Cryptococcus neoformans and *Cryptococcus gattii* are encapsulated, heterobasidiomycetous fungi that have progressed from being rare human pathogens, with just over 300 cases of cryptococcosis reported in the literature before 1955, to becoming a common worldwide opportunistic pathogen as immunocompromised human populations have dramatically increased over the past 2 decades.[2] Cryptococcosis crosses the entire spectrum of patient populations, from the apparently immunocompetent host without an underlying disease to those severely immunocompromised from infection with the human immunodeficiency virus (HIV), an organ transplantation, or a malignancy and its treatment.[3,4] Furthermore, it has a wide range of clinical presentations, which can vary from asymptomatic colonization of the respiratory airways to dissemination of infection into any part of the human body. *Cryptococcus* enters the host primarily through the lungs but has a special predilection for invading the central nervous system (CNS) of the susceptible host. Pulmonary infections are common and may have multiple clinical presentations and management issues. On the other hand, cryptococcal meningitis represents the primary life-threatening infection for this fungal pathogen and has required the most clinical attention.

HISTORY

The first identification of *Cryptococcus* from an environmental source was made by Sanfelice in 1894, from peach juice in Italy.[5] Within a year, Busse and Buschke[6] independently reported the first human case of cryptococcosis in a young woman who developed a chronic ulcer over the skin above her tibia, with yeasts identified in the tissue and later at autopsy. This yeast was also found to have spread to multiple organs in her body. By 1914, Versé described a human case of cryptococcal meningitis,[7] and in 1916 Stoddard and Cutler gave a complete description of the CNS pathology for this infection, including in their report that the yeast forms had surrounding areas of clearing within the tissue.[8] This finding was the first description of the signature structure for this yeast, the polysaccharide capsule. During the early years of clinical cryptococcosis, the names of this yeast were several and included *Saccharomyces neoformans, Cryptococcus hominis,* and *Torula histolytica*. In 1950 Benham attempted to categorize these poorly defined yeasts based on morphology, fermentation, and serologic studies.[9] She named one yeast *C. hominis* and its disease "cryptococcosis." The name was later changed to *C. neoformans* based on temporal priority because Sanfelice had first used the species name of *neoformans.* However, despite Benham's proposal, it took another 25 years before "cryptococcosis" became the primary nomenclature for this infection rather than "torulosis." In 1976 Kwon-Chung discovered and characterized the sexual stage of *Cryptococcus,* and the teleomorphs were named *Filobasidiella neoformans* and *Filobasidiella bacillispora.*[10] It was proposed in 2002 that there be two varieties—*C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *grubii* (serotype A)—and another species, *Cryptococcus gattii* (serotypes B and C).[11] In 2005 the genome sequence of *C. neoformans* was released,[12] and now hundreds of strains, including strains of *C. gattii,* have been sequenced for comparisons[13–15] as we have entered the genome age for fungal pathogen discoveries. With whole-genome sequence information of over a thousand strains, the *Cryptococcus* species complex has now been further divided into genotypes and species, and taxonomic issues are dynamic.[1,16,17]

MYCOLOGY

Life Cycle and Genetics

The life cycles of both *C. neoformans* and *C. gattii* involve two distinct forms: asexual and sexual. The asexual stage exists as encapsulated yeast cells that reproduce by simple, narrow-based budding. The haploid (occasionally diploid in nature), unicellular yeasts are the primary forms recovered from environmental sources and human infections. The asexual or yeast forms represent the primary structures seen in host tissue and recovered from cultures during clinical disease. However, this fungus has a more complex life cycle, with a bipolar mating system that can be observed under certain in vitro conditions and even on plants.[18] For example, yeasts exist in one of two mating types, "alpha" or "a." When two strains of opposite mating types are physically placed together on specific, nutrient-poor media such as V-8 juice agar, the cells undergo conjugation, producing filaments with true clamp connections. At the ends of these filaments, basidia form, and within these basidia, meiosis occurs and chains of basidiospores are produced. The 1- to 2-micron basidiospores, with their size and shape, have been hypothesized to be the infectious propagules.[19] They may deposit in the lung, where the spores rapidly convert to yeasts. However, the sexual stage at present remains a laboratory observation, and the sexual structures, such as basidiospores, have yet to be identified in nature, but clearly, sexual recombination appears to be occurring in nature.[20] Studies have made great progress in precisely understanding the molecular signaling networks that control the sexual cycle, and in some cases genes in these mating pathways have been linked to both morphogenesis and virulence of the yeast.[21]

In the 1980s, an interesting epidemiologic observation was made and confirmed by others that in most areas of the world, more than 95% of environmental and clinical *C. neoformans* isolates appear to contain only the alpha mating locus.[22] The reason for this genetic bias remains unclear, but two factors may be important. First, it has been discovered that under certain environmental conditions *C. neoformans* undergoes haploid fruiting.[23] Haploid fruiting occurs when haploid yeast strains under specific conditions produce hyphae and basidiospores without mating and the exchange of genetic information through meiosis. It is possible that this haploid fruiting with sporulation allows wider dissemination of the fungus in the environment and thus more environmental exposure, leading to more clinical disease. Second, it has now been shown that sexual reproduction can occur between partners with the same mating type,[24] and this allows recombination and improved fitness of progeny. The alpha mating strains are much more likely to produce haploid fruiting structures or perform same-sex matings than the "a" mating strains. An alternative explanation for the alpha mating locus bias is that the approximately 100-kilobase locus or its adjacent genomic areas contain virulence genes that make these mating-type strains more fit in the environment (or in the host). Initial studies with congenic strains of *C. neoformans* var. *neoformans* differing primarily in the mating locus did suggest that the alpha mating strain was more virulent in mice.[24] On the other hand, the alpha and "a" mating loci have been identified in *C. neoformans* var. *grubii,* and experiments with two congenic strains in this variety differing only in the mating locus appear to be identical in virulence.[25] It is still uncertain how much the mating loci types contribute to the virulence of this yeast, and the alpha mating–type bias is not yet precisely explained. However, as witnessed in the Vancouver Island *C. gattii* outbreak, sexual recombination in nature between strains can play an important role in the evolution of pathologic fitness for strains.[20] Furthermore, in certain areas within Botswana, environmental and clinical isolates do have similar numbers of both mating types,[14] so that simple exposure may be cause for the mating-type bias.

Taxonomy

The genus *Cryptococcus* comprises 19 species, loosely characterized as a variety of encapsulated yeasts. There continues to be occasional reports of human infections with several of these non-*neoformans* and non-*gattii* species, such as *Cryptococcus albidus* and *Cryptococcus laurentii.*[26–28] However, such clinical reports are uncommon, and the disease is occasionally poorly documented with these rare cryptococcal species. Therefore any human infection with a cryptococcal species other than *C. neoformans* or *C. gattii* needs rigorous histopathology and cultural proof of invasive disease.

For several decades, *C. neoformans* strains had been grouped into two varieties that included five serotypes based on their capsular structure. *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* included strains with serotypes A, D, and AD, and *C. gattii* contained strains with serotypes B and C. The serotype classification (A–D) describes antigenic differences in the structure of the polysaccharide capsule; these differences are detected by antibodies from rabbit sera[29] or by specific monoclonal antibodies.[30,31]

The stable taxonomic classification of these varieties and serotypes has now evolved through new genomic analyses, and several new changes have been proposed.[32] With the use of specific DNA typing methods and other physiologic factors, it has been proposed that the serotype A strains be classified into a separate variety, *C. neoformans* var. *grubii.*[33]

Serotype D isolates are to remain in the variety *neoformans.* The varietal status of serotype AD hybrid strains has not been proposed. It has also become clear that most of the serotype AD strains represent stable diploid strains, possibly occurring as incomplete genetic crosses between varieties *neoformans* and *grubii.* However, genetic mapping of A and D strains suggests that these varieties biologically diverged from each other more than 18 million years ago.[34] Thus with whole-genome sequencing of many cryptococcal strains, *C. neoformans* var. *grubii* (serotype A) can be divided into five genotypes (VNI, VNII, VNBI, VNBII, and VNIII), with a separate variety, *C. neoformans* var. *neoformans* (serotype D), as genotype VNIV. *C gattii* (serotypes B and C) has been proposed to be divided into five cryptic species: *Cryptococcus gattii*, *Cryptococcus bacillisporus*, *Cryptococcus deuterogattii, Cryptococcus tetragattii,* and *Cryptococcus decagattii* (genotypes VGI–VGV).[7]

As rapid advances in the understanding of genetic population diversity are made among the *Cryptococcus* complex during the genome era, taxonomic relationships and nomenclature will remain in some flux. It has been suggested that the group be designated "*Cryptococcus* complex species" until further widespread genetic and phenotypic studies are completed and analyzed.[16] However, at present, for clinicians the standard serotype classification used for half a century and the split into two varieties, var. *neoformans* and var. *grubii,* and two species, *C. neoformans* and *C. gattii,* still remain useful nomenclature for describing the clinical strain differences in epidemiology, pathogenesis, and clinical features. As further research continues, the taxonomic name of a strain may become less important to the clinician than the identification of its specific genetic structure. In this chapter, the terms "var. *grubii*" and "var. *neoformans*" will continue to be appended to the designation of serotype A and serotype D, respectively, even though serotyping sera are no longer available, because so much of the literature is based on serotype.

The anamorph (yeast or asexual stage) dominates clinical discussion of this encapsulated yeast. On the other hand, the teleomorph, with its more complex structure and its genome sequences, places this fungus within the basidiomycete family, and its teleomorph genus name is *Filobasidiella.* Thus the teleomorph of serotypes A and D strains is called *Filobasidiella neoformans* and the teleomorph of serotypes B and C strains is designated *Filobasidiella bacillispora;* however, these teleomorphic names are not used in clinical practice.

Identification

On most routine laboratory agar media, colonies of *C. neoformans* and *C. gattii* appear within 48 to 72 hours after plating a specimen. Some selective fungal media containing cycloheximide inhibit the growth of this yeast and thus should not be used. For blood cultures, the lysis-centrifugation method works well for isolating *Cryptococcus* but is no longer necessary because automated blood culture methods can reliably detect cryptococcemia, which is commonly observed in severely immunosuppressed patients such as patients with acquired immunodeficiency syndrome (AIDS) and disseminated disease.[35] In some populations of the world with high rates of HIV infection, cryptococcemia is a common finding in patients during the workup of fever with blood cultures. However, cryptococcemia rarely produces symptoms of hypotension or septic shock.

On agar plates, the yeast colony grows as a white-to-cream–colored, opaque colony several millimeters in diameter. The colonies typically become mucoid with prolonged incubation, reflecting increased polysaccharide capsule formation. Colonies occasionally develop sectors that differ in pigmentation or exhibit morphologic changes (e.g., smooth or wrinkled). In fact, *C. neoformans* has been shown to possess the ability to produce a morphologic switching colony phenotype, which explains the variety of colony shapes in some strains and emphasizes the plasticity of the cryptococcal genome.[36,37] The optimal environmental growth temperature for the majority of *C. neoformans* strains is between 30° and 35°C, with a maximum tolerated temperature for most strains at 40°C. Serotype A strains tend to tolerate higher environmental temperatures better than serotype D and serotype B/C strains.[38] *C. neoformans* and *C. gattii* strains generally grow well at 37°C, with doubling generation times of 3 to 6 hours, and this high-temperature growth characteristic is a primary virulence phenotype that separates them from other cryptococcal species that generally do not either grow or survive well at mammalian body temperatures and thus are rarely found to be human pathogens.

In the clinical laboratory, *C. neoformans* and *C. gattii* can be readily differentiated from other yeasts on the basis of their morphology and biochemical tests. The specific identification can be confirmed by a battery of biochemical tests available commercially in kits.[39,40] Recently the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry system has been effective in identifying and distinguishing both *C. neoformans* and *C. gattii* yeasts.[41] However, there are three direct tests that predict that a yeast may be *C. neoformans* or *C. gattii.* First, placing the yeast into an India ink preparation for microscopy may reveal the encapsulation of the yeast. The capsule is generally better seen in direct clinical specimens from the host and may not be as apparent in wet mounts made from in vitro cultures. This finding occurs because capsule production is induced by certain environmental cues, such as elevated carbon dioxide concentrations, serum, urea, or limited iron conditions. In fact, the mammalian host environment provides an ideal environment for capsule production.

Second, a rapid urease test is positive for most *Cryptococcus* species. *Cryptococcus* species, unlike *Candida* species, possess urease, an enzyme that hydrolyzes urea to ammonia and increases the ambient pH. A positive urease test can be detected in the laboratory within minutes.[42] Several nonpathogenic yeasts can produce abundant urease, and *Trichosporon* species may be weakly urease positive.

Third, *C. neoformans* is one of the few yeast species that possesses prominent activity of laccase,[43] an enzyme that allows the conversion of diphenolic compounds into melanin. Detection of this unique biologic characteristic is possible with media containing niger seed (birdseed), caffeic acid, or dopamine. Yeast colonies that turn brown to black on these special agars are identified as melanin positive. In a clinical specimen, such a yeast colony will likely be either *C. neoformans* or *C. gattii.* However, other cryptococcal species in the general environment also possess laccase, but these selective agar assays are still particularly helpful when attempting to identify pigmented cryptococcal colonies from environmental samples contaminated by other fungi or bacteria species, or both.

Histopathologically, *C. neoformans* and *C. gattii* have several characteristic features. In most clinical cases, *Cryptococcus* in tissue exhibits a prominent capsule. Microscopically, most clinical isolates appear as spherical, narrow-based, budding, encapsulated yeast cells in both tissue and culture. Short hyphal or pseudohyphal structures may exist in vivo, or under certain stress conditions in vitro, but these structures are rarely observed unless certain in vitro nutrient conditions for mating or haploid fruiting are met. The yeast cells vary in size from 5 to 10 microns in diameter, and they exhibit single or multiple buds. Because the buds are readily detached from their parental cells, the majority of yeast cells in both tissue and culture lack buds. In tissue, *Cryptococcus* has the ability to produce large (titan) cells of 50 to 100 μm, which possess features such as aneuploidy and large antiphagocytic capsules that promote their survival.[44] Finally, the size of the capsule under direct observation dynamically varies with the individual strain and its immediate environment.

There are three methods for identifying the four serotypes. First, commercial antibodies had been used to distinguish differences in the capsular structures but presently are not available. Second, there are known differences between the biochemical pathways of the serotypes. Most serotypes B and C (*C. gattii*) isolates assimilate glycine as a sole carbon source, whereas serotypes A and D (*C. neoformans*) isolates generally do not. An agar containing L-canavanine, glycine, and bromothymol blue (CGB) uses a color change to separate serotypes A and D from B and C. Third, analysis of DNA base composition is extremely accurate. Comparison of sequenced genomes of these serotypes shows an approximately 6% to 8% overall difference in nucleotide sequences between serotype A and D strains and an even greater difference between these strains and serotype B and C strains.[13] A variety of molecular methods, including random amplified polymorphic DNA (RAPD), karyotypes, polymerase chain reaction (PCR) fingerprinting, multilocus sequencing typing (MLST),[45] and direct sequencing of strains, can be used to readily identify an isolate as belonging to a certain serotype or clade.[46,47] Furthermore, strains are classified into

specific genotypes by PCR fingerprint patterns and sequence. There are presently multiple distinct genotypes with VNI, VNII, VNBI, VNBII, VNBIII, and VNIV for *C. neoformans* and VGI to VGV for *C. gattii,* and within these genotypes there are subgroup genotypes such as VGIIa to VGIIc, identified by MLST,[45] which possess epidemiologic and maybe pathobiologic relevance.[48] Along with genome sequencing, MALDI-TOF mass spectrometry has also become a precise and facile method for distinguishing cryptococcal strains in the laboratory.[41]

ECOLOGY

C. neoformans and *C. gattii* are saprobes in nature.[49] *C. neoformans* was first described in fruits, but after years of investigation it is clear that it also has an environmental niche or habitat associated with certain trees and rotting wood. A second consistent finding is that *C. neoformans* has frequently been isolated from soil contaminated by guano from birds.[50,51] On the other hand, *C. gattii* clearly has an environmental association with trees, from eucalyptus to a variety of coniferous trees, but not bird guano.[52]

Cryptococcus neoformans Serotypes A, D, and AD (*grubii* var. or *neoformans* var.)

In the 1950s, Emmons first isolated *C. neoformans* from soil and from the droppings and nests of pigeons.[50,51] Since the original reports, the fungus has been found in soil samples from around the world. However, the soils most enriched in *C. neoformans* are those that are frequented by birds, especially pigeons, turkeys, and chickens. Guano from other birds, such as canaries and parrots, has also yielded the yeast. Despite this consistent ecologic observation, the precise link between the yeast's natural habitat and the birds is still not certain. Occasionally, birds develop disease that involves *C. neoformans,* but this is relatively unusual. The resistance of birds to disease may result from their very high body temperature, which is not conducive to growth of *C. neoformans,* and possibly even their innate protective immunity. However, the yeasts may transiently colonize the gastrointestinal tract of the birds. The most common environmental niche for this species remains rotting vegetation or wood of certain trees, such as coniferous trees and particularly mopane trees. The birds may simply represent vectors, spreading the fungus from these vegetations into the soils and dusts of human traffic.

Cryptococcus gattii Serotypes B and C

Unlike *C. neoformans, C. gattii* has never been cultured from bird guano. Furthermore, there appears to be a certain geographic limitation to the occurrence of infections with this variety. With this knowledge base, investigators were initially able to culture *C. gattii* from vegetation around and associated with the river red gum trees (*Eucalyptus camaldulensis*) and forest red gum trees (*Eucalyptus tereticornis*) in Australia.[53] Because these trees were exported to other areas of the world where *C. gattii* is also observed, it was reasoned that *Eucalyptus* species may be a vector for infection. It was suggested that the poorly encapsulated yeasts or basidiospores might be released in relationship to the flowering of these trees, but this has not yet been proved. However, despite the association of these trees with *C. gattii,* the outbreak of cryptococcosis on Vancouver Island, British Columbia, revealed that other trees such as firs, maples, and oaks may also be an ecologic niche for specific strains of *C. gattii.*[54] It is proposed that these new geographic locations and specific environmental niches for *C. gattii* could be due to recent climate changes.[52]

Another ecologic factor that may be important to the human pathogenicity of this fungus is its association with other organisms. For example, it has been found in soil associated with a variety of bacteria, amebas, mites, worms, and sow bugs. The stress of this biotic area with its abundant predatory scavengers may have selected for a yeast species that can survive such harsh conditions. In fact, studies have shown that *C. neoformans* can survive within amebas, which in some respects may provide an environment similar to that in a human macrophage.[55] Furthermore, nonpathogenic cryptococci can act as food for the nematode *Caenorhabditis elegans,* but *C. neoformans* can actually kill the worm, and several invertebrate models (worms, grubs, and amebae) have been used in studies of cryptococcal pathogenesis and treatment experiments.[56,57]

EPIDEMIOLOGY

C. neoformans is not generally considered to be a routine constituent of the human microbiota. There are clinical reports of *Cryptococcus* being isolated from nonsterile body sites on patients with no signs or symptoms of cryptococcosis.[58] It can also be detected as a commensal in cats and dogs. Furthermore, endobronchial colonization is more frequently observed in humans in the presence of an underlying chronic pulmonary disease. When *C. neoformans* is isolated from nonsterile clinical specimens, the clinician must examine the patient for evidence of disease and analyze risk factors for the potential development of disease before planning further management strategies. Several methods have been used to study the existence of prior infection with *C. neoformans* without evidence for disease. Research has shown that patients with cryptococcosis have delayed hypersensitivity to cryptococcal antigens,[59] and the prevalence of positive skin test reactions in pigeon fanciers and laboratory workers engaged in research activities with this yeast has been reported to be high.[60] Unfortunately, there is no established skin test for routine clinical use in patients with cryptococcosis today, and this reduces our ability to assess the magnitude of this infection. However, most adults possess specific antibodies to *C. neoformans* antigens; for instance, in New York City, most children acquire antibodies to cryptococcal antigens before the age of 10 years.[61,62] These observations suggest that there are frequent asymptomatic infections. These studies examining serologic evidence of cryptococcal exposure in children do emphasize that in some respects there are certain areas of high exposure to this fungus and other geographic sites with much lower exposure.[63] Although exposure to this yeast is likely limited in certain areas of the world, cryptococcal disease has been reported worldwide.

The vast majority of patients with symptomatic disseminated cryptococcosis have a clearly identified underlying immunocompromised condition (Table 262.1). The most common underlying conditions worldwide include AIDS, prolonged treatment with corticosteroids, organ transplantation, advanced malignancy, diabetes, and sarcoidosis. In fact, occurrence of cryptococcosis may identify an underlying idiopathic CD4 lymphocytopenia[64] or the development of certain autoantibodies, or be associated with the use of the specific immune-modifying monoclonal antibodies, such as alemtuzumab, infliximab, etanercept, and adalimumab.[65,66] Finally, it has been estimated that approximately 20% of patients who have cryptococcosis without HIV infection have no apparent underlying disease or risk factor.[67] The genetic susceptibility to cryptococcal disease has been clearly determined in murine models but needs more studies in humans. However, recent studies on associations with polymorphisms in immunoglobulin genes and mannose-binding protein gene might be the start of understanding the group of patients with cryptococcal disease without an apparent immunosuppressive event,

TABLE 262.1 Conditions Known to Be or Possibly Associated with Predisposition to *Cryptococcus neoformans* Infections

HIV infection
Lymphoproliferative disorders
Sarcoidosis
Corticosteroid therapy
Hyper-IgM syndrome
Hyper-IgE syndrome
Autoantibodies to GM-CSF
Monoclonal antibodies (e.g., infliximab, entanercept, adalimumab, alemtuzumab)
Systemic lupus erythematosus[a]
HIV-negative CD4$^+$ T-cell lymphocytopenia
Diabetes mellitus[b]
Organ transplantation[a]
Peritoneal dialysis
Cirrhosis

[a]Immunosuppressive therapy may account for the predisposition.
[b]Diabetes mellitus has historically been considered a risk factor for cryptococcal infection. However, diabetes is a common disease, and it is unclear whether this condition is truly a specific risk factor for cryptococcosis.
GM-CSF, Granulocyte-macrophage colony-stimulating factor; *HIV,* human immunodeficiency virus; *IgE,* immunoglobulin E; *IgM,* immunoglobulin M.
Modified from Casadevall A, Perfect JR. Cryptococcus neoformans. *Washington, DC: ASM Press; 1998:410.*

or how these associations might add to the risk of an immunosuppressed individual.[68–70] Furthermore, recent studies have suggested a link between autoantibodies to the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) and risk for cryptococcosis.[71,72]

The best estimates for rates of cryptococcosis in the United States in the pre-AIDS era predicted an overall incidence of 0.8 case per 1 million persons per year. In 1992, during the peak of the AIDS epidemic in the United States, the rate reached almost 5 cases of cryptococcosis per 100,000 persons per year in several large cities. In the mid-1990s, before highly active antiretroviral therapy (ART) but with widespread use of fluconazole for oral candidiasis, the rate was reduced, and it stabilized in the cities at approximately 1 case per 100,000 persons per year.[73,74] With the widespread use of ART in developed countries by the beginning of the 21st century, the incidence of cryptococcosis has declined further and appears to have reached a stable number of new infections.[75] In the AIDS population in developed countries, it now generally represents an infection that identifies a disadvantaged patient or an untreated and undiagnosed HIV infection. Thus cryptococcosis in patients with AIDS identifies a group as having or wanting less access to medical care, or both.[76]

In less medically resourced countries with major ongoing epidemics of HIV, such as in sub-Saharan Africa, cryptococcosis reached high prevalence. Some reports estimate that 15% to 45% of individuals with advanced HIV infection succumb to cryptococcosis.[77,78] In a population-based surveillance study for cryptococcosis in an antiretroviral-naïve South African population, the overall incidence in HIV-infected patients was 95 cases per 100,000 patients, and those with AIDS had a rate of 14 cases per 1000 patients.[79] A recent sobering study in Botswana with generally excellent access to ART showed an initial drop in cases of cryptococcosis, but over the last 4 to 5 years the incidence has stabilized at a still very high level.[80] A general report from the US Centers for Disease Control and Prevention emphasized that at the peak of the AIDS epidemic, there were annually almost a million new cases of disseminated cryptococcosis with more than 600,000 deaths per year, and in sub-Saharan Africa mortality is higher than with infections caused by tuberculosis in similar areas.[3] In many African medical centers, cryptococcosis represents the most common cause of culture-proven meningitis, even surpassing *Neisseria meningitidis* and *Streptococcus pneumoniae* meningitis.[81] In fact, the risk of cryptococcosis appears higher for African-born individuals even when they move to industrialized nations.[82] Increasing cases of cryptococcosis have consistently followed the pattern of HIV infections, and in countries such as Thailand, blood cultures done before widely available ART frequently contained this yeast. However, with increased availability of ART in sub-Saharan Africa and Asia, the magnitude of these observations has changed.[83] An updated assessment of cryptococcosis during widespread ART now predicts over 200,000 cases per year with over 100,000 deaths per year from cryptococcosis.[84] However, the incidence of cryptococcosis will not be dramatically reduced further secondary to undiagnosed HIV infections, noncompliance with ART, or antiretroviral drug resistance.

The varieties or species of *Cryptococcus* that are identified as causing disease differ by geographic location and by whether the patient has a concomitant HIV infection. Before the AIDS epidemic, Kwon-Chung and Bennett found that at least 80% of clinical isolates worldwide were *C. neoformans* serotype A (var. *grubii*).[85] *Cryptococcus gattii* serotype B was almost exclusively found in tropical and subtropical areas, such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa. Serotype C was rare in all localities but seemed to follow the same geographic distribution as serotype B. However, with outbreaks in Vancouver and the Pacific Northwest in the United States and even isolated reports of *C. gattii* infections east of the Mississippi River and in Europe,[86,87] the epidemiology is clearly changing with this species.[52] *C. neoformans* serotype D was predominantly isolated from Europe, especially Denmark, Germany, Italy, France, and Switzerland, and some strains of this variety were found in the United States.[85,88,89] In AIDS patients, the vast majority of isolates are serotype A, although serotype D has constituted a significant percentage of isolates in several areas of France. A small, measurable portion of cases in AIDS patients have been reported to be caused by *C. gattii* and specifically by VGIII and VGIV genotypes.[90–92] On the other hand, the numbers of *C. gattii* infections remain small even in areas where this species was commonly observed to cause disease in the pre-AIDS era, and clinical presentations and outcomes remain similar to *C. neoformans* infections in a comparable risk group.[93,94]

Cryptococcosis has a measurable rate of infection in two other major risk groups: cancer patients and recipients of solid-organ transplants. Since the 1950s, it has been known that patients with lymphoproliferative disorders and certain hematologic malignancies, such as chronic lymphocytic leukemia, were at higher risk than the general population for cryptococcosis.[95–98] A retrospective analysis of case reports from a single large cancer center from 1989 to 1999 reported that the incidence of cryptococcosis was 18 cases per 100,000 admissions, and the occurrence is predicted to increase with further frequent use of cell-mediated immune inhibitors, such as alemtuzumab, ibrutinib, and fludarabine, in the management of certain malignancies.[99,100] On the other hand, checkpoint inhibitors to programmed cell death protein-1 for cancer treatment may actually be preventive with their actions against cryptococcosis.[101] Because of their profound and prolonged immunosuppression, organ transplant recipients have also been a prime target for this infection. In one cohort, cryptococcosis occurred in 2.8% of all solid-organ transplant recipients.[102] Kidney and liver transplant recipients appear to have the highest risk for cryptococcosis.[102–104] In contrast, in bone marrow transplant recipients, who have a high incidence of fungal infections, cryptococcosis is not common.[105] In rare circumstances, the transplanted organ (e.g., cornea, kidney, lung) has been shown to carry the cryptococcal infection into a susceptible recipient.[106–108] This clinical scenario represents one of the few instances of person-to-person transmission of this infection.

