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Fungal Infections

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The incidence of invasive fungal infections has increased dramatically over the past two decades, mostly due to an increase in the number of immunocompromised patients.[1-4] Patients who undergo chemotherapy for a variety of diseases, patients with organ transplants, and patients with the acquired immune deficiency syndrome have contributed most to the increase in fungal infections.[5] The actual incidence of invasive fungal infections in transplant patients ranges from 15% to 25% in bone marrow transplant recipients to 5% to 42% in solid organ transplant recipients.[6,7] The most frequently encountered are *Aspergillus* species, followed by *Cryptococcus* and *Candida* species. Fungal infections are also associated with a higher mortality than either bacterial or viral infections in these patient populations. This is because of the limited number of available therapies, dose-limiting toxicities of the antifungal drugs, fewer symptoms due to lack of inflammatory response, and the lack of sensitive tests to aid in the diagnosis of invasive fungal infections.[1] A study of patients with fungal infections admitted to a university-affiliated hospital indicated that community-acquired infections are becoming a serious problem; 67% of the 140 patients had community-acquired fungal pneumonia.[8] There is also an increase in nosocomial fungal infections.[9]

Fungi are eukaryotic, unicellular to multicellular organisms that have chitinous cell walls, and reproduce asexually, sexually, or both ways. Fungal cells are larger and genomically more complex than bacteria. Their cell wall contains polysaccharides, proteins, and sugars, and their antigens are rich in complex polysaccharides and glycoproteins. Plasma membranes of fungi contain ergosterol, which is the primary target for antifungals such as amphotericin B. Although there are more than 1.3 million fungal species in the environment, only about 150 are known to be pathogenic to humans. For detailed taxonomy of the fungi, several texts are available.[10-12] The virulence factors of fungi resemble those of bacteria, such as possession of a capsule, adhesion molecules, toxins, free radicals, etc. Thus, fungi can elicit similar tissue reactions as bacteria, including acute exudative, necrotizing, and granulomatous reactions. Pathogenicity of fungi depends on the virulence of the particular fungus, the infecting dose, the route of infection, and the immune status of the host. Other factors that influence the pathogenicity include the coexistence of other infections in the host, the organs affected, and other underlying diseases. There is no clear-cut evidence that fungal infections are contagious, with the exception of dermatophytoses, pityriasis versicolor, and candidiasis of the newborn. However, accidental inoculation or direct contamination of an open wound can result in transmission of the etiologic agent of infection.[13] Hence care should be taken to avoid direct contact when handling contaminated bodily discharges and tissues.

Most pulmonary infections begin in the lungs following inhalation of aerosolized fungi from the environment.[12,14] Once the fungi reach the lungs, the infection can remain localized, or it can disseminate to produce severe systemic disease that is often fatal. Rarely, the gastrointestinal tract and skin may be the primary focus of systemic infection with secondary involvement of the lungs, especially in immunocompromised patients.

The discussion of fungi in this chapter is confined to invasive pulmonary infections. Fungi that cause invasive pulmonary infection can be divided in two main groups: (1) primary or true pathogens, and (2) opportunistic pathogens. The primary or true pathogenic fungi (also referred to as endemic fungi) infect healthy, immunologically competent individuals.[15,16] These fungi can be very aggressive, and produce severe disseminated and fatal infections in immunocompromised patients. While endemic fungi such as *Histoplasma capsulatum* and *Coccidioides immitis* are the most common, a number of other fungi have emerged as important, though less common pathogens. *Penicillium marneffei, Fusarium* species, *Scedosporium* species, and *Malassezia* species are increasing in incidence as opportunistic infections in immunocompromised hosts, especially in those with

AIDS.[17,18] Each of these fungi can cause systemic life threatening infections. In contrast, the opportunistic fungi cause infections in critically ill or immunosuppressed patients. Almost all opportunistic fungi gain entry through the lungs, except endogenous *Candida* spp. The most common opportunistic mycoses include candidiasis, aspergillosis, cryptococcosis, zygomycosis, pseudallescheriasis, fusariosis, and trichosporonosis.

The dramatic increase in opportunistic fungal infection in the latter half of the 20th century is related to several factors.[17,19,20] The important factors include immunosuppression of transplant patients, chemotherapy for malignancies, broad-spectrum therapy for bacterial infections, and the long-term placements of catheters for various therapies. Other conditions that predispose to opportunistic infections include malignancies, especially leukemia and lymphomas, chronic lung diseases, abdominal surgery, burns, diabetes mellitus, Cushing's syndrome, malnutrition, uremia, alcohol abuse with cirrhosis, drug abuse, congenital immunodeficiency syndromes, and AIDS.[5,18,21] Epidemiologic factors such as an increasing aging population and migration of susceptible persons into highly endemic areas also contribute to the increase in opportunistic infections.[22]

Host factors responsible for increased susceptibility to fungal infections include (1) a decrease in the number or functional impairment of mature granulocytes and mononuclear phagocytes, (2) depressed B-lymphocyte (humoral) immunity resulting in decreased production of immunoglobulins and impaired opsonization, (3) depressed T-lymphocyte (cell-mediated) immunity, (4) abnormal host immune-regulation,[20,23,24] and (5) neutropenia. Other factors include disruption of mucosal and skin barriers, disorders of complement system, and hereditary immune dysfunctions.[23,24]

Fungal infections in severely immunocompromised patients present with clinical features that are often different from immunocompetent patients. The tissue response is also different. There may be little or no inflammatory response with even massive infection, and there may not be granuloma formation. Additionally, coexisting infections are very common; therefore, the possibility of multiple infectious agents should never be overlooked. Special stains or immunostains for fungi and other organisms should be routinely used for demonstrating fungi and other infectious agents in severely immunocompromised patients.

