marriott, T. Pfeiffer, D. Parr, and K. Byth.** 2000. Epidemiology and host- and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Australasian Cryptococcal Study Group. Clin. Infect. Dis. **31:**499-508. doi: 10.1086/313992.

34. **Cheng, P. Y., A. Sham, and J. W. Kronstad.** 2009. *Cryptococcus gattii* isolates from the British Columbia cryptococcosis outbreak induce less protective inflammation in a murine model of infection than *Cryptococcus neoformans*. Infect. Immun. **77:**4284-4294. doi: 10.1128/IAI.00628-09.
35. **Clipsham, R. C., and J. O. Britt Jr.** 1983. Disseminated cryptococcosis in a macaw. J. Am. Vet. Med. Assoc. **183:**1303-1305.
36. **Coenjaerts, F. E., A. M. Walenkamp, P. N. Mwinzi, J. Scharringa, H. A. Dekker, J. A. van Strijp, R. Cherniak, and A. I. Hoepelman.** 2001. Potent inhibition of neutrophil migration by cryptococcal mannoprotein-4-induced desensitization. J. Immunol. **167:**3988-3995.
37. **Cogliati, M., M. Allaria, A. M. Tortorano, and M. A. Viviani.** 2000. Genotyping *Cryptococcus neoformans* var. *neoformans* with specific primers designed from PCR-fingerprinting bands sequenced using a modified PCR-based strategy. Med. Mycol. **38:**97-103.
38. **Colom, M. F., S. Frases, C. Ferrer, A. Jover, M. Andreu, S. Reus, M. Sanchez, and J. M. Torres-Rodriguez.** 2005. First case of human cryptococcosis due to *Cryptococcus neoformans* var. gattii in Spain. J. Clin. Microbiol. **43:**3548-3550. doi: 10.1128/JCM.43.7.3548-3550.2005.
39. **Cox, G. M., J. Mukherjee, G. T. Cole, A. Casadevall, and J. R. Perfect.** 2000. Urease as a virulence factor in experimental cryptococcosis. Infect. Immun. **68:**443-448.
40. **Currie, B., T. Vigus, G. Leach, and B. Dwyer.** 1990. *Cryptococcus neoformans* var *gattii*. Lancet. **336:**1442.
41. **Currie, B. P., L. F. Freundlich, and A. Casadevall.** 1994. Restriction fragment length polymorphism analysis of *Cryptococcus neoformans* isolates from environmental (pigeon excreta) and clinical sources in New York City. J. Clin. Microbiol. **32:**1188-1192.
42. **Dannaoui, E., M. Abdul, M. Arpin, A. Michel-Nguyen, M. A. Piens, A. Favel, O. Lortholary, F. Dromer, and French Cryptococcosis Study Group.** 2006. Results obtained with various antifungal susceptibility testing methods do not predict early clinical outcome in patients with cryptococcosis. Antimicrob. Agents Chemother. **50:**2464-2470. doi: 10.1128/AAC.01520-05.
43. **Datta, K., K. H. Bartlett, R. Baer, E. Byrnes, E. Galanis, J. Heitman, L. Hoang, M. J. Leslie, L. MacDougall, S. S. Magill, M. G. Morshed, K. A. Marr, and *Cryptococcus gattii* Working Group of the Pacific Northwest.** 2009. Spread of *Cryptococcus gattii* into Pacific Northwest region of the United States. Emerg. Infect. Dis. **15:**1185-1191.
44. **De Bedout, C., N. Ordonez, B. L. Gomez, M. C. Rodriguez, M. Arango, A. Restrepo, and E. Castaneda.** 1999. In vitro antifungal susceptibility of clinical isolates of *Cryptococcus neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. Rev. Iberoam. Micol. **16:**36-39.
45. **del Poeta, M., D. L. Toffaletti, T. H. Rude, S. D. Sparks, J. Heitman, and J. R. Perfect.** 1999. *Cryptococcus neoformans* differential gene expression detected in vitro and in vivo with green fluorescent protein. Infect. Immun. **67:**1812-1820.
46. **Denning, D. W., R. W. Armstrong, B. H. Lewis, and D. A. Stevens.** 1991. Elevated cerebrospinal fluid pressures in patients with cryptococcal meningitis and acquired immunodeficiency syndrome. Am. J. Med. **91:**267-272.
47. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis. A study in 111 cases. Ann. Intern. Med. **80:**176-181.
48. **Diamond, R. D., J. E. May, M. Kane, M. M. Frank, and J. E. Bennett.** 1973. The role of late complement components and the alternate complement pathway in experimental cryptococcosis. Proc. Soc. Exp. Biol. Med. **144:**312-315.

49. **Dixit, A., S. F. Carroll, and S. T. Qureshi.** 2009. *Cryptococcus gattii*: An Emerging Cause of Fungal Disease in North America. Interdiscip. Perspect. Infect. Dis. **2009:**840452. doi: 10.1155/2009/840452.
50. **Dong, Z. M., L. Jackson, and J. W. Murphy.** 1999. Mechanisms for induction of L-selectin loss from T lymphocytes by a cryptococcal polysaccharide, glucuronoxylomannan. Infect. Immun. **67:**220-229.
51. **Dong, Z. M., and J. W. Murphy.** 1993. Mobility of human neutrophils in response to *Cryptococcus neoformans* cells, culture filtrate antigen, and individual components of the antigen. Infect. Immun. **61:**5067-5077.
52. **Dong, Z. M., and J. W. Murphy.** 1995. Effects of the two varieties of *Cryptococcus neoformans* cells and culture filtrate antigens on neutrophil locomotion. Infect. Immun. **63:**2632-2644.
53. **Dong, Z. M., and J. W. Murphy.** 1996. Cryptococcal polysaccharides induce L-selectin shedding and tumor necrosis factor receptor loss from the surface of human neutrophils. J. Clin. Invest. **97:**689-698. doi: 10.1172/JCI118466.
54. **Dong, Z. M., and J. W. Murphy.** 1997. Cryptococcal polysaccharides bind to CD18 on human neutrophils. Infect. Immun. **65:**557-563.
55. **Dromer, F., S. Mathoulin, B. Dupont, and A. Laporte.** 1996. Epidemiology of cryptococcosis in France: a 9-year survey (1985-1993). French Cryptococcosis Study Group. Clin. Infect. Dis. **23:**82-90.
56. **Dromer, F., S. Mathoulin, B. Dupont, L. Letenneur, and O. Ronin.** 1996. Individual and environmental factors associated with infection due to *Cryptococcus neoformans* serotype D. French Cryptococcosis Study Group. Clin. Infect. Dis. **23:**91-96.
57. **Dromer, F., C. Perronne, J. Barge, J. L. Vilde, and P. Yeni.** 1989. Role of IgG and complement component C5 in the initial course of experimental cryptococcosis. Clin. Exp. Immunol. **78:**412-417.
58. **Dromer, F., O. Ronin, and B. Dupont.** 1992. Isolation of *Cryptococcus neoformans* var. *gattii* from an Asian patient in France: evidence for dormant infection in healthy subjects. J. Med. Vet. Mycol. **30:**395-397.
59. **Dufait, R., R. Velho, and C. De Vroey.** 1987. Rapid identification of the two varieties of *Cryptococcus neoformans* by D-proline assimilation. Mykosen. **30:**483.
