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Cryptococcosis in the Era of AIDS—100 Years after the Discovery of *Cryptococcus neoformans*

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INTRODUCTION

One hundred years ago, *Cryptococcus neoformans* was discovered when this encapsulated yeast was isolated independently from peach juice in Italy by Sanfelice (439) and from the tibial lesion of a patient in Germany by Busse (58) and Buschke (57). Many of the early case reports were associated with patients with cancer. In 1905, Von Hansemann presented the first report of cryptococcal meningitis (191). Much of the pathology and the features of primary pulmonary cryptococcosis were described by Baker and Haugen (8). After several years, controversies regarding the nomenclature of *C. neoformans* were settled (16), as it had been given a variety of names, including *Saccharomyces neoformans*, *Blastomyces neoformans*, *Cryptococcus hominis*, and *Torula histolytica*. Outdated synonyms for cryptococcosis include torulosis, European blastomycosis, and torula meningitis. Reports of infection due to other species of *Cryptococcus* (*C. albidus* and *C. laurentii*) are extremely rare (203, 291).

There are two varieties of *C. neoformans* and four major serotypes, based on capsular epitopes. Nearly all infections of patients with AIDS involve strains of a single variety and serotype, although such strains vary at the phenotypic and genotypic levels (45, 78, 310, 497). This review will focus on the clinical manifestations and management of cryptococcosis in patients with AIDS and the etiologic agent of 99% of these cases—*C. neoformans* var. *neoformans* serotype A. We will also attempt to critically review controversial issues and current areas of research, especially those that pertain to the molecular basis of pathogenesis of *C. neoformans*, host defenses, and the management of cryptococcosis.

CRYPTOCOCCUS NEOFORMANS

The 19 species within the genus *Cryptococcus* are characterized as variously encapsulated budding yeasts. Despite reports of other pathogenic species, the major pathogen and subject of this review is the species *C. neoformans*, an encapsulated, usually spherical yeast of which two varieties are recognized, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. By using cross-absorbed rabbit polyclonal antisera, the capsular serotypes of *C. neoformans* were defined as serotypes A through D and serotype AD (147–149, 505). Rare isolates lack a capsule or possess a capsule but cannot be serotyped. As indicated in Table 1, isolates of *C. neoformans* var. *neoformans* may possess capsular serotypes A, D, or AD, and isolates of *C. neoformans* var. *gattii* are serotype B or C. In addition to their serotypes, the two varieties of *C. neoformans* also differ in certain biochemical properties, ecology, and epidemiology (Tables 1 and 2).

On routine laboratory media, colonies of *C. neoformans* develop within 36 to 72 h. They are white to cream colored and opaque and may be several millimeters in diameter. Colonies may also develop sectors that differ in pigmentation. The colonies are typically mucoid, and the amount of capsule can be

TABLE 1. Comparison of the two varieties of *C. neoformans*[a]

Feature	*C. neoformans* var. *neoformans*	*C. neoformans* var. *gattii*
Teleomorph	*Filobasidiella neoformans* var. *neoformans*	*Filobasidiella neoformans* var. *bacillispora*
Ecology	Soil and avian feces	Eucalyptus trees
Geographical distribution (natural isolates)	Worldwide	Tropical (Southern California, Australia, Southeast Asia, Africa)
Capsule	Yes	Yes
Phenol oxidase production	Yes	Yes
Malate assimilation	No	Yes
Feedback repression of creatinine deaminase	Yes	No
Canavanine susceptibility	Yes	No
Glycine assimilation (% of strains)	10–20	100
Serotypes	A, D, and AD	B and C

[a] Data are from references 139, 140, 143, 249, 252, 255, 257, 265, 269, 281, 396, and 397.

TABLE 2. Geographic distribution of *C. neoformans* serotypes in patients with cryptococcosis[a]

Group and geographic distribution of clinical isolates	Frequency of serotype (%)			
	A	D	B	C
Non-AIDS patients				
U.S. (except Southern California)	>80	4	4	2
Southern California	>40	<1	37	14
Worldwide: temperate	50–95	3–70	≈5	≈1
Worldwide: tropical	30–45	<1	55	≤15
AIDS patients				
Worldwide (except France)	99	<1	<1	<1
France	>80	17	0	0

[a] Data are from references 11, 130, 140, 251, 252, 257, 284, 358, 397, 501, and 507.

judged from the degree of colonial mucosity. Highly encapsulated colonies may coalesce and slowly trickle down a slant to puddle in the bottom of the tube or drip off the medium of inverted plates.

Microscopically, most clinical isolates appear as spherical, budding, encapsulated yeast cells in both tissue and culture (509). Rarely, short hyphal forms are seen, and filamentous variants have been isolated (145). The yeast cells vary in size from 5 to 10 μm in diameter and exhibit both single and multiple budding. Since the buds are readily detached from their parent cells, the majority of cells in tissue or culture may lack buds. The hallmark of *C. neoformans* is it capsule, which can be visualized in an India ink preparation (Fig. 1). The size of the capsule varies considerably.

Capsule size or thickness is determined by the genetics of the strain as well as by conditions of growth. For a given strain, capsule production in vitro can be optimized by cultivation on solid or liquid medium in the presence of 1% glucose, ≥1.0 μg of thiamine per ml, ≥1.0 mg of glutamate per ml, neutral pH, temperature of 37°C, elevated carbon dioxide, or decreased concentration of iron (135, 181, 280, 498). Conversely, capsule production can be decreased in vitro by growth at lower temperatures, under conditions of high osmolarity (16% glucose or 2.9% NaCl) or acid pH, or after storage in soil (135, 181, 213, 280, 498). Under the same conditions of growth, some strains characteristically produce large, medium, or minimal capsules. The observation that the effects of these growth conditions on capsule size are strain dependent provides additional evidence of phenotypic variation among isolates of *C. neoformans*. Nevertheless, most strains, even those that are consistently small-capsuled in vitro, develop large capsules during infection. Rarely, capsule-free isolates have been found in tissue (152, 259, 268, 311). The finding that isolates from patients with AIDS have smaller than average capsules has not been consistently observed (41).

Two decades ago, successful mating experiments by Kwon-Chung demonstrated that *C. neoformans* represents the asexual or anamorphic form of a heterothallic basidiomycete, which she named *Filobasidiella neoformans* (249, 250). Of the clinical and natural isolates of *C. neoformans* that are fertile, more than 95% belong to only one of the two mating types of *F. neoformans*—mating type α. In most cases, only the haploid anamorph is isolated from clinical or natural samples (292, 476).

Ecology, Sexual Reproduction, and Identification

Current information suggests that in nature, the two varieties of *C. neoformans* reside in separate environmental niches (Table 1). *C. neoformans* var. *neoformans* has long been isolatable from soil and avian habitats (269). *C. neoformans* thrives on and is enriched by the nitrogenous components associated with avian guano (142, 143). Birds do not become infected, probably because their relatively high body temperature is inimical to *C. neoformans*. However, birds are likely to distribute the yeasts in nature. In avian nesting areas, the yeast cells possess minimal capsules, and hence the cells are dry and easily aerosolized (53, 349, 431). In this state, the yeast cells are smaller and capable of being inhaled to the level of the alveolus (349, 415).

In recent years, a natural association has been recognized between *C. neoformans* var. *gattii* (serotype B) and flowering eucalyptus trees, such as the red river gum tree (*Eucalyptus camaldulensis*) (138–140). To date, all of the isolates recovered from eucalyptus trees have been serotype B. If the isolation of *C. neoformans* var. *neoformans* from avian environments reflects only colonization by enrichment, then the true ecology of serotypes A, D, and C remains to be discovered.

The extent, if any, to which sexual reproduction by *C. neoformans* occurs in nature is unknown. Certainly, the small, dry basidiospores would make effective infectious particles, and they have been suggested to be a source of infection (138, 140). As noted above, clinical isolates of *C. neoformans* are usually haploid and invariably of only one of the two mating types (the α type). Ordinarily, this finding would militate against the basidiospore's being an important source of infection. Since equal numbers of each mating type are produced during sexual reproduction, some patients would be expected to have the **a** mating type or cells of each type. However, most isolates recovered from nature also possess the α allele. Therefore, in the environment, either *C. neoformans* reproduces predominantly asexually or the **a** mating type does not survive as well as the α type. Interestingly, murine virulence has been linked to the α mating type, so this mating type, or a vicinal gene(s), is likely to be important for pathogenicity (253). Since studies of the population genetics, as were recently reported for strains of *Candida albicans* from patients with AIDS (416), have not been

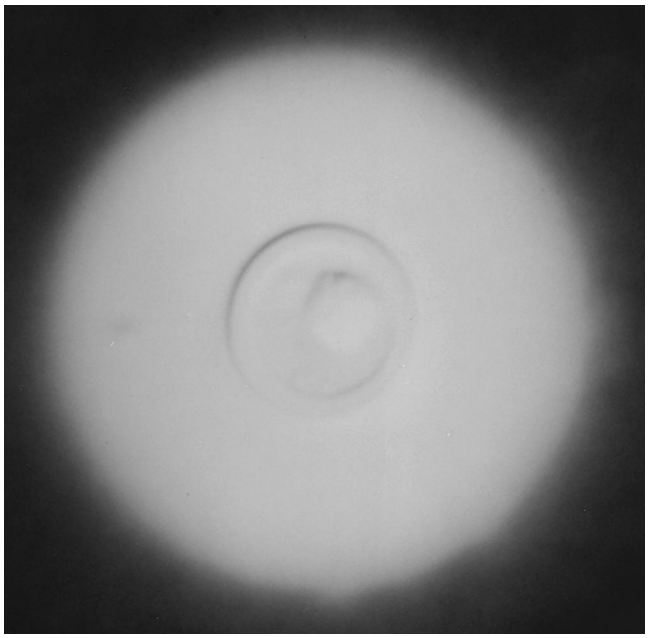

FIG. 1. India ink examination of CSF, demonstrating the heavily encapsulated yeast cells of *C. neoformans*. Magnification, ×600.

performed, it is not possible to estimate the extent of clonal or sexual reproduction that occurs naturally in *C. neoformans*.

As judged from the ability to readily isolate the major pathogenic variety of *C. neoformans* from soil and avian habitats, *C. neoformans* is ubiquitous in nature, yet the incidence of cryptococcosis is relatively low. Therefore, it is likely that many more people inhale the yeast cells (or basidiospores) than become ill. Indeed, an intact cell-mediated immunity (CMI) and competent phagocytes provide strong defenses against cryptococcosis. The actual extent of subclinical exposure is unknown because an adequate skin test antigen has not been developed for population surveys. However, limited serological studies indicate that most healthy individuals possess antibodies to *C. neoformans* (23, 231, 323).

All species of *Cryptococcus* are nonfermentative, hydrolyze starch, assimilate inositol, and produce urease. These characteristics, as well as the morphological features of the capsule and the rare presence of pseudohyphae (26, 145, 426), distinguish them from other clinically important yeasts. Each of the two varieties can be recognized by malate or glycine assimilation, resistance to canavanine, feedback repression of creatine deaminase, and other biochemical reactions (254, 255).

Some of the physiological tests that distinguish the two varieties of *C. neoformans* are indicated in Table 1. On appropriate media, more that 85% of isolates of *C. neoformans* var. *gattii* (serotypes B and C) assimilate glycine and malate and are resistant to concentrations of cycloheximide or canavanine that inhibit isolates of *C. neoformans* var. *neoformans* (serotypes A and D) (402). Most isolates of the latter variety are unable to use malate as a source of carbon or glycine as a sole carbon and nitrogen source. Both varieties use creatinine and are induced to synthesize creatinine deaminase, but only in isolates of *C. neoformans* var. *neoformans* is this enzyme repressed by the ammonia byproduct. Selective media containing creatinine, glucose, and pH indicators that exploit this difference have been devised to differentiate the two varieties. Isolates of both varieties exhibit comparable in vitro susceptibility to antifungal drugs (160).

Growth and Physiology

C. neoformans has minimal growth requirements of simple carbon and nitrogen sources, and even vitamin supplementation with thiamine may not be required for growth (47). The growth rate of *C. neoformans* depends on a variety of conditions. However, in most complete media at 37°C, the exponential doubling time varies among different strains from 2.5 to 6 h (314). *C. neoformans* generally does not grow as rapidly as yeasts such as *Saccharomyces cerevisiae* and *Candida albicans* under similar conditions. Unlike other species of *Cryptococcus*, the ability of *C. neoformans* to use low-molecular-weight nitrogenous compounds such as creatinine may partially explain its ecological niche in avian guano. It is inhibited by alkaline pH and high temperature.

Temperature is an important environmental signal for *C. neoformans* growth. Unlike *C. neoformans*, most other species of *Cryptococcus* are unable to grow at 37°C and are nonpathogenic. Indeed, the growth of *C. neoformans* is tightly regulated by temperature, since in vitro temperatures of 39 to 40°C will significantly slow its growth rate, and at this temperature, the yeasts undergo intracellular vacuolization and produce aberrant budding patterns and pseudohyphal structures. *C. neoformans* var. *gattii* is even more sensitive to high temperatures, and at 40°C most strains are killed within 24 h. The importance of temperature to pathobiology is further emphasized by temperature-sensitive mutants of *C. neoformans*, which do not grow at 37°C and are avirulent in animals regardless of their ability to form capsules or produce melanin (256).

Some of the physiological features of *C. neoformans* have been exploited for its identification and the study of differences among isolates. In the presence of urea, most strains readily excrete large amounts of urease, and its detection in vitro has led to a rapid urease test for the identification of *C. neoformans* (279, 526). A unique biochemical feature of *C. neoformans* is its ability to produce diphenol oxidases (see below), which may function as antioxidants and enhance survival of the yeast in the host (217, 219). The presence of this enzyme, which oxidizes a number of diphenolic substrates and leads to the production of melanin, has been used for identification and is considered a potential target for anticryptococcal chemotherapy (211, 215, 517).

As indicated above, differences in uptake of L-malic acid, repression of the inducible enzyme creatinine deaminase, and the ability to use glycine have been used to differentiate the two varieties in vitro, but there is no evidence that these different physiological characteristics relate to pathobiological differences between the varieties. However, an intact purine metabolic pathway has been found to be critical for infection with *C. neoformans*. When the purine pathway gene for phosphoaminoimidazole carboxylase (*ADE2*) is inactive, *C. neoformans* cannot grow and produce disease in an immunocompromised host (388). A similar requirement for pathogenicity has also been observed in *ade2* auxotrophs of *Candida albicans* (237). These findings suggest that if selective fungal inhibitors were found, this enzyme could be an important antifungal target.

The environmental conditions required for sexual reproduction in *C. neoformans* suggest that these events are under nutritional control. Generally, matings occur on nonenriched media, such as V-8 juice and hay-infusion agar, and at temperatures below 37°C. Actually, the best conditions for meiosis occur at room temperature and ambient pCO_2 concentrations. These observations suggest that mating is regulated by environmental signals, such as nitrogen starvation. Recent studies by Moore and Edman indicate that *C. neoformans* cells transcribe, process, and secrete pheromones in response to starvation (330). Recent advances in molecular biology now permit the investigation of these signals for gene regulation and expression during meiosis and their molecular mechanisms.

Two external physiological conditions have been linked to growth and regulation of the capsule in *C. neoformans*. Both the pCO_2 concentration and ferric iron availability dramatically influence growth and capsular size (181, 498). Synthesis of capsular polysaccharide and capsular size are increased at physiological pCO_2 and by reduced ferric iron. As capsular size increases, the growth rate decreases. Most strains of *C. neoformans* produce large capsules at physiological pCO_2 concentrations and small capsules when exposed to environmental pCO_2 levels. This environmental regulation of the capsule is consistent with the yeast's requirements for pathogenicity. That is, coming from nature, a small capsule is necessary for inhalation and deposition in the lungs, after which the development of a large capsule protects the yeast against a variety of host defenses. Since *C. neoformans* does not produce hydroxamate siderophores (218) but responds to iron in the environment, it probably has specific surface receptors for the acquisition of iron (220). These two environmental stimuli, pCO_2 and iron, are apparently sensed by *C. neoformans* and linked to essential regulatory mechanisms for its adaptation and survival in the host. These processes should become the focus of future research on the molecular mechanisms of *C. neoformans* pathobiology.

Virulence Factors

Strains of *C. neoformans* isolated from patients are able to grow at 37°C, produce a phenol oxidase enzyme, and are almost always encapsulated. The in vitro capsule size, whether small, medium, or large, is a stable characteristic of many strains of *C. neoformans*. Analysis of virulence usually involves experimental infection and a comparison of mortality rates or histopathology or a census of viable yeasts in selected organs. Strains of *C. neoformans* vary in virulence for animals, but virulence is not correlated with the amount of capsular polysaccharide (CPS) (135). Classical genetic analyses of strains have been impeded because many natural isolates and mutants are infertile or self-fertile.

The mechanisms of virulence are unresolved. Pathogenicity requires the production of phenol oxidase, growth at 37°C, and the presence of a capsule (256, 422). These properties are essential but not sufficient for virulence. The basis for the neurotropism of *C. neoformans* is unclear but may involve selective evasion of host defenses or enrichment in the target tissues (e.g., utilization of catecholamines, which are excellent substrates for phenol oxidases and are abundant in the central nervous system [CNS] and the adrenal glands, another target organ). The capsule or CPS potentiates infection, depresses inflammation, inhibits phagocytosis (55, 244), and suppresses both cellular and humoral immunity (46). A secreted mannoprotein induces specific T helper and T suppressor cells that modulate CMI responses in mice (205, 336, 344).