Currently Available Diagnostic Methods

Currently available laboratory methods for diagnosing invasive fungal infections include (1) isolation of fungi in the laboratory, (2) histologic evidence of invasion, and (3) molecular diagnostic techniques. A combination of these approaches is recommended, but is not always possible.[25]

Isolation of Fungi by Culture

Most of the fungi that cause invasive infection can be cultured on standard laboratory media. One of the major advancements over the past two decades has been in the culture and recovery of fungi from blood.[1,26] Biphasic systems were the first advancement in the broth-based culture system. This was followed by the development of lysis centrifugation, involving the incorporation of a tube containing an anticoagulant and a lytic agent, centrifugation, and placement of the pellet on solid media. Although labor intensive and relatively expensive, this method has become the "gold standard" for recovery of fungi from blood.[27,28] Automated blood culture systems have been developed, that are superior in their recovery of yeast from blood.[29] Despite these advances in technology, isolation of fungi from blood cultures is an insensitive marker for invasive fungal infections, and often takes several days to weeks for growth, resulting in delays in treatment. Fungemia was found in only 28% of patients with autopsy-proven invasive candidiasis in one series, and only 58% of patients with two or more visceral infections had fungemia.[30] Most of the fungi that cause invasive pulmonary infections can be cultured on standard media; however, culture and characterization may take up to 2 to 4 weeks.

When a fungus is isolated, the question may still remain whether the isolate is an invasive pathogen, normal flora not causing infection, or an environmental contaminant. Often the specimen may not be sent to the laboratory for culture and entirely submitted for histologic evaluation. Pathologists therefore have to rely on their ability to recognize and identify fungi in smears and tissue sections. The presence of a fungus growing in tissue provides indisputable evidence of invasive infection. Histologic studies can also confirm the presence of other concomitant infections by bacteria, viruses, protozoa, and parasites. Finally, no other diagnostic test can determine whether the host response signifies invasion or allergic reaction. Although certain patterns of host reaction suggest that mycosis exists, there are no absolute criteria that permit an etiologic diagnosis with as much certainty as tissue diagnosis.

Histologic Diagnosis of Fungal Infections

Although different methods of diagnosis are available for fungal infections, the presence of a fungus in the tissue sections provides indisputable evidence of invasive infection. In addition, histologic studies can also confirm the presence of coexisting infections by other fungi, viruses,

bacteria, protozoans, and helminths in the tissue. Histology also provides evidence of host response to invasive infection or allergic reactions, for example invasive vs. allergic bronchopulmonary aspergillosis. Because of their size, chemical composition, and morphologic diversity, many fungi can be identified in tissue sections by either conventional microscopic examination of hematoxylin and eosin (H&E) stained sections, or by the use of special stains. In tissue, fungi usually occur either as hyphae, budding yeast-like cells, endosporulating spherules, or a combination of these forms.[31] Certain fungi can be specifically identified because they have a distinctive morphology in tissue. When classic forms are observed, an etiologic diagnosis can be made: for example, adiaspiromycosis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis capsulatum, histoplasmosis duboisii, paracoccidioidomycosis, penicilliosis marneffei, protothecosis, rhinosporidiosis, and sporotrichosis. In this group of fungi only one species of fungus is the cause of each particular mycosis. Other mycoses are caused by any of the several species of a genus, all of which appear morphologically similar in tissue sections. Although these mycologic agents cannot be identified as to species by conventional histologic methods, the disease that they cause can be diagnosed generically; for example, aspergillosis, candidiasis, and trichosporonosis. Still other mycoses are caused by any of a number of fungi belonging to different genera. These fungi appear similar, if not identical to one another in tissue, and although it is not possible to identify the etiologic agent, the mycosis can be named. This group includes diseases such as phaeohyphomycosis and zygomycosis.

Several special histochemical stains can be used to demonstrate fungi in sections. Detailed description of these staining procedures, which are given in many excellent texts[32–36] and manuals,[37,38] are not included here. Hematoxylin and eosin is a versatile stain that enables the pathologist to evaluate the host response, including the Splendore-Hoeppli phenomenon, and to detect other fungi and microorganisms.[39] It is the stain of choice to confirm the presence of naturally pigmented fungi, and to demonstrate the nuclei of yeast-like cells. With H&E, however it is often difficult to distinguish poorly stained fungi from tissue components, even at higher magnifications. When sparse, fungi are easily overlooked in H&E sections. Special stains for fungi are therefore essential for the histopathologic evaluation of unexplained inflammatory processes.

Special Stains

Most fungi can be readily demonstrated with any of the special fungal stains, the most common of which are Gomori's methenamine silver (GMS), Gridley's fungus (GF), and periodic acid-Schiff (PAS) reaction procedures. However, GMS is preferred for screening because it gives better contrast and stains even degenerated and nonviable fungi that are sometimes refractory to the other two stains. It also stains algae (*Prototheca* and *Chlorella* spp.), cyst walls of *Pneumocystis jiroveci* and pathogenic free-living soil amebas, the spore coat of most microsporidian parasites, intracytoplasmic granular inclusions of cytomegalovirus, *Actinomyces israelii* and related species, *Nocardia* spp., most *Mycobacterium* spp., and nonfilamentous bacteria with polysaccharide capsules such as *Klebsiella pneumoniae* and *Streptococcus pneumoniae*. Prolonged staining in the silver nitrate solution may be required to adequately demonstrate degenerated fungal elements such as the yeastlike cells of *Histoplasma capsulatum* var. *capsulatum* in residual pulmonary granulomas.

The main disadvantage of special fungal stains is that they mask the natural color of pigmented fungi, making it impossible to determine whether a fungus is colorless, hyaline, or dematiaceous (pigmented). Such a determination is crucial in the histologic diagnosis of mycosis caused by dematiaceous fungi such as phaeohyphomycosis.[10] As a rule special fungal stains do not adequately demonstrate the inflammatory response to fungal invasion. To counteract this, a GMS-stained section can be counterstained with H&E for a simultaneous study of the fungus and the host response. Calcific bodies that are sometimes found in caseous granulomas are also stained with H&E, and can be mistaken for yeastlike fungi. This is especially true when calcific bodies are apposed to give the false impression of budding yeasts, or when the bodies are laminated to give the appearance of a capsule or thick cell wall. Best stains to avoid this misinterpretation are GMS and GF, because the chromic acid used as an oxidizer in these stains dissolves the calcium, leaving the calcific bodies unstained.