Sarcoidosis, with or without corticosteroid therapy, predisposes to cryptococcosis and can be a diagnostic dilemma. The lung, skin, bone, and CNS lesions of the two diseases overlap clinically and by histopathology. Despite uncertain pathophysiology, diabetes as an underlying disease or cofactor is frequently mentioned in those patients without HIV or transplant recipient risk factors in most reviews.

There has always been a small but consistently higher rate of cryptococcosis in males than in females, and at times there are subtle differences in immunologic responses between sexes, suggesting hormonal influences on cryptococcal disease.[109,110] Cryptococcosis can occur before puberty, but even in children with several known risk factors the incidence is uncommon, and in an area of high HIV prevalence only 2% of cryptococcosis cases were in children.[111] Interestingly, there have been several reports of cryptococcosis in children with a hyperimmunoglobulin M syndrome.[112,113] In adults, idiopathic $CD4^+$ T-cell lymphocytopenia may be identified by the development of disseminated cryptococcosis, and paradoxically this underlying condition with cryptococcosis actually may have a good prognosis for treatment outcome.[64,114] With much less precision, it has been suggested that smoking and outdoor activities may increase the risk of cryptococcosis,[73,115] and that recent intravenous drug abuse might provide risk even without HIV infection.[116]

There is general agreement that most cryptococcal infections are acquired primarily by inhalation of infectious propagules, but there are occasional cases of direct traumatic inoculation through contaminated environmental projectiles or laboratory/clinical accidents such as needlesticks.[117,118] However, neither the environmental source of infection nor the infectious form of *C. neoformans* has been precisely established in most cases of cryptococcosis. It is hypothesized that either dehydrated, poorly encapsulated yeast cells or basidiospores (<5 μm) are needed as infectious propagules for alveolar deposition in the lungs.[19] Studies at sites with contaminated soils or trees have found that the surrounding air contains the correct size of propagules for airway infection.[119–121] Molecular typing methods have confirmed that clinical isolates can be indistinguishable from environmental isolates.[122–124] Although associations between infection and environmental exposure have been reported for many of the classic dimorphic fungi, this association is rare for *C. neoformans.* However, the outbreak of *C. gattii* infections on Vancouver Island and the Pacific Northwest has convincingly linked human and animal infections to common environmental exposures.[54] There has not been a consistent seasonal association for the occurrence of cryptococcosis worldwide, although one study did observe more cases in the fall

and winter.[125] These uncertain observations likely reflect the influence of the host-reactivation pathophysiology of this disease in many cases, or differences in environmental humidity for aerosol production. Recent fundamental niche mediation models have begun to trace and map the best environmental conditions for certain cryptococcal species' survival.[126]

Human-to-human transmission of cryptococcosis has not been reported except in cases of contaminated transplant tissue.[51,106,107] Many species of animals, including dogs and cats, can develop cryptococcosis,[127–129] but there is infrequent evidence of zoonotic transmission between them and humans. In one case, *C. neoformans* isolated from the cage of a pet cockatoo was molecularly linked with the strain that caused infection in a transplant recipient who was exposed to the cage.[130] Also, several cryptococcal cases have been linked to intense bird exposures,[131] and even a possible nosocomial outbreak of cryptococcosis in an intensive care unit has been reported.[132]

PATHOGENICITY

The encapsulated yeast *C. neoformans* has been studied extensively for more than 50 years. In the past 2 decades, genetic and molecular biologic research, in concert with well-established and robust animal models, has rapidly increased our understanding of its pathobiology.[133] Progress in cryptococcal molecular biology has led to the use of karyotypes, repetitive elements, transposons, and whole-genome sequencing to identify yeast strains through a variety of analyses, including restriction fragment length polymorphism, RAPD, PCR fingerprints, and MLST. Recently, the entire sequenced genomes of many strains of *C. neoformans* and *C. gattii* have been published,[12,14,15,47,134,135] and with present sequencing methods many more strains will have their entire genomes sequenced and analyzed. Several transformation systems are available for introducing DNA into this yeast, and site-specific gene disruptions and replacements are routine; a comprehensive whole-genome deletion library has even been created.[136] Dozens of specific null mutants in a variety of pathways or for specific enzymes have been produced to examine their impact on the virulence composite of the yeast in several robust animal models,[133,137] including zebrafish, in which pathogenesis can visually be observed.[133,137,138] Furthermore, differential display PCR, complementary DNA subtraction techniques, serial analysis of gene expression, microarray analysis, and RNA sequencing have been used to document and understand *C. neoformans* transcriptional profiles.[55,139–142] Proteomic and metabolomic approaches have also been used to study its pathophysiology.[143–145] It is also observed that the cryptococcal genome shows plasticity and rapid changes, and variations can influence its virulence and resistance to drugs.[46,60,61,146–149] With the use of whole-genome sequencing and following persistent or relapse isolates in the human host, the rates and location of genetic and epigenetic changes have been reported in the human host.[150–152]

All these molecular tools have been employed to determine the components and mechanisms that make this yeast such an efficient and deadly pathogen. The following paragraphs describe its most prominent virulence phenotypes.

Capsule

The most distinctive feature of *C. neoformans* and *C. gattii* is a polysaccharide capsule containing an unbranched chain of α-1,3-linked mannose units substituted with xylosyl and β-glucuronyl groups. The serotype specificity appears to be determined by structural differences in the glucuronoxylomannan (GXM) related to the number of xylose residues and the degree of *O*-acetylation of hydroxyl groups. The capsular polysaccharide has a highly negative cell surface charge and is attached to the cell wall by α-1,3-glucan residues. However, it is easily released into the immediate growth media or tissue. Capsular thickness, which varies between isolates, is regulated by several environmental cues, including ambient partial pressure of carbon dioxide, serum, urea, and low iron concentrations, which increase capsular size in many strains. These environmental signals appear to augment the yeast's ability to produce disease and may help explain why the capsule may be small in in vitro cultures and the general environment but is much larger when observed within the mammalian host. Mutant cryptococci that are made to be hypocapsular or acapsular are dramatically less virulent than their parental strains in animal models.[153] However, infections caused by capsule-free or poorly encapsulated strains have been rarely observed in the mammalian host.[154,155]

The impact of the capsular polysaccharide on host immunity can be profound at many pathophysiologic levels. For example, it has been shown to produce the following effects on the host[156]: (1) it acts as an antiphagocytosis barrier, (2) it depletes complement, (3) it produces antibody unresponsiveness, (4) it dysregulates cytokine secretion, (5) it interferes with antigen presentation, (6) it produces brain edema, (7) it creates selectin and tumor necrosis factor receptor loss, (8) it allows a highly negative charge around yeast cells, (9) it extrudes itself into the intracellular environment with the potential for local toxicity on cellular organelles, and (10) it enhances HIV replication. Furthermore, it confers resistance to oxidative stress, which may improve its intracellular survival.[157] When the GXM is shed into the host environment, it affects host immunity at many levels, but fortunately, its detection in host fluids permits a very successful diagnostic test.

The biochemistry of this imposing structure remains poorly understood, but new insights continue to occur for this structure.[158] For example, multiple genes related to capsule synthesis have been identified.[159] Through creation of specific null mutants, it has been shown that any disturbance in efficient capsular synthesis (e.g., reduced formation, secretion, or elimination of the structure) attenuates the ability of the mutated yeast to produce disease.[153] Furthermore, there have been new insights into the many molecular signaling pathways that control expression of the capsule. For example, one critical pathway necessary for efficient capsular production uses a G protein that signals through a cyclic adenosine monophosphate–mediated pathway. Down-regulation of this pathway with a concomitant reduction in capsule size produces an attenuated virulence phenotype, but if a mutation in the pathway upregulates capsule production, the mutant yeast becomes hypervirulent.[160]

Melanin

The production of melanin is observed in many fungi, including some pathogenic species.[43] *C. neoformans* possesses a laccase, an enzyme that catalyzes the conversion of diphenolic compounds such as L-3,4-dihydroxyphenylalanine (DOPA), norepinephrine, epinephrine, and other related aromatic compounds to quinones, which rapidly autopolymerize to form melanin. The production of this pigment can help identify the yeast in the laboratory, but it is also a major virulence factor for the yeast. Laccase is bound to the inner aspect of the yeast's cytoplasmic membrane, and a site-directed mutant for the gene encoding for it has been created. This laccase-negative or albino mutant has been attenuated for virulence in animal models.[161]

One proposed mechanism by which melanin may protect the yeast is through its ability to act as an antioxidant, and it has been shown that yeast cells without the ability to form melanin are more susceptible to oxidative stress. Other potential mechanisms by which melanin protects the yeast from host damage involve the following: (1) cell wall support or integrity, (2) alteration in cell wall charge, (3) interference with T-cell response, (4) abrogation of antibody-mediated phagocytosis, and (5) protection from temperature changes and antifungal agents.

It remains unclear whether the catecholamine-rich CNS, with its excellent substrates for melanin formation, provides some tissue tropism or a rich environment that enhances this yeast's ability to produce disease. For instance, it has clearly been shown that melanin is formed in yeast cells within the brain.[162,163]

High-Temperature Growth

A basic trait of all pathogenic fungi is their ability to grow well at mammalian body temperature. For example, *C. neoformans* and *C. gattii* are the only cryptococcal species to grow consistently well at 37°C, and when mutants are made that cannot grow well at this body temperature, they are avirulent even when they possess the ability to make a capsule and produce melanin. There appears to be some evolutionary drift in high-temperature growth, in that isolates of *C. gattii* and serotype D *C. neoformans* (var. *neoformans*) generally appear to be more sensitive to growth inhibition and killing at high temperatures than serotype A

C. neoformans (var. *grubii*), and these less heat-tolerant yeast isolates quickly lose viability at temperatures of 40°C and above.[38]

There has been progress in understanding the genetic controls for high-temperature growth in *C. neoformans*. First, signaling pathways such as calcineurin, RAS, and trehalose have been associated with the yeast's ability to grow at mammalian body temperatures, and these are linked to its virulence composite.[164–167] It is also clear that vacuolar adenosine triphosphatase activity[168] is also important for high-temperature growth of this yeast. *C. neoformans* has evolved a series of molecular pathways and mechanisms to withstand host temperature stresses, and these thermal responses are a major reason it is a pathogen.[169]

Other Pathogenicity Factors

Detailed research has focused on the three classic virulence factors of *C. neoformans* (capsule, melanin, and growth at 37°C), but this complex pathogen has many other tools to produce disease. First, individual clinical or environmental strains vary in their ability to produce disease in animal models despite possessing all known major virulence factors. Second, strains can also rapidly change their virulence potential by passage through animals. Third, although many strains are clonal, there have been recombination events in nature to produce serotype AD hybrids and progeny that are more fit or virulent than the parental strains.[20] Fourth, future research will need to determine genetic or epigenetic controls over the subtle changes that make both environmental and clinical strains vary individually in their virulence potentials; even clinical isolates can vary in their virulence in animals despite already producing human disease.[170] The future challenge for research will be to link genetic changes in strains with clinical outcome.

A series of specific genetic loci have been associated with the virulence composite.[3] Phospholipase activity has been linked to virulence by a gene knockout of the phospholipase B1 gene (*PLB1*). Null mutants of *PLB1* are hypovirulent[171] and may have an impact on the immunologic response of the host to infection.[5] *C. neoformans* makes large amounts of urease, and if the *URE1* gene encoding for this enzyme is disrupted, infection with the mutant is attenuated in mice, where it has a blood-brain barrier entry defect,[172] but not in rabbits when inoculated directly into the CNS compartment.[6,173] A vacuolar adenosine triphosphatase gene is an example of a locus that appears to have an impact on several virulence phenotypes, and the absence of the encoding gene attenuates virulence of the strain.[9,168] There are now many examples in *C. neoformans* in which a gene or pathway controls multiple virulence phenotypes.[174] In addition to melanin as an antioxidant, several other genes, such as those for superoxide dismutase,[175] alternative oxidase,[176] and flavohemoglobin,[177] are associated with oxidative or nitrosative stress and have been linked to the virulence composite of this yeast.

It is clear that mechanisms for stress responses are important for the yeast to establish a robust infection, but there is also some redundancy in these systems because the yeast can at times still survive in the host without these protective features.[23] The sexual cycle and virulence of fungi are linked together in pathogenesis.[21] The alpha mating locus has been linked to the virulence of *C. neoformans* serotype D (var. *neoformans*) strains by the demonstration that the alpha mating strain was more virulent than its congenic "a" mating pair.[24] These results suggest that the mating locus, which is more than 100 kilobases in size, may contain some virulence genes. On the other hand, studies have found that congenic *C. neoformans* serotype A (var. *grubii*) strains had no apparent difference in virulence between "alpha" and "a" mating strains in several animal models.[178] This illustrates an important principle, which has also been shown with multiple genetic loci: virulence genes in one variety are not used by another variety or species, and vice versa. Thus both micro and major evolutionary drift may explain the variability and complexity of the entire virulence composite of *C. neoformans* and *C. gattii* strains.

HOST RESPONSES

Because serologic and skin hypersensitivity studies frequently identify cryptococcal infections and yet the incidence of cryptococcosis is low, it has been concluded that host immunity in humans is generally effective in controlling infection after initial exposure to this yeast.[179,180] In fact, the vast majority of cryptococcal infections are diagnosed in patients with a compromised cell-mediated immunity.[179] Furthermore, there is general agreement among clinicians that a strong cellular immune response producing granulomatous inflammation is essential for containment of infection.[180–183] Because granuloma formation is a result of a helper T cell 1 (Th1)–polarized response, cytokines such as tumor necrosis factor, interferon-γ, and interleukin (IL)-2 are required.[184,185] Proinflammatory cytokines, such as IL-12 and IL-18, and chemokines, such as monocyte chemotactic protein-1 and macrophage inflammatory protein-1α, are critically important for recruitment of inflammatory cells to the site of this infection.[186,187]

Several immune cell populations, such as natural killer cells, and certain types of lymphocytes have been shown to possess direct anticryptococcal effects. Human lymphocytes (CD4, CD8) inhibit growth of *C. neoformans* by direct contact.[188] A primary effector cell against *C. neoformans* is the macrophage, which produces anticryptococcal activity when it is "activated."[189] Other professional phagocytes, such as monocyte-derived macrophages, microglial cells, and polymorphonuclear neutrophils, may kill or at least significantly inhibit *C. neoformans,* but the alternative macrophage responses enhance infection.[190] It has been shown that a major factor in the infectivity of *C. neoformans* is its ability to survive inside cells and that this yeast can actually be extruded from professional phagocytes to infect other cells or be contained in vacuoles with virulence proteins.[191–193]

It is not only the state of cellular activation or the type of host cell but also the number of cells at the site of infection that appear to provide an effective host immune response. It is clear from natural history studies in patients with AIDS that the risk of cryptococcal disease dramatically increases as total CD4 lymphocyte counts drop below 50 to 100 cells/μL of blood.[194] In these patients, the paucity of inflammatory cells at the site of infection, such as the subarachnoid space, is impressive. Animal studies further confirm the importance of cell-mediated immunity by showing that T-cell–depleted mice are dramatically more susceptible to infection.[195]

A series of innate factors, such as the anticryptococcal activity of saliva and serum, may discourage active infection or disease with *C. neoformans.* Although surfactant A, dectin-1, and Toll-like receptors appear to have little influence on cryptococcosis,[196–198] there are other innate factors, such as surfactant D, that appear to have a critical impact on infection, and the yeast can actually co-opt this host material to improve its survival.[199] Phagocytosis of *C. neoformans* is optimally performed in the presence of complement or antibody. The intracellular fate of yeasts depends on cytokines such as interferon-γ or GM-CSF to improve intracellular inhibition or killing of the yeasts by either host oxidative or nonoxidative mechanisms. Human studies have consistently correlated a positive outcome with the presence and quantity of cytokines such as interferon-γ at the site of infection.[201] Human genome and immunogenetic studies in the future should reveal whether patients who have cryptococcosis, despite having an apparently normal immune system and no risk factors, might have subtle defects in innate or acquired immunity to this yeast. For example, it has already been shown that long-term survivors of cryptococcal meningitis have measurable persistent specific cell-mediated defects against *C. neoformans,* and recently it was shown that there is an association of common functional genetic polymorphisms in low-affinity Fcγ receptors with cases of cryptococcosis.[69,202,203]

It appears that *C. neoformans* has both extracellular and intracellular components or stages of infection. The results of histologic examination of tissues and fluids range from virtual absence of an inflammatory reaction to intense granulomatous inflammation with caseous necrosis. The immune reaction appears to be primarily a function of the host status, but the yeast can participate in the inflammatory response because switch variants in a single strain can produce vastly different histologic responses.[36] The immune response may be influenced by not only the shedding of polysaccharide into tissue but also other yeast factors such as mannitol,[204] melanin,[205] and prostaglandins,[206] which may have profound direct effects on immunomodulation of these infections.

There is substantial evidence that the humoral immunity arm can contribute to an effective immune response,[207,208] including an immunoglobulin M response.[209] Several groups have shown that preinfection monoclonal antibody strategies directed against the polysaccharide capsule can reduce the burden of yeasts and improve survival in animal models.

In fact, a polysaccharide–tetanus toxoid conjugate vaccine was shown to elicit high titers of antibody to the capsule and subsequently to protect against an intravenous inoculation of cryptococci in mice.[210] These antibodies provide for (1) efficient phagocytosis, (2) enhanced natural killer cell function, and (3) improvement in clearing capsular polysaccharide. Antibodies to other structural components such as melanin[211] and glucosylceramide in the cell wall[212] have also been able to improve the host's ability to fight infection. Sophisticated serologic studies of the host have suggested that there are both qualitative and quantitative differences in the individual types of immunoglobulins that may predispose to disseminated cryptococcal disease.[213] Finally, one pilot study in humans has been performed to determine the safety and pharmacodynamics of a monoclonal antibody for treatment of cryptococcosis.[214]

PATHOGENESIS

The pathogenesis of cryptococcosis is determined by three broad factors: (1) the status of the host defenses, (2) the virulence of the strain of *C. neoformans* and *C. gattii,* and (3) the size of the inoculum. The relative importance of each factor as a determinant of clinical disease remains uncertain, but it is clear that the complexities of these interactions together produce the ultimate presentation.

A reasonable scenario for the pathophysiology of cryptococcosis is that the susceptible host comes into contact with cryptococci from the environment through inhalation of infectious propagules. In the alveoli, the yeasts contact the alveolar macrophages, which recruit other inflammatory cells through cytokines/chemokines, and a proper Th1 response and granulomatous inflammation is elicited. The infection can then take one of three pathways:

1. In an immunosuppressed host, the yeast continues to proliferate and disseminate, causing clinical disease.
2. The effective immune response completely eliminates the yeast from the host.
3. The yeasts produce a small pulmonary lymph node complex and remain dormant in tissues but are not dead.

The third scenario may be a common occurrence. Baker, in elegant postmortem studies of asymptomatic individuals, showed the existence of pulmonary foci and hilar nodes containing yeasts in individuals with no antecedent complaints.[215,216] The yeasts remain dormant and the host is clinically asymptomatic until loss of local immunity occurs through, for example, corticosteroid use or progression of an HIV infection. Then the yeasts begin to replicate in the pulmonary lymph node complex and eventually disseminate into organs outside the lung. This pathophysiology is similar to the scenario proposed for reactivation of tuberculosis and histoplasmosis. Studies in France have given epidemiologic support for this concept of reactivation. In African expatriates who lived in Europe for many years before their development of cryptococcosis, the infecting strain possessed a genotype consistent with strains from an African origin.[217] Although this evidence is indirect, there is also little evidence that cryptococcosis arises from a recent exposure, and only rare case clusters following a group exposure have been reported.

In careful autopsy studies it has been shown to more accurately call the CNS pathogenesis features "meningoencephalitis," but because of common usage this chapter primarily uses the more restrictive term "meningitis."

CLINICAL MANIFESTATIONS

The two common sites for infection with this encapsulated yeast, the lung and the CNS,[218] were emphasized in a large review of cryptococcosis in HIV-negative patients. In this cohort, 109 patients (36%) were diagnosed with only pulmonary involvement and 157 (51%) presented with initial evidence of CNS disease.[67] Three other sites of infection (skin, prostate, and eye) have clinical features that are worthy of mention. However, it should be noted that *C. neoformans* has been found to infect any organ of the human body (Table 262.2), and in the severely immunosuppressed patient cryptococcosis may present with involvement of multiple body sites.

Cryptococcosis demonstrates a few differences depending on whether the patient has or does not have an underlying HIV infection.[125,219–222] HIV-infected patients present with more CNS and extrapulmonary infections, higher rates of positive India ink examinations, higher polysaccharide antigen titers, more frequent positive blood cultures, and fewer cerebrospinal fluid (CSF) inflammatory cells. These clinical distinctions are primarily a function of the severity of immunosuppression and the resulting high burden of yeasts. They most likely do not reflect a specific interaction between HIV and *C. neoformans* growth.

Lung

The respiratory tract is the most common portal of entry for this yeast, and symptoms range from asymptomatic colonization of the airway[223] to life-threatening pneumonia with evidence of an acute respiratory distress syndrome.[224–226] In at least one-third of normal hosts, the infection is asymptomatic on presentation and is detected by an abnormal chest radiograph. On the other hand, patients can present with acute symptoms of fever, chest pain, cough, weight loss, and sputum production.[227] Common and unusual pulmonary presentations are listed in Table 262.2. Cryptococcosis occasionally occurs with another pathogen; coinfections of the lung have been reported with tuberculosis, nocardiosis, and echinococcosis.[228–230] There are rare cases of cryptococcosis associated with pulmonary alveolar proteinosis and autoantibodies against GM-CSF.[231,232] Also, *C. neoformans* may be isolated from the sputum repeatedly over months and years in patients with prior chronic lung disease but no immunosuppression, no evidence of active pulmonary parenchymal disease, negative serum cryptococcal antigen, and negative fungal cultures from urine and CSF. These patients are considered to have chronic endobronchial colonization.

In normal hosts, chest radiographs commonly show well-defined, noncalcified single (Fig. 262.1) or multiple nodules. An initial presentation may be that of a radiographic lesion (or more than one) that is worrisome for a lung malignancy but then is proved by lung biopsy to be a cryptococcal infection. Other radiographic characteristics include indistinct masslike infiltrates, hilar lymphadenopathy, lobar infiltrates (Fig. 262.2), pleural effusions, and lung cavitation.[233] When infection is limited to the lung, the test for serum cryptococcal antigen is often negative. If there is pulmonary cryptococcosis with a positive test for serum cryptococcal antigen, it is prudent to consider an extrapulmonary source of infection, although workups may still be negative for another infection site (e.g., blood, skin, urine, CSF). When *C. neoformans* has been isolated from the lung in patients at high risk for dissemination due to an underlying immunosuppressive disease or treatment, a lumbar puncture should be considered to rule out CNS infection, even in the absence of symptoms. Although it is rare, early, asymptomatic spread to the CNS may be manifested only by a positive CSF fungal culture, with otherwise normal CSF and a negative antigen test. The number of cryptococci may be so low that several milliliters of CSF must be cultured for a positive culture to be obtained. Because positive CSF cultures are infrequent, some clinicians advocate treating previously normal patients with disease apparently limited to the lung who are asymptomatic and have no apparent underlying disease with long-term fluconazole and omitting the lumbar puncture.[234,235]

In the severely immunosuppressed host with AIDS or receiving high-dose corticosteroids, cryptococcal pneumonia can progress more rapidly (over days instead of weeks).[224,226] Unlike immunocompetent hosts, most immunosuppressed individuals have constitutional symptoms such as fever, malaise, chest pain, shortness of breath, and weight loss. In these patients, pneumonia can progress to features of acute respiratory compromise even without evidence of CNS involvement. However, because of the ability of the yeast to disseminate outside the primary lung focus to the CNS, these very-high-risk patients frequently present with a meningeal rather than a pulmonary syndrome. In AIDS patients, cryptococcal pneumonia may not be symptomatic, and more than 90% may present with concomitant CNS infection at the initial diagnosis. Chest radiographs in these immunocompromised hosts are similar in their range of presentations to those of immunocompetent hosts. However, alveolar and interstitial infiltrates are particularly common and thus might be confused with *Pneumocystis* infection. Because the severely immunosuppressed patient with pulmonary cryptococcosis and AIDS generally has a CD4 count substantially below 100 cells/μL, it is always prudent to consider the possibility of coinfection with other opportunists such as typical and atypical mycobacterium, cytomegalovirus, *Nocardia,* and *Pneumocystis.*

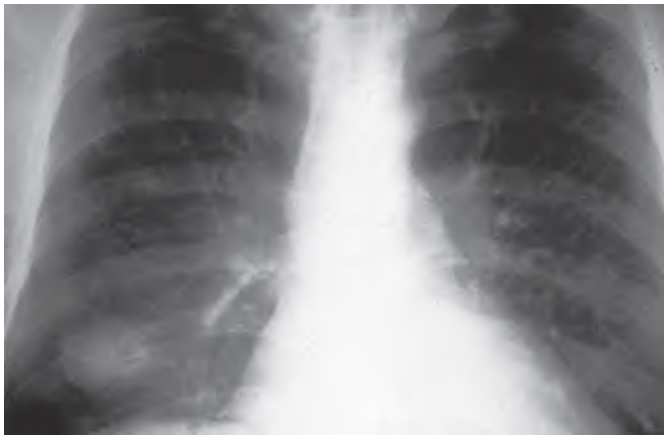

FIG. 262.1 Cryptococcal nodule. Previously healthy, asymptomatic patient with a right lung nodule.

FIG. 262.2 Cryptococcal pneumonia. Previously healthy patient with fever, cough, shortness of breath, and left lobar infiltrate.