The CPS is a long, unbranched polymer, an α-1,3-mannan with monosaccharide branches of xylose and glucuronic acid (22). Several antigenic serotypes of the CPS (A-D, AD, and untypeable) have been defined, but most isolates from both environmental samples and clinical specimens are serotype A. We have begun to study the biological and chemical properties of the CPS of strains of serotype A that differ in virulence (451–453).

Growth and responses in vivo. To initiate a systemic infection, any microorganism must be capable of growing within the host, and *C. neoformans* grows well at 37°C and can utilize numerous host substrates. At 41°C, *C. neoformans* is inhibited or killed, and this temperature restriction may be an important determinant of its pathogenicity. Only rare strains of other species of *Cryptococcus* are capable of in vitro growth at 37°C.

Elegant research on bacterial pathogenesis has clearly demonstrated that certain bacteria sense environmental signals that trigger a variety of virulence genes, which respond in a coordinately regulated manner (120, 156, 173, 315, 468, 518). In many circumstances, this response(s) permits the invading microorganism to grow or survive in a hostile environment. These mechanisms may be activated by specific molecules, such as phenolic compounds or sugars, as has been shown for phytopathogens (4, 324), or by environmental stimuli, such as iron concentration, temperature, osmolarity, pH, oxygen, carbon dioxide, and calcium, all of which participate in the regulation of virulence in a variety of bacteria (30, 52, 174, 299, 363, 433, 467, 481).

This environmental paradigm may be used to identify genes that are regulated at the site of infection and are essential for survival of *C. neoformans* in the host. By using the rabbit model of cryptococcal meningitis and comparing the expression of genes in yeasts that were grown in vivo and in vitro, genes have been isolated that are both upregulated and downregulated at the site of a CNS infection (382). The identification of these genes and elucidation of their regulation are in progress. An initial step in this strategy is the use of differential hybridization and cDNA subtractive techniques to isolate infection-specific genes. However, the importance of these genes in the pathobiology of *C. neoformans* will need to be analyzed by specific gene mutations and/or gene disruption experiments. In an elegant set of experiments, Mahan et al. developed a system to select bacterial virulence genes that are specifically induced in host tissues (293). This system could potentially be adapted to investigate *C. neoformans*. With the recent advances in the molecular biology of *C. neoformans*, the use of appropriate animal models, and the correlation between complementation of adenine prototrophy and restoration of virulence (388), a similar experimental approach would identify in vivo-induced promoters and lead to isolation of the virulence genes of *C. neoformans*.

CPS. The capsule of *C. neoformans* is clearly a virulence factor. In experimental cryptococcosis, capsule-free isolates or mutants are less virulent than encapsulated wild-type cells (162, 242). Human cases due to capsule-free or small capsuled isolates have a stronger host response, greater inflammation, and less severe disease (152, 268, 311). The size of the capsule, both in vitro and in vivo, varies among isolates, as does virulence, but virulence is not correlated with capsule size (135). The capsule has been implicated in numerous biological effects. A body of experimental evidence suggests that the capsule is required but not necessarily sufficient for optimal pathogenicity. Capsule-free yeast cells are ingested by various phagocytes to a much greater extent than are encapsulated cells (55, 243, 245), although ironically they are not killed as readily as encapsulated cells by monocytes or macrophages (273, 313).

Different strains of *C. neoformans* serotype A vary considerably in both animal virulence and capsule size in vitro. As noted, these two properties are not directly correlated. Nevertheless, the capsule is a crucial virulence factor. The glucuronoxylomannan (GXM) is probably responsible for many of the pathobiological properties attributed to the capsule. GXM has been shown, under the appropriate conditions, to specifically inhibit both phagocytosis of *C. neoformans* and the production of antibody to it. Differences in virulence among strains of serotype A have been correlated with resistance to killing by alveolar macrophages, but not with differences in capsule size or the extent of phagocytosis. As described below, GXM is a potent activator of the alternative complement pathway, which leads to the deposition of iC3b and other opsonins on and within the capsule (240).

A number of in vitro studies have examined the phagocytosis of yeast cells of *C. neoformans* by phagocytes under various conditions and explored the kinetics of this interaction and the influence of serum, complement, antibody, and yeast-to-phagocyte ratio. These in vitro experiments have employed a variety of phagocytes (neutrophils, monocytes, alveolar, and peritoneal macrophages) from different sources (mice, rats, guinea pigs, and humans) as well as different strains of *C. neoformans* (245, 246, 337). The extent to which yeast cells are ingested by macrophages is inversely related to the size of the capsule (36). However, different strains with similar capsule sizes, which are ingested to a comparable degree, differ in the extent to which they are killed by alveolar macrophages (37). The GXMs from different strains of serotype A vary in the ability both to bind to capsule-free yeast cells and to inhibit their phagocytosis (451, 452).

Phenol oxidase and melanin production. *C. neoformans* produces a unique phenol oxidase(s) that converts a variety of hydroxybenzoic substrates, including catecholamines such as 3,4-dihydroxyphenylalanine, into brown or black pigments, which impart a dark color to colonies or the medium (211, 401, 403). As noted above, this reaction has been used for the

accurate and rapid identification of *C. neoformans*. The contribution of phenol oxidase to cryptococcal virulence has not been elucidated, but there is experimental support for several possible hypotheses. The utilization by phenol oxidase of natural catecholamines (e.g., norepinephrine and dopamine) as substrates for melaninogenesis may somehow relate to the unexplained neurotropism of *C. neoformans* (217). For example, the end product of the activity of phenol oxidase, melanin, can function as an antioxidant, which may protect *C. neoformans* from oxidative host defenses (215–217, 219, 407).

Genetic analyses support the association of virulence with phenol oxidase production (256, 422). Polacheck and associates have shown that a mutant lacking phenol oxidase was killed by the epinephrine oxidative system in the presence of a transition metal ion and hydrogen peroxide (406). The wild type was resistant, which suggests that phenol oxidase may consume epinephrine and protect *C. neoformans* from this oxidative system in the CNS (406).

Other determinants of pathogenicity. In addition to the capsule and phenol oxidase, other features of *C. neoformans* may contribute to its virulence. An as yet undefined virulence attribute(s) is apparently linked to the α mating type locus (253). Secreted proteinases which may contribute to the breakdown of host tissue or humoral proteins have been described (48).

As noted above, the neurotropism of *C. neoformans* may be explained in part as an evasion of host defenses or the utilization of specific neuronal substrates. It is also possible that *C. neoformans* attaches via ligand-like binding to specific receptors present only on neuronal cells. For example, *C. neoformans* adheres in vitro to rat lung and glial cells; this adherence is trypsin sensitive and inhibitable by aminosugar and disaccharides, but not by components of the capsule (306).

Antigenic Composition

Culture filtrates of *C. neoformans* yield at least three well-characterized antigens: GXM, galactoxylomannan (GalXM), and a mannoprotein (344). The antigen responsible for CMI appears to be a mannoprotein, which is probably associated with the cell wall (79).

Serotypes. When appropriately immunized with whole yeast cells, rabbits will produce high titers of antibodies to the capsule. By cross-absorption of such antisera, the four distinct capsular serotypes (A to D) were defined (148, 149, 505). As described above, these serotypes differ in their association with the two varieties of *C. neoformans* (249, 254), their ecology, and their prevalence in disease (Tables 1 and 2). As mentioned, strains of serotype A can be isolated worldwide and are the most frequent cause of cryptococcosis in all patients, with or without AIDS. Strains of serotype D are more common in certain areas of Europe, notably France, Italy, and Denmark (130). Serotypes A and D have been isolated from soil and avian, especially pigeon, guano. Serotypes B and C have only recently been isolated from nature in association with eucalyptus trees in Australia and in San Francisco (139, 396) and bat guano in Brazil (265).

GXM. When *C. neoformans* is grown in vitro or during an infection in vivo, the CPS becomes solubilized and can be precipitated from the culture supernatant fluid or detected in body fluids. The purified capsular material is a high-molecular-weight polysaccharide. Hydrolysis of CPS from cells of any of the serotypes yields mannose, xylose, and glucuronic acid. Chemical studies support an α-1,3-linked polymannose backbone with β-linked monomeric branches of xylose and glucuronic acid. GXM is the major CPS of *C. neoformans* (76). The structures of the GXMs of the four serotypes differ in the degree of mannosyl substitution and the molar ratios of mannose, xylose, and glucuronic acid (78, 487, 488).

GXM resembles a T-cell-independent type 2 antigen (79, 131, 470). It is poorly immunogenic by itself, but if it is conjugated to a protein carrier, a strong antibody response develops, as occurs when the whole cell is the immunogen (66, 113, 179). GXM is a virulence factor, and several notable biological properties have been demonstrated—GXM is anti-inflammatory, antiphagocytic, and immunosuppressive. In mice, GXM has been shown to induce specific tolerance, both low-dose T-cell-dependent and high-dose T-cell-independent tolerance (79, 471). Among strains of the same serotype, structural differences have been reported (77, 78, 453, 488), and monoclonal antibodies have defined antigenic differences (15, 128, 457, 482).

GXM has long been recognized to activate the alternative complement pathway, leading to the opsonization of intact, encapsulated yeast cells with C3 (36). In recent elegant studies, Kozel and colleagues have associated activation of the alternative pathway with GXM and quantified the deposition of C3b and iC3b (240, 398, 524).

GalXM and mannoprotein. Culture filtrates of *C. neoformans* also yield smaller amounts of another polysaccharide, GalXM, as well as a mannoprotein, which appears to be the immunodominant antigen that is responsible for evoking CMI (79). GalXM is composed of mannose, xylose, and galactose (221). GalXM can be separated from mannoprotein because only the latter binds concanavalin A (79). In the murine model, Murphy has shown that a culture filtrate containing mannoprotein and other antigens can function as an immunomodulator and elicit a cascade of suppressor cells, which may explain the observation that many patients acquire and retain a specific immunological tolerance to *C. neoformans* (338, 344).

Other antigens. Recently, another exoantigen has been purified from culture filtrate material (188). This acidic glycoprotein contains mannose and has a mass of 115 kDa. It binds to a monoclonal antibody that reacts only weakly with the mannoprotein (188). On immunoblots, patient sera reacted strongly to this antigen.

Molecular Biology

To fully investigate the pathogenesis of cryptococcosis, it is necessary to understand the molecular biology of *C. neoformans*. During the past two decades, knowledge of eukaryotic molecular biology has been significantly developed around several fungal models, notably *S. cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, and *Aspergillus nidulans*. The molecular experience with these fungi can now be used to elucidate systems for the pathogenic fungi. In many respects, *C. neoformans* has the potential to become a model system for the molecular study of host-fungus dynamics. Indeed, animal models have been developed for *C. neoformans*, virulent phenotypes have been characterized, a genetic system has been described, and a foundation in molecular biology has been established (136, 240, 252, 253, 270, 271, 338).

Karyotypes, repetitive elements, and strain-specific probes. Until 5 years ago, knowledge of the molecular biology of *C. neoformans* was limited to DNA relatedness between varieties (6, 145). However, molecular studies have progressed rapidly in recent years. Initial studies focused on the analysis of restriction fragment length polymorphisms (RFLPs) with various repetitive elements (405, 458, 497) or mitochondrial DNA (496) to identify and classify isolates. This work has been extended by the use of PCR technology to fingerprint different strains rapidly and specifically (94, 310). Methods to separate

TABLE 3. Cloned genes of *C. neoformans*

Genes	Reference(s)
rDNA genes	419
Dihydrofolate reductase	450
URA5 (oritidine, monophosphate, pyrophosphorylase)	65, 137
URA3 (oritidine 5′, phosphate decarboxylase)	516
ADE1 (phosphoribosylaminoimidazole-succinocarboxamide synthetase)	516
ADE2 (phosphoribosylamino-imidazole carboxylase)	388
LEU2 (3-isopropyl malate dehydrogenase)	516
*HIS*3* (imidazoleglycerol-phosphate dehydratase)	362
CAP (capsule gene)	71
DPO-S (a phenol oxidase gene)	517
MTH-1 (? regulator of mannitol dehydrogenase)	384
NMT (*N*-myristoyl transferase)	286
ARF (ADP-ribosylation factor)	286
Actin	92
β-Tubulin	97
TRP1 (phosphoribosyl anthranilate isomerase)	383
*MAT*α locus	330
Thymidylate synthase	283
GPA1 (GTP-binding protein α subunit)	484

chromosomes of *C. neoformans* and thus produce a karyotype for each strain were also developed (381, 404). These results showed an impressive variation in chromosome sizes among strains, and this polymorphism was stable for each strain, both in vitro and in vivo. Thus, karyotypes and PCR fingerprints can be used to distinguish strains of *C. neoformans* var. *neoformans* for epidemiological purposes (310, 378).

Differences among karyotypes have also been used for epidemiological studies of *C. neoformans* var. *gattii*. A preliminary investigation revealed similar karyotypes among environmental isolates from different locations but different karyotypes among clinical strains (258). The mechanisms for this karyotypic variation among strains and the high frequency of karyotypic changes, which have also been seen among meiotic progeny from genetic crosses (91), warrant further investigation.

In the molecular diagnostic arena, a commercial probe with excellent sensitivity and specificity has been developed for the rapid identification of cultures of *C. neoformans* (208). For the potential identification of *C. neoformans* in clinical specimens, PCR has been used to amplify a portion of the fungal ribosomal DNA (rDNA) of *C. neoformans* from clinical material, which was subsequently digested with enzymes to distinguish it from the amplicons of other yeasts (202). The rDNA sequences of *Cryptococcus* and related species have also been subjected to molecular phylogenetic analyses (322, 500), and from these sequence data, additional primers were designed that specifically amplify rDNA from *C. neoformans* (320).

Gene cloning. The task of cloning *C. neoformans* genes has begun. A series of genes have been identified, and some have been sequenced. Table 3 lists the known cloned genes. Because of rapid changes in this field, this list is almost surely incomplete. The breadth of techniques used to clone these genes indicates the significant advances in the molecular biology of *C. neoformans*. (i) Genes have been cloned by heterologous gene probes to genomic libraries. (ii) cDNA libraries have been used to complement bacterial mutants, with recovery of the identified fungal transcript. (iii) Genes have been identified by their ability to be expressed and thus complement mutations in *S. cerevisiae*. (iv) Partial amino acid sequences of purified proteins have been identified, and from this information, oligonucleotides have been generated to clone the entire gene. (v) Complementation of a known mutation in *C. neoformans* has been performed with plasmid rescue of the gene. (vi) Differential gene expression has been used to identify genes responding to specific environmental conditions.

Of the genes of *C. neoformans* that have been sequenced, genes with and without introns have been identified. However, not enough genes have been sequenced to identify a characteristic promoter motif or polyadenylation signal for the majority of *C. neoformans* genes. Our experience with the cryptococcal *ADE2* gene has indicated that there are frequent allelic nucleotide sequence variations among strains, and in noncoding regions, widely divergent sequences can occur among strains. This allelic sequence polymorphism has been formally studied with the *URA5* gene, and extensive allelic variation in nucleotide sequence (differences of approximately 5 to 7%) among strains has been described (65). It remains unknown whether this allelic polymorphism is characteristic of many or only a few genes in *C. neoformans*.

Transformation. As important as the molecular tools of karyotyping, PCR fingerprinting, and gene cloning are for the advancement of molecular epidemiology, diagnosis, and phylogeny, the single most important technique for the study of molecular pathogenesis in *C. neoformans* has been the development of a transformation system to allow the transfer of DNA into and out of this yeast. The first transformation system in *C. neoformans* was reported by Edman and Kwon-Chung (137). Using electroporation, they complemented a *Ura5* mutant with the *URA5* gene. Efficiency of transformation was highest with linear DNA, which was extrachromosomal in the majority of transformants (495). Analysis of these transformants revealed that telomeres were frequently added to the ends of the vectors, which replicated extrachromosomally (136). With this discovery, Edman et al. constructed a vector containing both telomeres and a *C. neoformans* library for use in complementation experiments in *C. neoformans* in which this vector can now be rescued from *C. neoformans*. Since its introduction, this vector strategy has been used to clone several genes (71). These vectors may be improved with the addition of autonomous replication sequences and centromeres to further increase their gene dosage and/or stability.

A second transformation system uses biolistic DNA delivery and the *ADE2*-complementing allele (483). This method results in highly efficient transformation, with thousands of transformed colonies per plate. Approximately half the transformants are integrative, and a fourth of these show homologous recombination. These findings are encouraging for the ability to perform critical gene disruption experiments in *C. neoformans*. However, our current experience suggests that homologous recombination may be allele dependent, which is true for other fungi. Thus, to investigate certain genes, the yeast cell cycle and/or transcription of a particular gene may need to be controlled to permit efficient site-specific recombination and gene disruption for each targeted gene. Recently, our group, in collaboration with Lodge and Gordon, used biolistic transformation to disrupt the wild-type *N*-myristoyltransferase (*NMT*) gene of *C. neoformans* (285, 286), producing a temperature-sensitive mutation in this gene. Progress continues with other transformation systems that use dominant selection markers with sugars (galactose) or antibiotics (aminoglycosides) (93). When developed, these systems will further increase knowledge of the molecular versatility of *C. neoformans*.