Mucin stains, such as Alcian blue and Mayer's, or Southgate's mucicarmine, readily demonstrate the mucoid capsule of *Cryptococcus neoformans*. This staining reaction usually differentiates *Cryptococcus* from other fungi of similar morphology. In some cases, however, poorly encapsulated cryptococci in tissue sections may not stain positive with mucicarmine mucin stains.[3] Furthermore, mucin stains are not specific for *C. neoformans*; the cell walls of *Blastomyces dermatitidis* and *Rhinosporidium seeberi* are often colored to varying degrees with the mucin stains. However, the latter two fungi are nonencapsulated and morphologically distinct, and not ordinarily mistaken for cryptococci. The cell wall of *C. neoformans* contains silver reducing substances, possibly melanin precursors, and can be stained with the Fontana-Masson silver procedure for melanin.[40] This stain is especially useful in those cases of cryptococcosis with invasive yeast forms that do not have readily detectable capsules, the so-called dry variants. Such forms could possibly be

confused with nonencapsulated yeasts of similar morphology. Fontana-Masson and Lillie's ferrous iron stains for melanin can also be used to confirm and accentuate the presence of melanin or melanin-like pigments in the cell walls of poorly pigmented agents of phaeohyphomycosis in tissue sections.[41]

The cytoplasm of certain fungi in tissue sections, especially the yeastlike cells of *B. dermatitidis* and *H. capsulatum* var. *capsulatum* is variably acid fast.[42] However, these staining properties are inconsistent and should not be used for diagnosis. The cell walls of fungi, in general, are not acid fast. The morphologic features, size, and staining characteristics of some of the common fungi are presented in Table 10.1.

Autofluorescence

When examined under ultraviolet light, some fungi or fungal components in the H&E-stained tissue sections are autofluorescent.[43] Graham[44] reported bright green to yellow-green autofluorescence of *Aspergillus* species, *Candida* species, and *Coccidioides immitis*. When sections of these fungi were stained with the PAS reagent, bright yellow fungal autofluorescence against a deep red-orange background was observed.[45] Autofluorescence may help delineate sparse or poorly stained fungi in H&E-stained sections; however, this property is inconsistent and should not be used for definitive diagnosis. Most fungi in frozen or paraffin embedded tissue sections also stain nonspecifically with Calcofluor white, a cotton whitener that fluoresces under ultraviolet light.[46] This rapid and simple fluorescence procedure can be routinely used in the intraoperative examination of fresh-frozen tissues for fungi.

Immunoperoxidase Stains

This technique can be used to identify certain fungi in smears and in formalin-fixed, paraffin-embedded tissue sections.[47–49] This technique, however, up to now, has only limited diagnostic use.[50,51]

Immunofluorescence

Direct immunofluorescence (IF) can improve the diagnostic capability of conventional histopathology in the diagnosis of fungal diseases.[45,52] The IF procedure, which can

TABLE 10.1. Morphology and histologic stains for common pulmonary fungal infections

Organism	Tissue form		Histology stains that visualize organism			
	Size	Microscopic features	GMS/PAS	Mucin	H&E	Other
Histoplasma capsulatum	2–5 μm	Spherical to oval uniform yeast; narrow-based; single bud	+	–	–	
Blastomyces dermatitidis	8–15 μm	Double-contoured yeast forms with broad-based, single bud	+	–	+	
Cryptococcus neoformans	2–20 μm	Pleomorphic, narrow-necked single budding yeast forms with capsule	+	+	+/–	Fontana-Masson
Coccidioides immitis	Spherule: 60 μm	Spherules:		–	+	Fontana-Masson (occ)
		Mature	+/–			
		Immature	+/+			
	Endospores: 1–2 μm	Endospores	+/+	–	+/–	
Paracoccidioides brasiliensis	10–60 μm	Rounded yeast forms of variable sizes with double-contoured cell walls; multiple narrow-based buds "ship's wheel"	+	–	+	
Sporothrix schenckii	2–10 μm	Budding yeast forms: spherical, oval and elongated (cigar shaped)	+	–	+	Fontana-Masson (occ)
Candida albicans	Yeast 5–7 μm pseudohyphae 3–5 μm	Budding oval yeast forms, pseudohyphae and rare septate hyphae	+	–	+/–	Gram positive
Aspergillus spp.	Hyphae 3–6 μm	Septate, acute angle branching	+	–	+	
Phaeohyphomyces spp.	Hyphae	Pigmented hyphae	+	–	+/–	Fontana-Masson
Pseudallescheria boydii	Hyphae 2–5 μm	Septate, nonpigmented hyphae	+	–	+	
Penicillium marneffei	2.5–5 μm	Oval to elongated yeast, septate	+	–	+/–	
Fusarium spp.	3–8 μm	Septate hyphae	+	–	+/–	
Zygomyces spp.	10–25 μm	Mainly nonseptate hyphae, right-angle branching	+	–	+	

occ, occasional.

be performed on smears and on formalin-fixed, paraffin-embedded tissue sections, is very helpful in confirming a presumptive histologic diagnosis, especially when fresh tissues are not available for culture or when atypical forms of the fungus are seen. The Division of Mycotic Diseases, Center for Disease Control and Prevention, Atlanta, Georgia, and others have developed a broad battery of sensitive and specific reagents for detecting and identifying the more common pathogenic fungi. The IF procedure has several advantages. Final identification of an unknown fungus in replicate by using sections from the same block of tissue is possible within hours after H&E- and GMS-stained sections are initially examined. The need for time-consuming and costly culture procedures is often obviated by IF, and the hazards of handling potentially infectious materials are reduced when microorganisms are inactivated by formalin prior to IF staining. Prolonged storage of formalin-fixed tissues, either wet tissue or paraffin embedded, does not appear to affect the antigenicity of fungi. This antigenic stability makes possible retrospective studies of paraffin-embedded tissue blocks, and the shipment of specimens to distant reference laboratories for confirmatory identification. Details of the IF technique are given in several references.[53,54]