60. **Ellerbroek, P. M., A. I. Hoepelman, F. Wolbers, J. J. Zwaginga, and F. E. Coenjaerts.** 2002. Cryptococcal glucuronoxylomannan inhibits adhesion of neutrophils to stimulated endothelium in vitro by affecting both neutrophils and endothelial cells. Infect. Immun. **70:**4762-4771.
61. **Ellerbroek, P. M., L. H. Ulfman, A. I. Hoepelman, and F. E. Coenjaerts.** 2004. Cryptococcal glucuronoxylomannan interferes with neutrophil rolling on the endothelium. Cell. Microbiol. **6:**581-592. doi: 10.1111/j.1462-5822.2004.00384.x.
62. **Ellis, D., and T. Pfeiffer.** 1992. The ecology of *Cryptococcus neoformans*. Eur. J. Epidemiol. **8:**321-325.
63. **Ellis, D. H., and T. J. Pfeiffer.** 1990. Ecology, life cycle, and infectious propagule of *Cryptococcus neoformans*. Lancet. **336:**923-925.
64. **Ellis, D. H., and T. J. Pfeiffer.** 1990. Natural habitat of *Cryptococcus neoformans* var. *gattii*. J. Clin. Microbiol. **28:**1642-1644.
65. **Ensley, P. K., C. E. Davis, M. P. Anderson, and K. C. Fletcher.** 1979. Cryptococcosis in a male Beccari's crowned pigeon. J. Am. Vet. Med. Assoc. **175:**992-994.
66. **Escandon, P., A. Sanchez, M. Martinez, W. Meyer, and E. Castaneda.** 2006. Molecular epidemiology of clinical and environmental isolates of the *Cryptococcus neoformans* species complex reveals a high genetic diversity and the presence of the

molecular type VGII mating type a in Colombia. FEMS Yeast Res. **6:**625-635. doi: 10.1111/j.1567-1364.2006.00055.x.
67. **Fan, M., B. P. Currie, R. R. Gutell, M. A. Ragan, and A. Casadevall.** 1994. The 16S-like, 5.8S and 23S-like rRNAs of the two varieties of *Cryptococcus neoformans*: sequence, secondary structure, phylogenetic analysis and restriction fragment polymorphisms. J. Med. Vet. Mycol. **32:**163-180.
68. **Feldmesser, M., S. Tucker, and A. Casadevall.** 2001. Intracellular parasitism of macrophages by *Cryptococcus neoformans*. Trends Microbiol. **9:**273-278.
69. **Fell, J. W., T. Boekhout, A. Fonseca, G. Scorzetti, and A. Statzell-Tallman.** 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int. J. Syst. Evol. Microbiol. **50 Pt 3:**1351-1371.
70. **Feng, X., Z. Yao, D. Ren, and W. Liao.** 2008. Simultaneous identification of molecular and mating types within the Cryptococcus species complex by PCR-RFLP analysis. J. Med. Microbiol. **57:**1481-1490. doi: 10.1099/jmm.0.2008/003665-0.
71. **Fenwick, B., K. Takeshita, and A. Wong.** 1985. A moluccan cockatoo with disseminated cryptococcosis. J. Am. Vet. Med. Assoc. **187:**1218-1219.
72. **Fera, M. T., E. La Camera, and A. De Sarro.** 2009. New triazoles and echinocandins: mode of action, in vitro activity and mechanisms of resistance. Expert Rev. Anti Infect. Ther. **7:**981-998. doi: 10.1586/eri.09.67.
73. **Fromtling, R. A., H. J. Shadomy, and E. S. Jacobson.** 1982. Decreased virulence in stable, acapsular mutants of *Cryptococcus neoformans*. Mycopathologia. **79:**23-29.
74. **Galanis, E., and L. Macdougall.** 2010. Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999-2007. Emerg. Infect. Dis. **16:**251-257.
75. **Garcia-Hermoso, D., G. Janbon, and F. Dromer.** 1999. Epidemiological evidence for dormant *Cryptococcus neoformans* infection. J. Clin. Microbiol. **37:**3204-3209.
76. **Goldman, D. L., H. Khine, J. Abadi, D. J. Lindenberg, L. Pirofski, R. Niang, and A. Casadevall.** 2001. Serologic evidence for *Cryptococcus neoformans* infection in early childhood. Pediatrics. **107:**E66.
77. **Granados, D. P., and E. Castaneda.** 2005. Isolation and characterization of *Cryptococcus neoformans* varieties recovered from natural sources in Bogota, Colombia, and study of ecological conditions in the area. Microb. Ecol. **49:**282-290. doi: 10.1007/s00248-004-0236-y.
78. **Graybill, J. R., and L. Mitchell.** 1979. Host defense in Cryptococcosis. III. In vivo alteration of immunity. Mycopathologia. **69:**171-178.
79. **Griner, L. A., and H. A. Walch.** 1978. Cryptococcosis in columbiformes at the San Diego Zoo. J. Wildl. Dis. **14:**389-394.
80. **Grover, N., S. R. Nawange, J. Naidu, S. M. Singh, and A. Sharma.** 2007. Ecological niche of *Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii* in decaying wood of trunk hollows of living trees in Jabalpur City of Central India. Mycopathologia. **164:**159-170. doi: 10.1007/s11046-007-9039-2.
81. **Gueho, E., L. Improvisi, R. Christen, and G. S. de Hoog.** 1993. Phylogenetic relationships of *Cryptococcus neoformans* and some related basidiomycetous yeasts determined from partial large subunit rRNA sequences. Antonie Van Leeuwenhoek. **63:**175-189.
82. **Guerrero, A., and B. C. Fries.** 2008. Phenotypic switching in *Cryptococcus neoformans* contributes to virulence by changing the immunological host response. Infect. Immun. **76:**4322-4331. doi: 10.1128/IAI.00529-08.
83. **Guerrero, A., N. Jain, X. Wang, and B. C. Fries.** 2010. *Cryptococcus neoformans* variants generated by phenotypic switching differ in virulence through effects on macrophage activation. Infect. Immun. **78:**1049-1057. doi: 10.1128/IAI.01049-09.

84. **Guillot, L., S. F. Carroll, R. Homer, and S. T. Qureshi.** 2008. Enhanced innate immune responsiveness to pulmonary *Cryptococcus neoformans* infection is associated with resistance to progressive infection. Infect. Immun. **76:**4745-4756. doi: 10.1128/IAI.00341-08.
85. **Hagen, F., S. V. Assen, G. J. Luijckx, T. Boekhout, and G. A. Kampinga.** 2009. Activated dormant *Cryptococcus gattii* infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach. Med. Mycol. . doi: 10.3109/13693780903300319.
86. **Hajjeh, R. A., L. A. Conn, D. S. Stephens, W. Baughman, R. Hamill, E. Graviss, P. G. Pappas, C. Thomas, A. Reingold, G. Rothrock, L. C. Hutwagner, A. Schuchat, M. E. Brandt, and R. W. Pinner.** 1999. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. Cryptococcal Active Surveillance Group. J. Infect. Dis. **179:**449-454. doi: 10.1086/314606.