EPIDEMIOLOGY

Cryptococcosis has been considered a sporadic infection with a worldwide distribution. As noted above, the major variety, *C. neoformans* var. *neoformans*, is ubiquitous in the soil and in avian guano, which supply a reservoir of small, dry, inhalable yeasts. Spontaneous infections occur in nonhuman animals, particularly in cats; however, no transmission of cryptococcosis among animals or humans has been reported (150). Despite its rare isolation from the respiratory tract of healthy individuals, *C. neoformans* is not a member of the normal microbial flora of humans or animals (132). Furthermore, outbreaks of cryptococcosis in which more than one patient acquires the infection at the same time and place, presumably from exposure to a common source of the inoculum, do not occur (474).

Although cryptococcosis does occur in many patients who are not apparently compromised, most patients have a preexisting condition or disease. The more frequent underlying conditions, in order of decreasing association, are human immunodeficiency virus (HIV) infection, extensive treatment with corticosteroids, organ transplantations, chronic leukemias and lymphomas, and sarcoidosis. Because of the historical association of cryptococcosis with Hodgkin's disease, leukemia, and lymphoma, an etiological link between *C. neoformans* and cancer was suggested. The patients at highest risk illustrate the crucial importance of CMI for adequate resistance to cryptococcosis.

Even before the AIDS epidemic, cryptococcosis occurred much more often in males than in females. Curiously, despite large numbers of children at risk, the incidence of cryptococcosis in patients below the age of puberty is unaccountably rare.

Patients with AIDS

Cryptococcosis is a leading mycological cause of morbidity and mortality among AIDS patients (81, 239, 429, 464, 529). In many patients, cryptococcosis is the first indication of AIDS. The incidence of life-threatening cryptococcal infections among patients with AIDS has been estimated at 6 to 10% in the United States, western Europe, and Australia and 15 to 30% in sub-Saharan Africa (412). The incidence is particularly high in the southeastern United States and equatorial Africa (83, 412). During initial therapy, 10 to 25% of these patients die, and 30 to 60% succumb within 12 months (412).

As noted, almost all cases of cryptococcosis in patients with AIDS globally are due to *C. neoformans* var. *neoformans* serotype A. Even in geographic areas endemic for *C. neoformans* var. *gattii*, patients with AIDS are infected almost exclusively with *C. neoformans* var. *neoformans* serotype A. However, in France, which is one of the countries where serotype D has historically been more prevalent, among 273 cases that were analyzed from 1990 to 1992, 17% were serotype D, and 80% of these patients were infected with HIV (130).

Sections below will discuss the clinical manifestations and management of cryptococcosis in patients with AIDS.

HOST DEFENSES

The high prevalence of *C. neoformans* in nature and the relatively low frequency of cryptococcosis imply that many persons are probably exposed without developing symptoms. The actual frequency of subclinical infection is unknown because a skin test antigen with which to assess exposure is not available and the primary pulmonary infection does not usually leave marked residua, such as cavities or calcifications. The few reports of human skin testing with experimental antigens or measuring antibodies in healthy persons have indicated a variable level of reactivity and exposure to *C. neoformans* (23, 323, 430). Antibody levels are higher in sera from pigeon breeders (155). However, the cryptococcal antigens that have been used to detect delayed-type hypersensitivity, lymphocyte responsiveness, or antibodies appear to cross-react with other fungal antigens.

Cryptococcosis is initiated in the lung after inhalation of yeast cells of *C. neoformans*. Small ($\leq$5 μm in diameter), minimally encapsulated yeasts have been isolated from the air and avian environments (349, 415, 431). The predominance of yeasts of a single mating type among both clinical and natural isolates, including airborne isolates, tends to exclude the basidiospore ($\approx$3 μm) as a routine source of infection. Yeasts that are not expelled by the respiratory epithelia may penetrate to the alveoli.

In the alveolar spaces, the yeast cells are initially confronted by the alveolar macrophages (AMϕ). Whether active infection and disease follow this interaction depends largely on the competence of the cellular defenses of the host, as well as the number and virulence of the yeast cells. Cellular immune mechanisms normally mediate a successful host response through activation of macrophages. The CPS, the mannoprotein, and perhaps other yeast components are also capable of modulating, as well as subverting, the host responses. *C. neoformans* infects the lungs to a variable extent and may subsequently disseminate to the skin, bone, prostate, or, with predilection, the CNS. Regardless of the site of involvement, the histopathology is often minimal but may entail moderate cellular infiltration (mostly histiocytes) or granulomata.

The primary interaction between local AMϕ and inhaled yeast cells may be the crucial determinant of whether or not disease ensues. In vitro, resident AMϕ bind and ingest yeasts in the presence of fresh, normal serum. However, strains of *C. neoformans* vary in the extent to which they are able to attach to and become engulfed by AMϕ, and this variation is inversely related to the size of the yeast particle or thickness of the capsule (36). The intracellular fate of the yeasts is also strain dependent, but the susceptibility or resistance of a given strain is unrelated to the extent to which the strain is engulfed (37).

Phagocytosis (i.e., attachment and ingestion) of *C. neoformans* by AMϕ is optimized in vitro in the presence of normal serum containing opsonins such as C3 and anti-CPS immunoglobulin G (IgG) (36). In normal alveoli, AMϕ undoubtedly interact with inhaled yeast cells in the absence of optimal concentrations of opsonins, at least until an inflammatory response, if any, is induced (8). Interestingly, yeast cells of *C. neoformans* are bound to and killed by normal rat or human AMϕ to a limited but perhaps adequate extent in the absence of serum (35, 278). This mechanism involves a receptor on the AMϕ with affinity for mannose-rich determinants present on the yeast cell walls and unrelated to CPS (35). Indeed, normal serum contains an inhibitor of this binding to human AMϕ (278). Opsonin-independent phagocytosis was observed with capsule-free and small- or medium-capsuled strains of *C. neoformans*, but not with large-capsuled cells. Such serum-free phagocytosis may be of critical importance in the alveolar spaces, where only marginal concentrations of serum are initially present and the yeasts presumably have minimal capsules. In the normal host, this process may contain the yeast challenge and stimulate an effective CMI response before the yeasts are able to grow and synthesize appreciable quantities of CPS to undermine the host defenses. Of course, macrophages from HIV-infected persons have displayed general impairment (9, 427), and the specific anti-*C. neoformans* activities of hu-

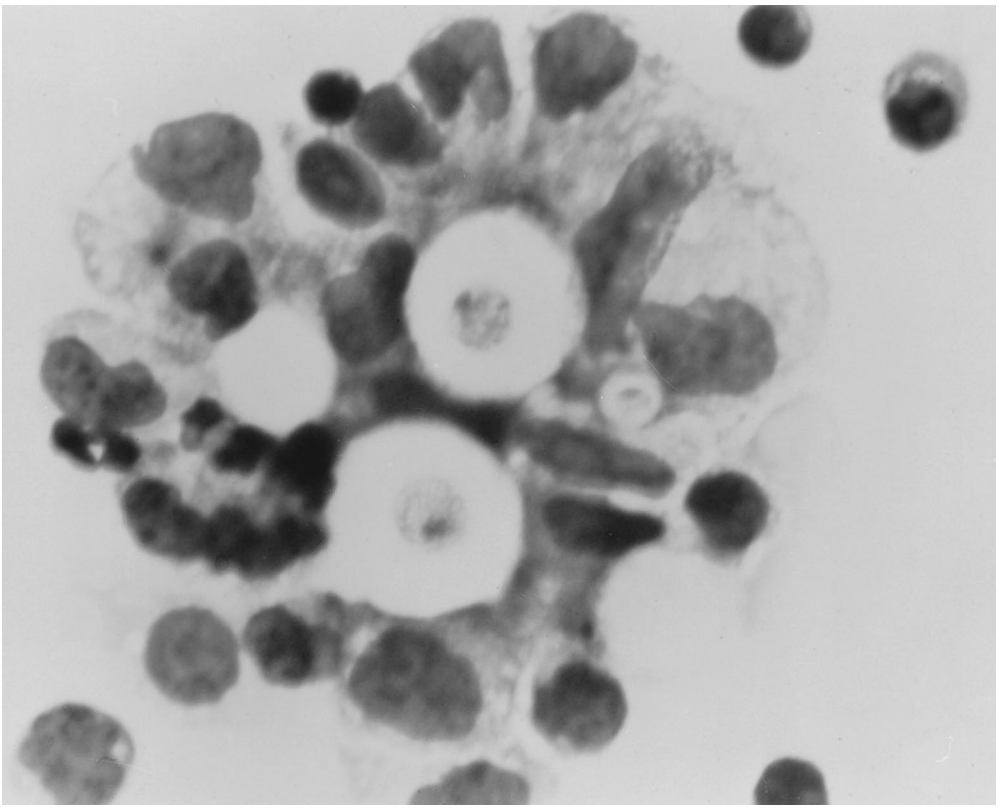

FIG. 2. Cytospin preparation of CSF from an immunocompetent rabbit with cryptococcal meningitis. The infection has induced a vigorous inflammatory response that will eventually lead to complete elimination of the yeasts from the subarachnoid space.

man AMϕ are depressed by the gp120 protein of HIV type 1 (HIV-1) (506).

From the lungs, *C. neoformans* may reach the bloodstream, where normal human neutrophils (polymorphonuclear leukocytes [PMN]), monocytes, and monocyte-derived macrophages have demonstrated effective in vitro killing of the yeast cells by intracellular and extracellular mechanisms (see Fig. 2) (119, 277, 313). Many years ago, healthy human serum and other fluids were shown to inhibit the in vitro growth of *C. neoformans* (418). Neither antibody nor complement, this inhibitory factor(s) was not completely purified, and its importance is unknown.

Pathogenesis

The pathogenesis of cryptococcosis is determined by three broad factors—the status of the host defenses, the virulence of the strain of *C. neoformans*, and the size of the inoculum. The relative importance of these three factors as determinants of active infection has not been clarified, nor has current research dissected the complexities of host defenses and virulence, each of which may involve multiple factors and interactions.

One fundamental concept of the pathogenesis of cryptococcosis places paramount importance on the integrity of the host defenses. This concept is supported by the reasonable assumption that, because of the ubiquity of *C. neoformans* in nature and the relatively low prevalence of symptomatic infection, the incidence of exposure far exceeds the incidence of disease. Therefore, most people are probably resistant, and only individuals with compromised host defenses are likely to develop cryptococcosis. However, this conclusion does not account for the occurrence of disease in apparently healthy individuals. Bulmer and Tacker have suggested that disease can be initiated in persons with normal defenses by exposure to an unusually large inoculum (56), which is similar to the situation with acute pulmonary coccidioidomycosis or histoplasmosis. An alternative, but not mutually exclusive theory is that strains of *C. neoformans* in nature differ in virulence, and the establishment of symptomatic infection, whether in healthy or compromised subjects, depends upon inhalation of more virulent yeast cells. Although current knowledge of the pathogenesis of cryptococcosis neither confirms nor excludes either possibility, successful resistance to cryptococcosis requires intact CMI. The crucial importance of CMI has been confirmed by experimental and clinical observations, and several excellent researchers are currently pursuing this area (see below).

Although the precise mechanisms of effective resistance to *C. neoformans* have not been established, persons with impaired CMI are unquestionably more susceptible, especially patients with AIDS, certain mononuclear phagocyte abnormalities (e.g., Hodgkin's disease), and a variety of cellular immunodeficiencies, either natural or induced by immunosuppressive treatment concomitant with organ transplantation or anticancer chemotherapy (121, 312, 374). Mice that are treated with cortisone (165) or antilymphocytic serum, as well as congenitally athymic mice (438), are more susceptible than normal control mice to challenge doses of *C. neoformans*. The importance of CMI is also supported by the apparent lack of protection associated with specific antibody (see below), the reported improvement following treatment with immunostimulators, and the successful transfer of protection with T cells.

As described earlier, several antigen preparations have been developed to detect delayed-type hypersensitivity and evaluate CMI (205, 336, 417). Lymphocyte studies have confirmed that during infection, specific CMI responses are depressed (194, 312), which may also account for the diminished antibody response (116).

Regarding the impact of the organism, strain variation in virulence has been documented, but this aspect of pathogenicity has not been fully explored. Kwon-Chung and her colleagues developed mutants of *C. neoformans* and performed classical genetic analyses that identified the production of CPS and the enzyme phenol oxidase as independent requisites for virulence (256, 422). Despite several studies of the pathobiology associated with CPS and phenol oxidase, the exact molecular mechanisms responsible for their virulence have not been clarified. Of the various host-*C. neoformans* interactions described, cryptococcal determinants of virulence have been studied the least, yet they may be the most important aspect of the pathogenicity of cryptococcosis. Future genetic and molecular approaches should define the important cryptococcal factors and their mechanisms of action.

Animal Models

Although *C. neoformans* is widely reported to cause both local and disseminated infection in a variety of animal species (10, 282, 425), studies with the more popular animal models of experimental cryptococcosis have tended to rely on a large inoculum of yeasts or some induced immune defects. Such models highlight the importance of host immunity and possibly host specificity. For example, although cryptococcosis is dramatically potentiated in humans by concomitant infection with HIV, disseminated cryptococcosis is not strongly associated with cats who are infected with a similar retrovirus, feline immunodeficiency virus (295, 296, 360). Other animal species are more susceptible to the development of disseminated cryptococcosis. Most strains of mice are highly susceptible to experimental infection with a small number of yeasts. In contrast, rabbits are relatively resistant to even large inocula unless they are severely immunosuppressed (154, 380). Pigeons and other birds are also resistant, perhaps because of their relatively high body temperature (281).

For experimental studies of host defenses, the route of inoculation affects pathogenesis and the character of the CMI responses. For example, murine models that use different methods to establish infection have been developed. Intraperitoneal injections are the most convenient, intracerebral injections are the most direct, and intravenous injections are the most standardized and quantitative. However, intratracheal inoculation best simulates natural infection.

Most of the studies examining the pathobiology of *C. neoformans* have used murine models of cryptococcosis, and mice have been used to evaluate the importance of the various components of the immune system. For example, upon challenge with *C. neoformans*, C5-deficient mice (DBA/2) quickly die from acute cryptococcal pneumonia, and with smaller inocula, they succumb to late progressive meningoencephalitis, unlike BALB/c mice, which survive longer (129, 421, 423). These differences illustrate the importance of the recruitment of phagocytes, which is severely reduced in animals without C5 (and the release of the chemotactic peptide C5a). Human infections with HIV and treatment of animals with corticosteroids demonstrate the importance of a quantitative cellular immune response at the site of infection. The beige mouse has defects in chemotaxis of both neutrophils and monocytes, impairment of phagosome-lysosome fusion, and reduced microbicidal activity (197, 298, 434, 436, 437). These mice also have a defect in the ability of natural killer (NK) cells to kill target cells. These multiple defects reduce the cellular reaction at the site of infection and delay the transition from acute to chronic inflammation. These murine models support the central importance of phagocytes and NK cells in defense against *C. neoformans*.

Animal models also illustrate the importance of specific CMI in cryptococcosis. Nude mice, which are homozygous for the recessive gene that renders them athymic as well as hairless, are more susceptible to *C. neoformans* than heterozygous littermates (69, 184, 354, 434, 435, 437). These studies confirm the fundamental role of T cells in controlling the infection, despite normal or even hyperactive phagocytic cells and antibody production.

Mice that have been depleted of $CD4^+$ and $CD8^+$ cells have allowed the study of these specific cell types and their importance to host resistance. In some respects, they mimic the HIV-infected host. The current data suggest that $CD4^+$ cells are essential for the control of the inflammatory host response to *C. neoformans* in the lung and may be essential to protect the host from dissemination. The actual effector cells that are responsible for specific fungicidal activity may be $CD8^+$ cells, NK cells, and phagocytes (198, 200, 207, 326). Mice with severe combined immunodeficiency are more susceptible to *C. neoformans* infection and have been studied to determine the effects of replacement of specific cell populations on cryptococcosis (198, 199). Animal studies have also demonstrated that resistance to *C. neoformans* is likely to be multigenic and that even loci such as the murine XID allele, which is related to antibody production, can be important in resistance to *C. neoformans* (297). However, these studies often do not consider that different strains of *C. neoformans* vary in virulence.

The murine model has had the greatest utility in the early comparative studies of antifungal therapies. Although mice present problems related to pharmacokinetics and sampling of specimens, their advantages for screening new therapies are low cost, availability of inbred populations, reproducible infections, and the availability of immunomodulators for testing.

Intravenous challenge of rats produces a more chronic pulmonary infection than seen in mice. In direct comparisons, in which comparable inocula of various strains of *C. neoformans* were given to groups of mice and rats, the rats developed more chronic infections, similar to cryptococcosis in humans. Some rats developed progressive pulmonary disease as well as meningoencephalitis. Furthermore, the relative virulence of a strain of *C. neoformans* was not necessarily the same in both rats and mice (318), which suggests that extrapolation of data among animal species may not be valid. Recent reports have described the histopathological and immunological features of pulmonary cryptococcosis in rats following intratracheal inoculation (100, 175).

Years of clinical experience with human cryptococcosis have indicated that corticosteroids clearly potentiate the course of cryptococcosis. Corticosteroids similarly enhance cryptococcosis in mice (289), guinea pigs (115, 165), rats (165), and rabbits (380). In rats and guinea pigs, a latent infection can be reactivated with corticosteroid therapy, similar to the pathological reactivation mechanism in humans (165). In guinea pigs and rabbits, corticosteroid-mediated enhancement of cryptococcosis has been shown to correlate with abrogation of the inflammatory response (115, 380). In the well-established rabbit model of cryptococcal meningitis (380), many features mimic human cryptococcosis: (i) immunosuppression is required; (ii) infection involves an immunologically sequestered site with respect to host proteins; (iii) severe cerebrospinal fluid (CSF)

leukopenia develops, as seen in AIDS patients; (iv) the infection is prolonged and eventually fatal; (v) dissemination to other organs occurs; and (vi) response to treatment regimens paralleled those in recent human trials. Through continuous sampling of CSF during infection, this model provides a biological window for studies on pathogenesis and treatment (368, 370–373, 377, 379, 380, 385, 390).