TABLE 262.2 Clinical Manifestations of Cryptococcosis

Central Nervous System	**Bone and Joints**
Acute, subacute, chronic meningitis	Osteolytic lesion (single or multiple sites)
Cryptococcomas of brain (abscesses)	Arthritis (acute/chronic)
Spinal cord granuloma	**Muscle**
Chronic dementia (from hydrocephalus)	Myositis
Lung	**Heart, Blood Vessels**
Nodules (single or multiple)	Cryptococcemia
Lobar infiltrates	Endocarditis (native and prosthetic)
Interstitial infiltrates	Mycotic aneurysm
Cavities	Myocarditis
Endobronchial masses	Pericarditis
Endobronchial colonization	Infected vascular graft
Acute respiratory distress syndrome	**Gastrointestinal Tract**
Mediastinal adenopathy	Esophageal nodule
Hilar adenopathy	Nodular or ulcerated lesions in stomach or intestines (may resemble Crohn disease)
Pneumothorax	Hepatitis
Pleural effusions/empyema	Peritonitis
Miliary pattern	Pancreatic mass
Skin	**Breast**
Papules and maculopapules	Breast abscess
Subcutaneous abscess	Lymph nodes
Vesicles	Lymphadenopathy
Plaques	**Thyroid**
Cellulitis	Thyroiditis
Purpura	Thyroid mass
Acne	**Adrenal Gland**
Draining sinuses	Adrenal insufficiency
Ulcers	Adrenal mass
Bullae	**Head and Neck**
Dermatitis herpetiformis–like	Gingivitis
Molluscum contagiosum–like	Sinusitis
Eye	Salivary gland enlargement
Papilledema	
Extraocular muscle paresis	
Keratitis	
Chorioretinitis	
Endophthalmitis	
Optic nerve atrophy	
Genitourinary Tract	
Prostatitis	
Renal cortical abscess	
Positive urine culture from occult source	
Genital lesions	

Modified from Casadevall A, Perfect JR. Cryptococcus neoformans. *Washington, DC: ASM Press; 1998:409.*

Central Nervous System

Most patients with cryptococcosis of the CNS present with signs and symptoms of subacute meningitis, such as headache, fever, cranial nerve palsies, lethargy, coma, or memory loss over several weeks (see Table 262.2).[218] Symptoms may not be typical, and patients may present with acute (several days) symptoms of severe headaches, with intermittent headaches, or even with no headache but with altered mental status.

HIV-infected patients with cryptococcal meningitis exhibit few differences at presentation from those without HIV. However, several clinical aspects may be more prominent in patients with AIDS.[67] First, the

burden of yeasts is generally higher, and this may be reflected in higher polysaccharide antigen titers, slower conversion of CSF to sterilization during treatment, and a tendency toward a higher incidence of increased intracranial pressure. Second, there is a greater likelihood of finding the yeast in extracranial locations during the initial workup. Third, the possibility is greater that a second CNS event may occur, such as infection with *Toxoplasma gondii* or development of a lymphoma. Fourth, the use of ART in AIDS patients has created a distinct immune reconstitution inflammatory syndrome (IRIS) in cryptococcal infections.[236–238] It appears in two forms: unmasking and paradoxical. In the unmasking form, after starting ART, some patients develop acute symptoms of cryptococcal meningitis or pain and swelling in peripheral, hilar, or mediastinal lymph nodes. In the paradoxical form, this syndrome may occur during treatment of cryptococcal meningitis in the first few weeks or months after ART is introduced. It appears to correlate with a significant drop in HIV load, but there may be only a modest rise in the number of CD4 cells.[239] It is hypothesized that as immunity improves with ART, silent or latent cryptococcal infections are made clinically apparent as inflammation is mobilized to interact with the yeasts or polysaccharide antigen. During treatment for cryptococcal meningitis, IRIS may be marked by increasing headaches, new neurologic signs, appearance of more inflammatory cells in the CSF, and possibly increased intracranial pressure.[240] Distinction between immune reconstitution and progressive infection can be difficult, but cultures from the CSF and lymph node aspirates are generally negative in IRIS, even though cryptococci may be present on a smear and cryptococcal antigen titers tend to be lowering.

There are still limited precise data that relate the severity of the meningitis to the particular infecting strain, but this is an area of active investigations.[20,170,241] In most cases the host defense responses determine the clinical manifestations. However, some clinical presentations may depend on the particular infecting strain. For example, in areas of the world where patients have infections with both *C. neoformans* serotypes A, D, and AD (var. *neoformans* and var. *grubii* hybrid) and *C. gattii,* cerebral cryptococcomas (Fig. 262.3) and hydrocephalus with or without large pulmonary mass lesions in immunocompetent hosts were found more commonly with the *C. gattii* infections.[242–244] Although patients infected with this species may have higher survival rates, a subgroup of patients with *C. gattii* infections have brain parenchymal lesions by scan, complications of hydrocephalus and increased intracranial pressure, cranial neuropathies, and a poor response to therapy with substantial morbidity. These observations suggest that some *C. gattii* strains may have a greater propensity for invading the brain parenchyma. Another example of a specific strain affecting disease production is from the Vancouver outbreak; it was found that a recombinant strain had been created in nature that was more virulent in animal models than one of the parental strains and, furthermore, this strain (genotype VGIIa) had taken over most of the environmental and clinical isolates within this outbreak.[20]

Skin

C. neoformans can produce almost any type of skin lesion (see Table 262.2). A common lesion is a papule or maculopapule with a soft or ulcerated center (Fig. 262.4). A draining sinus usually originates in an underlying bone lesion or occasionally a subcutaneous abscess. Some lesions in severely immunosuppressed patients are easily mistaken for molluscum contagiosum, and lesions can mimic acne vulgaris, squamous carcinoma, or basal cell carcinoma. After pulmonary and CNS sites of infection, the skin is the third most common organ for appearance of infection. Skin manifestations can be extraordinarily varied.[245–248] For example, in severely immunosuppressed patients, skin infections may present as a cellulitis (Fig. 262.5) or an abscess that mimics a bacterial skin infection in both appearance and rapidity of onset.[249,250] Because of the variety of skin manifestations, a correct diagnosis requires a biopsy with proper histopathology and culture. This is extremely important in the immunocompromised host.

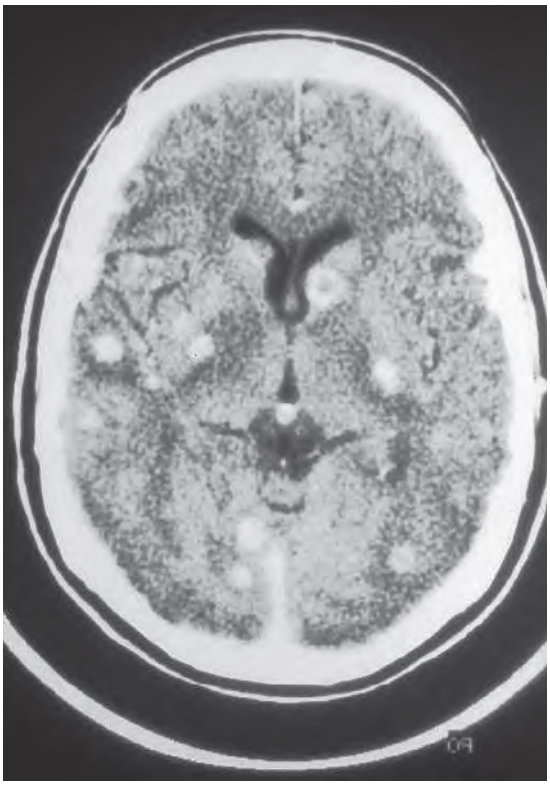

FIG. 262.3 **Computed tomography scan of previously healthy patient with multiple cryptococcomas.**

FIG. 262.4 **Forehead ulcer in a human immunodeficiency virus–infected host with *Cryptococcus neoformans* seen in histopathology.**

FIG. 262.5 **Severely immunosuppressed patient with cellulitis of the arm caused by *Cryptococcus neoformans*.**

In most cases, the skin lesions represent a sentinel finding for disseminated cryptococcal infection. In fact, severely immunosuppressed patients can present with both cutaneous cryptococcosis and another pathogenic fungus in the skin as a manifestation of disseminated fungal disease.[251] However, there is strong evidence that rare cases of skin cryptococcosis represent primary cutaneous cryptococcosis from direct inoculation or exposure rather than being a marker of disseminated disease. In a large retrospective review of patients with cutaneous findings, a series of immunocompetent patients had: (1) solitary skin lesion(s) on unclothed areas of the skin; (2) a history of skin injury, participation in outdoor activities, or exposure to bird droppings; (3) isolation of *C. neoformans;* and (4) no evidence of disseminated disease.[252] There are reports of direct inoculation of yeasts into skin by laboratory or clinical accidents and defined episodes of trauma.[117,118] In these cases, there has been no evidence for dissemination of infection from this body site of infection except for one case of a needlestick.[118]

Involvement of skin may be influenced by several factors. First, some strains of *C. neoformans* have been described as being dermatropic in animal models.[253] Second, an observation made in a cohort of solid-organ transplant recipients suggests that patients receiving tacrolimus appeared to develop a higher ratio of skin and soft tissue infections to CNS infections when compared with previous immunosuppressive regimens.[107] Because tacrolimus has anticryptococcal activity at temperatures of 37° to 39°C[254] but loses its anticryptococcal activity at environmental temperatures, the skin involvement might result from the lower temperatures at this body site.

Prostate

Like *Blastomyces* and *Mycobacterium tuberculosis, C. neoformans* can invade the prostate gland, and in most cases of cryptococcal infection this involvement is asymptomatic.[255] In fact, asymptomatic or silent prostate infection may first be identified during urologic surgery and may spread into the bloodstream during surgery.[256] For *C. neoformans,* this gland was considered an important site for sanctuary of this yeast from antifungal treatment in HIV-infected patients before ART.[257,258] Frequently, in follow-up of patients with AIDS and cryptococcal meningitis after initial antifungal therapy, cultures of urine (with or without prostatic massage) or seminal fluid were positive for the yeast. In many patients the location of relapse after therapy remains uncertain, but the prostate is clearly a site that requires prolonged therapy to clear infection in severely immunosuppressed patients. Besides the prostate, penile[259] and vulvar[260] lesions with *C. neoformans* have been reported, but there has been no evidence for conjugal spread of this yeast, and isolated cryptococcuria is a marker for disseminated disease.

Eye

In the early reviews of cryptococcal meningitis, ocular signs and symptoms were reported in 45% of the cases.[261] The most common manifestations are ocular palsies and papilledema. Small white retinal exudates, without overlying vitritis, are probably the next most common finding. In severely immunosuppressed patients, several features of ocular involvement have arisen. First, cryptococcal eye infections can occur simultaneously with other pathogens such as HIV and cytomegalovirus.[262] Second, the presence of extensive retinal lesions, particularly with vitritis, frequently leads to blindness, and only occasionally is it successfully managed.[263,264] Third, there are reports of catastrophic loss of vision without evidence for endophthalmitis.[265,266] In these cases of blindness, which may occur while receiving therapy, two pathogenic processes have been identified. First, there is a visual loss secondary to an optic neuritis produced by infiltration of the optic nerve with yeasts, and, as for endophthalmitis, there are few options for successful management. Second, other patients present with visual loss in one or both eyes during antifungal therapy. In these patients, symptoms are probably related to the development of cerebral edema and unrelieved high intracranial pressure. The probable pathogenesis is compression of the ophthalmic artery within the optic sheath. Treatment is decrease of CSF pressure by repeated lumbar punctures, CSF shunting, or perhaps slitting the optic sheath within the posterior orbit. Once blindness has occurred, return of visual acuity is rare. A central scotoma or optic atrophy may be the only sequela of cured ocular cryptococcosis.

Other Body Sites

C. neoformans can produce infection in most areas of the body (see Table 262.2), and several require further discussion. Cryptococcemia occurs during severe immunosuppression and when there is a high burden of yeasts in the body, and it is a common finding in advanced AIDS. Cryptococcemia rarely produces vascular instability, and only a few cases of native or prosthetic valve endocarditis have been described.[267]

Before the AIDS epidemic, bone lesions were reported in up to 5% of disseminated cases.[268] Bone lesions are typically one or more well-circumscribed osteolytic lesions in almost any bone and may have a contiguous soft tissue abscess ("cold abscess"). Bone lesions of sarcoidosis resemble cryptococcal lesions on radiographs but are more often on the hands or feet and have no contiguous soft tissue abscess.[269] In AIDS patients, yeasts may be found in bone marrow biopsy cultures.

Cryptococcal peritonitis can present in two distinct patient groups: (1) those receiving chronic ambulatory peritoneal dialysis and (2) those with underlying liver disease and cirrhosis.[270,271]

Rare body sites for cryptococcosis (less than a dozen reported cases) include genital and urinary tracts (renal cortical abscess, positive urine culture from an occult site); muscle (myositis); heart (native and prosthetic valve endocarditis, mycotic aortitis or aneurysm, myocarditis, pericarditis, vascular foreign body); thyroid (thyroiditis, mass); adrenal gland (adrenal insufficiency); head and neck (gingivitis, sinusitis, salivary gland enlargement); gastrointestinal tract (gastrointestinal nodules or ulcers); liver (hepatitis); breast (inflammatory mass); and lymph nodes (lymphadenopathy).

Immune Reconstitution Inflammatory Syndrome

IRIS and cryptococcosis are well described with HIV infection and its treatment,[237] but it also clearly occurs as a complication in solid-organ transplant recipients[272] and normal hosts.[273,274] IRIS may occur within a few days to a few months after the introduction of ART with manifestations of fever, organ inflammation (by symptoms, radiographs), increased intracranial pressure,[275] or a combination of these, with a pattern of immunologic biomarkers carefully described.[276,277] In solid-organ transplant recipients, it occurs with those on potent antirejection regimens that have been reduced after initiation of antifungal therapy. It occurs at a mean period of 6 weeks after starting antifungal therapy and may be associated with organ graft loss. An elegant series of cryptococcal spinal arachnoiditis cases was described with impressive features of a dysregulated immune system post–infectious inflammatory response syndrome and response to corticosteroid therapies.[278] IRIS in cryptococcosis with its dysregulated immune response needs to be identified by the clinician, because there are no specific tests for it, and the diagnosis relies on clinical guidelines and expert judgment.[279] It is so critically important to recognize IRIS because its management is different than for relapse or persistence of cryptococcosis.

LABORATORY DIAGNOSIS

Microscopic Examination

The simple procedure of mixing together India ink and biologic fluids to identify the 5- to 10-micron-diameter encapsulated yeasts remains a rapid and effective method for diagnosing cryptococcal meningitis (Fig. 262.6). Approximately 50% of non-AIDS patients with cryptococcal meningitis and over 80% of patients with AIDS have a positive India ink examination of the CSF. Experience is required to distinguish an encapsulated yeast from a lymphocyte with surrounding proteinaceous debris. India ink smears of urine, sputum, and bronchoalveolar lavage specimens are almost impossible to interpret. With calcofluor white and a fluorescent microscope, yeasts can be detected in a specimen when numbers are reduced. With routine histopathologic stains such as hematoxylin and eosin, the yeasts are surrounded by empty spaces, which reflect the capsule. The polysaccharide capsule can be identified with stains such as mucicarmine and alcian blue (Fig. 262.7), and its ability to produce melanin allows it to be stained with the Fontana-Masson stain. Gomori methenamine silver fungal stain identifies the narrow-based budding yeast in tissue (Fig. 262.8), and a Gram stain usually reveals a poorly stained gram-positive yeast. Both biopsies and

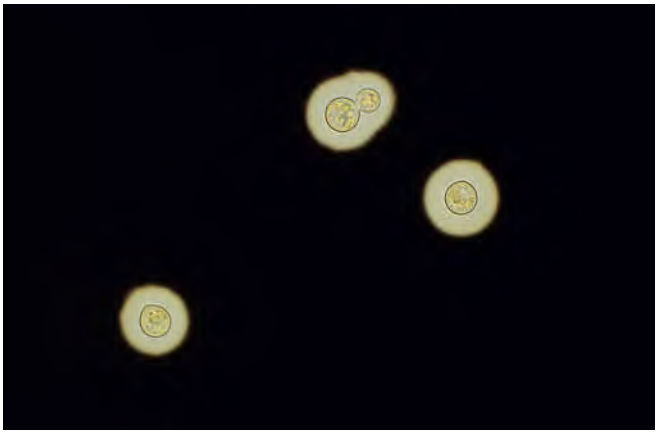

FIG. 262.6 India ink preparations from cerebrospinal fluid of patient with meningitis. Note encapsulated yeasts.

FIG. 262.8 Gomori methenamine silver stain shows narrow-based yeast with a faint capsule.

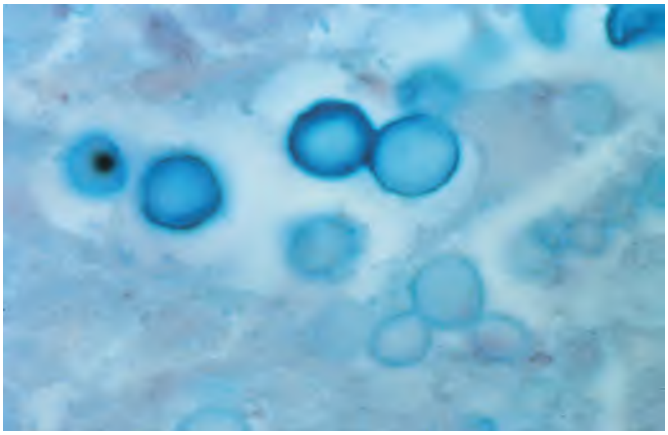

FIG. 262.7 Alcian blue stain of lung tissue from patient with cryptococcal pneumonia. Note blue stain of polysaccharide capsule.

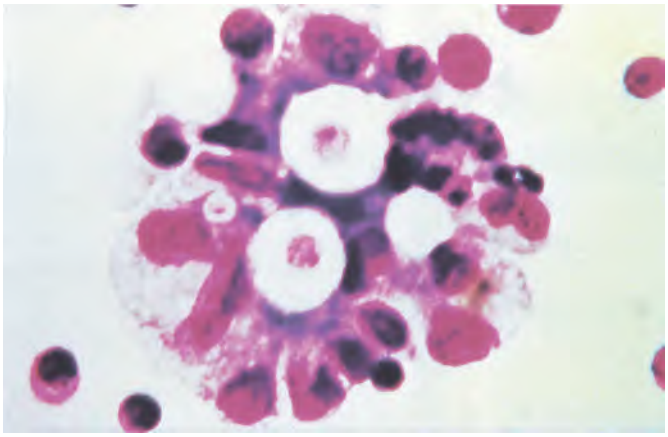

FIG. 262.9 Cytospin preparation of a host with cryptococcal meningitis showing encapsulated yeast surrounded by a mixed inflammatory reaction.

cytologies (Fig. 262.9) can be extremely helpful in the diagnosis of cryptococcosis.

Cultures

C. neoformans can grow on most bacterial and fungal media. Both automated and lysis centrifugation methods are effective in detecting cryptococcemia, and the finding of positive blood cultures has been more common during the AIDS epidemic. Most *C. neoformans* isolates from untreated patients can be detected in culture 3 to 7 days after the specimen is collected and placed into or on culture media. In some advanced CNS infections there can be more than a million colony-forming units of yeasts per milliliter of CSF. Quantitative CSF yeast counts have been used for evaluation of antifungal therapy.[280,281] Initially, this counting of viable CSF yeasts was an effective research tool to determine the impact of various treatments on the burden of yeasts, but recently this quantitative CSF culture strategy has begun to show clinical relevance and correlation with outcome.[282–284] It may become attractive to utilize in individual therapeutic strategies.[285]

Isolates can be identified by biochemical reactions,[39,40] MALDI-TOF mass spectrometry,[41] or DNA-based methods.[286,287] Other low-tech methods to presumptively identify the yeasts are to perform a rapid urease test or to inoculate the yeast onto Staib medium (birdseed agar),[288] DOPA, or caffeic acid media (in which colonies will produce melanin and turn brown to black). However, some other yeasts produce urease, and the highly mucoid colonies of some strains of *C. neoformans* may not produce enough melanin to be clearly positive.

The identification of *C. neoformans* and *C. gattii* can be made by several methods: (1) a color reaction on concanavalin-glycine-thymol agar, which distinguishes *C. neoformans* from *C. gattii*[289]; (2) an antibody kit for serotyping, which presently is not commercially available[290]; and (3) fingerprinting with DNA-based methods, which can further separate strains of the cryptococcal complex into multiple genotypes.[291–293] Reports show that the MALDI-TOF mass spectrometry platform can rapidly distinguish the two species.[294] However, with our present knowledge base it is not yet clinically apparent that the clinician needs to know the species, variety, or genotype to initially manage the patient with cryptococcosis.

Antigen Detection

The tests for detection of cryptococcal polysaccharide antigen in serum and CSF are extremely accurate for the diagnosis of invasive disease.[295] Both latex agglutination and enzyme-linked immunosorbent assay tests are greater than 90% sensitive and specific.[296] The Meridian CALAS latex agglutination test (Meridian Bioscience, Inc., Cincinnati, OH) has provided positive tests in sera and CSF, with negative Meridian Premier enzyme immunoassay (EIA) results, and usually from samples with lower antigen titers that follow treatment.[297] With the proper treatment of serum specimens (boiling and pronase/2-mercaptoethanol treatment), false-positive latex agglutination tests are not common.[298] False-positive latex agglutination tests are usually negative by EIA, and vice versa. An occasional false-positive test is observed when there is a cross-reactive antigen in the specimen, and this may occur with microorganisms such

as *Trichosporon asahii* (*beigelii*)[299] or other infections.[300] The false-negative tests may be present in early asymptomatic meningitis and in chronic, indolent meningitis. The clinical experience with, and preciseness of, antigen detection has been carefully studied and validated in sera and in CSF for clinical practice, and it is not recommended to detect polysaccharide antigen in the urine and bronchoalveolar lavage fluid, despite some reports to the contrary.[301] Recently, a new lateral flow assay (LFA) has been introduced into clinical practice. It is cheap, rapid, and simple. The LFA has been found to be more sensitive than the Meridian Premier EIA and at least as sensitive as the Meridian CALAS latex agglutination test.[302] The LFA has major indications for preemptive management of HIV-infected patients in resource-poor environments. A drop of fingerstick blood can be used to screen high-risk HIV-infected patients and can provide a bedside test with CSF.[303] Although dilutions of sera or CSF can be tested by LFA and titers determined, the quantitative results have differed from those obtained by latex agglutination.[304,305] Like other tests for cryptococcal polysaccharide antigen, false-positives can be observed in sera from the rare cases of *T. asahii* infection.[305,306]

A number of clinical issues are related to the use of cryptococcal polysaccharide antigen. Serum cryptococcal polysaccharide antigen tests have been used successfully to screen high-risk, febrile AIDS patients, particularly those with headache, in areas where the incidence of cryptococcal meningitis is high,[307] but these tests may be less useful in areas where the prevalence of infection is low.[308] In patients with cryptococcal infection of the lung who have a positive serum polysaccharide antigen test, there is heightened concern that the infection has become extrapulmonary. It is also unlikely that this high-molecular-weight molecule can cross the blood-CSF barrier, and thus its detection in CSF probably confirms the presence of the yeast in this compartment. In fact, there are occasional cases of meningitis in which CSF antigen is detected early in infection before there is a high enough yeast colony count for the routine clinical laboratory to detect a positive culture, particularly with small volumes of CSF. There are also isolated cases of serum cryptococcal polysaccharidemia in asymptomatic HIV-infected patients with negative fungal cultures from CSF and urine. Management of these asymptomatic antigenemia cases can be confusing, but in these high-risk patients it is wise to start preemptive antifungal therapy because many of them will eventually develop cryptococcosis, and their prognosis is poor.[309–312] Many preemptive strategies in multiple countries are being studied or instituted to save lives with early antifungal therapy intervention in cryptococcal antigen-positive HIV patients, and with the new LFA it is becoming more clinically feasible in resource-limited areas.[313] As a strategy in the clinic where lumbar punctures are limited, recent studies suggest that a positive serum antigen titer of 1:160 or greater reflects a clinically relevant risk of CNS involvement in asymptomatic patients and demands an aggressive approach to manage cryptococcal CNS disease.[314]

Despite its excellence as a diagnostic test, the cryptococcal polysaccharide antigen test is not sufficiently accurate to use in making specific decisions during treatment. In fact, serial polysaccharide antigen titers are imprecise and should not be used to develop treatment guidelines.[315] The cryptococcal polysaccharide antigen titer, however, does give general prognostic information. Initial high titers (≥1:1024) demonstrate a high burden of yeasts in the host, poor host immunity, and a greater chance of therapeutic failure. Furthermore, it is encouraging when consideration of an IRIS diagnosis is being made that the cryptococcal antigen titer is stable or dropping. Finally, cryptococcal antigen titers can be used in follow-up when maintenance antifungal regimens are discontinued in HIV-infected individuals. Antibodies to *C. neoformans* may be detected during infection and are helpful for epidemiologic studies, but because many of these patients with disease are immunosuppressed, the titers are inconsistent and not generally used for diagnostic or therapeutic decisions.

Radiology

The chest radiograph of pulmonary cryptococcosis can show a variety of characteristics, including local or diffuse infiltrates, nodules, hilar lymphadenopathy, cavitation, and pleural effusion(s).[316–320] In AIDS patients, the diffuse interstitial infiltrates may be confused with coexistent *Pneumocystis* infection.[321,322]

Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain are frequently used in the management of cryptococcal meningitis.[323–327] Approximately half of CT scans are normal during CNS infection. However, a CT scan can reveal hydrocephalus, gyral enhancement, or single or multiple nodules that may or may not be enhancing. Cryptococcomas may be single or multiple, and in some populations, such as those with *C. gattii* infection, they can occur in more than 25% of non-AIDS and apparently immunocompetent patients. In patients with AIDS, the CT scan differs only in that approximately one-third of patients demonstrate cortical atrophy from the underlying HIV infection. The MRI scans are more sensitive than CT scans for detecting abnormalities in cryptococcal meningitis. MRI findings can include numerous, clustered foci that are hyperintense on T2-weighted images and nonenhancing on postcontrast T1-weighted images in the basal ganglia or midbrain. Rarely, there may also be multiple miliary enhancing parenchymal and leptomeningeal nodules.

There are several points to emphasize with regard to CNS radiology. First, there is no pathognomonic scan, and patients with cryptococcal meningitis may simply present with evidence of idiopathic hydrocephalus.[328] Second, in AIDS patients, CNS parenchymal lesions may represent lymphoma or a second infection such as toxoplasmosis or nocardiosis. Third, follow-up scans may actually show worsening of lesions, with enlargement, new lesions, or persistence of cryptococcomas or more leptomeningeal enhancement. These findings are not necessarily a sign of treatment failure. They may simply represent enhancement by inflammation as microscopic yeast foci are being eliminated. Especially with the use of ART and the potential for immune reconstitution, these radiographs need to be judged carefully in the context of the patient's cultures and clinical signs and symptoms before deciding that the radiograph signifies treatment failure. Lesions on MRI scans may not decrease in size for months or years despite resolution of disease.[329]

MANAGEMENT

The management of cryptococcosis has been the subject of a series of evidence-based studies. Infectious Diseases Society of America guidelines have been established for therapy and were revised in 2010.[330,331] The general recommendations should be helpful to clinicians as a reference point for individual patient management, but there remain many unanswered questions, and unique decisions will need to be made in certain cases. It is clear that cryptococcal meningitis is uniformly fatal without antifungal treatment. However, before the availability of antifungal agents, there were several reports of patients who survived for years before succumbing to infection.[332] In contrast, with the severe immunosuppression of HIV infection and if adequate treatments are not available, a high percentage of untreated patients die within the first 2 weeks of hospitalization.[333] The rapidity of the progression is likely to depend on host factors. On the other hand, it has been shown that some individuals without an immunosuppressive underlying disease have asymptomatic endobronchial colonization with no detectable pulmonary lesions on CT scan, negative serum cryptococcal antigen, and negative fungal cultures from CSF and urine. These patients without disease may not need antifungal treatment. In a review of non-AIDS patients with positive pulmonary cultures for *C. neoformans,* approximately 20% did not receive treatment.[67] Previously, healthy patients with cryptococcosis confined to the lung healed spontaneously. However, because pulmonary cryptococcosis in immunosuppressed patients or those to be immunosuppressed can disseminate or become a chronic disease in the lung, all such patients should be treated.