87. **Hamilton, A. J., and M. D. Holdom.** 1997. Biochemical comparison of the Cu,Zn superoxide dismutases of *Cryptococcus neoformans* var. neoformans and *Cryptococcus neoformans* var. *gattii*. Infect. Immun. **65:**488-494.
88. **Harrison, T. S.** 2009. The burden of HIV-associated cryptococcal disease. AIDS. **23:**531-532. doi: 10.1097/QAD.0b013e328322ffc3.
89. **Heyderman, R. S., I. T. Gangaidzo, J. G. Hakim, J. Mielke, A. Taziwa, P. Musvaire, V. J. Robertson, and P. R. Mason.** 1998. Cryptococcal meningitis in human immunodeficiency virus-infected patients in Harare, Zimbabwe. Clin. Infect. Dis. **26:**284-289.
90. **Hibbett, D. S., M. Binder, J. F. Bischoff, M. Blackwell, P. F. Cannon, O. E. Eriksson, S. Huhndorf, T. James, P. M. Kirk, R. Lucking, H. Thorsten Lumbsch, F. Lutzoni, P. B. Matheny, D. J. McLaughlin, M. J. Powell, S. Redhead, C. L. Schoch, J. W. Spatafora, J. A. Stalpers, R. Vilgalys, M. C. Aime, A. Aptroot, R. Bauer, D. Begerow, G. L. Benny, L. A. Castlebury, P. W. Crous, Y. C. Dai, W. Gams, D. M. Geiser, G. W. Griffith, C. Gueidan, D. L. Hawksworth, G. Hestmark, K. Hosaka, R. A. Humber, K. D. Hyde, J. E. Ironside, U. Koljalg, C. P. Kurtzman, K. H. Larsson, R. Lichtwardt, J. Longcore, J. Miadlikowska, A. Miller, J. M. Moncalvo, S. Mozley-Standridge, F. Oberwinkler, E. Parmasto, V. Reeb, J. D. Rogers, C. Roux, L. Ryvarden, J. P. Sampaio, A. Schussler, J. Sugiyama, R. G. Thorn, L. Tibell, W. A. Untereiner, C. Walker, Z. Wang, A. Weir, M. Weiss, M. M. White, K. Winka, Y. J. Yao, and N. Zhang.** 2007. A higher-level phylogenetic classification of the Fungi. Mycol. Res. **111:**509-547. doi: 10.1016/j.mycres.2007.03.004.
91. **Hill, F. I., A. J. Woodgyer, and M. A. Lintott.** 1995. Cryptococcosis in a North Island brown kiwi (Apteryx australis mantelli) in New Zealand. J. Med. Vet. Mycol. **33:**305-309.
92. **Hill, J. O.** 1992. CD4+ T cells cause multinucleated giant cells to form around *Cryptococcus neoformans* and confine the yeast within the primary site of infection in the respiratory tract. J. Exp. Med. **175:**1685-1695.
93. **Hoag, K. A., M. F. Lipscomb, A. A. Izzo, and N. E. Street.** 1997. IL-12 and IFN-gamma are required for initiating the protective Th1 response to pulmonary cryptococcosis in resistant C.B-17 mice. Am. J. Respir. Cell Mol. Biol. **17:**733-739.
94. **Hoang, L. M., J. A. Maguire, P. Doyle, M. Fyfe, and D. L. Roscoe.** 2004. *Cryptococcus neoformans* infections at Vancouver Hospital and Health Sciences Centre (1997-2002): epidemiology, microbiology and histopathology. J. Med. Microbiol. **53:**935-940.
95. **Huffnagle, G. B.** 1996. Role of cytokines in T cell immunity to a pulmonary *Cryptococcus neoformans* infection. Biol. Signals. **5:**215-222.
96. **Huffnagle, G. B., G. H. Chen, J. L. Curtis, R. A. McDonald, R. M. Strieter, and G. B. Toews.** 1995. Down-regulation of the afferent phase of T cell-mediated pulmonary inflammation and immunity by a high melanin-producing strain of *Cryptococcus neoformans*. J. Immunol. **155:**3507-3516.

97. **Huffnagle, G. B., M. F. Lipscomb, J. A. Lovchik, K. A. Hoag, and N. E. Street.** 1994. The role of CD4+ and CD8+ T cells in the protective inflammatory response to a pulmonary cryptococcal infection. J. Leukoc. Biol. **55:**35-42.
98. **Huffnagle, G. B., J. L. Yates, and M. F. Lipscomb.** 1991. Immunity to a pulmonary *Cryptococcus neoformans* infection requires both CD4+ and CD8+ T cells. J. Exp. Med. **173:**793-800.
99. **Ikeda, R., and E. S. Jacobson.** 1992. Heterogeneity of phenol oxidases in *Cryptococcus neoformans*. Infect. Immun. **60:**3552-3555.
100. **Iqbal, N., E. E. DeBess, R. Wohrle, B. Sun, R. J. Nett, A. M. Ahlquist, T. Chiller, S. R. Lockhart, and *Cryptococcus gattii* Public Health Working Group.** 2010. Correlation of genotype and in vitro susceptibilities of *Cryptococcus gattii* strains from the Pacific Northwest of the United States. J. Clin. Microbiol. **48:**539-544. doi: 10.1128/JCM.01505-09.
101. **Irokanulo, E. O., A. A. Makinde, C. O. Akuesgi, and M. Ekwonu.** 1997. *Cryptococcus neoformans* var *neoformans* isolated from droppings of captive birds in Nigeria. J. Wildl. Dis. **33:**343-345.
102. **Iseki, M., M. Anzo, N. Yamashita, and N. Matsuo.** 1994. Hyper-IgM immunodeficiency with disseminated cryptococcosis. Acta Paediatr. **83:**780-782.
103. **J, K., BOEKHOUT Teun, F. W, and DIAZ Mara.** 2002. (1557) Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). **51:**3.
104. **Jain, N., and B. C. Fries.** 2008. Phenotypic switching of *Cryptococcus neoformans* and *Cryptococcus gattii*. Mycopathologia. **166:**181-188. doi: 10.1007/s11046-008-9137-9.
105. **Jain, N., L. Li, D. C. McFadden, U. Banarjee, X. Wang, E. Cook, and B. C. Fries.** 2006. Phenotypic switching in a *Cryptococcus neoformans* variety *gattii* strain is associated with changes in virulence and promotes dissemination to the central nervous system. Infect. Immun. **74:**896-903. doi: 10.1128/IAI.74.2.896-903.2006.
106. **Jenney, A., K. Pandithage, D. A. Fisher, and B. J. Currie.** 2004. Cryptococcus infection in tropical Australia. J. Clin. Microbiol. **42:**3865-3868. doi: 10.1128/JCM.42.8.3865-3868.2004.
107. **Jo, E. K., H. S. Kim, M. Y. Lee, M. Iseki, J. H. Lee, C. H. Song, J. K. Park, T. J. Hwang, and H. Kook.** 2002. X-linked hyper-IgM syndrome associated with Cryptosporidium parvum and *Cryptococcus neoformans* infections: the first case with molecular diagnosis in Korea. J. Korean Med. Sci. **17:**116-120.
108. **Kawakami, K.** 2004. Regulation by innate immune T lymphocytes in the host defense against pulmonary infection with *Cryptococcus neoformans*. Jpn. J. Infect. Dis. **57:**137-145.