The establishment of a variety of animal models with potential clinical relevance offers a solid foundation on which to explore and dissect immune factors, study the pathobiology of *C. neoformans*, and assess treatment with new antifungal agents and other treatment strategies. However, none of these models involves animals with AIDS, and the data on anti-*C. neoformans* defenses in HIV-infected humans are limited. HIV-infected patients are clearly susceptible to other mycoses well associated with impaired CMI, such as mucosal candidiasis and, in endemic locales, coccidioidomycosis and histoplasmosis. Conversely, patients with AIDS tend to have a relatively lower incidence of mycoses for which the neutrophil is the crucial host defense, such as systemic candidiasis, mucormycosis, and aspergillosis, at least in the early stages of AIDS (109).

CMI

Although several potentially important observations have been elucidated, the dynamics of the interaction between various components of *C. neoformans* and the panoply of host defenses remain incompletely understood. While there have been some definitive studies involving humans, most of the basic research has been done with experimental animal cells and systems, some of which are described in the previous sections. Space restrictions preclude an exhaustive review of the numerous studies on host defenses and immune responses to *C. neoformans*. Research in this area has been accelerating for the past 25 years, and recent reviews are available (241, 271, 337–339). For example, with a murine model, elegant studies from the laboratory of Murphy have explored the effects of NK cells on *C. neoformans* (339) and cryptococcal modulation of cellular immunity (338). The research of Kozel and colleagues has clarified the process of phagocytosis of *C. neoformans* by neutrophils and macrophages from both mice and humans, the mechanism of opsonization, and the antiphagocytic properties of the capsule (241). Levitz and associates have published a series of recent reports on the conditions for inhibition of growth of *C. neoformans* by various human leukocytes (271). For many years, Kwon-Chung and Bennett have been leaders in studying the clinical aspects of cryptococcosis and the biology of *C. neoformans* (252). Similarly, Diamond has investigated both clinical features and host defenses against cryptococcosis (115, 116).

Importance of CMI. Experimental and clinical evidence strongly implicates CMI as the crucial component of the immune system for adequate resistance to cryptococcosis. The high susceptibility of adults with AIDS provides compelling clinical evidence for the importance of intact CMI. The relatively low incidence of cryptococcosis in children with AIDS is especially provocative and suggests that something more than impaired CMI is required for susceptibility. (It is difficult to explain this discrepancy on the basis of differential exposure or other conditions.) In addition to patients with AIDS, as noted elsewhere, others with compromised CMI have long been recognized to be at risk for cryptococcosis.

Athymic (nude) mice, which lack CMI, are more susceptible to experimental cryptococcosis than their heterozygous littermates, and T-cell-depleted mice are more susceptible than control mice. In immunocompetent strains of mice, intratracheal challenge with *C. neoformans* induces an influx of both $CD4^+$ and $CD8^+$ T cells, both of which are required to restrict dissemination of the yeasts (198, 200, 206, 207).

Mechanisms of resistance. Protective CMI responses may be mediated via direct cytotoxic effects on *C. neoformans*, stimulation of protective antibody responses, or activation of effector cells, such as phagocytes (PMN, AMϕ, and monocytes), NK cells, or other cells. As noted, there is little support for nonantibody humoral factors in resistance. Evidence for the importance of phagocytosis, phagocyte-mediated inhibition of growth and killing, and antibody-mediated resistance are discussed below.

NK cells from mice have demonstrable anticryptococcal activity, albeit at relatively high NK-to-yeast cell ratios (339). In contrast, both human NK cells and lymphokine-activated killer cells failed to inhibit the growth of multiple strains of *C. neoformans*; however, human NK cells were inhibitory in the presence of small quantities of antibody (314). More recently, human interleukin-2 (IL-2)-activated T cells and NK cells have been shown to exhibit contact-dependent inhibition of *C. neoformans* (274, 275).

Data from studies with mice, rats, and humans suggest that in the lungs, the inflammatory lesions that are protective and contain the infection require AMϕ and both $CD4^+$ and $CD8^+$ T cells. Pulmonary T cells from infected mice respond in vitro to mitogen stimulation by secreting both Th1 (gamma interferon [IFN-τ] and IL-2) and Th2 (IL-4, IL-5, and IL-10) cytokines, which result in the infiltration of macrophages and granulocytes (206). Several studies indicate the importance of $CD8^+$ cells in restricting the spread of *C. neoformans* from the lungs and generating an effective CMI response (200, 325, 327). As suggested in these reports, patients with AIDS who have low $CD4^+$ cell counts may benefit from stimulation of their $CD8^+$ T cells. Undoubtedly, the proportions of responding Th1 and Th2 cells influence the degree of protection that develops. Murphy and her colleagues are beginning to dissect the relative expression and modulation of these inflammatory and helper cytokines in an in vivo murine system (51, 340).

Human lymphocytes stimulated by IL-2 inhibited the growth of *C. neoformans* by a mechanism that required direct contact (275). These cells were $CD4^+$, $CD8^+$, and $CD16/56^+$. A recent report demonstrated that human fetal astrocytes were activated in vitro by IL-1β and IFNγ to inhibit the growth of *C. neoformans*, and this inhibition was accompanied by the generation of reactive nitrogen intermediates (266). Clearly, the types of nonphagocytic effector cells, their roles, and their relative importance have yet to be completely delineated (275, 277, 342).

Phagocytosis

Phagocytosis (i.e., attachment and/or ingestion) of *C. neoformans* has been observed in vivo and extensively documented in vitro with several mammalian systems. The deposition of C3b and its degradative products is essential for optimal phagocytosis (36, 102, 245), but other factors may also be important (246). In vitro, exogenously added CPS binds specifically to acapsular yeast cells of *C. neoformans* to inhibit their phagocytosis (55, 245, 452). Following phagocytosis, PMN appear to be more cryptococcocidal than peripheral monocytes or peritoneal macrophages (PMϕ), although few studies have compared these cells directly or examined activated phagocytes (13, 33, 119, 157, 376, 512). Several studies have examined the phagocytosis of *C. neoformans* by AMϕ, which is the initial and perhaps most crucial host defense (3, 13, 36, 37, 273, 345, 512). The fate of phagocytized yeast cells

TABLE 4. Comparison of cryptococcosis in patients with and without AIDS[a]

Feature	With AIDS	Without AIDS
Laboratory findings		
Initial direct examination (India ink) of clinical material	Often positive (>80%), with numerous yeasts	May be positive for sparse numbers of encapsulated yeasts
Culture of clinical specimens for *C. neoformans*	Frequently positive (blood, CSF, urine)	CSF cultures usually positive; blood and urine cultures rarely positive
Prognostic value of latex agglutination test for capsular antigen	Antigen in serum and CSF may persist at very high titers for long periods, even after treatment	Antigen titers in CSF at start of therapy tend to reflect prognosis; serum titer usually negative or low
Pathology		
Sites of involvement	Lung, CNS, skin; more sites of extraneural dissemination	CNS; occasionally lung, bone, or skin
Histopathology	Many yeast cells; few inflammatory cells	Fewer yeasts; inflammation active, with some granulomatous reaction
Treatment	AMB and 5FC[b] plus long-term fluconazole	AMB and 5FC
Prognosis	Good to poor; relapses usually occur without suppressive therapy	Good to poor, depending on control of underlying disease

[a] Data are from references 83, 144, 239, 261, 429, and 529.
[b] 5FC, flucytosine.

of *C. neoformans* depends on the strain being tested (37, 119, 273). When more than one strain of *C. neoformans* has been examined, ingestion has been shown to be inversely related to the size of the capsule or the total volume of the yeast particle (36, 54, 246, 272, 273). However, regardless of the degree to which a strain is phagocytized, the proportion of yeasts that are killed is strain dependent and not related to particle or capsule size (37).

In vitro inhibition of growth or killing by normal phagocytes is optimal in the presence of serum opsonins, more pronounced at high ratios of effector cells to yeast cells, and influenced by the surface to which the phagocytes are attached (37, 276). In studying the consequences to *C. neoformans* after exposure in vitro to phagocytes and other effector cells, actual killing can be determined if yeast viability is measured within 4 h, before surviving yeasts have time to multiply. At longer periods, the inhibition of growth can be determined. Inhibition of growth, which is usually measured at 24 h, is strain dependent, but most strains of *C. neoformans* are relatively resistant to the anti-*C. neoformans* activity of nonactivated macrophage populations, including AMϕ, monocyte-derived macrophages, and macrophage-derived cell lines.

Activation of phagocytes with certain cytokines will enhance their in vitro anticryptococcal activity (75, 157, 272, 329, 376). For example, inhibition of growth of *C. neoformans* by rat AMϕ is increased by treatment with IFN-γ or recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulates growth of the AMϕ as well as increasing ingestion and killing of yeasts (75, 329). The combination of IFN-τ and GM-CSF resulted in more anticryptococcal activity than either cytokine alone (75). Complement-dependent phagocytosis of murine PMϕ is also enhanced by tumor necrosis factor alpha and GM-CSF (87). Recently, M-CSF has been shown to enhance transiently the fungistatic activity of murine PMϕ or AMϕ, but not the phagocytosis of *C. neoformans*; optimal inhibition was observed with the combination of activated macrophages and fluconazole (49, 50, 347).

For optimal growth inhibition of *C. neoformans* cells that invade the circulation, PMNs require C3 and utilize the complement receptors for opsonization (118). Studies with C5-deficient mice indicate that C5 promotes PMN-mediated killing (290).

Immunological Tolerance

The phenomenon of specific anergy, immunosuppression, or immunological tolerance following exposure to *C. neoformans*, CPS, or mannoprotein has been demonstrated in mice and extensively investigated by Murphy and her colleagues (341). As noted earlier, depending upon the dose and mode of antigen presentation, both antibody to GXM and CMI responses to mannoprotein may be suppressed (79). In mice, suppression of CMI and delayed hypersensitivity are induced by antigen and mediated by a cascade of antigen-specific T suppressor cells and factors (338). One of these T suppressor cell factors, which requires exposure to antigen for its elaboration, directly inhibits the phagocytosis of *C. neoformans* by a subset of macrophages (27, 28). These models may correspond to the specific lack of immunological responsiveness seen in humans (194, 195).

Antibodies and Protection

Although specific antibody is not by itself deleterious to *C. neoformans*, it may function as an opsonin (36, 345) or participate in antibody-dependent cellular cytotoxicity (114, 314, 343). Indeed, passive administration of anti-*C. neoformans* monoclonal antibodies has been shown to prolong the survival of mice and reduce the tissue census of yeast cells compared with control animals (127, 332). Anti-GXM monoclonal antibodies of the three major immunoglobulin classes were protective (334). The basis for antibody-mediated protection is not clear but could involve opsonization to increase phagocytosis of the yeasts, enhanced clearance of deleterious levels of GXM, or participation in antibody-dependent cellular cytotoxicity against *C. neoformans* (332, 334).

Interaction between *C. neoformans* and HIV

The incidence of cryptococcosis, which is a frequent life-threatening infection among patients with AIDS, is increasing in AIDS patients (81, 121, 144, 166, 239, 448, 528, 529). Cryptococcosis is the first manifestation of HIV infection in 26 to 45% of patients (81, 239), and recent data indicate that *C. neoformans* appears to potentiate HIV infection (357, 394). In AIDS patients, the clinical features of cryptococcosis differ somewhat from those of patients without AIDS (Table 4).

Patient tissues and body fluids usually reveal high titers of CPS, which is cleared very slowly and by an unknown mechanism(s) (144).

Almost every isolate of *C. neoformans* from AIDS patients that has been examined has been *C. neoformans* var. *neoformans* serotype A, as are 75% of isolates from patients without AIDS (40, 210, 446). Even in southern California, where infection with *C. neoformans* var. *gattii* (serotypes B and C) in patients without AIDS is ca. 40%, isolates from patients with AIDS are invariably *C. neoformans* var. *neoformans* (448).

It was reported that isolates of *C. neoformans* from AIDS patients were small-capsuled (41, 42), although the patients had high circulating titers of capsular antigen (41). This unconfirmed observation supports the theory that isolates of *C. neoformans* from patients with AIDS differ from other isolates. These researchers found that the AIDS isolates developed larger capsules in mice, but comparisons of virulence or other properties were not performed (42).

To date, the DNA-based methods used to biotype or fingerprint strains of *C. neoformans* have not detected strains that are particularly associated with AIDS patients or specific geographic regions, but more testing needs to be done (310, 405, 458). For example, by using a method of DNA fingerprinting, each of four patients with cryptococcosis (three with AIDS) was shown to have a separate, individual strain of *C. neoformans*, both initially and after relapse (459).

Intriguing preliminary reports highlight the relatively unexplored and complex interaction between *C. neoformans* and HIV-infected macrophages and lymphocytes. Human peripheral blood mononuclear cells (PBMC) or monocyte-derived macrophages were infected with a lymphocytotropic strain of HIV-1 and incubated with killed yeast cells, using an acapsular or encapsulated strain of *C. neoformans* (357). Measured at 7 and 10 days, the yield of HIV-1 was 10-fold higher when the PBMC were incubated with either yeast strain, indicating that enhancement of HIV replication did not require CPS. In the monocyte-derived macrophages, viral replication was inhibited by the acapsular strain, and the encapsulated yeasts had no effect (357). These results may vary with the strain of *C. neoformans* or HIV, because another laboratory reported that serotype A CPS alone enhanced the growth of HIV-1 in H9 cells and accelerated the recovery of virus from PBMC of an HIV-infected patient (395).

Conversely, the envelope protein of HIV-1 (gp120) has been shown to inhibit the in vitro anticryptococcal activity of human AMϕ (506). More recently, normal human AMϕ, PMϕ, or PBMC were infected in vitro with either a monocytotropic or lymphocytotropic strain of HIV-1; measured at weekly intervals, infection with either strain of HIV-1 had no effect on the ability of AMϕ to inhibit the growth of *C. neoformans*, whereas the monocytotropic strain of HIV but not the lymphocytotropic strain reduced the fungistatic activity of PMϕ and monocytes (60).

CLINICAL MANIFESTATIONS

The clinical manifestations of cryptococcosis tend to overlap between patients with AIDS and severely immunocompromised patients without AIDS. However, certain findings are generally more common in AIDS than non-AIDS patients. In AIDS, the sites of cryptococcal infection usually contain a higher burden of organisms and a paucity of inflammatory cells (see Table 4). Cryptococcosis in AIDS patients is characterized by a high frequency of positive blood and urine cultures, more sites of extraneural disease, a large number of yeast cells with few inflammatory cells in the CSF, and a higher incidence of clinical disease and relapse (121, 144, 239, 529).

Among patients with profound HIV- or corticosteroid-induced immunosuppression, the number of cryptococcal cases is increasing dramatically. In these situations, all areas of the body can be infected, including the adrenals, heart, liver, lymph nodes, joints, and kidneys (367). For example, bone has become a frequently reported site of extraneural infection (5, 180, 248, 331, 361, 521). However, this discussion will focus on five major sites of involvement that are particularly important in the diagnosis and management of cryptococcosis—the lungs, CNS, skin, prostate, and eye.

Lungs

As described earlier, the lung is invariably the portal of entry and initial site of infection for *C. neoformans*. For many years, microscopic pulmonary foci and hilar nodes containing yeasts have been recognized at autopsy (7, 8). This primary pulmonary lymph node complex, as in tuberculosis and histoplasmosis, may be asymptomatic but has the potential to disseminate or reactivate in immunosuppressed hosts.

Despite the asymptomatic nature of most cases, hundreds of cases of pulmonary cryptococcosis in apparently immunocompetent hosts have been reported in the medical literature (85, 117, 132, 190, 196, 209, 232, 302, 351, 359, 428, 489, 508, 527). Campbell's comprehensive review of the English literature almost 30 years ago remains the most complete description of primary pulmonary cryptococcosis (63). This review suggests that primary pulmonary cryptococcosis in the immunocompetent host may be asymptomatic in one-third of cases; i.e., the cause of an abnormal chest radiograph. However, most patients present with symptoms, such as cough (54%), chest pain (46%), sputum production (32%), weight loss (26%), fever (26%), and hemoptysis (18%) (63). Other symptoms are rare and include dyspnea, night sweats, and obstruction of the superior vena cava (267, 305). Several unusual manifestations have been reported. One patient had "allergic" cryptococcal pneumonia that was characterized by urticaria, hypotension, and dyspnea (170). Another case of cryptococcal pneumonia resembled Pancoast's tumor (317). Another patient developed bronchiolitis obliterans-organizing pneumonia (64). Pulmonary cryptococcosis in the immunocompetent host has occurred as a coinfection with both *Mycobacterium tuberculosis* (90, 228, 424) and echinococci (101). In one case, pulmonary tuberculosis developed several years after lung resection for cryptococcosis (424).