In Vitro Drug Susceptibility Analysis

Methods for in vitro susceptibility testing of *C. neoformans* and *C. gattii* have been modified and standardized, and the epidemiologic cutoff values for the cryptococcal complex to azoles have been established.[334,335] Most initial isolates have low minimal inhibitory concentrations (MICs) to amphotericin B, flucytosine, and azoles but high MICs to echinocandins. By in vitro susceptibility testing, isolates have been detected in treated patients who are resistant to flucytosine, azoles, and polyenes. In fact, there appears to be some correlation between MICs and clinical resistance,[336–338] and by molecular typing methods, most of the refractory cases represent relapse isolates rather than reinfection.[339,340] In fact, they

possess MICs that are similar to those of the primary isolates.[341] Some of the findings may be due to the known unstable drug heteroresistance characteristics of *C. neoformans* in vivo.[342] However, in some cases it was suggested by molecular techniques that reinfection occurred with a novel strain.[343,344] When MICs of isolates rise while the patient is being treated, or when the MICs were initially greater than 16 μg/mL for fluconazole or 128 μg/mL or greater for flucytosine, failure of treatment might possibly be related directly to drug resistance.[345] However, there is as yet no specific therapeutic MIC breakpoint for antifungal drugs validated for cryptococcosis. In fact, in HIV-associated cryptococcal meningitis within Africa where fluconazole is used to treat mucocutaneous candidiasis and as primary therapy for cryptococcal disease, positive relapse episodes have been associated with isolates that had reduced susceptibility to fluconazole (MIC ≥64 μg/mL).[150,346] *C. neoformans* strains that possess known drug resistance mechanisms—from drug target amplifications or changes in the ergosterol pathway to augmentation of drug efflux pumps through genetic mutations/duplications in ergosterol-target genes or high-level expression of drug efflux genes—have been identified.[342,345] Furthermore, plasticity in the genome can allow chromosome instability, such as aneuploidy and thus duplications of specific drug target(s) or efflux drug pump(s), to produce a lower response to fluconazole in vivo.[347] However, unstable drug resistance with aneuploidy formation during in vivo stress may be lost in vitro and thus might be missed with in vitro cultures and direct drug susceptibility testing.[60] In fact, the differences between in vivo and in vitro yeast cells with a plastic genome may contribute to the apparent fungistatic behavior of fluconazole in the treatment of high-burden yeast infections.

Treatment Strategies

In 2010 the Infectious Diseases Society of America updated their Clinical Practice Guidelines for the management of cryptococcal disease,[127] and their principles and direct recommendations are incorporated in the following discussion with updated opinions.

Cryptococcal Meningitis

Amphotericin B remains the cornerstone of induction therapy for cryptococcal meningitis, and from the early studies when it was used alone[348] to its use in combinations, it has performed reasonably well for this infection, with successes in the non-AIDS era of between 60% and 75%.[349,350] With polyene therapy, two issues have been noted. First, recent studies have suggested that higher daily doses of amphotericin B might be more effective in sterilization of the CSF.[351] A standard induction dose for amphotericin B deoxycholate has now been established at 0.7 mg/kg/day. Second, liposomal amphotericin B (AmBisome) at 3 to 6 mg/kg/day and probably amphotericin B lipid complex at 5 mg/kg/day have had treatment successes similar to that of amphotericin B deoxycholate, with reduced toxicity.[352,353] In patients with renal dysfunction or at risk for renal dysfunction, the lipid products of amphotericin B are frequently recommended. For example, they are used as primary therapy in transplant recipients.[331] In fact, lipid formulations of amphotericin B may also be advantageous in all patients with cryptococcal meningitis in that they are less likely to require adjustments of the induction regimen that can produce a negative impact on treatment outcome.[354,355] Also, in resource-limited areas, there is both animal[356] and now human experience[357] that supports limited doses of liposomal amphotericin B with azole in induction therapy as effective.

Flucytosine has been used alone in treatment of cryptococcal meningitis,[358] but frequent development of direct drug resistance on monotherapy has meant that it cannot be recommended as a single agent for treatment of this infection. It is primarily used in combination therapy with conventional amphotericin B or lipid formulations of amphotericin B,[349,350,359–362] and doses in patients with normal renal function are typically 100 mg/kg/day but will need to be adjusted in those with renal dysfunction. Drug levels should be monitored to keep 2-hour postdose levels under 100 μg/mL,[363] or careful follow-up of complete blood counts should be performed, to reduce the development of bone marrow depression in those with risk for this toxicity, such as patients with renal dysfunction or those receiving high doses of polyenes. One report concluded that adding flucytosine to amphotericin B reduced the rates of relapse during itraconazole maintenance compared with amphotericin B monotherapy,[364] and another retrospective report showed reduced rates of failing in severe cryptococcal meningitis with the combination compared with other regimens[365]; finally, a direct survival benefit from this combination therapy has been reported in two large randomized studies.[280,366] Azoles have been used effectively in the management of cryptococcal meningitis. For instance, fluconazole has been used extensively in cryptococcal meningitis because of its excellent CSF pharmacokinetics and long-term oral safety.[367–371] Clinical trials have shown that it penetrates well into CSF and is excellent for use in the consolidation and suppressive phases of cryptococcal meningitis management.[372,373] However, it tends to be fungistatic and is probably best used in the stage of infection in which there is a low burden of yeasts in the CSF, and thus is not recommended for the primary induction phase of therapy for meningitis when a polyene is available for use.

Itraconazole, despite its poor CSF penetration and inconsistent oral bioavailability, has been successfully used in the treatment of cryptococcal meningitis.[374,375] However, it has been shown to be inferior to fluconazole for the suppressive phase of treatment.[364] Its place in therapy for cryptococcal meningitis is probably as a less-than-ideal alternative to first-line therapy with fluconazole in the consolidation and suppressive phases of management if the drug levels are monitored. It is not recommended for primary induction-phase therapy. Other azoles have been studied for treatment of cryptococcosis, but miconazole and ketoconazole are no longer used. The relatively new triazoles—voriconazole, posaconazole, and isavuconazole—have been studied in a small number of refractory cases of cryptococcosis with moderate success,[314,376,377] but it is not clear whether they possess any advantage over fluconazole.

The antifungal class of β-glucan synthase inhibitors, such as caspofungin, micafungin, and anidulafungin, does not possess reliable anticryptococcal activity and is not to be used for management of cryptococcal infections.

Combination therapy for the management of cryptococcal meningitis has been extremely well studied. The combination of amphotericin B and flucytosine has become the standard therapy for meningitis, and in patients without AIDS it usually sterilizes CSF after 2 weeks of therapy. In fact, it clears CSF yeast counts significantly faster than amphotericin B alone, amphotericin B plus fluconazole, or all three agents together.[378] In areas without access to flucytosine, amphotericin B plus fluconazole has reasonable anticryptococcal induction success, and the 800-mg/day dose of fluconazole is probably superior to the 400-mg/day dose when added to amphotericin B.[379] Flucytosine and fluconazole together have been studied in animals and in open clinical trials for induction therapy with some benefit, and the combination is not inferior to the polyene combination regimens,[380] but this combination remains an alternative regimen when only oral therapy can be given pending further studies.[381] Finally, successful three-drug regimens for induction therapy have occasionally been successfully reported with a polyene, an azole, and flucytosine,[382] but the added benefit of a three-drug regimen has not consistently been proven to be better than the two-drug regimens.

A standard algorithm for the management of cryptococcal meningitis in patients with HIV is a three-stage regimen.[360] Induction-phase treatment is initiated with amphotericin B 0.7 mg/kg/day plus flucytosine 100 mg/kg/day for at least 2 weeks. In resource-limited environments, a 1-week induction combination of a polyene plus flucytosine appears to be effective, with a better outcome than prolonged polyene therapy.[380] Patients who have responded clinically may be switched to fluconazole 400 to 800 mg/day for 8 to 10 weeks as a consolidation phase. Finally, a suppressive phase is begun with fluconazole 200 mg once daily. The use of suppressive- or maintenance-phase therapy for cryptococcal meningitis became a concept during the pre-ART AIDS epidemic, when 50% to 60% of patients relapsed after therapy was stopped. With the use of fluconazole, daily suppression was better than that obtained with intermittent amphotericin B or itraconazole, and there was a reduction in the relapse rates to less than 5%.[372,373] Recent data in several studies have shown that administration of ART, with its ability to produce immune reconstitution (rising CD4 counts and lower HIV loads), allows antifungal therapy to be stopped after 1 to 2 years in patients with a CD4 count above 100/μL for at least 3 months, a nondetectable viral load, and a negative or low serum cryptococcal antigen.[383,384]

However, these patients should be followed off therapy with serum cryptococcal antigen titers for rising titers and/or development of new symptoms.

Patients without AIDS can be given either a 4-week induction-phase regimen of amphotericin B, with or without initial flucytosine, or, more commonly, the previously mentioned regimen used for AIDS patients of 2 weeks. If induction therapy includes only a polyene, this phase should be extended at least another 2 weeks. In transplant recipients with known renal toxicity issues, a lipid polyene formulation is favored with flucytosine for a 2-week induction period. These patients can then receive a consolidation phase of fluconazole 400 to 800 mg/day for 8 weeks and finally be placed on suppressive doses of fluconazole 200 mg/day. Criteria for stopping therapy are not well defined but include resolution of initial symptoms, negative CSF cultures from several milliliters of CSF, normal CSF glucose, and a prolonged asymptomatic period of 6 months to 1 year. A negative CSF or serum cryptococcal antigen does not appear to be required to discontinue therapy. Patients with continuing high-level immunosuppression may benefit from prolonged fluconazole therapy after these criteria are met because relapse rates are measurable. Because there was a 15% to 25% relapse rate before the AIDS epidemic, primarily occurring in the first year after stopping therapy, most patients will receive at least 12 months of suppressive fluconazole therapy.

The site of infection may modify the treatment recommendation.[330] Any presentation of disseminated cryptococcosis should probably follow the recommendations for cryptococcal meningitis. On the other hand, cryptococcosis confined to the lung in previously healthy persons responds well to fluconazole at 200 to 400 mg/day for 3 to 6 months.[67,368,369] Nonimmunosuppressed patients with endobronchial colonization but without radiologic evidence of pulmonary parenchymal disease do not require antifungal treatment. However, if the patient is symptomatic, immunocompromised, or at risk for immunosuppression, treatment should be started. CNS cryptococcomas are treated with a prolonged combination of amphotericin B and flucytosine for the induction phase and then tend to be treated for longer periods with fluconazole, but they rarely need surgical removal.[385] MRI scans of the brain may not show a decrease in lesion size for many months after treatment is started and even stopped. Edema around a lesion, if present, decreases more rapidly.[329]

Identifying a relapse or persistent infection can be difficult in patients with cryptococcal infections. The two clearest signs of relapse after at least 4 weeks of an established antifungal regimen, which suggest a change in management, are (1) development of new clinical signs and symptoms and (2) repeat positive cultures. The persistence of a positive India ink examination or changing versus fixed polysaccharide antigen titers are not precise indications of relapse. IRIS in cryptococcosis must be considered.[239] It is marked by a rapid return of an inflammatory response that may produce new symptoms, such as fever, headaches with or without increasing intracranial pressure, and increased number of host cells in the CSF. This syndrome may occur from several weeks to months to a year after beginning ART or after immunosuppressive dose reduction in transplant recipients. It is still not certain when it is best to initiate ART during the treatment of cryptococcal meningitis to prevent this syndrome, but recommendations range from 2 to 10 weeks after the start of antifungal therapy, and at present it is beneficial to not start ART during the induction phase of treatment.[331] In resource-limited settings ART may need to be started around the 4- to 5-week period. This recommendation is supported by the Cryptococcal Optimal ART Timing (COAT) trial, which showed a worse outcome with early ART administration (during the first 2 weeks of cryptococcal therapy) compared with delayed ART.[386] It is important to recognize that IRIS is not an indication of direct antifungal failure and might be improved with empirical corticosteroid therapy in seriously ill patients with CNS disease.

A critical management issue in cryptococcal meningitis is the role of increased intracranial pressure.[387,388] Patients with severe infection often present with CSF opening pressures in excess of 250 mm of CSF and rapidly progressing signs of cerebral edema. Clinicians need to particularly respond to high opening pressures and symptoms rather than a specific elevated opening pressure measurement. Symptoms include confusion, somnolence, severe headache, emesis, cranial nerve palsies, and fading vision. The pathophysiology for this elevated subarachnoid pressure even as antifungal treatment is started remains uncertain, but there is a suggestion of CSF outflow obstruction through the arachnoid villi by clumping of yeasts.[388] Furthermore, cerebral edema is obvious at autopsy, with uncal grooving, midbrain compression, and herniation of the cerebellar tonsils. Control of increased intracranial pressures with external drainage, such as by repeated lumbar punctures with large-bore needles and ventricular or lumbar drains, may be necessary during the early treatment phase, and frequent lumbar punctures may be especially important in resource-limited settings.[390,391] Persistent, symptomatic, high CSF pressures may warrant placement of a permanent CSF shunt. In retrospective reviews, corticosteroid treatment for elevated intracranial pressure without IRIS was not found to be generally useful.[387,388] In fact, a recent study confirms the lack of value for routine early use of corticosteroids in cryptococcal meningitis during induction therapy, with more disabilities, adverse events, and reduced antifungal killing of yeasts with the use of corticosteroids.[392] Thus cases need to be examined on an individual basis for use of corticosteroids during the diagnosis of IRIS. Unfortunately, despite interventions, blindness, permanent dementia, or death may still result.

It is vital to distinguish cerebral edema from classic hydrocephalus. The latter is diagnosed by the presence of dilated cerebral ventricles and dementia, with or without gait ataxia or urinary incontinence. CSF pressure may or may not be elevated. A loculated temporal horn of the lateral cerebral ventricle may present as a space-filling mass and cause transfalciform herniation. Symptoms of hydrocephalus need to be identified in the follow-up management period and can occur months after the initial diagnosis. A shunt for hydrocephalus can be placed successfully during effective therapy for cryptococcal meningitis.[328] No evidence suggests that a shunt placement after institution of appropriate antifungal therapy presents a foreign body that impairs cure, but it needs to be placed after the start of the antifungal regimen.

Every attempt to improve the immunity of the patient with cryptococcosis should be made. For example, a goal is to reduce the routine daily dose of prednisone to less than or equal to 20 mg/day during therapy. Adjunctive cytokine therapies, such as with granulocyte colony-stimulating factor, GM-CSF, and interferon-γ, have in vitro support, and preliminary human studies with interferon-γ as adjunctive therapy have been positive.[393,394] However, immunomodulation for management of cryptococcosis awaits further definitive clinical studies, and clinicians must be wary of rapidly pushing too far with immune stimulation into IRIS. At present, immunomodulation should probably be considered as adjunctive therapy for culture-positive refractory cases. Specific monoclonal antibodies for treatment remain in early clinical trials.[214] Finally, ART has a major influence on improving immunity and has made a significant impact on a patient's long-term prognosis with cryptococcal meningitis.[395] ART should be instituted and monitored in all HIV-infected patients after induction treatment for cryptococcal meningitis. It is essential to gain control of the HIV infection for long-term success.

PROGNOSIS

The most important prognostic factor for success in the treatment of cryptococcosis remains the ability to control the patient's underlying disease. In fact, it has been shown that cancer victims have shorter survival than patients with AIDS because of the inability to control their underlying neoplasm.[396] In another major group of patients with cryptococcosis, those who received solid-organ transplants, the results are conflicting. Studies have shown an outcome similar to that in patients without an underlying disease,[67] but another study reported a death rate of 42%.[366] Ironically, the group with the worst prognosis is the non-HIV, nontransplant recipient group, which likely represents the variety of underlying diseases or delay in making the diagnosis, or both.[397]

Several studies have examined the prognostic features of cryptococcal meningitis,[42,349,359] and a summary of the different populations, treatment modalities, and end point evaluations suggests that there are three major prognostic findings: (1) burden of yeasts at presentation, (2) poor inflammatory response, and (3) level of the patient's sensorium at presentation. For example, a poor prognosis is indicated by a strongly positive India ink examination, a high polysaccharide antigen titer (≥1:1024), and a poor inflammatory response in the CSF (<20 cells/μL).[398]

In one cohort, cryptococcosis was more severe in males, HIV-seropositive patients, and serotype A infection.[399] In a *C. gattii* cohort, both neurologic deterioration sufficient to require intensive care unit admission and the presence of underlying immunosuppressive diseases were associated with a poor prognosis.[400] Patients who present with a lucid sensorium have a better prognosis than those who are stuporous or in a coma. An abnormal brain imaging at baseline is associated with reduced survival. The prognosis is also influenced by the ability to manage the underlying disease and to detect and treat elevated intracranial pressure. For instance, poor prognostic underlying conditions are severe liver disease or a hematologic malignancy.[10] Multiple studies have identified the poor prognosis of patients with liver diseases, and this likely reflects both end-organ disease and delayed diagnosis.[401] Identification of these high-risk patients so that failure and relapse can be predicted may allow the clinician to design a specific antifungal regimen for this refractory subset. Although the host appears to be the primary factor in outcome, the infecting cryptococcal strain may have some impact on outcome, and more studies are warranted in this area.[402] In most cases in developed countries, the immediate mortality rate (at 6 months to a year) of cryptococcal meningitis unfortunately still remains at 10% to 25%.[103,403] In underdeveloped countries with limited resources, the mortality rate at 6 months can reach from 50% to 100%.[333]

PREVENTION

Four potential methods for preventing infection in high-risk patients exist. First, in the pre-ART era, fluconazole prophylaxis in patients with AIDS and CD4 counts under 100 cells/μL has been shown to be effective in reducing the incidence of cryptococcosis.[404–406] However, both the use of ART and concern about fluconazole resistance with its widespread use have reduced enthusiasm for this general approach in most settings. Second, active immunization with a vaccine in high-risk patients has been considered. A cryptococcal GXM–tetanus toxoid conjugate vaccine that protected mice has been developed,[407] and several new potential protective antigens have been identified.[408] However, human trials have not yet been conducted, and parameters for the use of a vaccine in nonimmunosuppressed populations with potential risk factors for disease are hard to define. Third, the use of protective serotherapy with specific monoclonal antibodies[409,410] could be considered in high-risk patients, but protection would require repeated injections. Fourth, a preemptive treatment strategy in areas of high cryptococcal prevalence and HIV infection, by using cryptococcal antigen screening with ART introduction, may be cost-effective in managing or preventing cryptococcal disease.[411] Finally, high-risk patients can attempt to avoid high-risk environments, such as sites where large numbers of yeasts might be aerosolized from bird droppings.

Key References

The complete reference list is available online at Expert Consult.

4. Maziarz EK, Perfect JR. Cryptococcosis. *Infect Dis Clin North Am*. 2016;30:179–206.
10. Kwon-Chung KJ. Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. *Mycologia*. 1976;68:821–833.
14. Desjardins CA, Giamberardino C, Sykes SM, et al. Population genomics and the evolution of virulence in the fungal pathogen *Cryptococcus neoformans*. *Genome Res*. 2017;27:1207–1219.
16. Kwon-Chung KJ, Bennett JE, Wickes BL, et al. The case for adopting the "species complex" nomenclature for the etiologic agents of cryptococcosis. *mSphere*. 2017;2.
17. Hagen F, Lumbsch HT, Arsic Arsenijevic V, et al. Importance of resolving fungal nomenclature: the case of multiple pathogenic species in the *Cryptococcus* genus. *mSphere*. 2017;2.
20. Fraser JA, Giles SS, Wenink EC, et al. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature*. 2005;437:1360–1364.
28. Khawcharoenporn T, Apisarnthanarak A, Mundy LM. Non-*neoformans* cryptococcal infections: a systematic review. *Infection*. 2007;35:51–58.
41. Posteraro B, Vella A, Cogliati M, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based method for discrimination between molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii*. *J Clin Microbiol*. 2012;50:2472–2476.
49. Levitz SM. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. *Rev Infect Dis*. 1991;13:1163–1169.
52. Bartlett K, Cheng P, Duncan C, et al. A decade of experience: *Cryptococcus gattii* in British Columbia. *Mycopathologia*. 2012;173:311–319.
55. Steenbergen JN, Shuman HA, Casadevall A. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc Natl Acad Sci*. 2001;98:15245–15250.
62. Goldman DL, Khine H, Abadi J. Serologic evidence for *Cryptococcus* infection in early childhood. *Pediatrics*. 2001;107:66.
67. Pappas PG, Perfect JR, Cloud GA, et al. Cryptococcosis in HIV-negative patients in the era of effective azole therapy. *Clin Infect Dis*. 2001;33:690–699.
70. Maskarinec SA, Johnson MD, Perfect JR. Genetic susceptibility to fungal infections: what is in the genes? *Curr Clin Microbiol Rep*. 2016;3:81–91.
80. Tenforde MW, Mokomane M, Leeme T, et al. Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: incidence of and temporal trends in cryptococcal meningitis. *Clin Infect Dis*. 2017.
81. Hakim JG, Gangaidzo IT, Heyderman RS. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *AIDS*. 2000;14:1401–1407.
84. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*. 2017.
99. Kontoyiannis DP, Peitsch WK, Reddy BT. Cryptococcosis in patients with cancer. *Clin Infect Dis*. 2001;32:145–150.
102. Husain A, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis*. 2001;7:375–381.
104. Pappas P, Alexander B, Andes D, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis*. 2012;50:1101–1111.
105. Kontoyiannis DP, Marr K, Park B, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. *Clin Infect Dis*. 2010;50:1091–1100.
111. Meiring S, Quan V, Cohen C, et al. A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. *AIDS*. 2012;26:2307–2314.
127. Faggi E, Gargani G, Pizzirani C, et al. Cryptococcosis in domestic mammals. *Mycoses*. 1993;36:165–170.
137. Perfect JR. *Cryptococcus neoformans:* a sugar-coated killer with designer genes. *FEMS Immunol Med Microbiol*. 2005;45:395–404.
143. Heitman J, Kozel T, Kwon-Chung KJ, et al. Cryptococcus neoformans: *From Human Pathogen to Model Yeast*. ASM Press; 2011:1–620.
150. Chen Y, Farrer RA, Giamberardino C, et al. Microevolution of serial clinical isolates of *Cryptococcus neoformans* var. *grubii* and *C. gattii*. *MBio*. 2017;8:e00166-17.
156. Casadevall A, Perfect JR. Cryptococcus neoformans. Washington: ASM Press; 1998:409.
170. Clancy CJ, Nguyen MH, Alandoerffer R, et al. *Cryptococcus neoformans* var. *grubii* isolates recovered from persons with AIDS demonstrate a wide range of virulence during murine meningoencephalitis that correlates with the expression of certain virulence factors. *Microbiology*. 2006;152(Pt 8):2247–2255.
179. Murphy JW. Cryptococcal immunity and immunostimulation. *Adv Exp Med Biol*. 1992;319:225–230.
183. Lee SC, Dickson DW, Casadevall A. Pathology of cryptococcal meningoencephalitis: analysis of 27 patients with pathogenetic implications. *Hum Pathol*. 1996;27:839–847.
192. Feldmesser M, Tucker SC, Casadevall A. Intracellular parasitism of macrophages by *Cryptococcus neoformans*. *Trends Microbiol*. 2001;9:273–278.
209. Rohatgi S, Pirofski LA. Host immunity to *Cryptococcus neoformans*. *Future Microbiol*. 2015;10:565–581.
210. Devi S, Scheerson R, Egan W. *Cryptococcus neoformans* serotype a glucuronoxylomanna protein conjugate vaccines: synthesis, characterization, and immunogenicity. *Infect Immun*. 1991;59:3700–3707.
215. Baker RD. The primary pulmonary lymph node complex of cryptococcosis. *Am J Clin Pathol*. 1976;65:83–92.
227. Warr W, Bates JH, Stone A. The spectrum of pulmonary cryptococcosis. *Ann Intern Med*. 1968;69:1109–1116.
238. Wiesner DL, Boulware DR. *Cryptococcus*-related immune reconstitution inflammatory syndrome (IRIS): pathogenesis and its clinical implications. *Curr Fungal Infect Rep*. 2011;5:252–261.
242. Mitchell DH, Sorrell TC, Allworth AM, et al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin Infect Dis*. 1995;20:611–616.
276. Boulware D, Meya D, Bergemann T, et al. Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: a prospective cohort study. *PLoS Med*. 2010;7:e1000384.
278. Panackal AA, Komori M, Kosa P, et al. Spinal arachnoiditis as a complication of cryptococcal meningoencephalitis in non-HIV previously healthy adults. *Clin Infect Dis*. 2017;64:275–283.
282. Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis*. 2014;58:736–745.
302. Hansen J, Slechta E, Gates-Hollingsworth M, et al. Large scale evaluation of the Immuno-Mycologics Inc. (IMMY) lateral flow and enzyme-linked immunoassays for the detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clin Vaccine Immunol*. 2013;20:52–55.
313. Cassim N, Coetzee LM, Schnippel K, et al. Estimating the cost-per-result of a national reflexed cryptococcal antigenaemia screening program: forecasting the impact of potential HIV guideline changes and treatment goals. *PLoS ONE*. 2017;12:e0182154.
331. Perfect JR, Dismukes WE, Pappas PG, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2010;50:291–322.
347. Sionov E, Lee H, Chang YC, et al. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog*. 2010;6:e1000848.
349. Bennett JE, Dismukes W, Duma RJ, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med*. 1979;301:126–131.
360. van der Horst C, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. *N Engl J Med*. 1997;337:15–21.
366. Day J, Chau TT, Wolbers M. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med*. 2013;368:1291–1302.
380. Malloy S, Kanyama C, Heyderman R A randomized controlled trial for the treatment of HIV-associated cryptocel meningites in African: oral fluconazole plus flucytosine or one week amphotericin B-based therapy vs two weeks amphotericin B-based therapy. The ACTA TRIAL Paris, 2017.
386. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med*. 2014;370:2487–2498.
387. Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. *Clin Infect Dis*. 2000;30:47–54.