Molecular Diagnostic Techniques

Several molecular diagnostic techniques are now available for identification and confirmation of fungi.[55,56] Polymerase chain reaction (PCR) assays for the diagnosis of invasive fungal infections have been developed.[57–60] One of the targets is a highly conserved area in the 18S ribosomal DNA gene. Information about this gene has facilitated the design of primers that are specific for a wide variety of medically important fungi. The PCR-based assays are very sensitive, with detection limits down to 1 colony-forming unit (CFU) per milliliter, and a sensitivity of 100% and specificity of 65% documented in prospective trials.[61] Specific oligonucleotide probes have been developed to identify fungi that display yeast-like morphology in vivo.[62] Universal fungal primers ITS1 and ITS4, directed to the conserved regions of recombinant DNA (rDNA) were used to amplify DNA from *H. capsulatum*, *B. dermatitidis*, *C. immitis*, *P. brasiliensis*, *P. marneffei*, *Sporothrix schenckii*, *C. neoformans*, five *Candida* species, and *Pneumocystis jiroveci*. The PCR amplifications were detected colorometrically in an enzyme immunoassay format. With the exception of minor cross-reactivity, all probes were found to be highly specific. The question of excessive false-positive results with this high degree of sensitivity is under investigation. In one study, PCR for *Aspergillus* on whole blood samples was found to be highly sensitive for the detection and prediction of invasive pulmonary aspergillosis.[63] In this study the sensitivity appeared to correlate with the certainty of diagnosis as proven by tissue invasion. In addition, a PCR assay using the LightCycler technology is now available, reducing the risk of contamination and thus false-positive results. The real-time LightCycler PCR assay used with automated DNA extraction from serum provides better reliability and safety than the standard PCR test.[64]

Serologic Diagnosis

Serologic detection is limited in immunocompromised patients, since these patients do not mount a good antibody response during infection. Much of the research has focused on identifying antigens or metabolites that can be detected in the blood or urine during invasive fungal infection. Some of the recent markers developed for the diagnosis of invasive infections include (1,3)–β-D-glucan, a cell wall component of yeast and filamentous fungi detectable in the blood during invasive fungal infection. The reported sensitivity and specificity of the assay has been good, ranging from 78% to 100% and 88% to 100%, respectively.[65,66] This assay can detect invasive *Aspergillus, Candida, Fusarium, Trichosporon, Saccharomyces,* and *Acremonium,* but not *Cryptococcus.* In addition, for the diagnosis of invasive candidiasis, antigens or metabolites so far identified include cell wall mannoprotein (mannan), heat-labile antigen, D-arabinose, and enolase. β-glucan and mannan are available for identification of *Candida* antigen.[67–69] Unfortunately, none of the assays has performed well enough or has predictive value to be used routinely.[70–73] Several assays have been developed for the detection of galactomannan, a polysaccharide antigen component of aspergillus cell wall in the serum and urine of patients with invasive aspergillosis.[74–76] An enzyme-linked immunosorbent assay (ELISA) assay for detection of galactomannan showed a sensitivity of 65% to 100% and specificity of 81% to 100%.[74,77] Although the results for therapeutic monitoring are encouraging, there is an 8% rate of false-positive results.[76]

Specific Fungal Diseases

The morphologic features of the different fungi may be used for presumptive and in some cases definitive histologic diagnosis of fungi in tissues (Fig. 10.1).

Histoplasmosis Capsulati

Histoplasmosis was first described by a United States army physician in Panama in 1906, and has been subsequently reported worldwide.[78] In the U.S., the infection is most common in the southeastern, mid-Atlantic, and central states, with approximately 500,000 new infections