The antemortem diagnosis of pulmonary cryptococcosis in immunocompetent hosts has been made by either excisional or incisional lung biopsy for histopathology and/or by culture, cytopathology, sputum culture, antigen testing, and chest radiographs (103, 158, 230, 335, 454). Several reviews of cryptococcal pneumonia have documented asymptomatic colonization of the respiratory tree by *C. neoformans* (190, 469, 489, 508). This condition generally occurs in patients with an underlying lung disease, such as chronic obstructive pulmonary disease. Consequently, the isolation of *C. neoformans* from the sputum of a patient lacking clinical symptoms or radiographic evidence of a pulmonary infiltrate must be interpreted cautiously, and the patient may not always require therapy. In the normal host, pulmonary *C. neoformans* usually does not disseminate; therefore, cultures of blood, urine, and CSF as well as antigen tests of serum or CSF will be negative. Nevertheless, all patients with cryptococcal pneumonia and/or pulmonary colonization should be evaluated for cryptococcal meningitis with a lumbar puncture, even in the absence of neurologic

signs or symptoms, because of the propensity of *C. neoformans* to invade the CNS (233, 267).

Chest radiographs of cryptococcal pneumonia in the normal host may reveal well-defined, noncalcified, single or multiple lung nodules; indistinct to mass-like infiltrates; hilar and mediastinal lymphadenopathy; occasionally pleural effusions; and, more rarely, cavitation (153, 209, 301, 335, 359, 525, 527). The most common radiographic findings are single or multiple peripheral nodules, and many cryptococcal infections are discovered when these nodules are aspirated or removed to exclude malignancy (235).

Other diagnostic tests are rarely useful in primary pulmonary cryptococcosis unless there is evidence of dissemination. The serum is occasionally positive for cryptococcal antigen, which should always be assessed in patients with pulmonary cryptococcosis (103, 222, 230, 486). The presence of cryptococcal antigen in the serum of patients with apparent pulmonary disease alone is potentially significant. A titer of cryptococcal antigen of $\geq$8 in serum in an asymptomatic patient with documented pulmonary involvement may portend a high burden of organisms in the lung and/or disseminated infection. In this situation, we would be more likely to consider specific antifungal treatment.

In the immunocompromised host, cryptococcal pneumonia may have a completely different and more rapid clinical course. In such patients, *C. neoformans* tends to disseminate rapidly from the lungs or to reactivate from a primary focus, to eventually establish infection within the CNS, and patients often present with a meningeal rather than a pulmonary syndrome. An exception is the patient with adult respiratory distress syndrome, in whom the initial manifestation can be overwhelming cryptococcal pneumonia (196, 232, 346).

The major groups of immunocompromised patients are those with HIV infection, cirrhosis, diabetes, Cushing's syndrome, sarcoidosis, leukemia, lymphoma, or sickle cell disease, those receiving treatment with glucocorticoids, and those who have had an organ transplant (29, 59, 80, 233, 247). In a classical retrospective review of pulmonary cryptococcosis, Kerkering et al. described 41 patients with pulmonary infection, of whom 34 had an underlying immunocompromising condition other than HIV (233). Twenty-nine of these patients developed disseminated disease, and all but one with disseminated disease was identified as immunocompromised. Unlike immunocompetent hosts, who may have an inapparent pneumonitis, 83% of the immunosuppressed patients in this series had constitutional symptoms. The most common presenting symptoms were fever (63%), malaise (61%), chest pain (44%), weight loss (37%), dyspnea (27%), night sweats (24%), cough (17%), hemoptysis (7%), and headache (7%). A definitive diagnosis was established by culture of sputum or specimens obtained by bronchoscopy, thoracentesis, open-lung biopsy, or needle aspiration. Dissemination to the meninges occurred in 25 patients within 2 to 20 weeks after the diagnosis of pulmonary cryptococcosis. All of the patients had abnormal chest radiographs. The most common findings were alveolar or interstitial infiltrates, followed by single or multiple coin lesions, masses, cavitary lesions, and pleural effusions. In this review, the outcome of infection clearly supported the recommendation that immunodeficient hosts with pulmonary cryptococcosis receive antifungal therapy (233).

Although pulmonary cryptococcosis is less common than meningitis as a presenting complaint in patients with AIDS, it has been well described. The manifestation of pulmonary cryptococcosis in patients with HIV infection differs somewhat from cryptococcosis that is associated with other types of immunocompromising conditions (59, 74, 84, 234, 510). In one review of pulmonary disease in patients with AIDS, *C. neoformans* was the etiologic agent in approximately 10% of the cases (473). Almost all patients with AIDS present with symptoms, such as fever (81%), cough (63%), dyspnea (50%), weight loss (47%), headache (41%), and occasionally pleuritic chest pain and hemoptysis (59, 84, 510). On the basis of two studies, dissemination, especially to the meninges or blood, occurred in 94% of patients with pulmonary disease (59, 510). The findings on physical examination are usually not reported in clinical reviews but may include lymphadenopathy, rales, tachypnea, and splenomegaly (59). Patients often have concurrent oral candidiasis, which emphasizes the association of this disease with a CD4 count of $<$200 cells per μl (95). Actually, most patients with cryptococcosis and AIDS have CD4 counts of $<$100 cells per μl. Patients may acquire another opportunistic infection concomitant with pulmonary cryptococcosis, notably with *Pneumocystis carinii*, *Mycobacterium avium-intracellulare*, cytomegalovirus, or *Histoplasma capsulatum* (59, 74, 84). Conversely, pulmonary cryptococcosis may follow as a consequence of steroid therapy for *P. carinii* pneumonia (260).

Diagnostic studies in HIV-infected patients should include measurement of arterial pO_2, chest radiography, cultures, and cryptococcal antigen tests. Mild to moderate hypoxemia can occur, although normal pO_2 to profound hypoxemia has been described in cryptococcal pneumonia (59, 84). These patients may also present with adult respiratory distress syndrome (346, 391, 449). Chest radiographs most often reveal interstitial infiltrates, either focal or diffuse, and lymphadenopathy; unlike in immunocompetent and other types of immunocompromised patients, nodular and alveolar infiltrates are quite rare (59, 84, 316). Large masses and pleural effusions are also unusual (59). Since the most common radiographic presentation is interstitial infiltrates, cryptococcal pneumonia can easily be confused with *P. carinii* pneumonia, which is the most frequent cause of interstitial infiltrates in patients with AIDS (287). However, *P. carinii* may be less frequent today because of prophylaxis with trimethoprim-sulfamethoxazole. Cryptococcal antigen detection by the latex agglutination test while awaiting culture results can be extremely helpful. Since patients frequently have disseminated cryptococcosis, cryptococcal antigen is often detected in the serum, CSF, or urine (59, 84). Pulmonary and extrapulmonary cultures are pivotal in making the diagnosis. Suitable specimens for culture include expectorated sputum, bronchoalveolar lavage, transbronchial lung biopsy or needle aspiration, and pleural fluid samples (59, 74, 294, 455). Among patients with AIDS and disseminated cryptococcosis, cultures of blood and CSF are generally positive, but occasionally, the diagnosis is established by cultures of bone marrow, skin lesions, or urine, preferably obtained from males after prostatic massage (39, 144, 519).

CNS

Most patients (70 to 90%) present with signs and symptoms of subacute meningitis or meningoencephalitis, such as headache, fever, lethargy, coma, personality changes, and memory loss over 2 to 4 weeks. However, many patients may not exhibit these classical diagnostic features. For example, patients may present with severe headaches for only a few days, intermittent headaches for months, or no headaches. Indeed, by providing an earlier diagnosis, the presence of a headache may be an important favorable prognostic factor for survival (124). Unlike in bacterial meningitis, meningeal symptoms such as nuchal rigidity are unusual, and fever is not always present. However, the clinician must consider cryptococcal meningitis in the differential diagnosis of any high-risk patient, such as those

who have HIV infection or are receiving corticosteroids, in whom there are any central neurological symptoms or signs. Occasionally, a silent CNS infection is diagnosed after the isolation of *C. neoformans* from another body site.

Overall, the symptoms and signs of meningitis in patients with and without AIDS are similar. However, there may be three areas in which cryptococcal meningitis differs somewhat in AIDS patients. First, the duration of symptoms and signs may be shorter in AIDS patients because of the high burden of organisms and poor inflammatory response in these patients. Conversely, some apparently nonimmunocompromised hosts with cryptococcal meningitis may have symptoms that persist for months; such patients may present with true chronic meningitis, with symptoms that are even more prolonged than those typical of the subacute course (2 to 4 weeks) seen with CNS tuberculosis. Second, patients with HIV infection, unlike those without this infection, more commonly develop a second site of infection that is readily detected, such as lung, skin, or blood, either before or at the time of diagnosis of CNS cryptococcosis. Third, HIV-infected patients have a greater likelihood of developing another opportunistic infection, such as with *Toxoplasma gondii*, or neoplasm. Focal neurological symptoms are not common on initial presentation (10%) with *C. neoformans*, and in patients with HIV infection, a second disease, such as another infection or tumor, should be considered when focal neurological lesions are identified. The majority of AIDS and non-AIDS patients with cryptococcal meningoencephalitis do not have evidence of cryptococcomas on brain radiographs. These slight differences between patients with and without AIDS are likely to reflect the degree of immunosuppression caused by AIDS rather than the HIV infection per se. A patient receiving massive doses of corticosteroids to prevent rejection of a transplanted organ may present with the same signs and symptoms as an HIV-infected individual with a CD4 count of <50 cells per μl.

There are few data that relate the severity of CNS disease to the infecting strain of *C. neoformans*, and the consensus has been that the state of the host defenses governs the clinical manifestations. However, the possibility that the strain influences the disease should not be completely dismissed. Speed recently reviewed the clinical experience from an area in Australia that attends to infections with both *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* (456). Cerebral cryptococcomas were found only in infections due to *C. neoformans* var. *gattii*. Although survival was better with this variety than with *C. neoformans* var. *neoformans*, the complications were greater, and they included problems of hydrocephalus and cranial nerve palsies. Strains of *C. neoformans* var. *gattii* generally infect apparently normal hosts and rarely infect AIDS patients (Table 2). Although these observations may represent only epidemiological factors of exposure, they may also suggest that different strains or varieties may have different capacities to cause CNS disease, depending on the immune status of the host. Clearly, in animals, there are well-documented strain-dependent differences in virulence.

Skin

Over the past 50 years, *C. neoformans* has been shown to cause almost every type of skin lesion (70, 282, 441, 445). Skin lesions may present as acneform lesions, purpura, papules, vesicles, nodules, tumors, abscesses, ulcers, superficial granulomas, plaques resembling ecchymosis, and sinus tracts. Recent reports of patients with AIDS and other high-risk patients have further expanded the variety of cutaneous manifestations to include herpetiformis (39), molluscum contagiosum-like lesions (88, 365), and cellulitis around an intravenous catheter resembling a bacterial infection (169, 300). This array of cutaneous presentations emphasizes the need to biopsy and obtain appropriate histopathological sections of all new skin lesions in high-risk patients. These sentinel lesions may be the first presentation of cryptococcosis, and cutaneous cryptococcosis almost always represents disseminated disease. However, primary cutaneous inoculation of *C. neoformans* has occurred during clinical and laboratory accidents; direct inoculation produces a papule at the site with or without a local immune reaction, such as regional lymphadenopathy (67, 172).

In a small number of high-risk patients, including those with HIV infection, secondary cryptococcal skin lesions may occur simultaneously with a cutaneous lesion due to another systemic mycosis (399). For example, we have treated a patient with AIDS who presented with concurrent cutaneous cryptococcosis and histoplasmosis, with spread from foci in the CNS and lungs, respectively. Therefore, in examining high-risk patients, skin lesions with different characteristics should be individually biopsied and processed.

Prostate

The prostate gland has long been recognized as a site for cryptococcal localization (44). The infection does not usually cause symptoms of prostatitis, but the yeasts have been isolated from prostatic tissue and blood after urological procedures (400). *C. neoformans* has even caused a penile ulcer (387) and a vulvar lesion (31), but there have been no reports of conjugal spread. The significance of genitourinary infection for clinical disease may reside in the prostate's potential as a protected sanctuary for this yeast during treatment. In careful follow-up of patients with HIV infection, cultures of routinely voided urine, urine obtained by postprostatic massage, and seminal fluid were frequently positive at the end of therapy (263, 462). Although it has not been proven, the prostate, in addition to the CNS, represents a potential reservoir of *C. neoformans* that may be responsible for clinical relapse of meningitis in AIDS patients. After treatment, certain high-risk male patients can be screened by culture of postprostatic massage urine samples to ensure that the yeasts have been eliminated. However, in patients with HIV infection, continuous therapy is recommended, and therefore sites of asymptomatic relapse may be less clinically relevant. In non-AIDS patients, the genitourinary source has been less well studied than in AIDS patients.

Eye

Ocular involvement with *C. neoformans* is far from rare (25). Prior to the HIV epidemic, one study found ocular signs and symptoms in approximately 45% of all patients with meningitis (356). Manifestations range from ocular palsies to involvement of the retina (96), which can also be simultaneously infected with other pathogens, such as HIV and cytomegalovirus (125). Ocular cryptococcosis presents the distinct possibility of visual loss. Indeed, most cases of cryptococcal endophthalmitis lead to severe visual loss, and only an occasional case is successfully managed (107). In approximately one-fourth of cases, eye involvement may present before the diagnosis of cryptococcal meningoencephalitis is made (96). Early diagnosis by aspiration of the vitreous body for culture or identification of *C. neoformans* at another site and rapid treatment are essential to preserving the patient's sight.

Recent reports have described catastrophic loss of vision in patients without evidence of endophthalmitis (223, 420). The funduscopic examination was either normal or revealed evidence of papilledema. Two pathogenic processes have been

suggested. Some patients have rapid visual loss in a period as short as 12 h and a clinical syndrome suggestive of optic neuritis, in which the optic nerve may be infiltrated with yeast cells. Few therapeutic measures have been successful for this form of cryptococcal visual loss. Another group of patients present with slow visual loss, which typically begins later in therapy and gradually progresses over weeks to months. In this group, symptoms may be related to increased intracranial pressure, and treatment of this condition with shunts or optic nerve fenestrations may halt the progression of visual loss.

The eye may also be a portal of entry for *C. neoformans*. There is a case report of the transmission of cryptococcosis from the donor of a corneal transplant to a recipient, who later developed meningitis (21). A case of cryptococcal keratitis following keratoplasty has also been described (392). In some patients, meningitis was identified only after eye involvement (24). These findings suggest that a potential portal of entry in some cases could be trauma to the eye with a foreign body contaminated with *C. neoformans*.

LABORATORY DIAGNOSIS

Direct Microscopic Examination

Clinical specimens—spinal fluid, tissue, sputum and respiratory samples, scrapings, punch biopsies, aspirates from cutaneous lesions, and other appropriate specimens—should be examined directly in an India ink preparation for the presence of yeast cells with capsules (Fig. 1). In wet preparations of fresh material, the yeast cell wall appears refractory, and the cells often contain spherical vacuoles in Brownian motion. The yeast cells are usually spherical and 5 to 7 μm in diameter. They typically appear without buds or with a single bud that is attached by a thin connection. The capsules may vary in thickness from a few micrometers to a width that equals or exceeds the diameter of the cell. In rare cases, cells may be observed that are outside the normal size range (from 2 to 15 μm), more ellipsoidal than spherical, multiply budding, or lacking capsules. CSF is best examined with India ink, which has the advantage of revealing the presence of a capsule. Aspirates and similar specimens can be effectively treated with a solution of calcofluor white and examined under a fluorescent microscope (187). Calcofluor is not specific for *C. neoformans*, as the cell walls of all fungi are stained and fluoresce brightly; however, it can be useful when few yeast cells are present.

In tissue sections, encapsulated yeasts appear to be surrounded by large empty spaces because of the poor staining of the CPS and distortion that results from sectioning. As noted, the inflammatory response in tissue is generally either minimal or granulomatous in character. In histologic sections, the cells of *C. neoformans* often appear collapsed and distorted. The capsule can be enhanced by staining with mucicarmine. Although it has been reported that yeast cells in the spinal fluid of patients with AIDS have smaller capsules (41), this observation is not made consistently.

Culture

C. neoformans can be isolated on most routine mycological or bacteriological media. For blood cultures, the most sensitive method appears to be the lysis-centrifugation procedure (479). In one analysis of this method, two blood cultures were sufficiently sensitive (>70%) to detect cryptococcemia in patients with AIDS (523). When nonsterile specimens are to be cultured, media containing cycloheximide should not be used because the organism is susceptible to this agent.

All species of *Cryptococcus* are nonfermentative, and most are encapsulated and similar in microscopic and colony morphology and produce extracellular starch and urease. Unlike most isolates of other species of *Cryptococcus*, *C. neoformans* is unique in its pathogenicity, the ability to grow at 37°C, and the production of phenol oxidase. Phenol oxidase leads to the formation of melanin, which can be demonstrated by the acquisition of a brown to black pigment when *C. neoformans* is grown on Staib's birdseed agar or caffeic acid medium (73, 252, 461). Many laboratories use a rapid urease test for the rapid recognition of *C. neoformans*. Isolates can be readily identified to the species level by several commercial systems on the basis of biochemical reactions (138, 466, 509) or by a DNA-based method (208, 320). A primary isolation medium has been described that contains a substrate for phenol oxidase to facilitate the recognition of *C. neoformans* among other microorganisms in nonsterile specimens, such as sputum and urine (110).

At the subspecies level, the two varieties of *C. neoformans* are differentiated by the color reaction when grown on canavanine-glycine-bromthymol blue agar (255). To serotype an isolate, there is a commercial kit consisting of monoclonal antibodies (Iatron, Tokyo, Japan) (210, 227). For epidemiological purposes, population studies, and pathobiological research, individual strains of *C. neoformans* can be biotyped or fingerprinted by DNA-based or enzymatic methods (45, 94, 458, 497). The DNA-based methods of strain identification are based on RFLP patterns of genomic or amplified DNA, specific DNA probes, and/or variations on the PCR method with single primers.