References

1. Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. *Fungal Genet Biol*. 2015;78:16–48.
2. Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV. *AIDS*. 2009;23:525–530.
3. Perfect JR, Casadevall A. Cryptococcosis. *Infect Dis Clin North Am*. 2002;16:837–874.
4. Maziarz EK, Perfect JR. Cryptococcosis. *Infect Dis Clin North Am*. 2016;30:179–206.
5. Sanfelice F. Contributo alla morfologia e biolgia dei blastomiceti che si sviluppano nei succhi di alcuni frutti. *Ann d'Igiene*. 1894;4:463–495.
6. Buschke A. Ueber eine durch coccidien hemorgerufene krankheit des menschen. *Dtsch Med Wochenschr*. 1895;21:14.
7. Versé M. Über einen Fall von generalisierter Blastomykose beim Menschen. *Ver Dtsch Path Ges*. 1914;17:275–278.
8. Stoddard JL, Cutler EC. Torula infections in man. Rockefeller Institute for Medical Research. *Monograph*. 1916;6:1–98.
9. Benham RW. Cryptococcosis and blastomycosis. *Ann N Y Acad Sci*. 1950;50:1299–1314.
10. Kwon-Chung KJ. Morphogenesis of *Filobasidiella neoformans,* the sexual state of *Cryptococcus neoformans*. *Mycologia*. 1976;68:821–833.
11. Kwon-Chung KJ, Boekhout T, Fell JW, et al. Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). *Taxon*. 2002;51:804–806.
12. Loftus BJ, Fung E, Roncaglia P, et al. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. *Science*. 2005;307:1321–1324.
13. D'Souza CA, Kronstad JW, Taylor G, et al. Genome variation in *Cryptococcus gattii,* an emerging pathogen of immunocompetent hosts. *MBio*. 2011;2:e00342-10.
14. Desjardins CA, Giamberardino C, Sykes SM, et al. Population genomics and the evolution of virulence in the fungal pathogen *Cryptococcus neoformans*. *Genome Res*. 2017;27:1207–1219.
15. Billmyre RB, Croll D, Li W, et al. Highly recombinant VGII *Cryptococcus gattii* population develops clonal outbreak clusters through both sexual macroevolution and asexual microevolution. *MBio*. 2014;5:e01494-01414.
16. Kwon-Chung KJ, Bennett JE, Wickes BL, et al. The case for adopting the "species complex" nomenclature for the etiologic agents of cryptococcosis. *mSphere*. 2017;2.
17. Hagen F, Lumbsch HT, Arsic Arsenijevic V, et al. Importance of resolving fungal nomenclature: the case of multiple pathogenic species in the *Cryptococcus* genus. *mSphere*. 2017;2.
18. Xue C, Tada Y, Dong X, et al. The human fungal pathogen *Cryptococcus* can complete its sexual cycle during a pathogenic association with plants. *Cell Host Microbe*. 2007;1:263–273.
19. Botts M, Giles S, Gates M, et al. Isolation and characterization of *Cryptococcus neoformans* spores reveal a critical role for capsule biosynthesis genes in spore biogenesis. *Eukaryot Cell*. 2009;8:595–605.
20. Fraser JA, Giles SS, Wenink EC, et al. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature*. 2005;437:1360–1364.
21. Nielsen K, Heitman J. Sex and virulence of human pathogenic fungi. *Adv Genet*. 2007;57:143–173.
22. Kwon-Chung KJ, Bennett JE. Distribution of "alpha" and "a" mating types of *Cryptococcus neoformans* among natural and clinical isolates. *Am J Med*. 1978;108:337–340.
23. Wickes BL, Mayorga ME, Edman U, et al. Dimorphism and haploid fruiting in *Cryptococcus neoformans:* association with the alpha-mating type. *Proc Natl Acad Sci*. 1996;93:7327–7331.
24. Kwon-Chung KJ, Edman JC, Wickes BL. Genetic association of mating types and virulence in *Cryptococcus neoformans*. *Infect Immun*. 1992;60:602–605.
25. Nielsen K, Cox GM, Wang P, et al. Sexual cycle of *Cryptococcus neoformans* variety grubii and virulence of congenic a and alpha isolates. *Infect Immun*. 2003;71:4831–4841.
26. Luna T, Lusins J. *Cryptococcus albidus* meningitis. *South Med J*. 1973;66:1230.
27. Kromery V, Kunova A, Mardiak J. Nosocomial *Cryptococcus laurentii* fungemia in a bone marrow transplant patient after prophylaxis with ketoconazole successfully treated with oral fluconazole. *Infection*. 1997;25:130.
28. Khawcharoenporn T, Apisarnthanarak A, Mundy LM. Non-neoformans cryptococcal infections: a systematic review. *Infection*. 2007;35:51–58.
29. Ikeda R, Shinoda T, Fukuzawa Y, et al. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. *J Clin Microbiol*. 1982;36:22–29.
30. Dromer F, Gueho E, Ronin O, et al. Serotyping of *Cryptococcus neoformans* by using a monoclonal antibody specific for capsular polysaccharide. *J Clin Microbiol*. 1993;31:359–363.
31. Cleare W, Casadevall A. The different binding patterns of two IgM monoclonal antibodies to *Cryptococcus neoformans* serotype A and D strains correlates with serotype classification and differences in functional assays. *Clin Diagn Lab Immunol*. 1998;5:125–129.
32. Kwon-Chung K, Boekhout T, Wickes B, et al. *Systematics of the Genus* Cryptococcus *and Its Type Species* C. neoformans. Cryptococcus *From Human Pathogen to Model Yeast*. ASM Press; 2011:3–16.
33. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. grubii: separate variety status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol*. 1999;37:838–840.
34. Xu J, Vilgalys R, Mitchell TG. Multiple gene genealogies reveal recent dispersion and hybridization in the human fungus, *Cryptococcus neoformans*. *Mol Ecol*. 2002;9:1471–1481.
35. Perfect JR, Durack DT, Gallis HA. Cryptococcemia. *Medicine (Baltimore)*. 1983;62:98–109.
36. Goldman DL, Fries BC, Franzot SP, et al. Phenotypic switching in the human pathogenic fungus, *Cryptococcus neoformans,* is associated with changes in virulence and pulmonary inflammatory response in rodents. *Proc Natl Acad Sci*. 1998;95:14967–14972.
37. Fries BC, Goldman DL, Casadevall A. Phenotypic switching in *Cryptococcus neoformans*. *Microbiol Infect Dis*. 2002;4:1345–1352.
38. Martinez LR, Garcia-Rivera J, Casadevall A. *Cryptococcus neoformans* var. *neoformans* (serotype D) strains are more susceptible to heat than *C. neoformans* var. *grubii* (serotype A strains). *J Clin Microbiol*. 2001;39:3365–3367.
39. el-Zaatari M, Pasarell L, McGinnis MR, et al. Evaluation of the updated Vitek yeast identification data base. *J Clin Microbiol*. 1990;28:1938–1941.
40. St. Germain G, Beauchesne D. Evaluation of the microscan rapid yeast identification panel. *J Clin Microbiol*. 1991;29:2296–2299.
41. Posteraro B, Vella A, Cogliati M, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based method for discrimination between molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii*. *J Clin Microbiol*. 2012;50:2472–2476.
42. Zimmer BL, Roberts GD. Rapid selective urease test for presumptive identification of *Cryptococcus neoformans*. *J Clin Microbiol*. 1979;10:380–381.
43. Langfelder K, Streibel M, John B, et al. Biosynthesis of fungal melanins and their importance for human pathogenic fungi. *Fungal Genet Biol*. 2003;38:143–158.
44. Crabtree J, Okagaki LWD, Strain A, et al. Titan cell production enhances the virulence of *Cryptococcus neoformans*. *Infect Immun*. 2012;80:3776–3785.
45. Meyer W, Aanensen D, Boekhout T, et al. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococus gattii*. *Med Mycol*. 2009;47:561–570.
46. Gillece J, Schupp J, Balajee S, et al. Whole genome sequence analysis of *Cryptococcus gattii* from the Pacific Northwest reveals unexpected diversity. *PLoS ONE*. 2011;6:e28550.
47. Kronstad J, Loftus BJ, Lodge JK. The cryptococcus genomes: tools for comparative genomics and expression. In: Heitman J, Kozel T, Kwon-Chung KJ, et al, eds. *Cryptococcus: From Human Pathogen to Model Yeast*. Washington, DC: ASM Press; 2011:115–126.
48. Meyer W, Castaneda A, Jackson S, et al. Molecular typing of Ibero American *Cryptococcus neoformans* isolates. *Emerg Infect Dis*. 2003;9:189–195.
49. Levitz SM. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. *Rev Infect Dis*. 1991;13:1163–1169.
50. Emmons CW. Isolation of *Cryptococcus neoformans* from soil. *J Bacteriol*. 1951;62:685–690.
51. Emmons CW. Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon. *Am J Hyg*. 1955;62:227–232.
52. Bartlett K, Cheng P, Duncan C, et al. A decade of experience: *Cryptococcus gattii* in British Columbia. *Mycopathologia*. 2012;173:311–319.
53. Ellis DH, Pfeiffer TJ. Ecology, life cycle, and infectious propagule of *Cryptococcus neoformans*. *Lancet*. 1990;336:923–925.
54. Stephen C, Lester S, Black W, et al. Multispecies outbreak cryptococcosis on southern Vancouver Island, British Columbia. *Can J Vet Res*. 2002;43:792–794.
55. Steenbergen JN, Shuman HA, Casadevall A. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc Natl Acad Sci*. 2001;98:15245–15250.
56. Mylonakis E, Casadevall A, Ausubel FM. Exploiting amoeboid and non-vertebrate animal model systems to study the virulence of human pathogenic fungi. *PLoS Pathog*. 2007;3:e101.
57. Mylonakis E, Ausubel FM, Perfect JR, et al. Killing of *Caenorhabditis elegans* by *Cryptococcus neoformans* as a model of yeast pathogenesis. *Proc Natl Acad Sci*. 2002;99:15675–15680.
58. Howard DH. The commensalism of *Cryptococcus neoformans*. *Sabouraudia*. 1973;11:171–174.
59. Schimpff SC, Bennett JE. Abnormalities in cell-mediated immunity in patients with *Cryptococcus neoformans* infection. *J Allergy Clin Immunol*. 1975;55:430–441.
60. Newberry WM Jr, Walter JE, Chandler JW Jr, et al. Epidemiologic study of *Cryptococcus neoformans*. *Ann Intern Med*. 1967;67:724–732.
61. Chen L-C, Goldman DL, Doering TL. Antibody response to *Cryptococcus neoformans* proteins in rodents and humans. *Infect Immun*. 1999;67:2218–2224.
62. Goldman DL, Khine H, Abadi J. Serologic evidence for *Cryptococcus* infection in early childhood. *Pediatrics*. 2001;107:66.
63. Davis J, Zheng WY, Glatman-Freedman A, et al. Serologic evidence for regional differences in pediatric cryptococcal infection. *Pediatr Infect Dis J*. 2007;26:549–551.
64. Zonios DI, Falloon J, Huang CY, et al. Cryptococcosis and idiopathic CD4 lymphocytopenia. *Medicine (Baltimore)*. 2007;86:78–92.
65. Nath DS, Kandaswamy R, Gruessner R, et al. Fungal infections in transplant recipients receiving alemtuzumab. *Transplant Proc*. 2005;37:934–936.
66. Tsiodras S, Samonis G, Boumpas DT, et al. Fungal infections complicating tumor necrosis factor alpha blockade therapy. *Mayo Clin Proc*. 2008;83:181–194.
67. Pappas PG, Perfect JR, Cloud GA, et al. Cryptococcosis in HIV-negative patients in the era of effective azole therapy. *Clin Infect Dis*. 2001;33:690–699.
68. Meletiadis J, Walsh TJ, Choi EH, et al. Study of common functional genetic polymorphisms of *FCGR2A, 3A* and *3B* genes and the risk for cryptococcosis in HIV-uninfected patients. *Med Mycol*. 2007;45:513–518.
69. Hu X, Wu J, Zhu L, et al. Association of Fcy receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. *PLoS ONE*. 2012;7:e42439.
70. Maskarinec SA, Johnson MD, Perfect JR. Genetic susceptibility to fungal infections: what is in the genes? *Curr Clin Microbiol Rep*. 2016;3:81–91.
71. Rosen LB, Freeman AF, Yang LM, et al. Anti-GM-CSF autoantibodies in patients with cryptococcal meningitis. *J Immunol*. 2013;190:3959–3966.
72. Saijo T, Chen J, Chen SC, et al. Anti-granulocyte-macrophage colony-stimulating factor autoantibodies are a risk factor for central nervous system infection by *Cryptococcus gattii* in otherwise immunocompetent patients. *MBio*. 2014;5:e00912-00914.
73. Hajjman A, Conn LA, Stephens DS. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. Cryptococcal Active Surveillance Group. *J Infect Dis*. 1999;179:449–454.
74. McNeil JI, Kan VL. Decline in the incidence of cryptococcosis among HIV-related patients. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1995;9:206–208.
75. van Elden LJ, Walenkamp AM, Lipovsky MM. Declining number of patients with cryptococcosis in the Netherlands in the era of highly active antiretroviral therapy. *AIDS*. 2000;14:2787–2788.
76. Mirza S, Phelan M, Rimland D, et al. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992-2000. *Clin Infect Dis*. 2002;36:789–794.
77. Clumeck N, Sonnet J, Taelman H, et al. Acquired immunodeficiency syndrome in African patients. *N Engl J Med*. 1984;310:492–497.
78. Van de Perre P, Lepage P, Kestelyn P. Acquired immunodeficiency syndrome in Rwanda. *Lancet*. 1984;2:62–65.
79. McCarthy KM, Morgan J, Wannemuehler KA, et al. Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. *AIDS*. 2006;20:2199–2206.
80. Tenforde MW, Mokomane M, Leeme T, et al. Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: incidence of and temporal trends in cryptococcal meningitis. *Clin Infect Dis*. 2017.
81. Hakim JG, Gangaidzo IT, Heyderman RS. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *AIDS*. 2000;14:1401–1407.

82. Dore GJ, Li Y, McDonald A, et al. Spectrum of AIDS-defining illnesses in Australia, 1992 to 1998: influence of country/region of birth. *J Acquir Immune Defic Syndr*. 2000;26:283–290.
83. Archibald LK, McDonald LC, Rheanpumikankit S. Fever and human immunodeficiency virus infection as sentinels for emerging mycobacterial and fungal bloodstream infections in hospitalized patients >15 years old, Bangkok. *J Infect Dis*. 1999;180:87–92.
84. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*. 2017.
85. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. *Am J Epidemiol*. 1984;120:123–140.
86. Hagen F, Colom MF, Swinne D, et al. Autochthonous and dormant *Cryptococcus gattii* infections in Europe. *Emerg Infect Dis*. 2012;18:1618–1624.
87. McCulloh RJ, Phillips R, Perfect JR, et al. *Cryptococcus gattii* genotype VGI infection in New England. *Pediatr Infect Dis J*. 2011;30:1111–1114.
88. Bennett JE, Kwon-Chung KJ, Howard DH. Epidemiology differences among serotypes of *Cryptococcus neoformans*. *Am J Epidemiol*. 1977;105:582–586.
89. Steenbergen JN, Casadevall A. Prevalence of *Cryptococcus neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var. *grubii* (serotype A) isolates in New York City. *J Clin Microbiol*. 2000;38:1974–1976.
90. Byrnes E, Li W, Ren P, et al. A diverse population of *Cryptococcus gattii* molecular type VHIII in southern Californian HIV/AIDS patients. *PLoS Pathog*. 2011;7: e1002205.
91. Nyazika TK, Hagen F, Meis JF, et al. *Cryptococcus tetragattii* as a major cause of cryptococcal meningitis among HIV-infected individuals in Harare, Zimbabwe. *J Infect*. 2016;72:745–752.
92. Byrnes E, Li W, Ren P, et al. A diverse population of *Cryptococcus gattii* molecular type VHIII in southern Californian HIV/AIDS patients. *PLoS Pathog*. 2011;7: e1002205.
93. Morgan J, McCarthy KM, Gould S, et al. *Cryptococcus gattii* infection: characteristics and epidemiology of cases identified in a South African province with high HIV seroprevalence, 2002-2004. *Clin Infect Dis*. 2006;43:1077–1080.
94. Steele KT, Thakur R, Nthobatsang R, et al. In-hospital mortality of HIV-infected cryptococcal meningitis patients with *C. gattii* and *C. neoformans* infection in Gaborone, Botswana. *Med Mycol*. 2010;48:1112–1115.
95. Collins VP, Gellhorn A, Trimble JR. The coincidence of cryptococcosis and disease of the reticulo-endothelial and lymphatic systems. *Cancer*. 1995;4:883–889.
96. Zimmerman LE, Rappaport H. Occurrence of cryptococcosis in patients with malignant disease of reticuloendothelial system. *Am J Clin Pathol*. 1954;24:1050.
97. Kaplan MH, Rosen PP, Armstrong D. *Cryptococcus* in a cancer hospital: clinical and pathological correlates in forty-six patients. *Cancer*. 1977;39:2265–2274.
98. Hutter RVP, Collins HS. The occurrence of opportunistic fungus infections in a cancer hospital. *Lab Invest*. 1962;11:1035–1045.
99. Kontoyiannis DP, Peitsch WK, Reddy BT. Cryptococcosis in patients with cancer. *Clin Infect Dis*. 2001;32:145–150.
100. Messina JA, Maziarz EK, Spec A, et al. Disseminated cryptococcosis with brain involvement in patients with chronic lymphoid malignancies on ibrutinib. *Open Forum Infect Dis*. 2017;4:ofw261.
101. Roussey JA, Viglianti SP, Teitz-Tennenbaum S, et al. Anti-PD-1 antibody treatment promotes clearance of persistent cryptococcal lung infection in mice. *J Immunol*. 2017;199:3535–3546.
102. Husain A, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis*. 2001;7:375–381.
103. Singh N, Gayowski T, Wagener MM, et al. Clinical spectrum of invasive cryptococcosis in liver transplant recipients receiving tacrolimus. *Clin Transpl*. 1997;11: 66–70.
104. Pappas P, Alexander B, Andes D, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis*. 2012;50:1101–1111.
105. Kontoyiannis DP, Marr K, Park B, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. *Clin Infect Dis*. 2010;50:1091–1100.
106. Beyt BE, Waltman SR. Cryptococcal endophthalmitis after corneal transplantation. *N Engl J Med*. 1978;298: 825–826.
107. Kanj SS, Welty-Wolf K, Madden J, et al. Fungal infections in lung and heart-lung transplant recipients, report of 9 cases and review of the literature. *Medicine (Baltimore)*. 1996;75:142–156.
108. Ooi BS, Chen BT, Lim CH, et al. Survival of a patient transplanted with a kidney infected with *Cryptococcus neoformans*. *Transplantation*. 1971;11:428–429.
109. Dromer F, Mathoulin-Pelissier S, Launay O, et al. Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study. *PLoSMed*. 2007;4:e21.
110. McClelland EE, Hobbs LM, Rivera J, et al. The role of host gender in the pathogenesis of *Cryptococcus neoformans* infections. *PLoS ONE*. 2013;8:e63632.
111. Meiring S, Quan V, Cohen C, et al. A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. *AIDS*. 2012;26:2307–2314.
112. Iseki M, Anzo M, Yamashita N, et al. Hyper-IgM immunodeficiency with disseminated cryptococcosis. *Acta Paediatr*. 1994;83:780–782.
113. Tabone MD, Leverger G, Landman J, et al. Disseminated lymphonodular cryptococcosis in a child with X-linked hyper-IgM immunodeficiency. *Pediatr Infect Dis J*. 1994;13:77–79.
114. Dev D, Basran GS, Slater D. Consider HIV negative immunodeficiency in cryptococcosis. *BMJ*. 1994; 308:1436.
115. Olson PE, Earhart KC, Rossetti RJ. Smoking and risk of cryptococcosis in patients with AIDS. *JAMA*. 1997; 277:629.
116. Shorman M, Evans D, Gibson C, et al. Cases of disseminated cryptococcosis in intravenous drug abusers without HIV infection: a new risk factor? *Med Mycol Case Rep*. 2016;14:17–19.
117. Casadevall AJ, Mukherjee J, Ruong R, et al. Management of *Cryptococcus neoformans* contaminated needle injuries. *Clin Infect Dis*. 1994;19:951–953.
118. Glaser JB, Garden A. Inoculation of cryptococcosis without transmission of the acquired immunodeficiency syndrome. *N Engl J Med*. 1985;313:264.
119. Powell KE, Dahl BA, Weeks RJ, et al. Airborne *Cryptococcus neoformans:* particles from pigeon excreta compatible with alveolar deposition. *J Infect Dis*. 1972;126:412–415.
120. Wiest PM, Flanigan T, Salata RA, et al. Serious infectious complications of corticosteroid therapy for COPD. *Chest*. 1989;95:1180–1183.
121. Ruiz A, Bulmer GS. Particle size of airborne *Cryptococcus neoformans* in a tower. *Appl Environ Microbiol*. 1980;41: 1225–1229.
122. Sorrell TC, Chen S, Ruma P, et al. Concordance of clinical and environmental isolates of *Cryptococcus neoformans* var. *gattii* by random amplification of polymorphic DNA analysis and PCR fingerprinting. *J Clin Microbiol*. 1996;34:1253–1260.
123. Currie BP, Freundlich LF, Casadevall A. Restriction fragment length polymorphism analysis of *Cryptococcus neoformans* isolates from environmental (pigeon excreta) and clinical sources in New York City. *J Clin Microbiol*. 1994;32:1188–1192.
124. Yamamoto Y, Kohno S, Koga H, et al. Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. *J Clin Microbiol*. 1995;33:3328–3332.
125. Sorvillo F, Beall G, Turner PA. Incidence and factors associated with extrapulmonary cryptococcosis among persons with HIV infection in Los Angeles County. *AIDS*. 1997;11:673–679.
126. Cogliati M, Puccianti E, Montagna MT, et al. Fundamental niche prediction of the pathogenic yeasts *Cryptococcus neoformans* and *Cryptococcus gattii* in Europe. *Environ Microbiol*. 2017;19:4318–4325.
127. Faggi E, Gargani G, Pizzirani C, et al. Cryptococcosis in domestic mammals. *Mycoses*. 1993;36:165–170.
128. Malik R, Dill-Mackey E, Martin P, et al. Cryptococcosis in dogs: a retrospective study of 20 consecutive cases. *J Med Vet Mycol*. 1995;33:291–297.
129. Malik R, Wigney DI, Muir DB, et al. Cryptococcosis in cats: clinical and mycological assessment of 29 cases and evaluation of treatment using orally administered fluconazole. *J Med Vet Mycol*. 1992;30:133–144.
130. Nosanchuk JD, Shoham S, Fries BC, et al. Evidence for zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised patient. *Ann Intern Med*. 2000;132:205–208.
131. Fessel WJ. Cryptococcal meningitis after unusual exposures to birds. *N Engl J Med*. 1993;328: 1354–1355.
132. Vallabhaneni S, Haselow D, Lloyd S, et al. Cluster of *Cryptococcus neoformans* infections in intensive care unit, Arkansas, USA, 2013. *Emerg Infect Dis*. 2015;21: 1719–1724.
133. Idnurm A, Williamson P. Genetic and genomic approches to *Cryptococcus* environmental and host responses. In: Heitman J, Kozel T, Kwon-Chung KJ, et al, eds. *Cryptococcus From Human Pathogen to Model Yeast*. Washington, DC: ASM Press; 2011:127–138.
134. Beale MA, Sabiiti W, Robertson EJ, et al. Genotypic diversity is associated with clinical outcome and phenotype in cryptococcal meningitis across Southern Africa. *PLoS Negl Trop Dis*. 2015;9:e0003847.
135. Loftus BJ, Fung E, Roncaglia P, et al. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. *Science*. 2005;307:1321–1324.
136. Liu OW, Chun CD, Chow ED, et al. Systematic genetic analysis of virulence in the human fungal pathogen *Cryptococcus neoformans*. *Cell*. 2008;135:174–188.
137. Perfect JR. *Cryptococcus neoformans:* a sugar-coated killer with designer genes. *FEMS Immunol Med Microbiol*. 2005;45:395–404.
138. Tenor JL, Oehlers SH, Yang JL, et al. Live imaging of host-parasite interaction in a zebrafish infection model reveals cryptococcal determinants of virulence and central nervous system invasion. *MBio*. 2015;6: e01425.
139. Del Poeta M, Toffaletti DL, Rude TH, et al. *Cryptococcus neoformans* differential gene expression detected *in vitro* and *in vivo* with green fluorescent protein. *Infect Immun*. 1999;67:1812–1820.
140. Jung KW, Yang DH, Maeng S, et al. Systematic functional profiling of transcription factor networks in *Cryptococcus neoformans*. *Nat Commun*. 2015;6:6757.
141. Chen YTD, Tenor JL, Litvintseva AP, et al. The *Cryptococcus neoformans* transcriptome at the site of human meningitis. *MBio*. 2014;pii: e01087-13.
142. Chow ED, Liu OW, O'Brien S, et al. Exploration of whole-genome responses of the human AIDS-associated yeast pathogen *Cryptococcus neoformans* var. *grubii:* nitric oxide stress and body temperature. *Curr Genet*. 2007;52: 137–148.
143. Heitman J, Kozel T, Kwon-Chung KJ, et al. Cryptococcus neoformans: *From Human Pathogen to Model Yeast*. ASM Press; 2011:1–620.
144. Clarke SC, Dumesic PA, Homer CM, et al. Integrated activity and genetic profiling of secreted peptidases in *Cryptococcus neoformans* reveals an aspartyl peptidase required for low pH survival and virulence. *PLoS Pathog*. 2016;12:e1006051.
145. Liew KL, Jee JM, Yap I, et al. In vitro analysis of metabolites secreted during infection of lung epithelial cells by *Cryptococcus neoformans*. *PLoS ONE*. 2016;11:e0153356.
146. Morrow CA, Lee RI, Chow EW. A unique chromosomal rearrangement in the *Cryptococcus neoformans* var. *grubii* type stain enhances key phenotypes associated with virulence. *MBio*. 2012;3:e00310-11(2012).
147. Sun S, Billmyre RB, Mieczkowski PA, et al. Unisexual reproduction drives meiotic recombination and phenotypic and karyotypic plasticity in *Cryptococcus neoformans*. *PLoS Genet*. 2014;10:e1004849.
148. Kronstad J, Saikia S, Nielson ED, et al. Adaptation of *Cryptococcus neoformans* to mammalian hosts: integrated regulation of metabolism and virulence. *Eukaryot Cell*. 2012;11:109–118.
149. Hu G, Wang J, Choi J. Chromosome copy number variation *Cryptococcus neoformans* influences virulence and occurs in isolates from AIDS patients. *BMC Genomic*. 2012;12:256.
150. Chen Y, Farrer RA, Giamberardino C, et al. Microevolution of serial clinical isolates of *Cryptococcus neoformans* var. *grubii* and *C. gattii*. *MBio*. 2017;8:e00166-17.
151. Rhodes J, Beale MA, Vanhove M, et al. A population genomics approach to assessing the genetic basis of within-host microevolution underlying recurrent cryptococcal meningitis infection. *G3 (Bethesda)*. 2017;7:1165–1176.
152. Ormerod KLMC, Chow EW, Lee IR, et al. Comparative genomics of serial isolates of *Cryptococcus neoformans* reveals gene associated with carbon utilization and virulence. *G3 (Bethesda)*. 2013;(g3. 113.005660v1).
153. Chang YC, Kwon-Chung KJ. Complementation of a capsule-deficiency mutation of *Cryptococcus neoformans* restores its virulence. *Mol Cell Biol*. 1994;14:4912–4919.
154. Bottone EJ, Wormser GP. Poorly encapsulated *Cryptococcus neoformans* from patients with AIDS. II: correlation of capsule size observed directly in cerebrospinal fluid with that after animal passage. *AIDS Res Hum Retrovirus*. 1986;2:219–225.
155. Farmer S, Komorowski R. Histologic response to capsule-deficient *Cryptococus neoformans*. *Arch Pathol*. 1973;96:383–387.
156. Casadevall A, Perfect JR. Cryptococcus neoformans. Washington: ASM Press; 1998:409.
157. Zaragoza O, Chrisman CJ, Castelli MV, et al. Capsule enlargement in *Cryptococcus neoformans* confers

resistance to oxidative stress suggesting a mechanism for intracellular survival. *Cell Microbiol*. 2008;10:2043–2057.