109. **Khan, Z. U., H. S. Randhawa, W. Chehadeh, A. Chowdhary, T. Kowshik, and R. Chandy.** 2009. *Cryptococcus neoformans* serotype A and *Cryptococcus gattii* serotype B isolates differ in their susceptibilities to fluconazole and voriconazole. Int. J. Antimicrob. Agents. **33:**559-563. doi: 10.1016/j.ijantimicag.2008.11.007.
110. **Khawcharoenporn, T., A. Apisarnthanarak, and L. M. Mundy.** 2007. Non-*neoformans* cryptococcal infections: a systematic review. Infection. **35:**51-58. doi: 10.1007/s15010-007-6142-8.
111. **Kidd, S. E., H. Guo, K. H. Bartlett, J. Xu, and J. W. Kronstad.** 2005. Comparative gene genealogies indicate that two clonal lineages of *Cryptococcus gattii* in British Columbia resemble strains from other geographical areas. Eukaryot. Cell. **4:**1629-1638. doi: 10.1128/EC.4.10.1629-1638.2005.
112. **Kidd, S. E., F. Hagen, R. L. Tscharke, M. Huynh, K. H. Bartlett, M. Fyfe, L. Macdougall, T. Boekhout, K. J. Kwon-Chung, and W. Meyer.** 2004. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British

Columbia, Canada). Proc. Natl. Acad. Sci. U. S. A. **101:**17258-17263. doi: 10.1073/pnas.0402981101.
113. **Kozel, T. R., and E. C. Gotschlich.** 1982. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. J. Immunol. **129:**1675-1680.
114. **Kozel, T. R., S. M. Levitz, F. Dromer, M. A. Gates, P. Thorkildson, and G. Janbon.** 2003. Antigenic and biological characteristics of mutant strains of *Cryptococcus neoformans* lacking capsular O acetylation or xylosyl side chains. Infect. Immun. **71:**2868-2875.
115. **Kozel, T. R., G. S. Pfrommer, A. S. Guerlain, B. A. Highison, and G. J. Highison.** 1988. Role of the capsule in phagocytosis of *Cryptococcus neoformans*. Rev. Infect. Dis. **10 Suppl 2:**S436-9.
116. **Kozel, T. R., A. Tabuni, B. J. Young, and S. M. Levitz.** 1996. Influence of opsonization conditions on C3 deposition and phagocyte binding of large- and small-capsule *Cryptococcus neoformans* cells. Infect. Immun. **64:**2336-2338.
117. **Kozel, T. R., M. A. Wilson, G. S. Pfrommer, and A. M. Schlageter.** 1989. Activation and binding of opsonic fragments of C3 on encapsulated *Cryptococcus neoformans* by using an alternative complement pathway reconstituted from six isolated proteins. Infect. Immun. **57:**1922-1927.
118. **Kwon-Chung, K., J. Bennett, and T. Theodore.** 1978. *Cryptococcus bacillisporus* sp. nov.: serotype BC of *Cryptococcus neoformans*. Int J Syst Bacteriol. 616-620.
119. **Kwon-Chung, K. J.** 1975. A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. Mycologia. **67:**1197-1200.
120. **Kwon-Chung, K. J.** 1976. A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. Mycologia. **68:**943-946.
121. **Kwon-Chung, K. J., and J. E. Bennett.** 1984. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. Am. J. Epidemiol. **120:**123-130.
122. **Kwon-Chung, K. J., J. E. Bennett, and J. C. Rhodes.** 1982. Taxonomic studies on *Filobasidiella* species and their anamorphs. Antonie Van Leeuwenhoek. **48:**25-38.
123. **Kwon-Chung, K. J., J. C. Edman, and B. L. Wickes.** 1992. Genetic association of mating types and virulence in *Cryptococcus neoformans*. Infect. Immun. **60:**602-605.
124. **Kwon-Chung, K. J., and A. Varma.** 2006. Do major species concepts support one, two or more species within *Cryptococcus neoformans*? FEMS Yeast Res. **6:**574-587. doi: 10.1111/j.1567-1364.2006.00088.x.
125. **Kwon-Chung, K. J., B. L. Wickes, J. L. Booth, H. S. Vishniac, and J. E. Bennett.** 1987. Urease inhibition by EDTA in the two varieties of *Cryptococcus neoformans*. Infect. Immun. **55:**1751-1754.
126. **Lagrou, K., J. Van Eldere, S. Keuleers, F. Hagen, R. Merckx, J. Verhaegen, W. E. Peetermans, and T. Boekhout.** 2005. Zoonotic transmission of *Cryptococcus neoformans* from a magpie to an immunocompetent patient. J. Intern. Med. **257:**385-388. doi: 10.1111/j.1365-2796.2005.01466.x.
127. **Lazera, M. S., M. A. Cavalcanti, L. Trilles, M. M. Nishikawa, and B. Wanke.** 1998. *Cryptococcus neoformans* var. *gattii* evidence for a natural habitat related to decaying wood in a pottery tree hollow. Med. Mycol. **36:**119-122.
128. **Lazera, M. S., F. D. Pires, L. Camillo-Coura, M. M. Nishikawa, C. C. Bezerra, L. Trilles, and B. Wanke.** 1996. Natural habitat of *Cryptococcus neoformans* var. *neoformans* in decaying wood forming hollows in living trees. J. Med. Vet. Mycol. **34:**127-131.
129. **Lazera, M. S., M. A. Salmito Cavalcanti, A. T. Londero, L. Trilles, M. M. Nishikawa, and B. Wanke.** 2000. Possible primary ecological niche of *Cryptococcus neoformans*. Med. Mycol. **38:**379-383.
130. **Lee, S. C., Y. Kress, M. L. Zhao, D. W. Dickson, and A. Casadevall.** 1995. *Cryptococcus neoformans* survive and replicate in human microglia. Lab. Invest. **73:**871-879.

131. **Levitz, S. M.** 2002. Receptor-mediated recognition of *Cryptococcus neoformans*. Nippon. Ishinkin Gakkai Zasshi. **43:**133-136.
132. **Lim, T. S., and J. W. Murphy.** 1980. Transfer of immunity to cryptococcosis by T-enriched splenic lymphocytes from *Cryptococcus neoformans*-sensitized mice. Infect. Immun. **30:**5-11.
133. **Lin, X., and J. Heitman.** 2006. The biology of the *Cryptococcus neoformans* species complex. Annu. Rev. Microbiol. **60:**69-105. doi: 10.1146/annurev.micro.60.080805.142102.
134. **Lipovsky, M. M., G. Gekker, S. Hu, L. C. Ehrlich, A. I. Hoepelman, and P. K. Peterson.** 1998. Cryptococcal glucuronoxylomannan induces interleukin (IL)-8 production by human microglia but inhibits neutrophil migration toward IL-8. J. Infect. Dis. **177:**260-263.
135. **Litvintseva, A. P., R. Thakur, L. B. Reller, and T. G. Mitchell.** 2005. Prevalence of clinical isolates of *Cryptococcus gattii* serotype C among patients with AIDS in Sub-Saharan Africa. J. Infect. Dis. **192:**888-892. doi: 10.1086/432486.