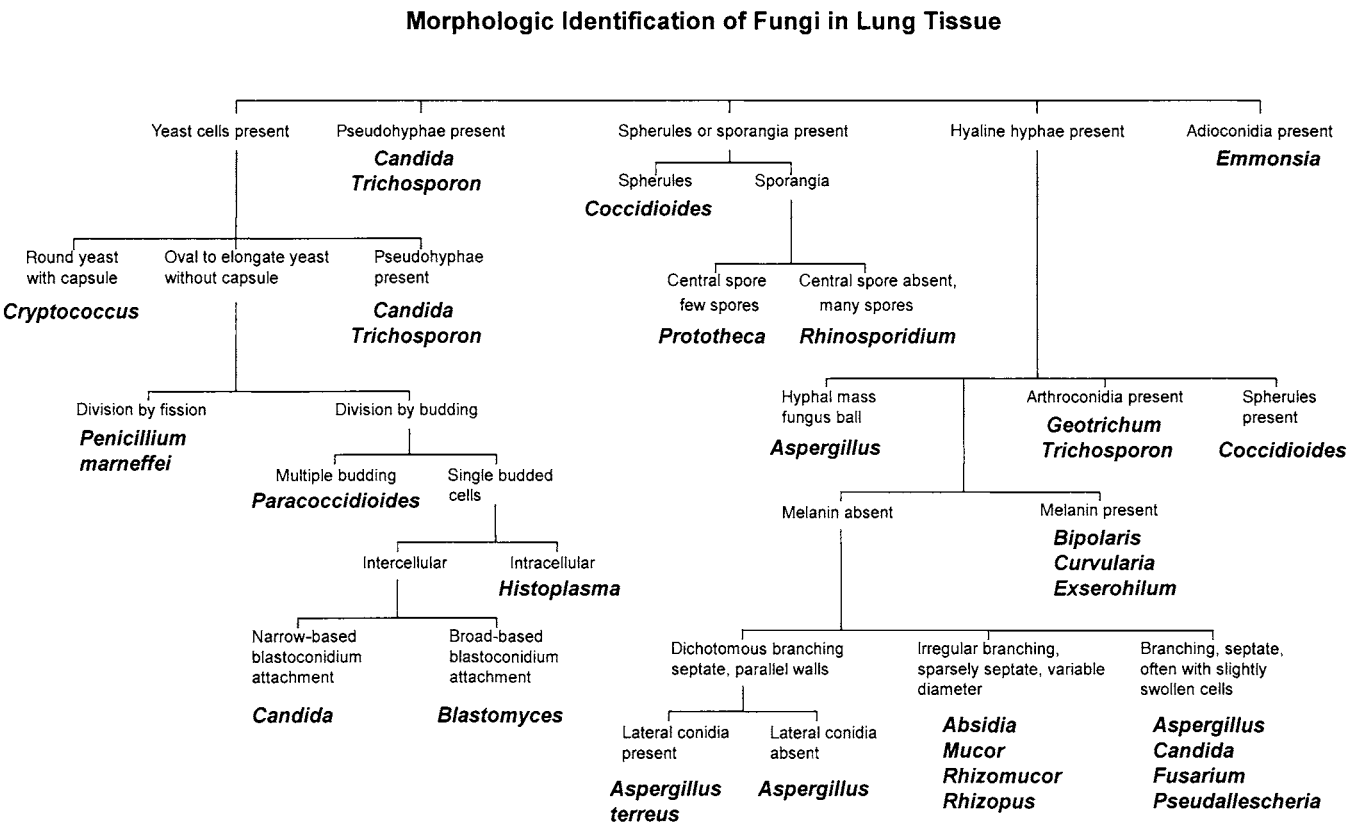

FIGURE 10.1. A flow chart for morphologic diagnosis of fungi in tissues.

occurring each year.[79,80] The Mississippi and Ohio River valleys are highly endemic areas. Other endemic countries in the Western Hemisphere include Guatemala, Mexico, Peru, and Venezuela.

Histoplasmosis is a respiratory infection caused by inhalation of infectious conidia or mycelial fragments of *H. capsulatum* var. *capsulatum*.[80] Avian and chiropteran habitats such as chicken coops, roosting shelters, caves, and attics favor growth and multiplication of the fungus in soil enriched with fecal material. The clinical spectrum of acute histoplasmosis varies according to the extent of exposure, presence of underlying lung disease, immunity to the fungus, and the host immune status. With a low exposure dose, most healthy individuals remain asymptomatic; however, with heavy exposure, a severe and potentially fatal pulmonary infection may develop.

In this chapter, the term *histoplasmosis capsulati* refers to infection by the classic, small-celled, *capsulatum* variety of *H. capsulatum*. Disease caused by the large-celled African, or *H. duboisii* variety of fungus is discussed separately, because it is a distinct clinical and pathological entity. Histoplasmosis duboisii is seen in humans and nonhuman primates in the African continent, where both varieties of histoplasmosis exist. The two varieties are indistinguishable in culture and can be identified only by the different size of their yeast forms in tissue.[80] There is no evidence that either mycosis is contagious.[10]

Microbiology

H. capsulatum var. *capsulatum* is a dimorphic fungus. It grows as a mold in soil and in culture at 25°C, and as budding yeast 2 to 4 μm in diameter in human tissues and in culture at 37°C on enriched media. On Sabouraud glucose agar, mycelial-form colonies are downy, white to golden brown, and produce two types of conidia: large (8 to 14 μm), thick-walled, tuberculate macroconidia with digitate protuberances; and small (2 to 4 μm), smooth-surfaced microconidia.[80]

Pathogenesis

The pathogenesis of histoplasmosis has been clearly established.[81–83] When infective soil or other particulate matter is disturbed, aerosolized conidia are inhaled and germinate in the alveolar ducts and alveoli. The yeast-like cells of the fungus are formed and then can proliferate, enter the lymphatics, and then enter the circulation via the lymphatics. Once in circulation, the fungus can disseminate to various organs and become sequestered in the phagocytic macrophages of the liver, spleen, lymph

nodes, and bone marrow, where organisms are phagocytosed and removed from the circulation. Dissemination is usually clinically silent and self-limited, and may result in the development of granulomas and calcifications in the infected tissues, especially the liver and spleen, often incidentally found at biopsy or autopsy.[84,85] Within 10 to 14 days after exposure, cellular immunity to *H. capsulatum* develops at the site of primary and disseminated infection with formation of granulomas, caseation, and later fibrosis.[81,82] Calcification of the necrotic granulomas may occur in the lungs, liver, spleen, and the hilar lymph nodes. Progressive disseminated infection occurs in the absence of effective cellular immunity, at the extremes of age, and in those with heavy exposure.[86,87] Endogenous reinfection may also result in disseminated infection in immunosuppressed patients.[88–93] In those with preexisting cellular immunity to *H. capsulatum*, a heightened immune response occurs, accounting for a shortened clinical course, despite a diffuse, intense inflammation of the pulmonary parenchyma.[84,91,92]

Clinical Features

Almost 75% of new infections are asymptomatic, self-limited pulmonary infections that heal without treatment. The remaining 25% of patients develop symptomatic disease that may present as (1) acute pulmonary disease, (2) disseminated disease, (3) chronic pulmonary disease, or (4) fibrosing mediastinitis. The clinical sequelae following inhalation of *H. capsulatum* conidia are shown in Figure 10.2.