158. Klutts JS, Doering TL. Cryptococcal xylosyltransferase 1 (Cxt1p) from *Cryptococcus neoformans* plays a direct role in the synthesis of capsule polysaccharides. *J Biol Chem*. 2008;283:14327–14334.
159. Haynes B, Skowyra M, Spencer S, et al. Toward an integrated model of capsule regulation in *Cryptococcus neoformans*. *PLoS Pathog*. 2011;7:e1002411.
160. Alspaugh JA, Perfect JR, Heitman J. *Cryptococcus neoformans* mating and virulence are regulated by the G-protein gamma subunit GPA1 and cAMP. *Genes Dev*. 1997;11:3206–3217.
161. Salas SD, Bennett JE, Kwon-Chung KJ, et al. Effect of the laccase gene, CNLAC1, on virulence of *Cryptococcus neoformans*. *J Exp Med*. 1996;184:377–386.
162. Nosanchuk JD, Rosas AL, Lee SC. Melanisation of *Cryptococcus neoformans* in human brain tissue. *Lancet*. 2000;355:2049–2050.
163. Rosas AL, Nosanchuk JD, Feldmesser M. Synthesis of polymerized melanin by *Cryptococcus neoformans* in infected rodents. *Infect Immun*. 2000;68:2845–2853.
164. Odom A, Muir S, Lim E, et al. Calcineurin is required for virulence of *Cryptococcus neoformans*. *EMBO J*. 1997;16: 2576–2589.
165. Alspaugh JA, Cavallo LM, Perfect JR, et al. RAS1 regulates filamentation, mating, and growth at high temperature of *Cryptococcus neoformans*. *Mol Microbiol*. 2000;36:352–365.
166. Petzold EW, Himmelreich U, Mylonakis E, et al. Characterization and regulation of the trehalose synthesis pathway and its importance in the pathogenicity of *Cryptococcus neoformans*. *Infect Immun*. 2006;74: 5877–5887.
167. Ngamskulrungroj P, Himmelreich V, Breger JA, et al. The trehalose pathway: an integral part of virulence composit for *Cryptococcus gattii*. *Infect Immun*. 2009;77:4584–4596.
168. Erickson T, Liu L, Gueylkian A, et al. Multiple virulence factors of *Cryptococcus neoformans* are dependent on VPH1. *Mol Microbiol*. 2001;42:1121–1131.
169. Perfect JR. *Crytococcus neoformans:* the yeast that likes it hot. *FEMS Yeast Res*. 2006;6:463–468.
170. Clancy CJ, Nguyen MH, Alandoerffer R, et al. *Cryptococcus neoformans* var. *grubii* isolates recovered from persons with AIDS demonstrate a wide range of virulence during murine meningoencephalitis that correlates with the expression of certain virulence factors. *Microbiology*. 2006;152(Pt 8):2247–2255.
171. Cox GM, McDade HC, Chen SC, et al. Extracellular phospholipase activity is a virulence factor for *Cryptococcus neoformans*. *Mol Microbiol*. 2001;39: 166–175.
172. Shi M, Li S, Zheng C, et al. Real-time imaging of trapping and urease-dependent transmigration of *Cryptococcus neoformans* in mouse brain. *J Clin Invest*. 2010;120:1683–1693.
173. Cox GM, Mukherjee J, Cole GT, et al. Urease as a virulence factor in experimental cryptococcosis. *Infect Immun*. 2000;68:443–448.
174. Pukkila-Worley R, Gerrald Q, Kraus P, et al. Transcriptional network of multiple capsule and melanin genes governed by the *Cryptococcus neoformans* cyclic AMP cascade. *Eukaryot Cell*. 2005;4:190–201.
175. Giles SS, Batinic-Haberle I, Perfect JR, et al. *Cryptococcus neoformans* mitochondrial superoxide dismutase: an essential link between antioxidant function and high-temperature growth. *Eukaryot Cell*. 2005;4:46–54.
176. Akhter S, McDade HC, Gorlach JM, et al. Role of alternative oxidase gene in pathogenesis of *Cryptococcus neoformans*. *Infect Immun*. 2003;71:5794–5802.
177. de Jesus-Berrios M, Liv L, Nussbaum JC, et al. Enzymes that counteract nitrosative stress promote fungal virulence. *Curr Biol*. 2003;13:1963–1968.
178. Del Poeta M, Cruz MC, Cardenas ME, et al. Synergistic antifungal activities of bafilomycin A(1), fluconazole, and the pneumocandin MK-0991/caspofungin acetate (L-743,873) with calcineurin inhibitors FK506 and L-685,818 against *Cryptococcus neoformans*. *Antimicrob Agents Chemother*. 2000;44:739–746.
179. Murphy JW. Cryptococcal immunity and immunostimulation. *Adv Exp Med Biol*. 1992;319:225–230.
180. Levitz SM. Overview of host defenses in fungal infections. *Clin Infect Dis*. 1992;14:S37–S42.
181. Schwartz DA. Characterization of the biological activity of *Cryptococcus* infections in surgical pathology. *Ann Clin Lab Sci*. 1988;18:388–397.
182. Lipscomb MF. Lung defenses against opportunistic infections. *Chest*. 1989;96:1393–1399.
183. Lee SC, Dickson DW, Casadevall A. Pathology of cryptococcal meningoencephalitis: analysis of 27 patients with pathogenetic implications. *Hum Pathol*. 1996;27: 839–847.
184. Aguirre K, Havell EA, Gibson GW, et al. Role of tumor necrosis factor and gamma interferon in acquired resistance to *Cryptococcus neoformans* in the central nervous system of mice. *Infect Immun*. 1995;63:1725–1731.
185. Kawakami K, Tohyama M, Teruya K. Contribution of interferon-gamma in protecting mice during pulmonary and disseminated infection with Cryptococcus neoformans. *FEMS Immunol Med Microbiol*. 1996;13:133–140.
186. Huffnagle GB, Strieter RM, McNeil LK. Macrophage inflammatory protein-1 alpha (MIP-alpha) is required for the efferent phase of pulmonary cell-mediated immunity to a *Cryptococcus neoformans* infection. *J Immunol*. 1997;159:318–327.
187. Huffnagle GB, Traynor TR, McDonald RA. Leukocyte recruitment during pulmonary *Cryptococcus neoformans* infection. *Immunopharmacology*. 2000;48:231–236.
188. Hill JO. 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*. 1992;175:1685–1695.
189. Levitz SM. Macrophage-*Cryptococcus* interactions. In: Zwilling BS, Eisenstein TK, eds. *Macrophage-Pathogen Interactions*. New York: Marcel Dekker; 1994:533–543.
190. Leopold Wager CM, Wormley FL Jr. Classical versus alternative macrophage activation: the ying and the yang in host defense against pulmonary fungal infections. *Mucosal Immunol*. 2014;7:1023–1035.
191. Feldmesser M, Kress Y, Novikoff P, et al. *Cryptococcus neoformans* is a facultative intracellular pathogen in murine pulmonary infection. *Infect Immun*. 2000;68:4225–4237.
192. Feldmesser M, Tucker SC, Casadevall A. Intracellular parasitism of macrophages by *Cryptococcus neoformans*. *Trends Microbiol*. 2001;9:273–278.
193. Alvarez M, Casadevall A. Cell-to-cell spread and massive vacuole formation after *Cryptococcus neoformans* infection of murine macrophages. *BMC Immunol*. 2007;8:16.
194. Crowe SM, Carlin JB, Stewart KI, et al. Predictive value of CD4 lymphocyte numbers for the development of opportunistic infections and malignancies in HIV-infected persons. *J Acquir Immune Defic Syndr*. 1991;4:770–776.
195. Wozniak KL, Young ML, Wormley FL Jr. Protective immunity against experimental pulmonary cryptococcosis in T cell-depleted mice. *Clin Vaccine Immunol*. 2011;18:717–723.
196. Giles SS, Zaas AK, Reidy MF, et al. *Cryptococcus neoformans* is resistant to surfactant protein A mediated host defense mechanisms. *PLoS ONE*. 2007;2:e1370.
197. Nakamura K, Kinjo T, Saijo S, et al. Dectin-1 is not required for the host defense to *Cryptococcus neoformans*. *Microbiol Immunol*. 2007;51:1115–1119.
198. Nakamura K, Miyagi K, Koguchi Y, et al. Limited contribution of Toll-like receptor 2 and 4 to the host response to a fungal infectious pathogen, *Cryptococcus neoformans*. *FEMS Immunol Med Microbiol*. 2006;47:148–154.
199. Geunes-Boyer S, Beers M, Perfect J, et al. Surfactant protein D facilitates *Cryptococcus neoformans* infection. *Infect Immun*. 2012;80:2444–2453.
200. Deleted in review.
201. Siddiqui AA, Brouwer AE, Wuthiekanun V, et al. IFN-gamma at the site of infection determines rate of clearance of infection in cryptococcal meningitis. *J Immunol*. 2005;174:1746–1750.
202. Henderson DK, Bennett JE, Huber MA. Long-lasting specific immunologic unresponsiveness associated with cryptococcal meningitis. *J Clin Invest*. 1982;69:1185–1190.
203. Rohatgi SGS, Kuniholm MH, Schultz H, et al. Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. *MBio*. 2013;(e00573-13).
204. Wong B, Perfect JR, Beggs S, et al. Production of the hexitol D-mannitol by *Cryptococcus neoformans* in vitro and in rabbits with experimental meningitis. *Infect Immun*. 1990;58:1664–1670.
205. Williamson PR. Biochemical and molecular characterization of the diphenol oxidase of *Cryptococcus neoformans:* identification as a laccase. *J Bacteriol*. 1994;176:656–664.
206. Noverr MC, Phare SM, Toews GB, et al. Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect Immun*. 2001;69:2957–2963.
207. Vecchiarelli A, Casadevall A. Antibody-mediated effects against *Cryptococcus neoformans:* evidence for interdependency and collaboration between humoral and cellular immunity. *Res Immunol*. 1998;149:321–333.
208. Rohatgi S, Pirofski L. Molecular characterization of the early B cell response to pulmonary *Cryptococcus neoformans* infection. *J Immunol*. 2012;189:5820–5830.
209. Rohatgi S, Pirofski LA. Host immunity to *Cryptococcus neoformans*. *Future Microbiol*. 2015;10:565–581.
210. Devi S, Scheerson R, Egan W. *Cryptococcus neoformans* serotype a glucuronoxylomanna protein conjugate vaccines: synthesis, characterization, and immunogenicity. *Infect Immun*. 1991;59:3700–3707.
211. Rosas AL, Nosanchuk JD, Casadevall A. Passive immunization with melanin-binding monoclonal antibodies prolongs survival in mice with lethal *Cryptococcus neoformans* infection. *Infect Immun*. 2001;69:3410–3412.
212. Rodriques ML, Travassos LR, Miranda KR. Human antibodies against a purified glucosylceramide from *Cryptococcus neoformans* inhibit cell budding and fungal growth. *Infect Immun*. 2000;68:7049–7060.
213. Fleuridor R, Lyles RH, Pirofski L. Quantitative and qualitative differences in the serum antibody profiles of human immunodeficiency virus-infected persons with and without *Cryptococcus neoformans* meningitis. *J Infect Dis*. 1999;180:1526–1535.
214. Larsen RA, Pappas PG, Perfect J, et al. Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. *Antimicrob Agents Chemother*. 2005;49:952–958.
215. Baker RD. The primary pulmonary lymph node complex of cryptococcosis. *Am J Clin Pathol*. 1976;65:83–92.
216. Salyer WR, Salyer DC, Baker RD. Primary complex of *Cryptococcus* and pulmonary lymph nodes. *J Infect Dis*. 1974;130:74–77.
217. Garcia-Hermoso D, Janbon G, Dromer F. Epidemiological evidence for dormant *Cryptococcus neoformans* infection. *J Clin Microbiol*. 1999;37:3204–3209.
218. Perfect JR. Cryptococcosis. *Infect Dis Clin North Am*. 1989;3:77–102.
219. Kovacs JA, Kovacs AA, Polis M, et al. Cryptococcosis in the acquired immunodeficiency syndrome. *Ann Intern Med*. 1985;103:533–538.
220. Zuger A, Louie E, Holzman RS, et al. Cryptococcal disease in patients with acquired immunodeficiency syndrome: diagnostic features and outcome of treatment. *Ann Intern Med*. 1986;104:234–240.
221. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. *N Engl J Med*. 1989;321:794–799.
222. Clark RA, Greer D, Atkinson W, et al. Spectrum of *Cryptococcus neoformans* infection in 68 patients infected with acquired immunodeficiency virus. *Rev Infect Dis*. 1990;12:768–777.
223. Duperval R, Hermans PE, Brewer NS, et al. Cryptococcosis, with emphasis on the significance of isolation of *Cryptococcus neoformans* from the respiratory tract. *Chest*. 1977;72:13–19.
224. Henson DJ, Hill AR. Cryptococcal pneumonia: a fulminant presentation. *Am J Med*. 1984;228:221.
225. Kent TH, Layton JM. Massive pulmonary cryptococcosis. *Am J Clin Pathol*. 1962;38:596–604.
226. Murray RJ, Becker P, Furth P, et al. Recovery from cryptococcemia and the adult respiratory distress syndrome in the acquired immunodeficiency syndrome. *Chest*. 1988;93:1304–1307.
227. Warr W, Bates JH, Stone A. The spectrum of pulmonary cryptococcosis. *Ann Intern Med*. 1968;69:1109–1116.
228. Kahn FW, England DM, Jones JM. Solitary pulmonary nodule due to *Cryptococcus neoformans* and *Mycobacterium tuberculosis*. *Am J Med*. 1985;78:677–681.
229. Riley E, Cahan WG. Pulmonary cryptococcosis followed by pulmonary tuberculosis: a case report. *Am Rev Respir Dis*. 1972;106:594–599.
230. Dalgleish AG. Concurrent hydatid disease and cryptococcosis in a 16-year-old girl. *Med J Aust*. 1981;2:144–145.
231. Rosen LBFA, Yang LM, Jutivorakool K, et al. Anti-GM-CSF autoantibodies in patients with cryptococcal meningitis. *J Immunol*. 2013;190: 3959–3966.
232. Saijo T, Chen J, Chen SC, et al. Anti-granulocyte-macrophage colony-stimulating factor autoantibodies are a risk factor for central nervous system infection by *Cryptococcus gattii* in otherwise immunocompetent patients. *MBio*. 2014;5:e00912-00914.
233. Feigin DS. Pulmonary cryptococcosis: radiologic-pathologic correlates of its three forms. *AJR Am J Roentgenol*. 1983;141:1263–1272.
234. Aberg JA, Mundy LM, Powderly WG. Pulmonary cryptococcosis in patients without HIV infection. *Chest*. 1999;115:734–740.
235. Baddley JW, Perfect JR, Oster RA, et al. Pulmonary cryptococcosis in patients without HIV infection: factors associated with disseminated disease. *Eur J Clin Microbiol Infect Dis*. 2008;27:937–943.
236. Woods ML, MacGinley R, Eisen DP, et al. HIV combination therapy: partial immune restitution

unmasking latent cryptococcal infection. *AIDS*. 1998;12:1491–1494.

237. Meya DB, Manabe YC, Boulware DR, et al. The immunopathogenesis of cryptococcal immune reconstitution inflammatory syndrome: understanding a conundrum. *Curr Opin Infect Dis*. 2016;29:10–22.
238. Wiesner DL, Boulware DR. Cryptococcus-related immune reconstitution inflammatory syndrome (IRIS): pathogenesis and its clinical implications. *Curr Fungal Infect Rep*. 2011;5:252–261.
239. Jenny-Avital ER, Abadi M. Immune reconstitution cryptococcosis after initiation of successful highly active antiretroviral therapy. *Infect Immun*. 2002;35:128–133.
240. Cinti SK, Armstrong WS, Kaufman CA. Recurrence of increased intracranial pressure with antiretroviral therapy in an AIDS patient with cryptococcal meningitis. *Mycoses*. 2003;44:497–501.
241. Wiesner DL, Moskalenko O, Corcoran JM, et al. Cryptococcal genotype influences immunologic response and human clinical outcome after meningitis. *MBio*. 2012;3.
242. Mitchell DH, Sorrell TC, Allworth AM, et al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin Infect Dis*. 1995;20:611–616.
243. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin Infect Dis*. 1995;21:28–34.
244. Chen S, Sorrell T, Nimmo G, et al. Epidemiology and host and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. *Clin Infect Dis*. 2000;31:499–508.
245. Borton LK, Wintroub BU. Disseminated cryptococcosis presenting as herpetiform lesions in a homosexual man with acquired immunodeficiency syndrome. *J Am Acad Dermatol*. 1984;10:387–390.
246. Schupbach CW, Wheeler CE, Briggaman RA. Cutaneous manifestations of disseminated cryptococcosis. *Arch Dermatol*. 1976;112:1734–1744.
247. Concus AP, Helfand RF, Imber MJ, et al. Cutaneous cryptococcosis mimicking *Molluscum contagiosum* in a patient with AIDS. *J Infect Dis*. 1988;158:897–898.
248. Pema K, Diaz J, Guerra LG, et al. Disseminated cutaneous cryptococcosis: comparison of clinical manifestations in the pre-AIDS and AIDS eras. *Arch Intern Med*. 1994;154:1032–1034.
249. Gauder JP. Cryptococcal cellulitis. *JAMA*. 1977;237:672–673.
250. Mayers DL, Martone WJ, Mandell GL. Cutaneous cryptococcosis mimicking gram-positive cellulitis in a renal transplant patient. *South Med J*. 1981;74:1032.
251. Pierard GE, Pierard-Franchimont C, Estrada JA, et al. Cutaneous mixed infections in AIDS. *Am J Dermatopathol*. 1990;12:63–66.
252. Neuville S, Dromer F, Morin O, et al. Primary cryptococcosis: a distinct clinical entity. *Clin Infect Dis*. 2003;36:347.
253. Chung S, Mondon P, Chang YC, et al. *Cryptococcus neoformans* with a mutation in the tetra tricopeptide repeat-containing gene, CCN1, causes subcutaneous lesions but fails to cause systemic infection. *Infect Immun*. 2003;71:1988–1994.
254. Odom A, Del Poeta M, Perfect J, et al. The immunosuppressant FK506 and its non-immunosuppressive analog L-685,818 are toxic to *Cryptococcus neoformans* by inhibition of a common target protein. *Antimicrob Agents Chemother*. 1997;41:156–161.
255. Braman RT. Cryptococcosis (Torulopsis) of prostate. *Urology*. 1981;17:284–286.
256. Plunkett JM, Turner BI, Tallent MB. Cryptococcal septicemia associated with urologic instrumentation in a renal allograft recipient. *J Urol*. 1981;125:241–242.
257. Larsen RA, Bozzette S, McCutchan A, et al. Persistent *Cryptococcus neoformans* infection of the prostate after successful treatment of meningitis. *Ann Intern Med*. 1989;111:125–128.
258. Staib F, Seibold M, Lage M, et al. *Cryptococcus neoformans* in the seminal fluid of an AIDS patient: a contribution to the clinical course of cryptococcosis. *Mycoses*. 1989;32:171–180.
259. Perfect JR, Seaworth B. Penile cryptococcosis with a review of mycotic infections of the penis. *Urology*. 1985;25:528–531.
260. Blocher KS, Weeks JA, Noble RC. Cutaneous cryptococcal infection presenting as vulvar lesion. *Genitourin Med*. 1987;63:341–343.
261. Okun E, Butler WT. Ophthalmologic complications of cryptococcal meningitis. *Arch Ophthalmol*. 1964;71:52–57.
262. Doft BH, Curtin VT. Combined ocular infection with cytomegalovirus and cryptococcosis. *Arch Ophthalmol*. 1982;100:1800–1803.
263. Crump JR, Elner SG, Elner VM, et al. Cryptococcal endophthalmitis: case report and review. *Clin Infect Dis*. 1992;14:1069–1073.
264. Denning DW, Armstrong RW, Fishman M, et al. Endophthalmitis in a patient with disseminated cryptococcosis and AIDS who was treated with itraconazole. *Rev Infect Dis*. 1991;13:1126–1130.
265. Johnston SR, Corbett EL, Foster O, et al. Raised intracranial pressure and visual complications in AIDs patients with cryptococcal meningitis. *J Infect*. 1992;24:185–189.
266. Rex JH, Larsen RA, Dismukes WE, et al. Catastrophic visceral loss due to *Cryptococcus neoformans* meningitis. *Medicine (Baltimore)*. 1993;72:207–224.
267. Casadevall A, Perfect JR. Cryptococcus neoformans. Washington: ASM Press; 1998:432–438.
268. Behrman RE, Masci JR, Nicholas P. Cryptococcal skeletal infections: case report and review. *Rev Infect Dis*. 1990;12:181–190.
269. Ross JJ, Katz JD. Cryptococcal meningitis and sarcoidosis. *J Infect Dis*. 2002;34:937–939.
270. Yinnon AM, Solages A, Treanor JJ. Cryptococcal peritonitis: report of a case developing during continuous ambulatory peritoneal dialysis and review of the literature. *Clin Infect Dis*. 1993;17:736–741.
271. Sungkanuparph S, Vibhagool A, Pracharktam R. Spontaneous cryptococcal peritonitis in cirrhotic patients. *J Postgrad Med*. 2002;48:201–202.
272. Singh N, Lortholary O, Alexander BD, et al. An immune reconstitution syndrome-like illness associated with *Cryptococcus neoformans* infection in organ transplant recipients. *Clin Infect Dis*. 2005;40:1756–1761.
273. Singh N, Perfect JR. Immune reconstitution syndrome and exacerbation of infections after pregnancy. *Clin Infect Dis*. 2007;45:1192–1199.
274. Ecevit IZ, Clancy CJ, Schmalfuss IM, et al. The poor prognosis of central nervous system cryptococcosis among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy. *Clin Infect Dis*. 2006;42:1443–1447.
275. Sungkanuparph S, Filler S, Chetchotisakd P, et al. Cryptococcal immune reconstitution inflammatory syndrome after antiretroviral therapy in AIDS patients with cryptococccal meningitis: a prospective multicenter study. *Clin Infect Dis*. 2009;49:931–934.
276. Boulware D, Meya D, Bergemann T, et al. Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: a prospective cohort study. *PLoS Med*. 2010;7:e1000384.
277. Boulware D, Hullsiek K, Puronen C, et al. Higher levels of CRP, D-dimer, IL-6 and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. *J Infect Dis*. 2011;203:1637–1646.
278. Panackal AA, Komori M, Kosa P, et al. Spinal arachnoiditis as a complication of cryptococcal meningoencephalitis in non-HIV previously healthy adults. *Clin Infect Dis*. 2017;64:275–283.
279. Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. *Lancet Infect Dis*. 2007;7:395–401.
280. Day J, Chau TT, Wolbers M. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med*. 2013;368:1291–1302.
281. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: feasibility and power of quantitative CSF cultures to determine fungicidal activity. *Lancet*. 2004;363:1764–1767.
282. Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis*. 2014;58:736–745.
283. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. *Clin Infect Dis*. 2007;45:76–80.
284. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clin Infect Dis*. 2009;49:702–709.
285. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clin Infect Dis*. 2009;49:702–709.
286. Huffnagle KE, Gander RM. Evaluation of Gen-probe's *Histoplasma capsulatum* and *Cryptococcus neoformans* ACCU probes. *J Clin Microbiol*. 1993;31:419–421.
287. Mitchell TG, Freedman EZ, White TJ, et al. Unique oligonucleotide primers in PCR for identification of *Cryptococcus neoformans*. *J Clin Microbiol*. 1994;32:253–255.
288. Staib F. *Cryptococcus neoformans* und *Guizotia abyssinica* (Syn. *G. oleifera*) Farbreaktion fur *C. neoformans*. *Zbl Hyg*. 1962;148:466–475.
289. Kwon-Chung KJ, Polacheck I, Bennett JE. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (serotypes B and C). *Ann Intern Med*. 1985;103:533–538.
290. Ikeda R, Shinoda R, Fukazawa Y, et al. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application of serotyping of clinical isolates. *J Clin Microbiol*. 1982;16:22–29.
291. Crampin AC, Matthews RC, Hall D, et al. PCR fingerprinting *Cryptococcus neoformans* by random amplification of polymorphic DNA. *J Med Vet Mycol*. 1993;31:463–465.
292. Spitzer ED, Spitzer SG. Use of a dispersed repetitive DNA element to distinguish clinical isolates of *Cryptococcus neoformans*. *J Clin Microbiol*. 1992;30:1094–1097.
293. Varma A, Kwon-Chung KJ. DNA probe for strain typing of *Cryptococcus neoformans*. *J Clin Microbiol*. 1992;30:2960–2967.
294. Firacative C, Trilles L, Meyer W. MALDI-TOF MS enables the rapid identification of the major molecular types within the *Cryptococcus neoformans*/*C. gattii* species complex. *PLoS ONE*. 2012;7:e37566.
295. Goodman JS, Kaufman L, Loening MG. Diagnosis of cryptococcal meningitis: detection of cryptococcal antigen. *N Engl J Med*. 1971;285:434–436.
296. Kauffman CA, Bergman AG, Severance PJ, et al. Detection of cryptococcal antigen. Comparison of two latex agglutination tests. *Am J Clin Pathol*. 1981;75:106–109.
297. Panackal AA, Dekker JP, Proschan M, et al. Enzyme immunoassay versus latex agglutination cryptococcal antigen assays in adults with non-HIV-related cryptococcosis. *J Clin Microbiol*. 2014;52:4356–4358.
298. Snow RM, Dismukes WE. Cryptococcal meningitis: diagnostic value of cryptococcal antigen in cerebrospinal fluid. *Arch Intern Med*. 1975;135:1155–1157.
299. McManus EJ, Jones JM. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcus neoformans* capsular polysaccharides in serum from a patient with disseminated trichosporon infection. *J Clin Microbiol*. 1985;21:681–685.
300. Chanock SJ, Toltzis P, Wilson C. Cross-reactivity between *Stomatococcus mucilaginosus* and latex agglutination for cryptococcal antigen. *Lancet*. 1993;342:1119–1120.
301. Baughman RP, Rhodes JC, Dohn MN, et al. Detection of cryptococcal antigen in bronchoalveolar lavage fluid: a prospective study of diagnostic utility. *Am Rev Respir Dis*. 1992;145:1226–1229.
302. Hansen J, Slechta E, Gates-Hollingsworth M, et al. Large scale evaluation of the Immuno-Mycologics Inc. (IMMY) lateral flow and enzyme-linked immunoassays for the detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clin Vaccine Immunol*. 2013;20:52–55.
303. Williams DA, Kiiza T, Kwizera R, et al. Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: a diagnostic accuracy study. *Clin Infect Dis*. 2015;61:464–467.
304. Binnicker MJ, Jespersen DJ, Bestrom JE, et al. Comparison of four assays for the detection of cryptococcal antigen. *Clin Vaccine Immunol*. 2012;19:1988–1990.
305. McMullan B, Halliday C, Sorrell T, et al. Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. *PLoS ONE*. 2012;7:e49541.
306. Rivet-Dañon D, Guitard J, Grenouillet F, et al. Rapid diagnosis of cryptococcosis using an antigen detection immunochromatographic test. *J Infect*. 2015;70:499–503.
307. Desmet P, Kayembe KD, DeVroey C. The value of cryptococcal serum antigen screening among HIV positive AIDS patients in Kinshasa, Zaire. *AIDS*. 1989;3:77–78.
308. Hoffman S, Stenderup J, Mathiesen LR. Low yield of screening for cryptococcal antigen by latex agglutination assay on serum and cerebrospinal fluid from Danish patients with AIDS or ARC. *Scand J Infect Dis*. 1991;23:697–702.
309. Feldmesser M, Harris C, Reichberg S, et al. Serum cryptococcal antigen in patients with AIDS. *Clin Infect Dis*. 1996;23:827–830.
310. Manfredi R, Moroni A, Mazzoni A, et al. Isolated detection of cryptococcal polysaccharide antigen in cerebrospinal fluid samples from patients with AIDS. *Clin Infect Dis*. 1996;23:849–850.
311. Jarvis JN, Lawn SD, Vogt M, et al. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis*. 2009;48:856–862.