136. **Litvintseva, A. P., R. Thakur, R. Vilgalys, and T. G. Mitchell.** 2006. Multilocus sequence typing reveals three genetic subpopulations of *Cryptococcus neoformans* var. *grubii* (serotype A), including a unique population in Botswana. Genetics. **172:**2223-2238. doi: 10.1534/genetics.105.046672.
137. **Liu, O. W., C. D. Chun, E. D. Chow, C. Chen, H. D. Madhani, and S. M. Noble.** 2008. Systematic genetic analysis of virulence in the human fungal pathogen *Cryptococcus neoformans*. Cell. **135:**174-188. doi: 10.1016/j.cell.2008.07.046.
138. **Loftus, B. J., E. Fung, P. Roncaglia, D. Rowley, P. Amedeo, D. Bruno, J. Vamathevan, M. Miranda, I. J. Anderson, J. A. Fraser, J. E. Allen, I. E. Bosdet, M. R. Brent, R. Chiu, T. L. Doering, M. J. Donlin, C. A. D'Souza, D. S. Fox, V. Grinberg, J. Fu, M. Fukushima, B. J. Haas, J. C. Huang, G. Janbon, S. J. Jones, H. L. Koo, M. I. Krzywinski, J. K. Kwon-Chung, K. B. Lengeler, R. Maiti, M. A. Marra, R. E. Marra, C. A. Mathewson, T. G. Mitchell, M. Pertea, F. R. Riggs, S. L. Salzberg, J. E. Schein, A. Shvartsbeyn, H. Shin, M. Shumway, C. A. Specht, B. B. Suh, A. Tenney, T. R. Utterback, B. L. Wickes, J. R. Wortman, N. H. Wye, J. W. Kronstad, J. K. Lodge, J. Heitman, R. W. Davis, C. M. Fraser, and R. W. Hyman.** 2005. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science. **307:**1321-1324. doi: 10.1126/science.1103773.
139. **Lopez-Martinez, R., and L. R. Castanon-Olivares.** 1995. Isolation of *Cryptococcus neoformans* var. *neoformans* from bird droppings, fruits and vegetables in Mexico City. Mycopathologia. **129:**25-28.
140. **Ma, H., J. E. Croudace, D. A. Lammas, and R. C. May.** 2006. Expulsion of live pathogenic yeast by macrophages. Curr. Biol. **16:**2156-2160. doi: 10.1016/j.cub.2006.09.032.
141. **Ma, H., J. E. Croudace, D. A. Lammas, and R. C. May.** 2007. Direct cell-to-cell spread of a pathogenic yeast. BMC Immunol. **8:**15. doi: 10.1186/1471-2172-8-15.
142. **Ma, H., F. Hagen, D. J. Stekel, S. A. Johnston, E. Sionov, R. Falk, I. Polacheck, T. Boekhout, and R. C. May.** 2009. The fatal fungal outbreak on Vancouver Island is characterized by enhanced intracellular parasitism driven by mitochondrial regulation. Proc. Natl. Acad. Sci. U. S. A. **106:**12980-12985. doi: 10.1073/pnas.0902963106.
143. **Ma, H., and R. C. May.** 2009. Virulence in Cryptococcus species. Adv. Appl. Microbiol. **67:**131-190. doi: 10.1016/S0065-2164(08)01005-8.
144. **MacDougall, L., S. E. Kidd, E. Galanis, S. Mak, M. J. Leslie, P. R. Cieslak, J. W. Kronstad, M. G. Morshed, and K. H. Bartlett.** 2007. Spread of *Cryptococcus gattii* in British Columbia, Canada, and detection in the Pacific Northwest, USA. Emerg. Infect. Dis. **13:**42-50.
145. **Malik, R., M. B. Krockenberger, G. Cross, R. Doneley, D. N. Madill, D. Black, P. McWhirter, A. Rozenwax, K. Rose, M. Alley, D. Forshaw, I. Russell-Brown, A. C.**

Johnstone, P. Martin, C. R. O'Brien, and D. N. Love. 2003. Avian cryptococcosis. Med. Mycol. **41:**115-124.
146. **Marquis, G., S. Montplaisir, M. Pelletier, S. Mousseau, and P. Auger.** 1985. Genetic resistance to murine cryptococcosis: increased susceptibility in the CBA/N XID mutant strain of mice. Infect. Immun. **47:**282-287.
147. **McClelland, E. E., D. L. Granger, and W. K. Potts.** 2003. Major histocompatibility complex-dependent susceptibility to *Cryptococcus neoformans* in mice. Infect. Immun. **71:**4815-4817.
148. **Merkel, G. J., and B. A. Scofield.** 1993. Conditions affecting the adherence of *Cryptococcus neoformans* to rat glial and lung cells in vitro. J. Med. Vet. Mycol. **31:**55-64.
149. **Merkel, G. J., and B. A. Scofield.** 1994. Comparisons between in vitro glial cell adherence and internalization of non-encapsulated and encapsulated strains of *Cryptococcus neoformans*. J. Med. Vet. Mycol. **32:**361-372.
150. **Mershon, K. L., A. Vasuthasawat, G. W. Lawson, S. L. Morrison, and D. O. Beenhouwer.** 2009. Role of complement in protection against *Cryptococcus gattii* infection. Infect. Immun. **77:**1061-1070. doi: 10.1128/IAI.01119-08.
151. **Meyer, W., D. M. Aanensen, T. Boekhout, M. Cogliati, M. R. Diaz, M. C. Esposto, M. Fisher, F. Gilgado, F. Hagen, S. Kaocharoen, A. P. Litvintseva, T. G. Mitchell, S. P. Simwami, L. Trilles, M. A. Viviani, and J. Kwon-Chung.** 2009. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. Med. Mycol. **47:**561-570. doi: 10.1080/13693780902953886.
152. **Meyer, W., A. Castaneda, S. Jackson, M. Huynh, E. Castaneda, and IberoAmerican Cryptococcal Study Group.** 2003. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg. Infect. Dis. **9:**189-195.
153. **Meyer, W., K. Marszewska, M. Amirmostofian, R. P. Igreja, C. Hardtke, K. Methling, M. A. Viviani, A. Chindamporn, S. Sukroongreung, M. A. John, D. H. Ellis, and T. C. Sorrell.** 1999. Molecular typing of global isolates of *Cryptococcus neoformans* var. *neoformans* by polymerase chain reaction fingerprinting and randomly amplified polymorphic DNA-a pilot study to standardize techniques on which to base a detailed epidemiological survey. Electrophoresis. **20:**1790-1799. doi: 2-2.
154. **Min, K. H., and K. J. Kwon-Chung.** 1986. The biochemical basis for the distinction between the two *Cryptococcus neoformans* varieties with CGB medium. Zentralbl. Bakteriol. Mikrobiol. Hyg. A. **261:**471-480.
155. **Mirshafiey, A., M. Chitsaz, M. Attar, F. Mehrabian, and A. R. Razavi.** 2000. Culture filtrate of *Cryptococcus neoformans* var. *gattii* (CneF) as a novel anti-inflammatory compound in the treatment of experimental septic arthritis. Scand. J. Immunol. **52:**278-284.
156. **Mitchell, D. H., T. C. Sorrell, A. M. Allworth, C. H. Heath, A. R. McGregor, K. Papanaoum, M. J. Richards, and T. Gottlieb.** 1995. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. Clin. Infect. Dis. **20:**611-616.