Serology

The serologic test for the diagnosis of cryptococcosis is both specific and sensitive. During infection, the CPS becomes solubilized in the body fluids, and this antigen can be detected and quantified with specific rabbit anti-*C. neoformans* antiserum. The most commonly employed method for either screening or titration is a latex agglutination test. The reagents for the test are commercially available in kit form. Latex particles are coated with the specific hyperimmune rabbit immunoglobulin and mixed with dilutions of patient CSF, serum, or urine (32). A positive agglutination at a dilution of 1:4 strongly suggests cryptococcal infection. Titers of ≥8 usually indicate active disease, and most patients with AIDS have higher antigen titers. Controls include latex particles coated with normal rabbit globulin to detect nonspecific agglutination, which occurs in serum with rheumatoid factor (17). To eliminate interfering substances such as rheumatoid factor, serum can be pretreated with a reducing agent or proteolytic enzyme. Most laboratories digest the serum with pronase and then boil it for 5 min. Depending upon the manufacturer, latex agglutination test kits detect as little as 10 ng of CPS per ml. Diagnosis by antigen detection is more sensitive (sensitivity, ≈95%) than either culture (≈75%) or direct microscopic examination by India ink (≈50%).

On rare occasions, the latex agglutination test may yield spurious results in either serum or spinal fluid. Most false-positive results are caused by the presence of rheumatoid factor, which is eliminated by treating the specimen with pronase or dithiothreitol or boiling it with EDTA (177, 182). Rarely, false-positive tests may occur when a cross-reactive antigen is present, such as the polysaccharide of *Trichosporon beigelii* or another microorganism (62, 72, 303). False-positive tests can also be caused by contamination of the specimen with a minute amount of agar or agarose, which may occur if the same pipette that is used to inoculate media for culture is reintroduced into

the spinal fluid (38, 193). Recently, false-positive latex agglutination tests of sera from HIV-infected patients were eliminated with 2-mercaptoethanol but not with pronase (515).

Patients with HIV infection and primary pulmonary cryptococcosis (without dissemination) may have false-negative tests for serum antigen (475). However, in a prospective study of bronchoalveolar lavage (BAL) fluid from patients with HIV, all eight patients who developed cryptococcal pneumonia had positive antigen titers of ≥8 in their BAL specimens (12). Although there were four false-positive reactions at a titer of 8, BAL titers of ≥8 in symptomatic patients may be diagnostic of pulmonary cryptococcosis (12).

Among patients with cryptococcal meningoencephalitis, false-negative results are rare, albeit well documented (19, 99, 189, 201). False-negative tests may be caused by (i) low levels of antigen, (ii) the presence of immune complexes, (iii) high titers of antigen (prozone), or (iv) infection with a poorly encapsulated or nonencapsulated strain of *C. neoformans*. Since serum and spinal fluid specimens are routinely screened directly before positive specimens are diluted for titration of antigen, some false-negative results have been attributed to a prozone effect. The prozone-like effects due to excess antigen or immune complexes can be eliminated by diluting the specimen or treating it with pronase, respectively (189). In a study of sera and CSF from AIDS patients, pronase treatment of serum eliminated false-negative results, but pronase was not required for CSF (189).

Four commercial kits are readily available for latex agglutination testing in the United States (from International Biological Laboratories, Cranbury, N.J.; American Micro Scan, Mahwah, N.J.; Immuno-Mycologics, Norman, Okla.; and Meridian Diagnostics, Cincinnati, Ohio). Since the sensitivity, specificity, and titers detected have been shown to vary among these kits, intralaboratory controls are essential (99, 189, 522). Each laboratory should employ a kit from only one manufacturer and check each new lot with reference reagents. A number of studies have confirmed the overall value of the latex agglutination test of serum and CSF for the diagnosis of cryptococcosis in patients with AIDS (43, 112, 159, 164, 189, 350). However, latex agglutination testing of BAL and urine samples may recognize pulmonary and disseminated cryptococcosis, respectively, with greater sensitivity in AIDS patients than does serum testing (12).

Several enzyme immunoassays (EIAs) have also been developed to detect either antigen or antibody. In comparison with the latex agglutination test, reading EIAs is less subjective, the EIA is unaffected by prozone reactions, and the EIA may detect antigen earlier and in smaller amounts. The EIA does not react with rheumatoid factor, and hence the specimen does not require pronase treatment. However, EIAs require more time to perform than the latex agglutination test, which can be completed in several minutes. Both tests detect all cryptococcal serotypes. A recently evaluated commercial EIA (Premier; Meridian, Cincinnati, Ohio), which employs a monoclonal antibody that recognizes all four serotypes, has been shown to offer excellent sensitivity and specificity (164). In recent comparisons of EIA and latex agglutination procedures from the same manufacturer (Meridian), agreement on screening tests was good (97.8%) (159). Of the 13 discrepant results, the EIA reported three false-positive test results that became negative on retesting and four false-negative results, one of which was positive on retesting. The latex agglutination test produced one false-negative result. For serum and spinal fluid samples, the EIA titers were generally higher (159). Another, smaller comparison of the latex agglutination and EIA methods reported somewhat less agreement (92%) but concluded that there were fewer false-positive results with the EIA (238). These conclusions were recently confirmed in a comparison of four LA procedures with the EIA method (478).

Antibodies to *C. neoformans* are usually not detectable during active cryptococcosis. CPS in the serum may combine with circulating antibody as well as inhibit its synthesis. However, with clinical recovery, the serum may be positive for antibody. Anti-*C. neoformans* antibodies can be titrated in dilutions of serum by (i) agglutination of charcoal particles coated with CPS, (ii) an indirect immunofluorescent antibody test, or (iii) the yeast cell agglutination test, which can be performed in tubes or microtiter wells, with a killed suspension of a small-capsuled to moderately encapsulated strain of *C. neoformans* (23, 176, 178, 505).

Molecular Identification

Several DNA-based methods of identification have recently been developed (321). Although conventional mycological methods are sufficient to identify *C. neoformans* at the species level, molecular methods have been used in epidemiological studies to identify the variety, serotype, or individual strain of *C. neoformans*. Strains can be identified by using pulsed-field gel electrophoresis or contour-clamped homogeneous-field gel electrophoresis to separate chromosomes and distinguish electrophoretic karyotypes of *C. neoformans* (130, 258, 378, 381, 404, 459, 516).

Several probes have been studied that distinguish the variety, serotype, or strain of *C. neoformans*. Genomic DNA can be digested with an appropriate restriction endonuclease, separated by electrophoresis, blotted, and hybridized with the species- or variety-specific probes CND1.7 and CND1.4, respectively (405). Other probes that distinguish individual strains include the plasmid probe UT-4p (130, 497), the *URA5* gene (98, 459), a repetitive element, CNRE-1 (98, 458–460), and simple DNA repeats (192).

PCR amplification of specific DNA from clinical material has proven successful with other microorganisms and can be expected to become a standard procedure in clinical microbiology laboratories (393). Species-specific probes and primers have been developed (208, 320). Strains can also be identified by the RFLP patterns of genomic and mitochondrial DNA (65, 98, 202, 459, 496, 500). Strains are much more quickly identified by banding patterns generated with the techniques of randomly amplified polymorphic DNA (94, 192) or by PCR fingerprinting (307, 309, 310, 319, 321), in which a simple repeat is used as a single primer for amplification. Figure 3 illustrates the method of PCR fingerprinting which we have used to identify the variety, serotype (A, D, or B/C), and individual strains of *C. neoformans*.

In addition to DNA-based methods of identification, strains can be distinguished by multilocus enzyme analysis, in which cytoplasmic extracts are subjected to starch gel electrophoresis and stained for 10 different enzymes (45). From the electrophoretic mobilities of these enzymes, the variety and serotype of *C. neoformans* can be identified. Variations in the structure of CPS among serotypes have also been documented (78, 488).

Radiology

Computed tomography (CT) and magnetic resonance (MR) scans have become important diagnostic and management tools in cryptococcal meningitis. In developed countries, one of these scans is performed on most patients with cryptococcal meningitis. The radiographic appearance of CNS cryptococcosis can vary among groups with different types of immunosuppression and underlying diseases, but it seems reasonable to

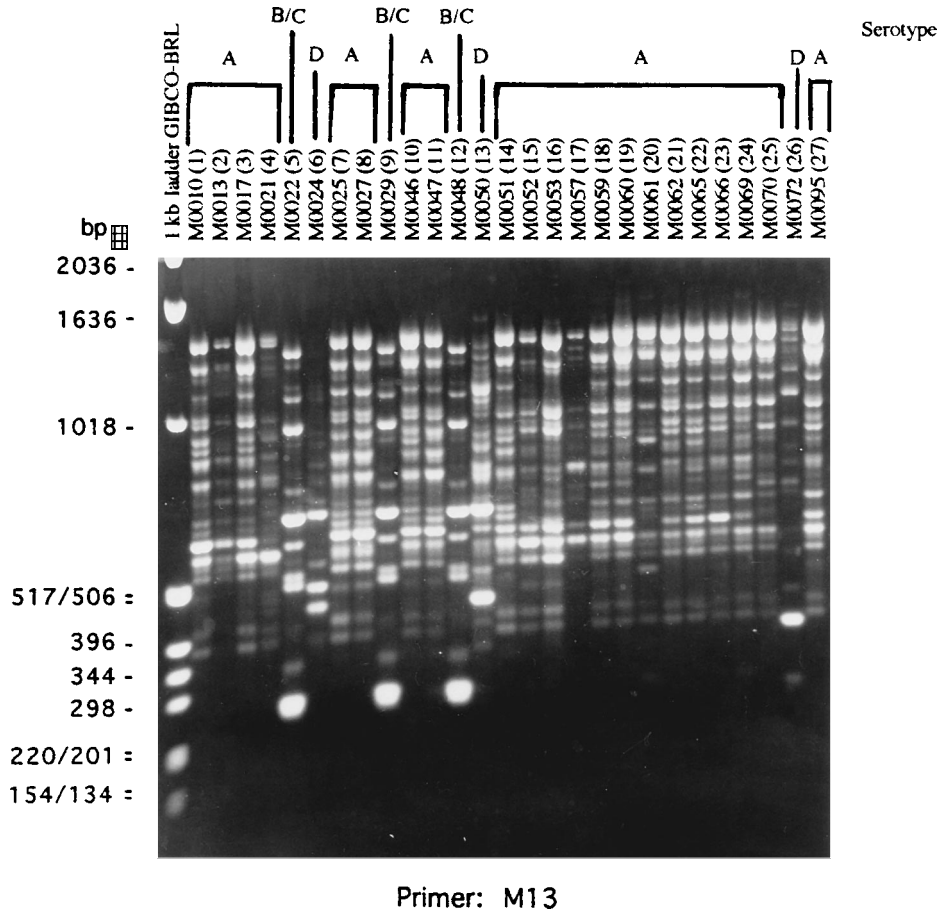

FIG. 3. Electrophoretic separation of PCR fingerprints of clinical isolates of *C. neoformans* obtained by amplifying genomic DNA with the M13 core sequence (5′ GAGGGTGGCCGGTTCT 3′) as a single primer (308, 310). The first lane contains a 1-kb ladder; the other lanes contain clinical isolates of *C. neoformans* from different patients. To identify serotypes, PCR band patterns were compared with those of reference strains of *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gattii* (serotypes B and C), and the results are indicated by the letters above the brackets. It is possible to recognize variety-, serotype-, and strain-specific bands. Reprinted from reference 309 with permission of the publisher.

discuss the presentations of those with and without AIDS. CT findings among non-AIDS patients with meningitis can reveal hydrocephalus, gyral enhancement, and multiple nodules, which are both enhancing and nonenhancing (89). In one study, these relative presentations either were normal (50%) or revealed hydrocephalus (25%), gyral enhancement (15%), or focal nodules (15%) (477). The cryptococcomas can be either single or multiple (442) and occur in up to 25% of patients (288). Cryptococcoma-like lesions with septa can even be found in the intraventricular spaces around the choroid plexus of the trigone; the spinal cord and its roots can be involved (355, 366). In patients with HIV infection, approximately half also had a normal scan, but in contrast to non-AIDS patients, 34% had diffuse cortical atrophy and 9% had hydrocephalus. Similar to non-AIDS patients, 11% had focal mass lesions (411). The mass lesions range from single to multiple enhancing nodules, either solid or ring-like, to multiple nonenhancing, low-density lesions in the basal ganglia and thalamus, which likely represent gelatinous pseudocysts (168). On MR, these hypodense lesions may be shown to be septic rather than infarcts. It is likely that the differences in CT presentations between the two patient groups represent the HIV infection of the brain causing atrophy and the profound effect of this infection on the poor inflammatory response within the CNS.

MR scans appear to be more sensitive than CT for detecting abnormalities. When CT scans are normal, MR scans may show significant abnormalities. MR findings include numerous clustered tiny foci that are hyperintense on T2-weighted images and nonenhancing on postcontrast T1-weighted images within basal ganglia and midbrain representing Virchow-Robin spaces, which do not enhance with gadopentetate dimeglumine. The MR may also show multiple miliary enhancing parenchymal and leptomeningeal nodules with gadopentetate dimeglumine (480). Although dilated Virchow-Robin spaces can be observed among all age groups, the perivascular spaces appear larger with age. Indeed, the MR finding of dilated Virchow-Robin spaces in a high-risk patient suggests cryptococcal meningitis (511). However, if these findings are present on MR, the clinical diagnosis is likely to be established by lumbar puncture, because this radiographic feature probably represents large numbers of yeast cells packed into these spaces or partially blocking the outflow of CSF. Hence, the yeasts should be seen in and cultured from spinal fluid.

Regarding radiographic examinations during cryptococcal meningitis, certain features suggest cryptococcal disease, but none are pathognomonic. Like other mycoses, cryptococcosis can present initially on the CT or MR scan as an apparent idiopathic hydrocephalus (212). In AIDS patients, toxoplasmosis or lymphoma may mimic some of these findings. In our

experience, some non-AIDS patients with cryptococcal meningitis have a concomitant CNS infection or tumor. In addition, among non-AIDS patients, focal parenchymal lesions may actually increase in size and/or number over the first few months of therapy. This does not necessarily indicate a failure of treatment. We suspect that it represents enhancement by inflammatory cells within these granulomas as microscopic foci are cleared. For patients who are clinically stable, we have simply continued fluconazole treatment for an extended course, and over a year, these lesions gradually resolved.

The other important radiographic site for cryptococcosis is the lung. As previously noted, the chest radiograph of an immunocompetent host with pulmonary cryptococcosis may depict a variety of features, including nodules, pneumonic infiltrates, hilar lymphadenopathy, and pleural effusions (153, 209, 235, 301, 335, 525, 527). In an asymptomatic patient, a typical initial finding is the solitary nodule, and generally these lesions are not calcified. In patients with AIDS and *C. neoformans* pneumonia, the chest radiograph is different and reveals either diffuse or focal interstitial infiltrates, with or without lymphadenopathy (59, 84, 316). This radiographic picture can be confused with that of *P. carinii* pneumonia, which may or may not be a coinfection (287). In the severely ill with HIV infection, chest radiographs may also resemble those of patients with adult respiratory distress syndrome (391, 449).

TREATMENT

In Vitro Susceptibility Testing

For most initial isolates of *C. neoformans*, the MICs of amphotericin B (AMB), flucytosine, and the azoles are low (171, 447). The methods for in vitro testing of antifungal agents against a variety of yeasts, including *C. neoformans*, have been standardized for media, inoculum, and endpoint determinations (146, 161, 348). Because of its relatively slow growth rate and the fact that some strains do not grow well in RPMI medium, *C. neoformans* will usually require 48 to 72 h before a final determination of the MIC can be made. A reliable microtiter method with BYNB-7 medium has been developed (171). As more experience is acquired with in vitro susceptibility testing for *C. neoformans*, MIC testing will become useful for clinical decisions.

The resistance of *C. neoformans* to flucytosine has been reported to be low (<2% of strains) (408). In the early studies with flucytosine therapy used alone for cryptococcal meningitis, a rising MIC for the isolate was correlated with clinical relapse (491, 492). This prediction of resistance based on in vitro susceptibility testing was also confirmed by relapses and failures during clinical treatment for pulmonary cryptococcal infections with flucytosine alone (233, 491).

AMB is uniformly active against initial *C. neoformans* isolates. The MICs are generally low and exhibit little variation. Remarkably, only an occasional case has involved an AMB-resistant strain detected by in vitro susceptibility testing (34). With the current treatment regimens, even in severely immunosuppressed patients, most treatment failures are not related to the development of direct polyene resistance. Although the standardization of a minimum fungicidal concentration has not been well established for *C. neoformans*, compared with flucytosine and the presently available azoles, AMB appears to possess in vitro fungicidal activity at relevant drug concentrations.