Acute Pulmonary Histoplasmosis

Patients are often asymptomatic,[84] with a positive skin test as the only indication of exposure. Asymptomatic, skin test positive patients have normal chest radiographs in 90% of cases, and lack detectable serum histoplasma-specific antibodies in 80% of cases.[94] Heavily exposed patients develop fever, chills, headache, myalgia, arthralgia, anorexia, and cough and chest pain between 1 to 4 weeks after exposure.[84,91,92] A primary complex consisting of hilar lymphadenopathy and a single small area of pulmonary infiltration may be seen in the chest radiograph; however, cavitation is rare.[95] Symptoms typically resolve in a few days to 2 weeks, but fatigue and asthenia may persist for months in severe infection. Pathologically, the acute lesion is characterized initially by bronchopneumonia with neutrophil infiltration, followed by macrophages, lymphocytes, and plasma cells; granuloma formation with distinctive giant cells occurs at 2 weeks and beyond. Caseous necrosis in the granulomas occurs later during the second month.[84] Chest radiographs may show patchy, nodular pulmonary infiltrates, and nodules measuring 1 to 4 cm in diameter, but rarely cavitation.[96,97] Pleural effusions may be seen, but organisms are rarely isolated from the pleural fluid.[98] The time course for resolution of the pulmonary lesions or the effect of antifungal therapy on the resolution has not been adequately studied. Anecdotal experience suggests that infiltrates clear in 2 to 4 months, while adenopathy may persist for years.[84]

Disseminated Histoplasmosis

This disease is seen in patients with impaired cell-mediated immunity, such as associated with hematologic malignancies, chemotherapy, solid organ transplants, AIDS, very young children, and sometimes in apparently healthy adults who may develop transient immunosuppression from a coexisting viral infection.[17,87,91,92,97] Hematogenous dissemination is characterized either by slowly

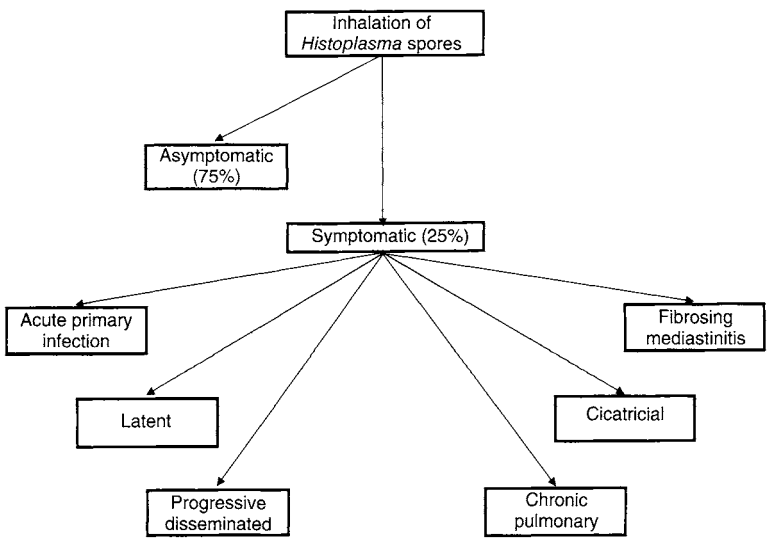

FIGURE 10.2. Clinical sequelae of inhalation of *Histoplasma capsulatum* conidia.

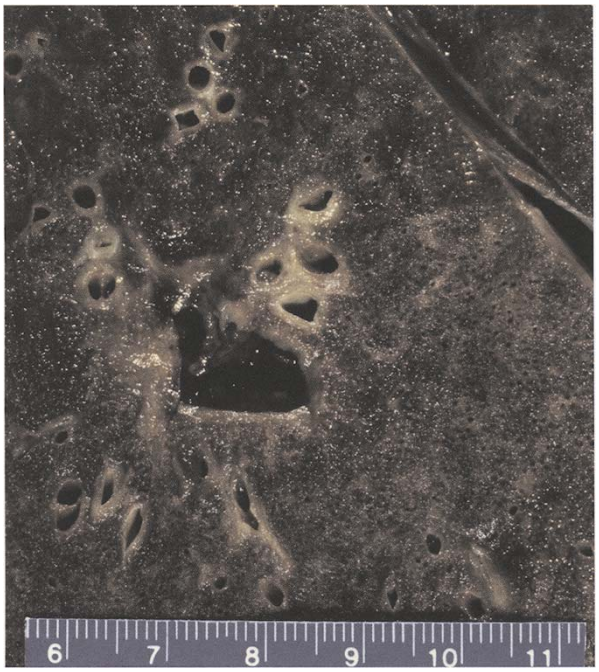

FIGURE 10.3. Disseminated histoplasmosis. Lung section from a patient with AIDS shows a central cavity and multiple pale pneumonic lesions.

progressive spread of infection to multiple organs, or by fulminating infection with rapid deterioration and death. The mortality rate can be up to 80% without antifungal therapy.[83,84,92,97] Clinically, patients have fever, chills, productive cough, hemoptysis, dyspnea, weight loss, malaise, headache, drowsiness, diarrhea, generalized lymphadenopathy, hepatosplenomegaly, purpura, and oropharyngeal and intestinal ulcerations.[87,92,99] Chest radiographs often show diffuse interstitial infiltrates, but rarely may be normal.[85,100] Lungs at autopsy may show edema, congestion, and granulomas (Fig. 10.3). Bone marrow suppression with cytopenia may be seen due to heavy parasitization of the monocyte macrophages. Patients may rarely develop adrenal insufficiency, and endocarditis.[101–103]

Chronic Pulmonary Histoplasmosis

This is primarily a disease of adults, in whom symptoms are similar to other chronic progressive pulmonary infections such as tuberculosis reinfection.[85,104] Four radiologic patterns have been described: infiltrative, cavitary, fibrosis with emphysema, and the residual solitary nodule or histoplasmoma.[85,100] The infiltrative pattern is frequently apical, appearing as unilateral mottled densities that may progress to become bilateral. In approximately 50% of cases, there may be thin-walled cavitations. The cavitary lesions represent endogenous reinfection similar to that seen in tuberculosis. The fibrotic lesions may be severe, and sometimes associated with emphysema and bronchiectasis (Fig. 10.4). The histoplasmoma ("coin" lesion) often resembles carcinoma, especially when it is noncalcified and enlarges progressively on sequential chest roentgenographs (Fig. 10.5).[104,105] These are often rounded and subpleural, they show stippled or concentric calcifications, and they are accompanied by enlarged and calcified hilar lymph nodes. Granulomatous and sclerosing mediastinitis or perihilar fibrosis can result from direct extension of infection from hilar lymph nodes (Figs. 10.6 and 10.7).[106,107] Calcified lymph nodes may erode through the adjacent bronchial wall as broncholiths (see Fig. 5.32 in Chapter 5).