157. **Mitchell, T. G., and J. R. Perfect.** 1995. Cryptococcosis in the era of AIDS--100 years after the discovery of *Cryptococcus neoformans*. Clin. Microbiol. Rev. **8:**515-548.
158. **Monari, C., F. Bistoni, and A. Vecchiarelli.** 2006. Glucuronoxylomannan exhibits potent immunosuppressive properties. FEMS Yeast Res. **6:**537-542. doi: 10.1111/j.1567-1364.2006.00072.x.
159. **Monari, C., T. R. Kozel, F. Bistoni, and A. Vecchiarelli.** 2002. Modulation of C5aR expression on human neutrophils by encapsulated and acapsular *Cryptococcus neoformans*. Infect. Immun. **70:**3363-3370.

160. **Morera-Lopez, Y., J. M. Torres-Rodriguez, T. Jimenez-Cabello, and T. Baro-Tomas.** 2005. *Cryptococcus gattii*: in vitro susceptibility to the new antifungal albaconazole versus fluconazole and voriconazole. Med. Mycol. **43:**505-510.
161. **Morgan, J., K. M. McCarthy, S. Gould, K. Fan, B. Arthington-Skaggs, N. Iqbal, K. Stamey, R. A. Hajjeh, M. E. Brandt, and Gauteng Cryptococcal Surveillance Initiative Group.** 2006. *Cryptococcus gattii* infection: characteristics and epidemiology of cases identified in a South African province with high HIV seroprevalence, 2002-2004. Clin. Infect. Dis. **43:**1077-1080. doi: 10.1086/507897.
162. **Nakamura, Y., R. Kano, S. Watanabe, and A. Hasegawa.** 2000. Molecular analysis of CAP59 gene sequences from five serotypes of *Cryptococcus neoformans*. J. Clin. Microbiol. **38:**992-995.
163. **Neilson, J. B., R. A. Fromtling, and G. S. Bulmer.** 1977. *Cryptococcus neoformans*: size range of infectious particles from aerosolized soil. Infect. Immun. **17:**634-638.
164. **Nielsen, K., G. M. Cox, A. P. Litvintseva, E. Mylonakis, S. D. Malliaris, D. K. Benjamin Jr, S. S. Giles, T. G. Mitchell, A. Casadevall, J. R. Perfect, and J. Heitman.** 2005. *Cryptococcus neoformans* {alpha} strains preferentially disseminate to the central nervous system during coinfection. Infect. Immun. **73:**4922-4933. doi: 10.1128/IAI.73.8.4922-4933.2005.
165. **Nishikawa, M. M., O. D. Sant'Anna, M. S. Lazera, and B. Wanke.** 1996. Use of D-proline assimilation and CGB medium for screening Brazilian *Cryptococcus neoformans* isolates. J. Med. Vet. Mycol. **34:**365-366.
166. **Nosanchuk, J. D., A. L. Rosas, S. C. Lee, and A. Casadevall.** 2000. Melanisation of *Cryptococcus neoformans* in human brain tissue. Lancet. **355:**2049-2050. doi: 10.1016/S0140-6736(00)02356-4.
167. **Nosanchuk, J. D., S. Shoham, B. C. Fries, D. S. Shapiro, S. M. Levitz, and A. Casadevall.** 2000. Evidence of zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised patient. Ann. Intern. Med. **132:**205-208.
168. **Okoli, I., C. A. Oyeka, K. J. Kwon-Chung, B. Theelen, V. Robert, J. Z. Groenewald, D. C. McFadden, A. Casadevall, and T. Boekhout.** 2007. *Cryptotrichosporon anacardii* gen. nov., sp. nov., a new trichosporonoid capsulate basidiomycetous yeast from Nigeria that is able to form melanin on niger seed agar. FEMS Yeast Res. **7:**339-350. doi: 10.1111/j.1567-1364.2006.00164.x.
169. **Olszewski, M. A., M. C. Noverr, G. H. Chen, G. B. Toews, G. M. Cox, J. R. Perfect, and G. B. Huffnagle.** 2004. Urease expression by *Cryptococcus neoformans* promotes microvascular sequestration, thereby enhancing central nervous system invasion. Am. J. Pathol. **164:**1761-1771.
170. **Osterholzer, J. J., R. Surana, J. E. Milam, G. T. Montano, G. H. Chen, J. Sonstein, J. L. Curtis, G. B. Huffnagle, G. B. Toews, and M. A. Olszewski.** 2009. Cryptococcal urease promotes the accumulation of immature dendritic cells and a non-protective T2 immune response within the lung. Am. J. Pathol. **174:**932-943. doi: 10.2353/ajpath.2009.080673.
171. **Pal, M.** 1989. *Cryptococcus neoformans* var. *neoformans* and munia birds. Mycoses. **32:**250-252.
172. **Park, B. J., K. A. Wannemuehler, B. J. Marston, N. Govender, P. G. Pappas, and T. M. Chiller.** 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. **23:**525-530. doi: 10.1097/QAD.0b013e328322ffac.
173. **Peachey, P. R., P. O. Gubbins, and R. E. Martin.** 1998. The association between cryptococcal variety and immunocompetent and immunocompromised hosts. Pharmacotherapy. **18:**255-264.

174. **Peetermans, W., H. Bobbaers, J. Verhaegen, and J. Vandepitte.** 1993. Fluconazole-resistant *Cryptococcus neoformans* var *gattii* in an AIDS patient. Acta Clin. Belg. **48:**405-409.
175. **Perfect, J. R.** 2006. *Cryptococcus neoformans*: the yeast that likes it hot. FEMS Yeast Res. **6:**463-468. doi: 10.1111/j.1567-1364.2006.00051.x.
176. **Perfect, J. R., W. E. Dismukes, F. Dromer, D. L. Goldman, J. R. Graybill, R. J. Hamill, T. S. Harrison, R. A. Larsen, O. Lortholary, M. H. Nguyen, P. G. Pappas, W. G. Powderly, N. Singh, J. D. Sobel, and T. C. Sorrell.** 2010. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. Clin. Infect. Dis. **50:**291-322. doi: 10.1086/649858.
177. **Petter, R., B. S. Kang, T. Boekhout, B. J. Davis, and K. J. Kwon-Chung.** 2001. A survey of heterobasidiomycetous yeasts for the presence of the genes homologous to virulence factors of Filobasidiella neoformans, CNLAC1 and CAP59. Microbiology. **147:**2029-2036.
178. **Pfeiffer, T. J., and D. H. Ellis.** 1992. Environmental isolation of *Cryptococcus neoformans* var. *gattii* from Eucalyptus tereticornis. J. Med. Vet. Mycol. **30:**407-408.
179. **Pirofski, L. A., and A. Casadevall.** 1996. *Cryptococcus neoformans*: paradigm for the role of antibody immunity against fungi? Zentralbl. Bakteriol. **284:**475-495.
180. **Polacheck, I., and K. J. Kwon-Chung.** 1980. Creatinine metabolism in *Cryptococcus neoformans* and *Cryptococcus bacillisporus*. J. Bacteriol. **142:**15-20.
181. **Randhawa, H. S., T. Kowshik, and Z. U. Khan.** 2003. Decayed wood of Syzygium cumini and Ficus religiosa living trees in Delhi/New Delhi metropolitan area as natural habitat of *Cryptococcus neoformans*. Med. Mycol. **41:**199-209.