The systemic azole compounds, especially miconazole, ketoconazole, fluconazole, and itraconazole, have all been used clinically to treat cryptococcosis. Their use has accelerated since the advent of the AIDS epidemic. Each of these azoles has in vitro inhibitory activity against most primary clinical isolates of *C. neoformans*. Although fluconazole may be slightly less potent than the others on a weight basis, this appears to have no clinical relevance. The AIDS epidemic and the widespread use of azoles in this persistently immunosuppressed population have precipitated an increase in lumenal infections due to azole-resistant *Candida* species. In vitro MIC testing can potentially predict the clinical resistance or failure of treatment in this group (61). However, most clinical relapses with *C. neoformans* that have been studied were due to strains for which the MICs of the azoles were similar to those for the primary isolate (68); furthermore, molecular typing of the isolates indicated that the cases were true relapses rather than reinfections (459). These relapses in patients with AIDS who were receiving ongoing azole therapy did not represent the emergence of resistant strains, but they emphasize the importance of the host defenses in eradicating the pathogen. Although the majority of isolates from cases of relapse do not demonstrate in vitro resistance, there have been reports in which the MIC of fluconazole for a clinical isolate was correlated with its resistance to the drug in experimental infection (499). Others report an occasional relapse isolate with a higher MIC of fluconazole than for the original isolate (364). Overall, despite the widespread use of azoles, the apparent incidence of azole resistance in cryptococcosis is low. One explanation is that some azole-resistant *C. neoformans* mutants are simply less virulent than the wild-type strains (214). The extent of azole cross-resistance among strains of *Candida* and *C. neoformans*, either in vitro or in vivo, among the various azoles has not yet been accurately determined.

Therefore, at this time, susceptibility testing for *C. neoformans* may be less useful for primary isolates. Most isolates will be uniformly susceptible to the major classes of antifungal agents. However, we strongly recommend that each initial isolate of *C. neoformans* be stored for at least 1 year after isolation so that the MIC can be compared with that for any future isolate from cases of relapse. In comparing the primary and subsequent isolates, a significant increase in the MIC (>4-fold) of the drug of treatment would suggest that a component of the treatment failure was related to cryptococcal drug resistance and that a new therapeutic drug regimen may be warranted.

The in vitro susceptibility testing of drug combinations for *C. neoformans* is poorly standardized, and thus clinical conclusions are even more uncertain than with single agents. However, a general overview of in vitro and in vivo studies suggests that there is a positive interaction for the following combinations: AMB-flucytosine, AMB-azole, AMB-rifampin, flucytosine-azole, and even the triple combination of AMB-flucytosine-azole (304, 409). These combinations against *C. neoformans* have not commonly exhibited in vitro antagonism. Since the combination of AMB and flucytosine has been so useful clinically, in vitro susceptibility testing is recommended in conjunction with future clinical investigations of combination therapies for cryptococcosis. Direct comparisons between this well-studied combination and all new combinations should be made prior to clinical trials.

Antifungal Agents in Animal Models

Animal models have been an important tool in the development of drugs for cryptococcosis. In vitro susceptibility testing provides an initial screening of candidate agents but cannot always predict efficacy in treating severely immunocompromised hosts with unique sites of infection and reliance on certain drug pharmacokinetics. Even the most prevalent inva-

sive mycoses—candidiasis and cryptococcosis—require collaborative clinical studies, which may require years to complete and must be carefully directed. The animal model allows a focused evaluation of the agent under controlled circumstances and with proper assessment of dose, pharmacokinetics, and specific endpoint evaluations. The results in animal models for cryptococcosis have encouraged the development of drugs in clinical practice today and discouraged further development of drugs that have displayed marginal or no advantage over present agents. Animal studies in cryptococcosis remain the bridge between drug screens and human trials.

Although no experimental model can exactly reproduce human disease, any model used should approximate clinical relevance. Early studies on the immunology of cryptococcosis used the guinea pig and the rat, but eventually the mouse became the favorite animal model. Mice are inexpensive, well-characterized immunologically, and genetically stable, and similar to immunocompromised patients, most strains are exquisitely susceptible to disseminated infection. In developing the murine model for treatment studies, inocula have been given by intrarespiratory, intravenous, and intracerebral routes. Treatment with most of the new and established agents has now been studied in murine models; their value in comparative studies with new agents or regimens is now well established. With mice, the investigator has the ability to deplete specific cell populations, such as CD4 cells, to make the findings relevant to AIDS patients. Since a major emphasis for the development of new therapeutic regimens should be the study of immune-based treatment regimens, murine models will be essential. Cloned biological immune factors such as IFN-γ and GM-CSF can be tested in mice as therapeutic agents.

The immunosuppressed rabbit model of cryptococcal meningitis has also been used extensively (370). The clinical relevance of this model was previously discussed. A major advantage of this model is the ability to perform comparative trials of different antifungal agents or regimens with a relatively small number of animals. It can also be used to discover new molecular targets of drugs, as discussed in the molecular biology section. Two examples are cited to illustrate the utility of this model to predict treatment outcome, which were not apparent from MIC testing or simple pharmacokinetics. First, AMB was the most rapidly fungicidal single agent in the model. This success occurred despite the extremely low (2 to 4 ng/ml) levels of AMB detected in the CSF of treated animals; similar to humans, the level would not have in vitro inhibitory activity against *C. neoformans* (373). A recent clinical trial in patients with AIDS confirmed the results of the studies in rabbits by demonstrating that AMB treatment sterilized the CSF faster than fluconazole treatment (432). These findings suggest that AMB acts similarly in both humans and rabbits, either through accumulation within the meningeal membrane or through its immunomodulating activities. Secondly, a similar success was observed for fluconazole- and itraconazole-treated rabbits with cryptococcal meningitis (385). These similar, positive treatment results occurred despite high CSF concentrations of fluconazole and unmeasurable CSF levels of itraconazole. These agents were later shown to display similar CSF pharmacokinetics in humans. In humans, cryptococcal meningitis has also been successfully treated with itraconazole (111, 502, 504). The basis for itraconazole's effectiveness in CNS infection is unresolved. However, this highly lipophilic compound accumulates in large concentrations on and within host cells (386), and this may promote its delivery to the yeasts through migrating host cells or exposure to the meningeal surface. These examples illustrate the importance of studying candidate drugs or regimens for cryptococcosis within a clinically relevant animal model.

Antifungal Agents

AMB. After more than 35 years of use, AMB has changed disseminated cryptococcosis from a uniformly fatal infection to a curable infection (440). Prior to the AIDS epidemic, studies of the treatment of cryptococcal meningitis showed a success rate of 60 to 70% (18, 124, 440, 490). A prospective study using AMB at 0.4 mg/kg/day for 10 weeks recorded a success rate of 68% in non-AIDS patients (18). While this figure is likely the most accurate for AMB alone in non-AIDS patients, when patients with AIDS were treated with AMB at 0.5 mg/kg/day for 10 weeks, the success rates dropped to 40% (432). However, these success rates are dependent on many factors, including study criteria for cure, the patient's underlying disease or stage of illness, and the dose and duration of AMB therapy. These success rates should therefore be used only for comparison with the single most successful agent in the treatment of cryptococcal meningitis. Individual patients may have better or worse outcomes, which depend more on various prognostic factors than on specific treatment regimens.

AMB has been used successfully in all forms of cryptococcosis, from pneumonia to meningitis, but the only comparative trials have been in cases of meningitis. Because of its toxicity, AMB therapy has been reduced in dose and duration by successfully using it in combination with flucytosine. Formulations of AMB with lipid have been developed to foster its delivery, reduce toxicity, and enable higher doses to be tolerated. Recently, cryptococcal meningitis has been treated with several lipid preparations—liposomal AMB (14, 86, 183), AMB colloidal dispersion (204), AMB lipid complex (ABLC) (82, 186, 389), and AMB injected into Intralipid (224). These preparations reduce the organ toxicity and allow much larger doses of AMB to be given. However, the studies have involved only small numbers of patients and animal models and are not yet conclusive. In the rabbit model, when comparable doses of either AMB or ABLC were used for treatment, ABLC was not as potent as AMB, but with much higher doses of ABLC (10-fold), which has lower toxicity than AMB, sterilization of the CSF occurred faster than with the lower dose of AMB (389). Therefore, it will be extremely important for clinicians to examine carefully the therapeutic-toxic dose ratio with each lipid formulation to optimize success.

In an attempt to optimize therapy for cryptococcal meningitis, AMB has also been used locally at the site of infection. Since AMB penetrates the CNS poorly, intraventricular AMB has been used successfully in severe cases of cryptococcal meningitis under the premise that local administration will increase its concentration within the CNS (410). However, this approach commonly leads to CNS complications (443) and must be balanced by the availability of at least two agents, flucytosine and fluconazole, which rapidly achieve high concentrations in the CNS after routine systemic administration.

Flucytosine. Flucytosine is a pyrimidine antagonist with established antifungal activity against *Candida* species, dematiaceous fungi, and *C. neoformans*. Flucytosine was initially used to treat cryptococcal meningitis and, with its excellent penetration into the CSF, enjoyed moderate success. However, because a high number of organisms develop with cryptococcal meningitis (the yeast census may exceed 10^6 CFU/ml of CSF), selection of resistant organisms was likely; mutation rates in the pyrimidine pathway are frequent, and mutations at several points in the pathway may lead to resistance. The relapse rate of cryptococcal meningitis with flucytosine-resistant isolates

approached 30 to 40%. Although flucytosine use was successful for managing cryptococcal pneumonia, occasional cases of cryptococcal meningitis occurred after treatment of pneumonia with flucytosine alone in severely immunocompromised hosts. Hence, single-drug therapy with flucytosine is less appealing when a high burden of organisms is anticipated. Subsequently, flucytosine has been successfully combined with AMB to take advantage of their different pharmacokinetics and synergistic mechanisms of action. With this potentially additive or synergistic combination, there has been less concern about development of resistance to flucytosine, and the reduced dose of AMB needed has diminished its toxicity.

Some clinicians believe that the use of flucytosine in cryptococcal meningitis causes intolerable side effects, such as leukopenia and gastrointestinal disturbances. This problem has been particularly emphasized in patients with AIDS (81). The toxicity of flucytosine frequently develops within the first 2 weeks of therapy. Several guidelines may assist clinicians with the use of flucytosine. First, the dose of 150 mg/kg/day used in the early studies for cryptococcal meningitis may be too high for some individuals. In patients with normal renal function, initial doses of 100 mg/kg/day will achieve acceptable drug concentrations. Second, in patients with cryptococcal meningitis, the serum flucytosine levels should be maintained at <100 μg/ml at 2 h after administration to reduce the potential risk of life-threatening leukopenias (463). It is reasonable to recommend that all patients receiving prolonged high doses of flucytosine for cryptococcal meningitis should have their drug levels monitored initially and with any change in renal function.

Miconazole. Miconazole is rarely used today for cryptococcosis. It offers no advantages over newer azoles and is more toxic. Of historical interest, treatment of cryptococcal meningitis with miconazole has produced both successes and failures (472, 513). Intraventricular miconazole has also proven successful against cryptococcal meningitis (185).

Ketoconazole. Standard doses of ketoconazole alone were not successful in treating cryptococcal meningitis (375), and with the development of newer triazoles, ketoconazole is not likely to become a significant therapeutic option for cryptococcal meningitis. However, for nonmeningeal cryptococcosis in patients without severe immunosuppression, ketoconazole should be effective. Although there are few reports of its effective administration for pulmonary cryptococcosis (123), ketoconazole is likely to be a cost-effective option for these infections.

Fluconazole. Because of its excellent pharmacokinetics in the CSF, fluconazole was evaluated in the treatment of cryptococcal meningitis (134, 465). In a trial in which patients with AIDS and cryptococcal meningitis were treated with AMB or fluconazole, the outcomes at 10 weeks were similar; the success rate of fluconazole was 34%, and that of AMB was 40% (432). However, with fluconazole, there was a trend toward earlier deaths and a longer period before sterilization of the CSF. This study supported fluconazole as a therapeutic option in the initial management of cryptococcal meningitis in patients with AIDS. Although 400 mg/day has been the usual dose for meningitis, higher doses have occasionally been successful in salvage therapy for prior failures (20). The optimal dose of fluconazole for initial therapy remains uncertain but is probably in the range of at least 400 to 800 mg/day. This triazole is fungistatic in vitro and appears to suppress the infection in vivo in AIDS patients, for whom the duration of therapy is considered indefinite. Along with proper dosage, several important questions remain regarding fluconazole therapy in cryptococcosis. For instance, how should fluconazole be used in non-AIDS patients with cryptococcal meningitis? Early results suggest that it is successful as initial therapy in 65 to 75% of such patients (133), but it has not yet been compared with AMB regimens. Because of its availability as an oral preparation and excellent safety profile, fluconazole could be used for longer courses of treatment in non-AIDS patients. Would prolonged treatment improve its success rate? What is its antifungal activity when given simultaneously with AMB? Despite its recent widespread use, further studies of fluconazole in cryptococcosis are required to define the optimal conditions for its administration.

Itraconazole. Despite its poor penetration into the CSF of humans and animals, a substantial number of patients with cryptococcal meningitis have been successfully managed with itraconazole (106, 111, 493, 502–504). Since direct comparisons of initial treatment with fluconazole and itraconazole therapy have not been completed at this time, a judgment about which is the better triazole cannot be made. Both may give similar outcomes for initial treatment of cryptococcosis, particularly if the absorption of itraconazole is ensured, but fluconazole may be better for suppressive therapy.

Combination therapy. For almost 20 years, combination therapy with AMB and flucytosine has been used in the treatment of cryptococcal meningitis. The first large comparative trial of AMB (0.3 mg/kg/day) and flucytosine (150 mg/kg/day) for 6 weeks versus AMB (0.4 mg/kg/day) for 10 weeks produced similar outcomes, but the combination resulted in more rapid sterilization of the CSF and reduced nephrotoxicity as a result of the lower dose of AMB (18). A follow-up study found that combination therapy for 4 versus 6 weeks produced comparable success if patients had favorable prognostic factors (124). However, it was clear that severely immunosuppressed individuals, such as renal transplant patients, would fail the short-course regimen. With the advent of AIDS and the high frequency of corticosteroid-induced infections, there has been less enthusiasm for short-course combination therapy of cryptococcal meningitis.

In two small studies of AIDS patients, the combination of AMB and flucytosine appeared to be even better than a single azole, such as fluconazole (264) or itraconazole (105). In most studies, more than 90% of patients, including those with AIDS, had sterile CSF after 2 weeks of treatment with AMB and flucytosine (18, 81, 83, 124, 144, 239, 264, 490, 529). These findings stimulated an ongoing comparison among AIDS patients, in which combination therapy or AMB alone is given for 2 weeks, after which the patients are randomized to receive either itraconazole or fluconazole treatment indefinitely. In non-AIDS patients, present investigations are now using a similar management schedule of induction with combination therapy and maintenance with an azole for a defined period of time. Studies are also in progress comparing combination therapy with higher doses of AMB (0.7 mg/kg/day) and lower doses of flucytosine (100 mg/kg/day) than were used in previous studies for 2 weeks, followed by high doses of fluconazole (800 mg/day) for 8 to 12 weeks. The optimal duration of treatment with this regimen remains uncertain. It is ironic that one of the best-studied combination antimicrobial regimens continues to be investigated for its optimal use.

There has also been interest in a completely oral combination regimen in the treatment of cryptococcal meningitis. Although triazoles have been used successfully as single agents in AIDS patients, there are scant data for their use in non-AIDS patients, in whom therapy is not anticipated to be life-long. Both animal data (2) and early human trials (225, 262a) have supported the combination of flucytosine and fluconazole. These agents have different mechanisms of antifungal action but achieve similar penetration into the CSF. Studies to deter-

mine the impact of this combination regimen on patient care have been initiated.

A third combination of agents, polyene and azole, has always been questioned because of the potential for antagonism. This theory of antagonism is based on the primary mechanisms of action of these agents, some in vitro studies, and results of treatment in an animal model of aspergillosis. However, in the rabbit model of experimental cryptococcal meningitis, AMB and ketoconazole had an additive effect (370). Any potential benefit of AMB combined with a triazole, such as fluconazole or itraconazole, remains undetermined, since there have been no controlled human studies. Present data show no apparent antagonism when AMB is used prior to suppressive therapy with fluconazole. Despite some recent positive experience, combined therapy with a polyene-azole or triple therapy including a polyene-azole-flucytosine (226) should be used only with careful clinical monitoring, since proper dosing and defined success rates remain unknown.

Prognosis

The most important prognostic factor for the success or failure of treatment for cryptococcal meningitis continues to be the ability to control the patient's underlying disease. Whether it is cancer (229), AIDS, high-dose corticosteroid treatment, or organ transplant (167, 444), if the underlying disease or condition cannot be controlled, the ability to successfully treat cryptococcal meningitis is limited. When one group reviewed their comparative experience of cryptococcosis in AIDS and cancer patients, they actually found a better prognosis in patients with AIDS (514). The median overall survival for patients with AIDS was 9 months, compared with 2 months for patients with neoplastic disease. Similarly, 78% of AIDS patients and 43% of patients with neoplastic disease were considered to have had their infection controlled. This finding probably reflects the ability to control the underlying disease rather than the infection with *C. neoformans*. Another major prognostic factor affecting the outcome is the burden of organisms on initial presentation of the patient, as evaluated by large numbers of yeasts on India ink examinations, elevated polysaccharide antigen titers, and poor inflammatory reactions (<20 cells per μl). The presence of many organisms may identify patients who either have a reduced ability to control the early stages of infection or are at high risk for relapse. A third significant factor is the mental status of the patient on admission. Patients with a lucid sensorium on admission have a better prognosis than patients who are stuporous or in coma. However, the mental status can deteriorate rapidly during therapy, and this depressed sensorium may be related to increased intracranial pressure.