312. Meya D, Manabe Y, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with CD4+ cell count< or = 100 cells/microL who start HIV therapy in resource-limited settings. *Clin Infect Dis*. 2010;51:448–455.
313. Cassim N, Coetzee LM, Schnippel K, et al. Estimating the cost-per-result of a national reflexed cryptococcal antigenaemia screening program: forecasting the impact of potential HIV guideline changes and treatment goals. *PLoS ONE*. 2017;12:e0182154.
314. Letang E, Muller MC, Ntamatungiro AJ, et al. Cryptococcal antigenemia in immunocompromised human immunodeficiency virus patients in rural Tanzania: a preventable cause of early mortality. *Open Forum Infect Dis*. 2015;2:ofv046.
315. Powderly WG, Cloud GA, Dismukes WE, et al. Measurement of cryptococcal antigen in serum and cerebrospinal fluid: value in the management of AIDS-associated cryptococcal meningitis. *Clin Infect Dis*. 1994;18:789–792.
316. Hunt KK Jr, Enquist RW, Bowen TE. Multiple pulmonary nodules with central cavitation. *Chest*. 1976;69:529–530.
317. Khoury MB, Godwin JD, Ravin CE, et al. Thoracic cryptococcosis: immunologic competence and radiologic appearance. *AJR Am J Roentgenol*. 1984;141:893–896.
318. McAllister CK, Davis CE Jr, Ognibene AJ, et al. Cryptococcal pleuro-pulmonary disease: infection of the pleural fluid in the absence of disseminated cryptococcosis—Case report. *Milit Med*. 1984;149: 684–686.
319. Young EJ, Hirsh DD, Fainstein V, et al. Pleural effusions due to *Cryptococcus neoformans:* a review of the literature and report of two cases with cryptococcal antigen determinations. *Am Rev Respir Dis*. 1980;121:743–746.
320. Zlupko GM, Fochler FJ, Goldschmidt ZH. Pulmonary cryptococcosis presenting with multiple pulmonary nodules. *Chest*. 1980;77:575.
321. Clark RA, Greer DL, Valainis GT, et al. *Cryptococcus neoformans* pulmonary infection in HIV-1-infected patients. *J Acquir Immune Defic Syndr*. 1990;3:480–485.
322. Miller WT, Edelman JM. Cryptococcal pulmonary infection in patients with AIDS: radiographic appearance. *Radiology*. 1990;175:725–728.
323. Cornell SH, Jacoby CG. The varied computed tomographic appearance of intracranial cryptococcosis. *Radiology*. 1982;143:703–707.
324. Tan CT, Kuan BB. *Cryptococcus* meningitis, clinical–CT scan considerations. *Neuroradiology*. 1987;29:43–46.
325. Long JA, Herdt JR, DiChiro G, et al. Cerebral mass lesions in torulosis demonstrated by computer tomography. *J Comput Assist Tomogr*. 1980;4: 766–769.
326. Poprich MJ, Arthur RH, Helmer E. CT of intracranial cryptococcosis. *AJR Am J Roentgenol*. 1990;154:603–606.
327. Wehn SM, Heinz R, Burger PC. Dilated Virchow-Robin spaces in cryptococcal meningitis associated with AIDS: CT and MR findings. *J Comput Assist Tomogr*. 1989;13: 756–762.
328. Ingram CW, Haywood HB, Morris VM, et al. Cryptococcal ventricular peritoneal shunt infection: clinical and epidemiological evaluation of two closely associated cases. *Infect Immun*. 1993;14:719–722.
329. Hospenthal D, Bennett JE. Persistence of cryptococcomas on neuroimaging. *Clin Infect Dis*. 2000;31:1303–1306.
330. Saag MS, Graybill JR, Larsen RA, et al. Practice guidelines for the management of cryptococcal disease. Infectious Disease Society of America. *Clin Infect Dis*. 2000;30:710–718.
331. Perfect JR, Dismukes WE, Pappas PG, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2010;50:291–322.
332. Campbell GD, Currier RD, Busey JF. Survival in untreated cryptococcal meningitis. *Neurology*. 1981;31:1154–1157.
333. Mwaba P, Mwansa J, Chintu C, et al. Clinical presentation, natural history and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgrad Med*. 2001;77:769–773.
334. Ghannoun MA, Ibrahim AS, Fu Y, et al. Susceptibility testing of *Cryptococcus neoformans:* a microdilution technique. *J Clin Microbiol*. 1992;30:2881–2886.
335. Espinel-Ingroff A, Aller AI, Canton E, et al. *Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distriutions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole. *Antimicrob Agents Chemother*. 2012;56:5898–5906.
336. Valez JD, Allendoerfer R, Luther M, et al. Correlation of in vitro azole susceptibility with in vivo response in a murine model of cryptococcal meningitis. *J Infect Dis*. 1993;168:508–510.
337. Aller AI, Martin-Manzuelos E, Lozano F, et al. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. *Antimicrob Agents Chemother*. 2000;44:1544–1548.
338. Witt MD, Lewis RJ, Larsen RA, et al. Identification of patients with acute AIDS-associated cryptococcal meningitis who can be effectively treated with fluconazole: the role of antifungal susceptibility testing. *Clin Infect Dis*. 1996;22:322–328.
339. Spitzer ED, Spitzer SG, Freundlich LF, et al. Persistence of initial infection in recurrent *Cryptococcus neoformans* meningitis. *Lancet*. 1993;341:595–596.
340. Van Wyk MGN, Mitchell TG, Litvintseva AP. Multilocus sequence typing of serially collected isolates of *Cryptococcus* from HIV-infected patients in South Africa. *J Clin Microbiol*. 2014;52:1921–1931.
341. Casadevall A, Spitzer ED, Webb D, et al. Susceptibilities of serial *Cryptococcus neoformans* isolates from patients with recurrent cryptococcal meningitis to amphotericin B and fluconazole. *Antimicrob Agents Chemother*. 1993;37:1383–1386.
342. Sionov ECY, Garraffo HM, Kwon-Chung KJ. Heteroresistance to fluconazole in *Cryptococcus neoformans* is intrinsic and associated with virulence. *Antimicrob Agents Chemother*. 2009;53:2804–2815.
343. Haynes KA, Sullivan DJ, Coleman DC, et al. Involvement of multiple *Cryptococcus neoformans* strains in a single episode of cryptococcosis and reinfection with novel strains in recurrent infection demonstrated by random amplification of polymorphic DNA and DNA fingerprinting. *J Clin Microbiol*. 1995;33:99–102.
344. Haynes KA, Sullivan DJ, Coleman DC, et al. Involvement of multiple *Cryptococcus neoformans* strains in a single episode of cryptococcosis and reinfection with novel strains in recurrent infection demonstrated by random amplification of polymorphic DNA and DNA fingerprinting. *J Clin Microbiol*. 1995;33:99–102.
345. Perfect JR, Cox GM. Drug resistance in *Cryptococcus neoformans*. *Drug Resist Updat*. 1999;2:259–269.
346. Bicanic T, Harrison T, Niepieklo A, et al. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. *Clin Infect Dis*. 2006;43:1069–1073.
347. Sionov E, Lee H, Chang YC, et al. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog*. 2010;6:e1000848.
348. Sarosi GA, Parker JD, Doto IL, et al. Amphotericin B in cryptococcal meningitis: long-term results of treatment. *Ann Intern Med*. 1969;71:1079–1087.
349. Bennett JE, Dismukes W, Duma RJ, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med*. 1979;301:126–131.
350. Utz JP, Garrigues IL, Sande MA, et al. Therapy of cryptococcosis with a combination of flucytosine and amphotericin B. *J Infect Dis*. 1975;132:368–373.
351. deLalla F, Pellizzer G, Vaglia A. Amphotericin B as primary therapy for cryptococcosis in patients with AIDS: reliability of relatively high doses administered over a relatively short period. *Clin Infect Dis*. 1995;20:263–266.
352. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (Ambisome) compared with amphotericin B followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. *AIDS*. 1997;11:1463–1471.
353. Hamil RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and amphotericin B for the treatment of AIDS-associated acute cryptococcal meningitis: randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis*. 2010;51:225–232.
354. Bratton EWEHN, Chastain CA, Lee MS, et al. Approaches to antifungal therapies and their effectiveness among patients with cryptococcosis. *Antimicrob Agents Chemother*. 2013;57:2485–2495.
355. Bratton EW, El Husseini N, Chastain CA, et al. Approaches to antifungal therapies and their effectiveness among patients with cryptococcosis. *Antimicrob Agents Chemother*. 2013;57:2485–2495.
356. Lestner J, McEntee L, Johnson A, et al. Experimental models of short courses of liposomal amphotericin B for induction therapy for cryptococcal meningitis. *Antimicrob Agents Chemother*. 2017;61.
357. Jarvis J, Leeme T, Chafie A. AMBITION-cm High Dose Liposomal Amphotericin B for HIV-related Cryptococcal Meningitis. Conf. Retroviruses and Opportunistic Infections. (R01) Seattle, 2017.
358. Utz JP, Shadomy S, McGehee RF. Flucytosine. *N Engl J Med*. 1972;286:777–778.
359. Dismukes WE, Cloud G, Gallis HA, et al. Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. *N Engl J Med*. 1987;317:334–341.
360. van der Horst C, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. *N Engl J Med*. 1997;337:15–21.
361. DeGans J, Portegies P, Tiessens G. Itraconazole compared with amphotericin B plus flucytosine in AIDS patients with cryptococcal meningitis. *AIDS*. 1992;6:185–190.
362. Larsen RA, Leal MAE, Chan LS. Fluconazole compared with amphotericin B plus flucytosine for cryptococcal meningitis in AIDS. *Ann Intern Med*. 1990;113:183–187.
363. Stamm AM, Diasio RB, Dismukes WE, et al. Toxicity of amphotericin B plus flucytosine in 194 patients with cryptococcal meningitis. *Am J Med*. 1987;83:236–242.
364. Saag MS, Cloud GA, Graybill JR, et al. A comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis. *Clin Infect Dis*. 1999;28:291–296.
365. Dromer F, Bernede-Bauduin C, Guillemot D, et al. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. *PLoS ONE*. 2008;3:e2870.
366. Day J, Chau TT, Wolbers M. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med*. 2013;368:1291–1302.
367. Stern JJ, Hartman BJ, Sharkey P, et al. Oral fluconazole therapy for patients with the acquired immunodeficiency syndrome and cryptococcosis: experience with 22 patients. *Am J Med*. 1988;85:477–480.
368. Dromer F, Mathoulin S, Dupont B, et al. Comparison of the efficacy of amphotericin B and fluconazole in the treatment of cryptococcosis in human immunodeficiency virus-negative patients: retrospective analysis of 83 cases. *Clin Infect Dis*. 1996;22(suppl 2):s154–s160.
369. Yamaguchi H, Ikemoto H, Watanabe K, et al. Fluconazole monotherapy for cryptococcosis in non-AIDS patients. *Eur J Clin Microbiol Infect Dis*. 1996;15:787–792.
370. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. *N Engl J Med*. 1992;326:83–89.
371. Berry AJ, Rinaldi MG, Graybill JR. Use of high dose fluconazole as salvage therapy for cryptococcal meningitis in patients with AIDS. *Antimicrob Agents Chemother*. 1992;36:690–692.
372. Bozette SA, Larsen RA, Chiu J, et al. A placebo-controlled trial of maintenance therapy with fluconazole after treatment of cryptococcal meningitis in the acquired immunodeficiency syndrome. *N Engl J Med*. 1991;324: 580–584.
373. Powderly WG, Saag MS, Cloud GA, et al. A controlled trial of fluconazole or amphotericin B to prevent relapse of cryptococcal meningitis in patients with the acquired immunodeficiency syndrome. *N Engl J Med*. 1992;326: 793–798.
374. Denning DW, Tucker RM, Hanson LH, et al. Itraconazole therapy for cryptococcal meningitis and cryptococcosis. *Arch Intern Med*. 1989;149:2301–2308.
375. Viviani MA, Tortorano AM, Langer M, et al. Experience with itraconazole in cryptococcosis and aspergillosis. *J Infect*. 1989;18:151–165.
376. Perfect JR, Marr KA, Walsh TJ, et al. Voriconazole treatment for less common, emerging or refractory fungal infections. *Clin Infect Dis*. 2003;36:1122–1131.
377. Pitisuttithum P, Negroni R, Graybill JR, et al. Activity of posaconazole in the treatment of central nervous system fungal infections. *J Antimicrob Chemother*. 2005;56: 745–755.
378. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: feasibility and power of quantitative CSF cultures to determine fungicidal activity. *Lancet*. 2004;363:1764–1767.
379. Pappas PGCP, Larsen RA, Manosuthi W, et al. A phase II randomized trial of amphotericin B alone or combined with fluconazole in the treatment of HIV-associated cryptococcal meningitis. *Clin Infect Dis*. 2009;48:1775–1783.
380. Malloy S, Kanyama C, Heyderman R A randomized controlled trial for the treatment of HIV-associated cryptoccl meningites in African: oral fluconazole plus flucytosine or one week amphotericin B-based therapy vs two weeks amphotericin B-based therapy. The ACTA TRIAL Paris, 2017.
381. Larsen RA, Bozzette SA, Jones BE, et al. Fluconazole combined with flucytosine for treatment of cryptococcal meningitis in patients with AIDS. *Clin Infect Dis*. 1994;19:741–745.
382. Jackson AT, Nussbaum J, Phulusa J, et al. A phase ll randomized controlled trial adding oral flucytosine to high-dose fluconazole with short-course amphotericin B, for cryptococcal meningitis. *AIDS*. 2012;26:1363–1370.
383. Martinez E, Garcia-Viejo MA, Marcos MA. Discontinuation of secondary prophylaxis for

cryptococcal meningitis in HIV-infected patients responding to highly active antiretroviral therapy. *AIDS*. 2000;14:2615.

384. Vibhagool A, Sungkanuparph S, Mootsikapun P, et al. Discontinuation of secondary prophylaxis for cryptococcal meningitis in human immunodeficiency virus–infected patients treated with highly active antiretroviral therapy: a prospective, multicenter randomized study. *Clin Infect Dis*. 2003;36:1329–1331.
385. Fujita NK, Reynard M, Sapico FL, et al. Cryptococcal intracerebral mass lesions. *Ann Intern Med*. 1981;94: 382–388.
386. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med*. 2014;370:2487–2498.
387. Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. *Clin Infect Dis*. 2000;30:47–54.
388. Denning DW, Armstrong RW, Lewis BH, et al. Elevated cerebrospinal fluid pressures in patients with cryptococcal meningitis and acquired immunodeficiency syndrome. *Am J Med*. 1991;91:267–272.
389. Deleted in review.
390. Van Gemert HM, Vermeulen M. Treatment of impaired consciousness with lumbar punctures in a patient with cryptococcal meningitis and AIDS. *Clin Neurol Neurosurg*. 1991;93:257–258.
391. Rolfes MA, Hullsiek KH, Rhein J, et al. The effect of therapeutic lumbar puncture on acute mortality from cryptococcal meningitis. *Clin Infect Dis*. 2014;59: 1607–1614.
392. Beardsley J, Wolbers M, Kibengo F, et al Adjunctive corticosteroids in HIV-associates cryptococcal meningitis: a randomized controlled trial in African and Southeast Asian adults [abstract]. 55th Interscience Conference on Antimicrobials and Chemotherapy. San Diego, September 17-22, 2015.
393. Pappas PG, Bustamante B, Ticona E, et al. Recombinant interferon-gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. *J Infect Dis*. 2004;189:2185–2191.
394. Jarvis J, Meintjes G, Rebe K, et al. Adjunctive interferon-γ immnotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS*. 2012;26:1105–1113.
395. Jongwutiwes U, Kiertiburanakul S, Sungkanuparph S. Impact of antiretroviral therapy on the relapse of cryptococcosis and survival of HIV-infected patients with cryptococcal infection. *Curr HIV Res*. 2007;5:355–360.
396. White M, Cirrincione C, Blevins A, et al. Cryptococcal meningitis with AIDS and patients with neoplastic disease. *J Infect Dis*. 1992;165:960–966.
397. Bratton E, El Husseini N, Chastain C, et al. Comparison and temporal trends of three groups with cryptococcosis: HIV-infected, solid organ transplant, and HIV-negative/ non-transplant. *PLoS ONE*. 2012;7:e43582.
398. Anekthananon T, Manosuthi W, Chetchotisakd P, et al. Predictors of poor clinical outcome of cryptococcal meningitis in HIV-infected patients. *Int J STD AIDS*. 2011;22:665–670.
399. Dromer F, Mathoulin-Pelissier S, Launay O, et al. Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study. *PLoS Med*. 2007;4:e21.
400. Chen S, Slavin M, Heath C, et al. Clinical manifestation of *Cryptococcus gattii* infection: determinants of neurological sequelae and death. *Clin Infect Dis*. 2012;55:789–798.
401. Spec A, Raval K, Powderly WG. End-stage liver disease is a strong predictor of early mortality in cryptococcosis. *Open Forum Infect Dis*. 2016;3:ofv197.
402. Weiner D, Moskalenko O, Corcoran JM, et al. MA. Cryptococcal genotype influences immunologic response and human clinical outcome after meningitis. *MBio*. 2012;3:e00196.
403. Chayakulkeeree M, Perfect JR. Cryptococcosis. *Infect Dis Clin North Am*. 2006;20:507–544.
404. Nightingale SD, Cal SX, Peterson DM, et al. Primary prophylaxis with fluconazole against systemic fungal infections in HIV-positive patients. *AIDS*. 1992;6:191–194.
405. Powderly WG, Finkelstein DM, Feinberg J, et al. A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. *N Engl J Med*. 1995;332:700–705.
406. Chetchotisakd P, Sungkanuparph S, Thinkhamrop B, et al. A multicentre, randomized, double-blind, placebo-controlled trial of primary cryptococcal meningitis prophylaxis in HIV-infected patients with severe immune deficiency. *HIV Med*. 2004;5:140–143.
407. Devi SJ, Scheerson R, Egan W, et al. *Cryptococcus neoformans* serotype A glucuronoxylomannan protein conjugate vaccines: synthesis, characterization, and immunogenicity. *Infect Immun*. 1991;59:3700–3707.
408. Specht CA, Lee CK, Huang H, et al. Vaccination with recombinant *Cryptococcus* proteins in glucan particles protects mice against cryptococcosis in a manner dependent upon mouse strain and cryptococcal species. *MBio*. 2017;8:e01872-17.
409. Mukherjee J, Pirofski LA, Scharff MD, et al. Antibody-mediated protection in mice with lethal intracerebral *Cryptococcus neoformans* infection. *Proc Natl Acad Sci USA*. 1993;90:3636–3640.
410. Mukherjee J, Zuckier LS, Scharff MD, et al. Therapeutic efficacy of monoclonal antibodies to *Cryptococcus neoformans* glucuronoxylomannan alone and in combination with amphotericin B. *Antimicrob Agents Chemother*. 1994;38:580–587.
411. Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomized controlled trial. *Lancet*. 2015;385:2173–2182.

263 *Histoplasma capsulatum* (Histoplasmosis)

George S. Deepe, Jr.

SHORT VIEW SUMMARY

Definition
- Histoplasmosis is the most frequent cause of fungal respiratory infection and has a broad spectrum of clinical manifestations ranging from a self-limited, acute, influenza-like illness to a progressive disseminated infection that is life-threatening.

Epidemiology
- The fungus is typically found in the midwestern and southeastern United States and in Central and South America.
- Indigenous cases have been reported worldwide.
- The fungus thrives in decaying bird guano (starlings and blackbirds) and bat guano.
- Patients with acquired immunodeficiency syndrome (AIDS) or who are receiving immunosuppressive drugs, including tumor necrosis factor-α inhibitors, are predisposed to disseminated infection.

Microbiology
- The fungus exists in the mycelial form in nature.
- The mycelia produce two sizes of conidia—microconidia and macroconidia. The former are thought to induce disease because they are small enough to reach the bronchioles and alveoli.
- Conversion to the yeast phase is driven by temperature.

Diagnosis
- Serology is useful for all but AIDS patients. Complement fixation titers of 1:32 or greater are indicative of active disease. The presence of the H immunodiffusion band signifies active disease. An M band does not discriminate between current or remote infection.
- The *Histoplasma* antigen test, either from urine or from serum, is particularly useful in diagnosis of extrapulmonary disease. It is also useful for following response to therapy.
- Tissue and buffy coat examination is useful for detecting the organism.
- See Table 263.2 for diagnostic tests.

Therapy
- Treatment regimens are as follows:
  - Acute pulmonary histoplasmosis: mild to moderate—no treatment or itraconazole 200 mg 3 times a day for 3 days followed by 200 mg twice a day for 6 to 12 weeks; moderate to severe—lipid-formulated amphotericin B, 3 to 5 mg/kg or deoxycholate amphotericin B, 0.7 to 1 mg/kg, for 1 to 2 weeks, followed by itraconazole for a total of 10 to 11 weeks; chronic cavitary—itraconazole as described above
  - Disseminated disease: moderate to severe—same as moderate-to-severe pulmonary disease; in children, can use deoxycholate amphotericin B, 1 mg/kg for 4 to 6 weeks
  - Rheumatologic manifestations—nonsteroidals
  - Mediastinal lymphadenitis—no treatment or, if symptomatic, itraconazole as described above
- See Table 263.3 for treatment options.

Prevention
- For prophylaxis for immunosuppressed patients, itraconazole 200 mg daily is administered.
- Construction sites, spelunking, and remodeling of unoccupied homes or farm habitat should be avoided.

Histoplasma capsulatum is one of the more common causes of infection in the US Midwest and Southeast. Histoplasmosis, acquired through inhalation of mycelial fragments and microconidia, is most often self-limiting but can cause potentially lethal infection in patients with preexisting conditions. It remains a frequent cause of opportunistic infection in patients whose immune system is impaired by pharmaceutic agents or by the human immunodeficiency virus (HIV). This accelerating trend is unlikely to abate because the reservoir of *H. capsulatum* (soil) will never disappear.

HISTORY

The discovery of *H. capsulatum* was made in December 1905, when Samuel Darling, a pathologist stationed in Panama, examined visceral tissues and bone marrow from a young man from Martinique whose death was originally attributed to miliary tuberculosis.[1] Peering through his microscope, Darling was struck by the presence of many small bodies, most of which were intracellular. Having been influenced by reports from Leishman and Donovan, he mistakenly thought that this organism was a protozoan. Because it lacked a kinetoplast, Darling assumed that it was a different *Leishmania* species. He termed this new species *Histoplasma capsulatum* because it seemingly exhibited a capsule. It was not until 1912, after reviewing tissue specimens, that da Rocha Lima suggested that the organism resembled a yeast rather than a protozoan.[2] More than 20 years later, the organism was finally isolated on artificial medium and observed to grow as a mold at room temperature and as a yeast at 37°C.[3]

For many years, the presence of pulmonary calcifications had become synonymous with healed tuberculosis by physicians. Amos Christie, a pediatrician at Vanderbilt University, dispelled that dictum.[4,5] The presence of cutaneous reactivity to a skin test reagent, prepared from the mycelial phase of the organism, in an infant with disseminated histoplasmosis prompted large-scale testing during the 1930s. This endeavor unearthed the surprising finding that histoplasmosis was highly prevalent in the Ohio and Mississippi River Valleys.[5] Moreover, many cases of presumed tuberculosis that were based on the presence of calcified nodules on chest radiographs were determined to be histoplasmosis instead.[6] Eventually, many individuals residing in tuberculosis sanatoriums in the midwestern and southeastern United States were recognized to have been mistakenly admitted. They had histoplasmosis, not tuberculosis. Some of these individuals contracted tuberculosis while housed in open wards with patients who had active pulmonary tuberculosis.