182. **Randhawa, H. S., T. Kowshik, K. Preeti Sinha, A. Chowdhary, Z. U. Khan, Z. Yan, J. Xu, and A. Kumar.** 2006. Distribution of *Cryptococcus gattii* and *Cryptococcus neoformans* in decayed trunk wood of Syzygium cumini trees in north-western India. Med. Mycol. **44:**623-630. doi: 10.1080/13693780600860946.
183. **Raso, T. F., K. Werther, E. T. Miranda, and M. J. Mendes-Giannini.** 2004. Cryptococcosis outbreak in psittacine birds in Brazil. Med. Mycol. **42:**355-362.
184. **Rhodes, J. C.** 1985. Contribution of complement component C5 to the pathogenesis of experimental murine cryptococcosis. Sabouraudia. **23:**225-234.
185. **Rivera, J., O. Zaragoza, and A. Casadevall.** 2005. Antibody-mediated protection against *Cryptococcus neoformans* pulmonary infection is dependent on B cells. Infect. Immun. **73:**1141-1150. doi: 10.1128/IAI.73.2.1141-1150.2005.
186. **Rosario, I., M. Hermoso de Mendoza, S. Deniz, G. Soro, I. Alamo, and B. Acosta.** 2005. Isolation of Cryptococcus species including *C. neoformans* from cloaca of pigeons. Mycoses. **48:**421-424. doi: 10.1111/j.1439-0507.2005.01153.x.
187. **Ruiz, A., and G. S. Bulmer.** 1981. Particle size of airborn *Cryptococcus neoformans* in a tower. Appl. Environ. Microbiol. **41:**1225-1229.
188. **Ruiz, A., R. A. Fromtling, and G. S. Bulmer.** 1981. Distribution of *Cryptococcus neoformans* in a natural site. Infect. Immun. **31:**560-563.
189. **Ruiz, A., J. B. Neilson, and G. S. Bulmer.** 1982. A one year study on the viability of *Cryptococcus neoformans* in nature. Mycopathologia. **77:**117-122.
190. **Safrin, R. E., L. A. Lancaster, C. E. Davis, and A. I. Braude.** 1986. Differentiation of *Cryptococcus neoformans* serotypes by isoenzyme electrophoresis. Am. J. Clin. Pathol. **86:**204-208.
191. **Sahu, A., T. R. Kozel, and M. K. Pangburn.** 1994. Specificity of the thioester-containing reactive site of human C3 and its significance to complement activation. Biochem. J. **302 (Pt 2):**429-436.

192. **Salkin, I. F., and N. J. Hurd.** 1982. New medium for differentiation of *Cryptococcus neoformans* serotype pairs. J. Clin. Microbiol. **15:**169-171.
193. **Salkowski, C. A., and E. Balish.** 1991. Susceptibility of congenitally immunodeficient mice to a nonencapsulated strain of *Cryptococcus neoformans*. Can. J. Microbiol. **37:**834-839.
194. **Sanfelice F.** 1894. Contributo alla morfologia e biologia dei blastomiceti che si sviluppano nei succi di alcuni fruttii. Ann Ig. 463-495.
195. **Schneerson-Porat, S., A. Shahar, and M. Aronson.** 1965. Formation of histiocyte rings in response to *Cryptococcus neoformans* infection. J. Reticuloendothel. Soc. **2:**249-255.
196. **Schwartz, D. A.** 1988. Characterization of the biological activity of Cryptococcus infections in surgical pathology. The budding index and carminophilic Index. Ann. Clin. Lab. Sci. **18:**388-397.
197. **Schwarz, P., G. Janbon, F. Dromer, O. Lortholary, and E. Dannaoui.** 2007. Combination of amphotericin B with flucytosine is active in vitro against flucytosine-resistant isolates of *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **51:**383-385. doi: 10.1128/AAC.00446-06.
198. **Scorzetti, G., J. W. Fell, A. Fonseca, and A. Statzell-Tallman.** 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Res. **2:**495-517.
199. **Seaton, R. A., A. J. Hamilton, R. J. Hay, and D. A. Warrell.** 1996. Exposure to *Cryptococcus neoformans* var. *gattii*--a seroepidemiological study. Trans. R. Soc. Trop. Med. Hyg. **90:**508-512.
200. **Severo, C. B., M. O. Xavier, A. F. Gazzoni, and L. C. Severo.** 2009. Cryptococcosis in children. Paediatr. Respir. Rev. **10:**166-171. doi: 10.1016/j.prrv.2009.06.009.
201. **Shahar, A., Y. Kletter, and M. Aronson.** 1969. Granuloma formation in cryptococcosis. Isr. J. Med. Sci. **5:**1164-1172.
202. **Shao, X., A. Mednick, M. Alvarez, N. van Rooijen, A. Casadevall, and D. L. Goldman.** 2005. An innate immune system cell is a major determinant of species-related susceptibility differences to fungal pneumonia. J. Immunol. **175:**3244-3251.
203. **Shibuya, K., A. Hirata, J. Omuta, M. Sugamata, S. Katori, N. Saito, N. Murata, A. Morita, K. Takahashi, C. Hasegawa, A. Mitsuda, T. Hatori, and H. Nonaka.** 2005. Granuloma and cryptococcosis. J. Infect. Chemother. **11:**115-122. doi: 10.1007/s10156-005-0387-x.
204. **Shrestha, R. K., J. K. Stoller, G. Honari, G. W. Procop, and S. M. Gordon.** 2004. Pneumonia due to *Cryptococcus neoformans* in a patient receiving infliximab: possible zoonotic transmission from a pet cockatiel. Respir. Care. **49:**606-608.
205. **Sorrell, T. C., A. G. Brownlee, P. Ruma, R. Malik, T. J. Pfeiffer, and D. H. Ellis.** 1996. Natural environmental sources of *Cryptococcus neoformans* var. *gattii.* J. Clin. Microbiol. **34:**1261-1263.
206. **Sorrell, T. C., S. C. Chen, P. Ruma, W. Meyer, T. J. Pfeiffer, D. H. Ellis, and A. G. Brownlee.** 1996. Concordance of clinical and environmental isolates of *Cryptococcus neoformans* var. *gattii* by random amplification of polymorphic DNA analysis and PCR fingerprinting. J. Clin. Microbiol. **34:**1253-1260.
207. **Speed, B., and D. Dunt.** 1995. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. Clin. Infect. Dis. **21:**28-34; discussion 35-6.
208. **Speed, B. R., J. Kaldor, B. Cairns, and M. Pegorer.** 1996. Serum antibody response to active infection with *Cryptococcus neoformans* and its varieties in immunocompetent subjects. J. Med. Vet. Mycol. **34:**187-193.
209. **Spitzer, E. D., and S. G. Spitzer.** 1992. Use of a dispersed repetitive DNA element to distinguish clinical isolates of *Cryptococcus neoformans*. J. Clin. Microbiol. **30:**1094-1097.

210. **Springer, D. J., and V. Chaturvedi.** 2010. Projecting global occurrence of *Cryptococcus gattii*. Emerg. Infect. Dis. **16:**14-20.