Prior to the AIDS epidemic, Diamond and Bennett (116) identified specific clinical features that correlated with failure or relapse during treatment of cryptococcal meningitis with AMB. Patients who died during therapy were more likely to have (i) an initial positive India ink examination, (ii) high CSF opening pressure, (iii) low CSF glucose, (iv) low CSF leukocytes (<20 leukocytes [WBC] per μl), (v) cryptococci isolated from extraneural sites, (vi) absence of anticryptococcal antibody, (vii) initial CSF or serum cryptococcal antigen titer of >32, and (viii) corticosteroid therapy or lymphoreticular malignancy. Patients who relapsed after treatment were characterized by (i) abnormal CSF glucose concentration for ≥4 weeks of therapy, (ii) low initial CSF leukocyte count, (iii) cryptococci isolated from extraneural sites, (iv) absence of anticryptococcal antibody, (v) posttreatment CSF or serum cryptococcal antigen titer of ≥8, (vi) no significant decrease in CSF and serum antigen titer during therapy, and (vii) daily corticosteroid therapy equivalent to 20 mg of prednisone or more after completion of therapy. Although some of these features have not been specifically identified in recent studies with AMB, flucytosine, and fluconazole, these factors cogently identify patients with a high burden of organisms and a poor inflammatory response. We believe they are still useful in the general assessment of prognosis.

Among non-AIDS patients with cryptococcal meningitis, studies with AMB and flucytosine treatment indicated a better prognosis if there was a normal mental status and/or headache at presentation and a CSF leukocyte count of >20 WBC per μl. In a large comparison of AMB and fluconazole in AIDS patients, significant pretreatment predictors of death during treatment included abnormal mental status, a CSF antigen titer of >1:1,024, and CSF leukocyte count of <20 WBC per μl. There is also the retrospective observation that the development of diastolic hypertension at the beginning of or during treatment was associated with death (151). This diastolic hypertension was also correlated with an increased CSF opening pressure at the start of or during treatment. In our experience during the treatment phase, poor prognostic findings included the development of systemic hypertension, new cranial neuropathies, worsening mental status, or recurrence of headaches. Most patients had evidence of severe intracranial pressure elevations when lumbar punctures were performed.

It is essential to continue to identify specific subsets of patients who are at high risk for failure or relapse during standard therapy. These patients will require the most carefully designed and individualized treatment strategies to improve their chances of survival. There are no prognostic guidelines for nonmeningeal cryptococcosis except that immunocompromised patients should be treated with systemic antifungal agents. However, the definition of who is immunocompromised is not always precise.

Concepts of Cryptococcosis in AIDS and Non-AIDS Patients

Site of infection. Most clinical experience has been with meningitis, and all other sites have been much less studied. Because of the unique propensity of *C. neoformans* for invading the CNS, it remains good practice to exclude meningitis by performing a lumbar puncture on all patients whenever *C. neoformans* is isolated from another body site. In most cases, treatment regimens that are successful for meningitis will also be adequate for invasive disease in other organs. However, there are two sites that require special attention.

First, although it remains uncertain, some clinical data suggest that the prostate may serve as a protective site for *C. neoformans* during treatment. In AIDS patients, in whom this phenomenon was first described, the significance of prostatic foci is diminished, since most receive indefinite suppressive therapy to prevent relapse. However, in non-AIDS patients, isolation of *C. neoformans* from the "normal" prostate or urine samples obtained after prostatic massage may direct clinicians to prolong treatment with oral agents, such as the triazoles, to ensure sterilization of this asymptomatic site.

Second, the treatment of pulmonary cryptococcosis remains poorly studied and requires better specific guidelines. There are only a few, small series of reported cases that were treated with azoles or flucytosine alone. Despite the lack of explicit guidelines, several comments about management are warranted. Immunosuppressed patients, such as those who have HIV or underlying cancer or are receiving corticosteroids, should be treated even if asymptomatic because the risk of

dissemination is high. All patients who are symptomatic should be treated. A more controversial but defensible view is to treat all patients from whom *C. neoformans* is isolated from the lung, since it is uncertain, even for those who are "colonized," whether or not they will receive corticosteroids at some time for disorders such as chronic obstructive pulmonary disease. Since present therapies can be given orally with minimal toxicity, this may be a reasonable approach. However, it is less certain that immunocompetent patients who have histological evidence of *C. neoformans* in the lung from a biopsy specimen but negative cultures of the tissue will respond to drug therapy. In the immunocompetent patient, individualized decisions and careful follow-up are the most appropriate strategies. If treatment is considered, severe pulmonary infections are probably best managed with AMB, with or without flucytosine. For those infections that do not appear life-threatening, the choices of fluconazole, itraconazole, and the less expensive ketoconazole are reasonable. Flucytosine can probably be used as well if the burden of organisms is low and the patient is not severely immunosuppressed. The optimal length of therapy is unknown, but generally most patients are treated for 1 to 6 months.

Most cases of torulomas or cryptococcal abscesses within the brain parenchyma can be successfully managed with systemic antifungal agents. Some lesions larger than 3 cm may require surgical removal to be managed successfully (163), but each case must be individualized.

Immune status of host. Every attempt should be made to improve the immunity of the host during treatment. On a practical basis, this generally means reducing the immunosuppressive agents. Ideally, corticosteroid treatment should be stopped. However, in many instances this is impossible, and a reasonable goal has been to achieve ≤20 mg of prednisone per day. Cyclosporine may have some anticryptococcal activity (328), although it is also likely to have a negative impact on clearing *C. neoformans* infections (236, 340, 372) and needs to be carefully monitored. With the use of new organ transplantations, such as liver and heart, which unlike kidneys cannot be sacrificed, the management problem of balancing immunosuppression and antifungal treatment becomes even more difficult. Antifungal drug interactions in this immunosuppressed population need to be carefully assessed, since agents such as cyclosporine, dilantin, azoles, rifampin, and AMB are being used together (378).

In AIDS patients, if the immune status at the time of cryptococcosis remains unaltered (i.e., CD4 counts of ≤100 cells per μl), complete eradication of *C. neoformans* with present fungistatic regimens has not been a consistently achievable goal. The high rate of recurrence has led to the concept of indefinite suppressive therapy.

Length of treatment and follow-up. In AIDS patients, the length of therapy and follow-up is indefinite. There is also a group of AIDS patients with quiescent disease (412). These patients have cultures that are positive for prolonged periods during initial therapy, but they remain asymptomatic. A detailed examination of the prognosis and follow-up of these patients is necessary to understand whether rapid sterilization of the CSF is an essential goal in treatment.

With the high relapse rates, AIDS patients are presently receiving antifungal therapy for life. More long-term studies and improved fungicidal regimens are needed in this group as the underlying infection evolves. However, some AIDS patients, even with current regimens, are probably cured, but unfortunately, they cannot be identified by present clinical data.

In non-AIDS patients, treatment regimens as short as 4 to 10 weeks have been acceptable for cryptococcal meningitis. However, it will be interesting to study whether the relapse rates can be further reduced by prolonged treatment of 3 to 6 months with the new, safer oral triazoles. Prognostic factors suggested by Diamond and Bennett (116) and the Mycoses Study Group trials remain important guides in determining the risk for relapse in these patients, and all patients should be closely followed over the first 6 months to 1 year posttherapy, when most relapses occur. Relapses should be considered and retreatment instituted with new or recurrent neurological symptoms and/or when *C. neoformans* is once again cultured from the CSF. A positive India ink test or high antigen titer by itself is insufficient for diagnosing clinical relapse and initiating retreatment. A normal lumbar puncture 1 year after cessation of therapy is a good indication that a cure has been achieved. Successfully treated patients may have a low-grade CSF pleocytosis for up to 6 months after treatment, but the CSF will be normal in most cases by 1 year posttreatment.

Primary isolates of *C. neoformans* can be stored for 1 year so that, if necessary, the MIC for relapse isolates can be compared with that for the initial isolates. Although most relapse isolates remain susceptible to the agent(s) used for initial treatment, drug-resistant isolates have occurred in both AIDS and non-AIDS patients. There is no consensus on the best management of such patients who relapse, but most have responded to the same regimens given for longer periods of time and at higher doses.

Suppressive therapy. As the incidence of cryptococcal meningitis increased in HIV-infected patients, the relapse rates were observed to soar after therapy was stopped. Relapse rates as high as 50 to 60% and the resultant shortening of life expectancy led to the concept of a chronic suppressive regimen. Studies have now been completed demonstrating that oral fluconazole therapy (200 mg/day) over a year can significantly reduce relapse rates to <5% (43, 414). This regimen has also proven superior to AMB (1.0 mg/kg intravenously once per week) for suppression. Itraconazole has also been useful for suppression (104), although at similar doses (200 mg/day), itraconazole is less effective than fluconazole (352). Even though the strategy of suppression has become standard practice, it has actually proven to be of benefit for approximately 1 year posttreatment. It is important to analyze the long-term (>1 year) consequences of this suppressive regimen on relapse rates, survival from underlying disease, and fungal resistance patterns of colonizing yeast flora in these patients.

Role of intracranial pressure. The management of increased intracranial pressure during cryptococcal meningitis remains a poorly studied but potentially important area of management. With the AIDS epidemic, many clinicians observed the rapid deterioration and death of patients recently started on therapy. These patients developed symptoms of increased intracranial pressure, such as systemic hypertension, decreased sensorium, cranial neuropathies, sudden visual loss, and increasing headaches. They generally have normal CT scans without prominent cerebral edema. The exact pathophysiologic mechanisms are uncertain, but it is reasonable to postulate that the elevated subarachnoid pressures are caused by reduced CSF outflow due to increased outflow resistance (108). Although it is speculative, the possibility exists that a large burden of yeasts and CPS may block CSF uptake by the arachnoid villi and lymphatic drainage. It is also possible that treatment may exacerbate this blockage by increasing the number of dead, clumped organisms which plug the resorptive membranes. Similar cases probably occurred prior to the AIDS era, but with HIV infection, the immune defect is so profound that large numbers of organisms ($>10^6$ CFU/ml) and high concentrations of CPS

accumulate in the subarachnoid space to help augment the severe and often sudden elevation of intracranial pressure.

Clinicians must be aware of the intracranial pressure, both initially at diagnosis and with any neurological deterioration during therapy, since death and other sequelae can occur rapidly. With initial signs and symptoms of elevated intracranial pressure, a lumbar puncture can produce dramatic relief of symptoms (494). After this maneuver, the best strategy is unclear, but it likely includes external drainage with a ventricular shunt or lumbar drain. If it is controlled in the acute stage, this problem will usually correct itself and does not become a chronic management issue. The use of corticosteroids has been suggested to reduce cerebral edema and the use of acetazolamide has been suggested to reduce production of CSF, but it has not been determined whether there is any benefit from these therapies. This potential problem demands expert neurosurgical assistance and aggressive management of the patient's intracranial pressures for a favorable impact and reduction of deaths during the first 2 weeks of treatment.

Since patients with cryptococcal meningitis may also present with subacute hydrocephalus, ventricular shunts may be necessary. In most cases, after antifungal therapy has been started, shunts can be inserted without a deleterious effect on the infection. Most shunts that are already in place before antifungal therapy will need to be removed to ensure cure, but individual cases may respond to systemic antifungal therapy alone (212).

Antigen titers. CPS antigen titers are excellent as diagnostic tests, but their use in treatment decisions is less clear and at times confusing (413). The initial titers probably correlate directly with the quantity of organisms in the subarachnoid space, as has been shown in animals (520). However, once treatment is started, the kinetics of cryptococcal polysaccharide antigen elimination is uncertain. Interestingly, despite its large molecular size, purified CPS, when inoculated into the subarachnoid space of normal rabbits, is rapidly eliminated after 1 to 2 days (369). Also, antigen levels in the serum do not contribute to antigen titers in the CSF (144). Therefore, persistently high CSF antigen titers during infection must reflect either a block in elimination of the antigen or continuous production of large amounts of antigen within the CNS. Regardless, neither specific antigen titers nor a drop in the titer should be used as a guide for the duration of treatment. Furthermore, as noted earlier, variations among antigen test kits will produce different endpoints. Because of this imprecision of the latex agglutination test, titers should not be used to direct treatment decisions or provide evidence of relapse (122, 412). Some patients may have antigen titers in the CSF that persist for months after the conclusion of successful treatment. Nevertheless, it is an encouraging sign when no antigen can be detected in the CSF of a treated patient.

Over the last few years, a new syndrome, isolated cryptococcal polysaccharidemia, has been described (350, 412). In high-risk patients, such as those infected with HIV, empirical screens of serum have been performed for evidence of CPS. In some areas of the world, such screening has been useful for diagnosis (112), and in other areas, the incidence of infection in high-risk patients is too low to justify screening for antigen (201). In the United States, this test has been used frequently as a screening test for febrile patients with HIV, since most AIDS patients with cryptococcal meningitis have detectable antigen in their serum. Patients at high risk for cryptococcosis have been identified with positive serum CPS antigen titers but negative clinical evaluation for cryptococcosis, including negative cultures of blood, urine, and CSF samples. The management of these patients is highly controversial. Our recommendation is to treat these patients if antigen titers are repeatedly positive in a patient at high risk for cryptococcosis, such as with HIV infection. The ability to use oral triazoles at a possibly earlier stage of illness outweighs the potential side effects of these drugs in noninfected patients. However, additional prospective evaluation of this subgroup of patients is needed because, although probable, it is not certain whether they are truly infected.

Antifungal drugs and cytokines. There is a clear and urgent need to continue to develop new, safe, specific fungicidal antimicrobial agents for cryptococcosis. The most recent, promising, preclinical group of antifungal agents, which inhibit synthesis of the cell wall polysaccharide β-glucan, do not have significant activity against *C. neoformans*. However, there remains the promise that studies in plants, chemical screens, or targeted drug research will lead to the discovery of new agents. The present challenge is to judiciously exploit the antifungal agents available, but also to attempt actively to improve the host immune response. Disseminated cryptococcosis is usually associated with immunosuppression, and the body of evidence in vitro and in animal models indicates that biological response modifiers or antibodies can have a positive effect on cryptococcosis. As discussed earlier, GM-CSF, IL-2, and IFN-γ can, directly or indirectly, stimulate host cells to inhibit or kill *C. neoformans*. There is also a growing roster of other cytokines, such as IL-12, which may benefit the host during infection with *C. neoformans*.

Specific monoclonal antibodies and serum therapy may have a positive effect on the prevention or treatment of experimental cryptococcosis (126, 127, 332, 333). More human studies will determine where these new agents can be used as adjunctive therapy with antifungal agents. These immunomodulators would be most beneficial following intravenous or intracisternal administration. The current experimental data support the initiation of clinical trials in this area. A preliminary trial with GM-CSF as adjunctive therapy with AMB for cryptococcal meningitis in AIDS patients suggested that sterilization of the CSF was accelerated in the presence of GM-CSF (485). Although this study was too small to draw specific conclusions about treatment, it represents an approach and research design with biological or immune enhancers that needs to be encouraged.

Prevention

Antifungal prophylaxis. Cryptococcosis is difficult to prevent because of its sporadic nature, occurrence during severe immunosuppression, and the meager epidemiological data on prevalence of infection in specific areas. However, in the AIDS era, and considering the less than satisfactory results of treatment, prevention has now been reexamined.

There is the possibility of antifungal prophylaxis. The first trial to evaluate prophylaxis with fluconazole to prevent cryptococcosis among patients with AIDS suggested a positive benefit (353), and a recently completed randomized trial has confirmed this benefit (1,. The importance of proving the efficacy and successful outcome of antifungal prophylaxis for cryptococcosis is essential before general recommendations can be made. Antifungal prophylaxis in certain high-risk groups may be useful, but it needs to be carefully considered. The analysis of any protocol will also need to consider costs, consequences to the underlying disease, prevalence of infection in the group at risk, and potential development of drug-resistant yeast flora.

Vaccine. Active immunization in the form of a vaccine is an ideal strategy for prevention among high-risk patients. For *C. neoformans*, polysaccharide-based vaccines have always had

poor immunogenicity. However, a cryptococcal GXM-tetanus toxoid conjugate vaccine has now been developed which appears to be highly immunogenic and to elicit high-affinity IgG antibodies that appear to show protection in murine models (113). This conjugate vaccine will need to be studied in both immunocompromised and immunocompetent patient populations. A major concern is the fact that some of the patients at highest risk may be too immunosuppressed to mount an adequate response. However, early administration of the vaccine in certain groups before immunosuppression develops should be possible. The protection afforded mice treated with the vaccine makes it the best candidate for trials in humans.

Humanized monoclonal antibodies. The technological ability to convert protective mouse antibodies to human derivatives portends the commercial development of protective and therapeutic antibodies. For prevention, passive therapy may be more difficult because the risk of infection is prolonged, and multiple reinfusions will be necessary. However, prophylactic gamma globulin has been used in pediatric AIDS patients to reduce infection. Furthermore, efforts continue to increase the half-life of the antibodies. These antibodies may also be useful as adjunctive therapy with other antifungal agents and/or to help eliminate cryptococcal polysaccharide from the host, since polysaccharide has been shown to enhance the growth of HIV in host cells (357, 395).

FUTURE PROSPECTS

One hundred years after its discovery, because of the AIDS epidemic, *C. neoformans* is causing more suffering now than at any time in history. For every million persons with AIDS, 50,000 to 100,000 or more will contract cryptococcosis, yet the current research on *C. neoformans* is broader and more intensive than ever. By the year 2000, we are optimistic that the epidemic of cryptococcosis will be under control, perhaps as a result of the development of a novel vaccine, effective immunotherapy, improvement in the delivery of current antifungal drugs, or methods of intervention based on knowledge of the virulence factors or genes of *C. neoformans*.

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