Radiologic abnormalities, when combined with clinical findings, often allow for a presumptive diagnosis of histoplasmosis capsulati.[85,100,108] A large radiologic study of the primary complex in histoplasmosis concluded that larger and more numerous calcifications in the lung and hilar nodes strongly suggest histoplasmosis rather than tuberculosis; the primary pulmonary focus is usually solitary in the latter.[109] Other findings in histoplasmosis included miliary ("buckshot") calcifications, pleural thickening, and enlarged hilar nodes (Fig. 10.7). Uncommon chest radiographic findings in progressive infection include broncholithiasis, vena cava obstruction, esophageal compression, pericardial calcifications, and sclerosing mediastinitis. In a large series of chest radiographs of 770 schoolchildren in a highly endemic area for histoplasmosis capsulati, it was observed that 90% of the

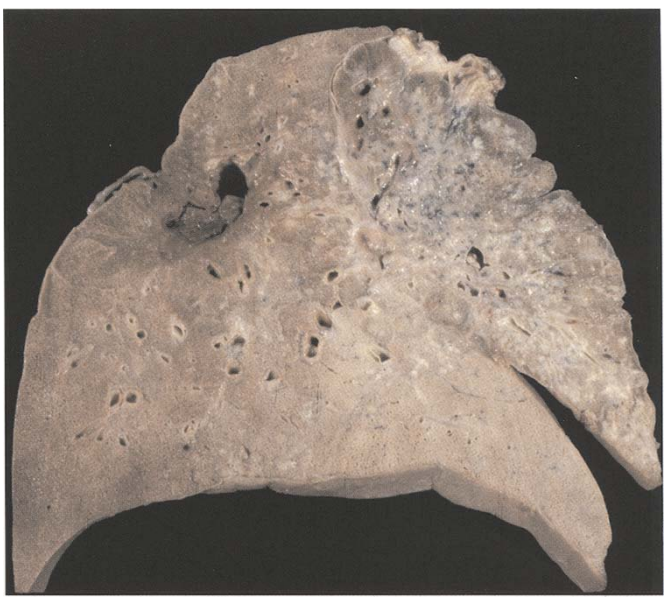

FIGURE 10.4. Chronic fibrocavitary histoplasmosis. There is a fibrotic and shrunken upper lobe with pleural fibrosis and bronchiectasis. A cavity with central necrosis is present in the superior segment of the lower lobe.

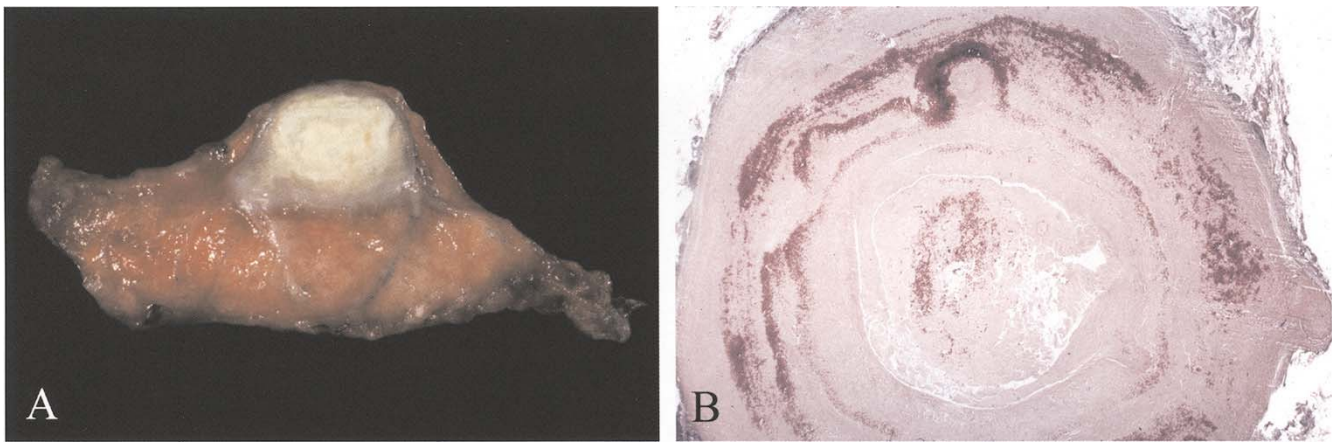

FIGURE 10.5. Histoplasmoma. **A.** Subpleural fibrocaseous nodule with thick fibrous capsule. **B.** Concentric calcific rings in the necrotic portion of the lesion.

children had a positive skin test for histoplasmin by age 14, and 75% of these had a normal chest radiograph.[110] The most common radiographic findings were solitary pulmonary calcification, unilateral pulmonary and hilar calcification (the primary complex), and rarely multiple pulmonary calcifications or bilateral hilar lymphadenopathy.

Fibrosing Mediastinitis

This is a benign disorder caused by proliferation of collagen within the mediastinum. Although most cases represent an excessive fibrotic response to a prior episode of histoplasmosis,[111,112] cases secondary to tuberculosis, zygomycosis, and Langerhans' cell histiocytosis are reported.[113–116] Approximately 80% of cases occur in persons between the ages of 20 and 40 years. The most common symptoms include cough, dyspnea, hemoptysis, pleurisy, and sweating. This rare manifestation of histoplasmosis is characterized by extensive fibrosis, resulting in entrapment and invasion of structures adjacent to mediastinal lymph nodes.[106] The heart, great vessels, and the esophagus may be involved. The disease is slowly progressive in most patients and often ultimately fatal.