211. **Stephen, C., S. Lester, W. Black, M. Fyfe, and S. Raverty.** 2002. Multispecies outbreak of cryptococcosis on southern Vancouver Island, British Columbia. Can. Vet. J. **43:**792-794.
212. **Subramaniam, K., B. Metzger, L. H. Hanau, A. Guh, L. Rucker, S. Badri, and L. A. Pirofski.** 2009. IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. J. Infect. Dis. **200:**244-251. doi: 10.1086/599318.
213. **Subramaniam, K. S., K. Datta, M. S. Marks, and L. A. Pirofski.** 2010. Improved survival of mice deficient in secretory immunoglobulin M following systemic infection with *Cryptococcus neoformans*. Infect. Immun. **78:**441-452. doi: 10.1128/IAI.00506-09.
214. **Sukroongreung, S., K. Kitiniyom, C. Nilakul, and S. Tantimavanich.** 1998. Pathogenicity of basidiospores of *Filobasidiella neoformans* var. *neoformans*. Med. Mycol. **36:**419-424.
215. **Tabone, M. D., G. Leverger, J. Landman, C. Aznar, L. Boccon-Gibod, and G. Lasfargues.** 1994. Disseminated lymphonodular cryptococcosis in a child with X-linked hyper-IgM immunodeficiency. Pediatr. Infect. Dis. J. **13:**77-79.
216. **Tay, S. T., M. Y. Rohani, T. S. Soo Hoo, and H. Hamimah.** 2009. Epidemiology of cryptococcosis in Malaysia. Mycoses. . doi: 10.1111/j.1439-0507.2009.01750.x.
217. **Tintelnot, K., K. Lemmer, H. Losert, G. Schar, and A. Polak.** 2004. Follow-up of epidemiological data of cryptococcosis in Austria, Germany and Switzerland with special focus on the characterization of clinical isolates. Mycoses. **47:**455-464. doi: 10.1111/j.1439-0507.2004.01072.x.
218. **Torres-Rodriguez, J. M., E. Alvarado-Ramirez, and R. Gutierrez-Gallego.** 2008. Urease activity in *Cryptococcus neoformans* and *Cryptococcus gattii*. Rev. Iberoam. Micol. **25:**27-31.
219. **Tucker, S. C., and A. Casadevall.** 2002. Replication of *Cryptococcus neoformans* in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc. Natl. Acad. Sci. U. S. A. **99:**3165-3170. doi: 10.1073/pnas.052702799.
220. **Van Dam, M. G., R. A. Seaton, and A. J. Hamilton.** 1998. Analysis of HLA association in susceptibility to infection with *Cryptococcus neoformans* var. *gattii* in a Papua New Guinean population. Med. Mycol. **36:**185-188.
221. **Van Duin, D., A. Casadevall, and J. D. Nosanchuk.** 2002. Melanization of *Cryptococcus neoformans* and Histoplasma capsulatum reduces their susceptibilities to amphotericin B and caspofungin. Antimicrob. Agents Chemother. **46:**3394-3400.
222. **Vanbreuseghem, R., and M. Takashio.** 1970. An atypical strain of *Cryptococcus neoformans* (San Felice) Vuillemin 1894. II. *Cryptococcus neoformans* var. *gattii* var. nov. Ann. Soc. Belges Med. Trop. Parasitol. Mycol. **50:**695-702.
223. **Vanden Bossche, H., F. Dromer, I. Improvisi, M. Lozano-Chiu, J. H. Rex, and D. Sanglard.** 1998. Antifungal drug resistance in pathogenic fungi. Med. Mycol. **36 Suppl 1:**119-128.
224. **Varma, A., and K. J. Kwon-Chung.** 1992. DNA probe for strain typing of *Cryptococcus neoformans*. J. Clin. Microbiol. **30:**2960-2967.
225. **Velegraki, A., V. G. Kiosses, A. Kansouzidou, S. Smilakou, A. Mitroussia-Ziouva, and N. J. Legakis.** 2001. Prospective use of RFLP analysis on amplified *Cryptococcus neoformans* URA5 gene sequences for rapid identification of varieties and serotypes in clinical samples. Med. Mycol. **39:**409-417.

226. **Velegraki, A., V. G. Kiosses, H. Pitsouni, D. Toukas, V. D. Daniilidis, and N. J. Legakis.** 2001. First report of *Cryptococcus neoformans* var. *gattii* serotype B from Greece. Med. Mycol. **39:**419-422.
227. **Viviani, M. A., M. Cogliati, M. C. Esposto, K. Lemmer, K. Tintelnot, M. F. Colom Valiente, D. Swinne, A. Velegraki, R. Velho, and European Confederation of Medical Mycology (ECMM) Cryptococcosis Working Group.** 2006. Molecular analysis of 311 *Cryptococcus neoformans* isolates from a 30-month ECMM survey of cryptococcosis in Europe. FEMS Yeast Res. **6:**614-619. doi: 10.1111/j.1567-1364.2006.00081.x.
228. **Washburn, R. G., B. J. Bryant-Varela, N. C. Julian, and J. E. Bennett.** 1991. Differences in *Cryptococcus neoformans* capsular polysaccharide structure influence assembly of alternative complement pathway C3 convertase on fungal surfaces. Mol. Immunol. **28:**465-470.
229. **White, M. H., and D. Armstrong.** 1994. Cryptococcosis. Infect. Dis. Clin. North Am. **8:**383-398.
230. **Wickes, B. L.** 2002. The role of mating type and morphology in *Cryptococcus neoformans* pathogenesis. Int. J. Med. Microbiol. **292:**313-329.
231. **Wickes, B. L., T. D. Moore, and K. J. Kwon-Chung.** 1994. Comparison of the electrophoretic karyotypes and chromosomal location of ten genes in the two varieties of *Cryptococcus neoformans*. Microbiology. **140 (Pt 3):**543-550.
232. **Wright, L., W. Bubb, J. Davidson, R. Santangelo, M. Krockenberger, U. Himmelreich, and T. Sorrell.** 2002. Metabolites released by *Cryptococcus neoformans* var. *neoformans* and var. *gattii* differentially affect human neutrophil function. Microbes Infect. **4:**1427-1438.
233. **Wuczkowski, M., V. Passoth, B. Turchetti, A. C. Andersson, M. Olstorpe, A. Laitila, B. Theelen, M. van Broock, P. Buzzini, H. Prillinger, K. Sterflinger, J. Schnurer, T. Boekhout, and D. Libkind.** 2010. Description of *Holtermanniella takashimae* sp. nov., *Holtermanniella* gen. nov. and proposal of the order Holtermanniales to accommodate Tremellomycetous yeasts of the Holtermannia clade. Int. J. Syst. Evol. Microbiol. . doi: 10.1099/ijs.0.019737-0.
234. **Young, B. J., and T. R. Kozel.** 1993. Effects of strain variation, serotype, and structural modification on kinetics for activation and binding of C3 to *Cryptococcus neoformans*. Infect. Immun. **61:**2966-2972.
235. **Zaragoza, O., M. L. Rodrigues, M. De Jesus, S. Frases, E. Dadachova, and A. Casadevall.** 2009. The capsule of the fungal pathogen *Cryptococcus neoformans*. Adv. Appl. Microbiol. **68:**133-216. doi: 10.1016/S0065-2164(09)01204-0.