ECOLOGY AND EPIDEMIOLOGY

Cases of histoplasmosis have been reported from every continent except Antarctica. *H. capsulatum* is a soil-based fungus that has been isolated from many regions of the world and is most often associated with river valleys; as mentioned earlier, the most highly endemic regions are the Ohio and Mississippi River Valleys (Fig. 263.1).[6] The conditions that favor the growth of this fungus in soil are a mean temperature of 22°C to 29°C, an annual precipitation of 35 to 50 inches, and a relative humidity of 67% to 87%. These conditions are typically found in the temperate

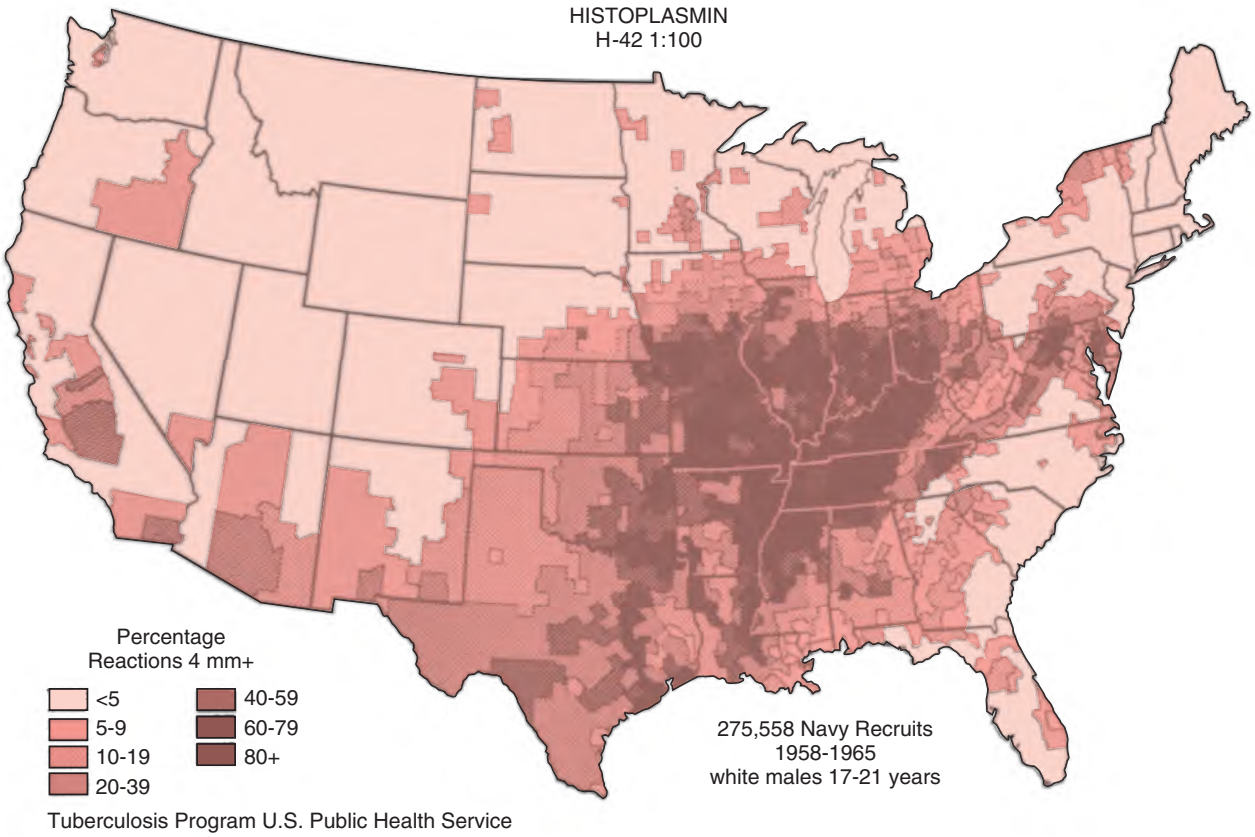

FIG. 263.1 Histoplasmin reactivity in the continental United States among naval recruits. *(From Edwards LB, Acquaviva FA, Livesay VT, et al. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States.* Am Rev Respir Dis. *1969;99:1–111.)*

zone between latitudes 45 degrees north to 30 degrees south.[7] The organism is usually found within 20 cm of the surface, and it prefers soil that is acidic, has a high nitrogen content, and is moist. In areas where avians roost, the fungus is found most often where the guano is decaying and mixed with soil.[8] In such areas, infectious particles can exceed 10^5/g of soil. Fresh guano is less likely to contain any infectious particles. There is a strong association between the presence of bird and bat guano and the presence of *H. capsulatum*. In fact, the first isolation of the organism from an environmental source was from an area adjacent to a chicken house. Birds are not infected by the fungus, and attempts to isolate *H. capsulatum* from their cloaca have been unsuccessful. Bats, on the other hand, carry the fungus in their gastrointestinal tracts and shed it.[9]

Disruption of the soil by excavation or construction is one of the most common means of releasing infectious elements that are inhaled and eventually settle into the lungs. Individuals involved in recreational or work activities that expose them to disrupted soil are at highest risk for infection. Persons at risk include spelunkers who roam caves where bats reside and those who are engaged in agriculture, outdoor construction, or rehabilitation of buildings that have been inhabited by birds or bats. Human-to-human transmission via the pulmonary route has not been reported.

H. capsulatum contains five to seven chromosomes. Differences in numbers of chromosomes are evident among strains. Originally, the organism could be distinguished by two chemotypes, but the advent of molecular biology has improved methods to distinguish strains of *H. capsulatum*. Eight clades of this fungus have been identified through molecular analysis[10]—two North American, two Latin American, and one each of Australian, Indonesian, Eurasian, and African clades. The spread of this fungus appears to have originated from Latin America between 3 and 13 million years ago. Interesting to note, many of the isolates recovered from acquired immunodeficiency syndrome (AIDS) patients in St. Louis were found to be in clade 1, and these isolates are much less virulent in mice.[11] Genetic differences can be associated with varied clinical manifestations. *H. capsulatum* from specific regions of South America often produce skin lesions, whereas isolates from North America do not. The findings suggest that *H. capsulatum* is highly diverse at the genetic level, perhaps based on the fact that the fungus undergoes sexual recombination in nature, thus allowing for exchange of genetic material.

The impact of histoplasmosis on health care costs is not precisely known because it is not a reportable disease. Hospitalizations for histoplasmosis in the United States showed an upward trend from 2001 to 2012. Over this time period, in-hospital mortality was 4.9%. The median cost for hospitalization was approximately $72,000, and total charges in 2012 were estimated to be $371 million.[12] Another analysis of mortality spanning the years 1980 to 1997 demonstrated that the incidence of mortality in the United States from histoplasmosis ranged from 0.1 to 0.15 per 100,000 population.[13] Earlier data indicated that disease develops in males more frequently than in females by a 4:1 ratio. This information was likely skewed because of the association of chronic pulmonary histoplasmosis with smoking, which for many years was a male-dominated activity. That sex bias has not been observed in recent years.

MYCOLOGY

H. capsulatum is classified as a member of the family Ascomycetes and has a heterothallic form designated *Ajellomyces capsulatum* (see Chapter 255). Mating types (+) and (−) have been described, and when combined onto sporulating medium, they produce fruiting bodies containing asci. Isolates from patients carry the (−) mating type two to seven times more frequently than the (+) type, although the ratio of mating types in soil is 1:1.[14]

The organism has two morphotypes: the mycelial and yeast phases. The former is present at ambient temperature, and the latter at 37°C or higher. The saprobic or mycelial phase can be divided into two colony

types: brown (B) and albino (A). The A type grows more rapidly in culture and loses the capability to produce spores after prolonged subculturing. The B type generates a brown pigment. Yeast cells from the B type are more virulent in mice than those from the A type.

The basic elements of the nutritional needs of the organism are poorly defined because of the lack of a standardized medium. The organism requires vitamins, thiamine, biotin, and iron. Sulfhydryl groups in the form of cysteine or cystine are necessary for growth and maintenance of the yeast phase. The mycelial and yeast phases differ in their requirements for calcium. Chelation of this element from medium inhibits the growth of the mycelial but not the yeast phase.[15]

Microscopic evaluation of the mycelial phase reveals two types of conidia. Macroconidia are large ovoid bodies that span 8 to 15 µm in diameter. The surface is decorated with slender protrusions that are referred to as *tuberculate.* Microconidia are small, smooth oval bodies with a diameter ranging from 2 to 5 µm (Fig. 263.2). These forms are believed to be the infective phase because their size is small enough to lodge in the terminal bronchioles and alveoli.

The transition from the saprobic to the yeast phase is a critical step in infectivity of the fungus. On exposure to 37°C, the organism undergoes genetic, biochemical, and physical alterations that result in the production of yeast cells that are uninucleate. These forms are small, typically 2 to 5 µm in diameter, and reproduce by multipolar budding (Fig. 263.3). The stimulus for the transition is heat, and the shift in temperature may be sensed by a change in the fluidity of the yeast membrane. Analysis of the conversion using microarrays has revealed numerous alterations in gene expression. The shift was associated with induction of genes contributing to conidiation, cell polarity, and melanin.[16] Using insertional mutagenesis, a transcription factor termed *Ryp1* has been found to be essential for growth of yeast cells at 37°C. In addition, three other genes, *Ryp2, Ryp3, Ryp4,* that are crucial for the transition have been identified. Hence, there is a complex regulatory network that dictates the conversion to yeast cells when the temperature is elevated.[17] Three biochemical stages have been identified during the conversion after exposure to 37°C. Stage 1 is characterized by an uncoupling of oxidation-phosphorylation and a decrease in RNA and protein synthesis. In stage 2, no respiration is detectable, and in stage 3 there is a resumption of respiration. Chitin and α- and β-glucan content differ between the two phases.

Within tissues, yeast cells may possess a morphologic appearance that differs from the usual ovoid shape. Misshapen or large yeasts have been observed in tissues and epithelial cells. These allomorphs may contain less α-1,3-glucan and appear to be less virulent in mice than oval-shaped yeasts.

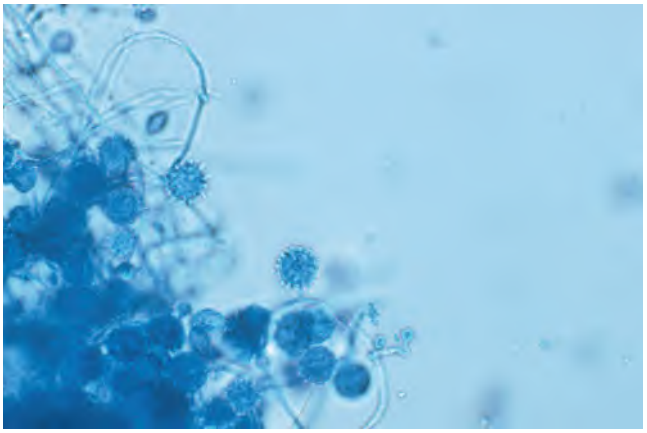

FIG. 263.2 Mycelial phase of *Histoplasma capsulatum.* Both macroconidia and microconidia are evident.

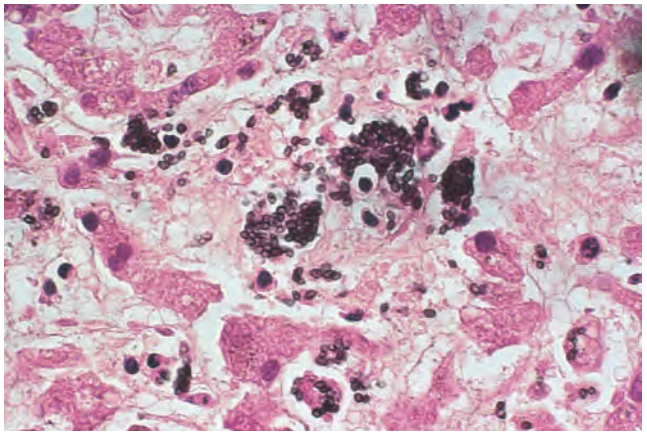

FIG. 263.3 Yeast cells of *Histoplasma capsulatum* in a section of liver. (Gomori methenamine silver, ×1000.)

PATHOGENESIS

The study of the pathogenesis of this fungus has accelerated as a result of technologic advances, including a transformation system to delete genes; silencing RNA; and insertional mutagenesis using *Agrobacterium tumefaciens.* These tools create the foundation for examining the influence of genes or gene regulators on the pathobiology of *H. capsulatum.*[18,19]

The transition from the mycelial to the yeast phase is the most critical determinant in the establishment of infection.[20] This contention is supported by several findings. First, it is rare to find mycelial particles in tissues of humans or mammals with established infection. Rather, yeast cells are commonly detected. Second, exposure of *H. capsulatum* mycelia to *p*-chloromercuriphenylsulfonic acid (PCMS), a sulfhydryl inhibitor, irreversibly blocks the conversion to yeasts but does not alter growth of yeasts or mycelia. PCMS-treated mycelia fail to infect animals.

Iron and zinc are vital elements required for survival of *H. capsulatum.*[21] The organism acquires iron from the intracellular environment by three means: release of iron-scavenging siderophores, production of a ferric reductase, and modulation of pH to remove iron from transferrin. α-1,3-Glucan has been found to be a key virulence factor in the pathogenesis of *H. capsulatum.*[18] Synthesis of this carbohydrate, which is regulated by an amylase, blocks β-glucan binding to Dectin-1 and thereby suppresses generation of important proinflammatory cytokines.

After conidia settle into the alveoli, they bind to the CD11-CD18 family of integrins and are engulfed by neutrophils and macrophages.[22] It is likely that the conversion of mycelia to the yeast phase transpires, at least partially if not entirely, intracellularly. The duration of the phase transition ranges from hours to days. After transformation of conidia into yeasts in the lungs, yeasts migrate, presumably intracellularly, to local draining lymph nodes and subsequently to distant organs rich in mononuclear phagocytes (e.g., liver, spleen). The yeasts grow within resting macrophages. Activation of cellular immunity is necessary for restricting growth, and in primary infection this arm of immunity matures by 2 weeks.

Innate Immunity

In experimental pulmonary infection, neutrophils constitute one of the prominent cell populations that emigrate early into infected foci of lungs.[23] These cells are capable of inhibiting the growth of yeast cells. Constituents from the azurophilic granules express fungistatic activity, and defensins also inhibit the growth of yeast cells.[24] Neutrophils mount a respiratory burst in response to the fungus, but the oxygen intermediates are trapped intracellularly. Despite the burst, there is little evidence that toxic oxygen intermediates contribute to the anti-*Histoplasma* activity of these phagocytes. Resistance to these molecules is mediated in large part by superoxide dismutase 3, a copper/zinc-dependent enzyme.[25]

Macrophages and dendritic cells are the principal effector cells in host resistance to this fungus.[22,26] The fate of yeast cells in each of these cell populations differs. Yeasts proliferate within resting mononuclear phagocytes, but this form is killed by dendritic cells. As noted, macrophages engulf yeast via CD11-CD18 receptors, whereas dendritic cells use the fibronectin receptor. Engagement of two disparate receptors may explain in part the different fates within these cell populations. The central importance of monocytes or macrophages, or both, in controlling *H. capsulatum* is highlighted by the discovery of individuals

with congenital monocytopenia (*GATA 2* deficiency) who present with disseminated histoplasmosis.[27]

In murine macrophages, a high percentage of yeast cells are located within phagolysosomes. Binding to the CD11-CD18 receptors and subsequent entry into human macrophages are mediated by heat shock protein 60 expressed on the surface of yeast.[28] The fungus must contend with the adverse contents (e.g., acid proteinases) of this intensely hostile environment. A mechanism whereby yeasts survive is by alkalinization of the phagolysosome.[29] Yeast cells raise the pH of the phagocytic compartment from 6 to 6.5. One reason for maintaining the pH within a narrow range is that yeast cells require iron to grow, and if the pH exceeds 6.5, they cannot acquire iron from the host.[22]

Nitric oxide produced by activated murine macrophages is a major mediator of anti-*Histoplasma* activity. The ability of this nitrogen intermediate to oxidize iron may explain its potent fungicidal activity.[30] However, its influence in human infection remains unknown because human macrophages infected with *H. capsulatum* have not been reported to produce nitric oxide.

Macrophages from HIV-infected individuals manifest defective activity in their interaction with *H. capsulatum*. These cells bind fewer yeasts than cells from uninfected individuals, and a direct correlation exists between the $CD4^+$ T-cell count and the capacity of macrophages to bind yeast cells. On entry into cells, yeasts grow more rapidly within macrophages from HIV-infected individuals or in macrophages that have been infected in vitro with a macrophage-tropic strain of HIV. The envelope glycoprotein 120 from the virus is responsible for the inhibition of binding yeasts to macrophages[22] but not the altered growth characteristics of the yeasts in phagocytes.

Adaptive Immunity

Within the elements of the acquired immune response, T cells are pivotal in clearance of the fungus. Experimental studies indicate that neither B cells nor antibodies influence host resistance, although the data are limited. $CD4^+$ cells are extremely important in controlling primary infection in mice.[31] The central role of this T-cell subset in this species is supported by the finding that in HIV-infected individuals, most cases of histoplasmosis develop when the $CD4^+$ cell count is lower than 200/μm.[32] Mice deficient in $CD8^+$ cells are impaired in their ability to reduce the fungal burden, but they can eventually eliminate the fungus.[31] Vaccination of mice with yeast cells gives rise to a $CD8^+$ interleukin (IL)-17^+ T-cell population that confers protective immunity. The outgrowth of this population provides an additional arm to adaptive immunity that can combat infection.[33] In secondary infection, the absence of $CD4^+$ or $CD8^+$ cells diminishes the efficiency of yeast elimination, but mice survive. The loss of protective immunity develops only when both subsets are eliminated.

The primary contribution of T cells to host defense is the release of cytokines that eventually activate mononuclear phagocytes. Neutralization of endogenous interferon-γ (IFN-γ) or mice congenitally deficient in this lymphokine are exceptionally susceptible to infection.[31] Other cytokines in mice that are necessary for host clearance are IL-12 and tumor necrosis factor-α (TNF-α). Blockade of endogenous production of either of these leads to the death of mice. The effect of IL-12 is mediated through the induction of IFN-γ.[31] Interesting to note, IL-12 is important in primary infection but not in reexposure histoplasmosis.[31] TNF-α and IFN-γ are both necessary for controlling primary infection, and the former is required for secondary infection.[31] Their importance in humans has been highlighted by the finding that TNF antagonists or individuals with defective IFN-γ signaling manifest an enhanced susceptibility to disseminated infection.[34,35]

In vitro, recombinant IFN-γ activates murine peritoneal macrophages to inhibit the growth of yeast cells. Macrophages from other tissue sources are either nonresponsive to this stimulus or require costimulation with lipopolysaccharide.[21] The anti-*Histoplasma* action of IFN-γ is mediated by limiting iron acquisition, and this effect can be reversed through exposure to additional iron.[29] Human macrophages, on the other hand, do not respond to human recombinant IFN-γ to inhibit yeast cell growth.[22] The cytokines that activate these cells are macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and IL-3.[22] Granulocyte-macrophage colony-stimulating factor appears to inhibit intracellular growth of yeast cells by limiting access to zinc in phagosomes.[36] Although the infection is limited by cell-mediated immunity, tissues are not sterilized. Tissues of infected individuals contain yeasts, some of which may remain viable for many years. The dormant organisms pose little risk unless the individual becomes immunosuppressed as a result of potent immunosuppressive agents used to combat various clinical conditions or immunosuppressive viruses such as HIV. The metabolic state of *H. capsulatum* in tissues is unknown. It is likely that some of the yeasts remain viable because individuals who moved from endemic to nonendemic areas many years ago may have reactivated infection. The cascade of immunologic events that leads to activation of this form of the infection remains largely unknown. A murine model of reactivation histoplasmosis has been developed, and it should facilitate studies of the organism and the host in this form of infection. In mice, $CD4^+$, $CD8^+$, and a Thy-1.2, $CD4^-$, $CD8^-$ cell must be eliminated to achieve progressive infection. B cells also appear to be important in the severity of reactivation disease.

Genetic Predisposition to Disease

The emerging field of human genetic susceptibility to infectious diseases has extended to histoplasmosis. Several underlying genetic alterations are now known to predispose to more aggressive histoplasmosis. The X-linked hyper–immunoglobulin M syndrome is associated with defective IL-17 generation, and the majority of these individuals with disseminated histoplasmosis manifest gastrointestinal invasion, thus indicating the central influence of IL-17 on mucosal host defenses.[37] Disseminated histoplasmosis in individuals with mutations in the IFN-γ receptor and IL-12 pathway has been recognized.[38] Others have reported that patients with disseminated histoplasmosis or coccidioidomycosis have a gain-of-function mutation in the transcription factor signal transducer and activator of transcription 1 (STAT1), which is important in the signaling cascade of type 1 and type 2 IFNs.[39] Although these cases are uncommon, one should consider the possibility of a genetic predisposition in individuals in whom preexisting immunologic defects are not known.

Granulomas

The hallmark of the tissue response to this fungus is the development of caseating or noncaseating granulomas in which calcium may be deposited (Fig. 263.4). The granuloma consists of an admixture of mononuclear phagocytes and lymphocytes, principally T cells. The putative function of the granuloma is to contain fungal growth. Although IFN-γ and TNF-α are important in the generation of granulomas formed in response to other microbes, neutralization of these two cytokines does not prevent their formation in response to *H. capsulatum*. The organization of the *Histoplasma* granuloma has been characterized in mouse livers and lungs. $CD4^+$ and $CD8^+$ cells are present in the granulomas of mice. T-cell composition is polyclonal, and these cells are the source of IFN-γ and IL-17, whereas macrophages are the principal source of TNF-α.[40]

Organized granulomatous inflammation is typically observed in self-limited disease. Conversely, in progressive disseminated histoplasmosis

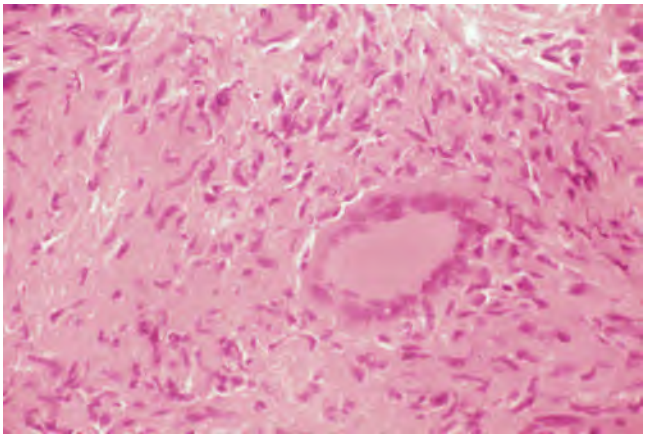

FIG. 263.4 Granuloma in the lung of a patient with histoplasmosis.

TABLE 263.1 Spectrum of *Histoplasma capsulatum*–Induced Disease

MANIFESTATIONS	ACUTE PULMONARY DISEASE	CHRONIC CAVITARY PULMONARY DISEASE	PROGRESSIVE DISSEMINATED DISEASE
Clinical	Often asymptomatic	Fever, productive cough, chest pain	Fever, weight loss, hepatosplenomegaly, hematologic disturbances[a]
Immunologic			
Positive skin test	>90%	70%–90%	30%–55%
Lymphocyte transformation	+++	+ to +++	±
Antibody to *Histoplasma capsulatum*[b]	25%–85%[c]	75%–95%	70%–90%
Antigenuria	20%[c]	40%	60%–90%
Pathologic			
Positive culture from lungs	<25%	5%–70%	50%–70%
Histology	Caseating and noncaseating granulomas, few yeasts, giant cells	Noncaseating granulomas, interstitial fibrosis, necrosis, yeasts, cavities, few to moderate yeasts	Diffuse macrophage proliferation, abundant few giant cells

[a]Hematologic disturbances include anemia, leukopenia, and thrombocytopenia.
[b]Complement fixation titer of greater than or equal to 1:8.
[c]Higher incidence in those with symptomatic infection.
+, Indicates a proliferative response to antigen or mitogen that is 3- to 5-fold higher than background; ++, 5- to 10-fold higher than background; +++, more than 10-fold higher.
From Deepe GS Jr, Bullock WE. Histoplasmosis: a granulomatous inflammatory response. In: Gallin JI, Goldstein IM, Synderman R, eds. Inflammation: Basic Principles and Clinical Correlates. *2nd ed. New York: Raven Press; 1992:943.*

(PDH), the more common histopathologic appearance of tissue is a massive influx of macrophages with scattered lymphocytes. Well-circumscribed granulomas are infrequently present, and the lack of an organized inflammatory response is indicative of a perturbed cellular immune response. Occasionally, the inflammatory response in mediastinal lymph nodes is exaggerated, resulting in excessive granuloma formation followed by fibrosis. The progressive scarring may affect the patency of the airways and major blood vessels.[41]

Delayed-Type Hypersensitivity

In experimental infection, either cutaneous or in vitro delayed-type hypersensitivity responses to *H. capsulatum* antigens are detected approximately 2 weeks after exposure.[31] In humans, delayed-type hypersensitivity responses manifest within 3 to 6 weeks after exposure.[42] These values are simply approximations because the precise time at which individuals are exposed in endemic areas is exceptionally difficult to determine. Reexposure to *H. capsulatum* in previously sensitized individuals is characterized by a more rapid tissue response. This finding is not surprising because *H. capsulatum* induces a memory response in which the immune system reacts in a much shorter time frame.

Infection with *H. capsulatum* produces a broad array of clinical and pathologic manifestations that must be recognized in order to diagnose and treat individuals afflicted with this fungus correctly. The clinicopathologic manifestations are summarized in Table 263.1.

PULMONARY HISTOPLASMOSIS

Acute Infections

Acute Primary Infection

Symptoms

The vast majority of primary infections (>90%) go unrecognized medically. Usually, they are asymptomatic or result in mild influenza-like illness for which individuals do not seek medical attention. However, there is a small proportion of patients who become overtly ill. The major determinant for the development of symptoms is likely to be the inoculum size, although differences in strain virulence cannot be excluded.[43,44] Other contributing factors include age and underlying diseases. Thus, older adults, children younger than 2 years, and individuals whose immune systems are compromised are more likely to develop progressive disseminated disease symptoms.

In those who become ill, the typical incubation time is 7 to 21 days, and most individuals manifest symptoms by day 14.[43,44,45] Fever that may reach 42°C, headache, nonproductive cough, chills, and chest pain are the most common symptoms noted. Chest pain is described usually as a substernal discomfort, although in an outbreak in children it was more often located in the anterior chest.[45] Pleuritic chest pain is uncommon. The chest pain is believed to be caused by enlargement of the mediastinal or hilar lymph nodes, or both. Malaise, weakness, fatigue, and myalgias are observed in a distinctly smaller percentage of patients. Most symptoms resolve within 10 days, but they can persist for several weeks if there is an exposure to a heavy inoculum. Acute pulmonary infection can be accompanied by a number of rheumatologic manifestations. Arthralgias, erythema nodosum, and erythema multiforme are present in approximately 6% of patients, most of whom are women.[46] In some, these manifestations of histoplasmosis may be the presenting complaint. Frank arthritis is distinctly uncommon.

FIG. 263.5 Chest radiograph of patient with acute pulmonary histoplasmosis.

Clinical Findings

Physical findings in acute pulmonary histoplasmosis are minimal. Crackles may be detected on auscultation of the lungs and, rarely, hepatosplenomegaly. The common radiographic features are characterized by a patchy pneumonitis that eventually calcifies and hilar lymphadenopathy (Fig. 263.5). If a heavy exposure has transpired, numerous patches of pneumonitis that calcify may develop, and these produce the so-called buckshot appearance on the chest radiograph.[35] Pleural effusions are