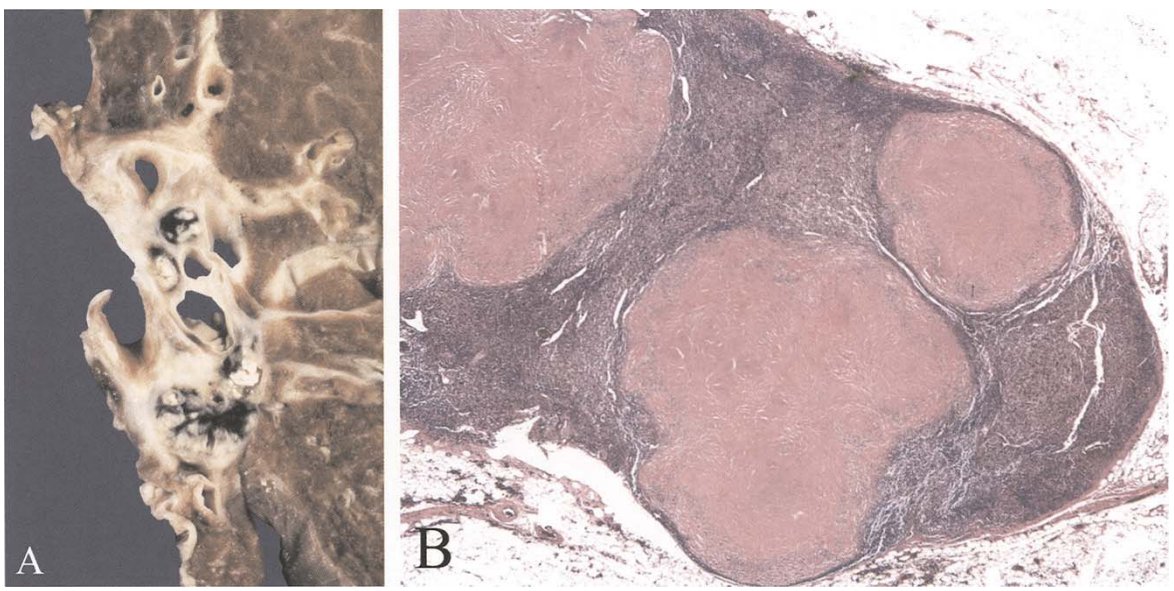

FIGURE 10.6. Hilar fibrosis. **A.** Chronic histoplasmosis with multiple fibrocaseous and calcified peribronchial lymph nodes and perihilar fibrosis. **B.** Fibrocaseous granulomas in hilar lymph node.

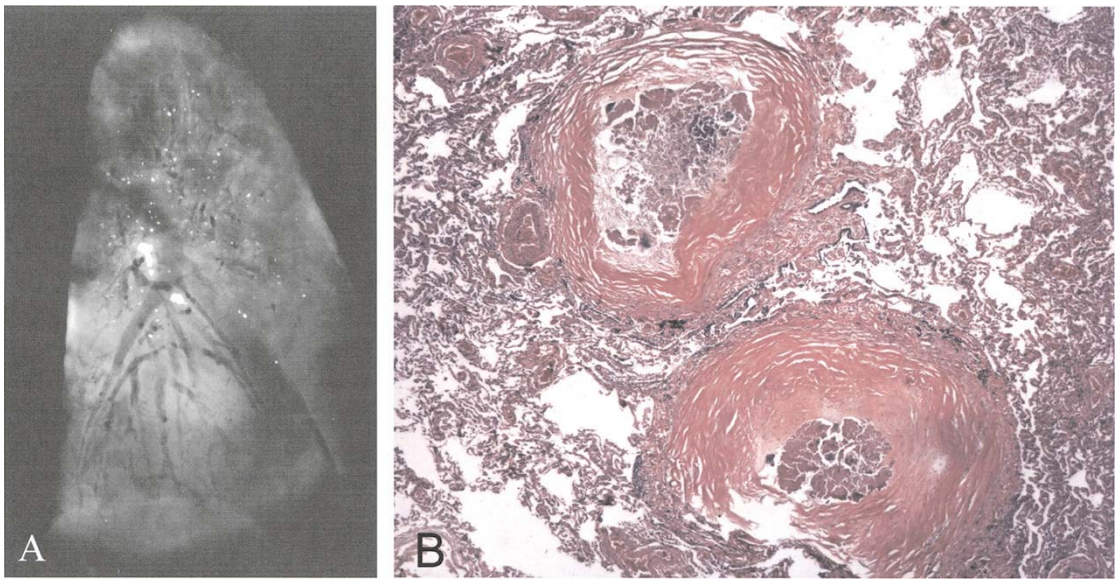

FIGURE 10.7. Calcifications. **A.** Specimen x-ray depicts large central hilar calcifications and numerous parenchymal miliary ("buckshot") calcifications. **B.** Dense fibrosis and calcification of miliary parenchymal histoplasma lesions.

Pathology

The characteristic histologic lesion of histoplasma infection is a granuloma, with or without central necrosis surrounded by lymphocytes, macrophages, and multinucleated giant cells (Fig. 10.8). The tissue form of the fungus is an oval 2- to 5-μm yeast that lacks a capsule and multiplies by budding at the end of the parent cell. The yeast often occurs in clusters because of the initial intracellular confinement within the mononuclear phagocytes, and the subsequent proliferation of the yeast within the phagocytic cells.[117] In H&E-stained sections, the basophilic cytoplasm is retracted from the thin, poorly stained cell wall, creating a clear space (halo) that gives the false impression of an unstained capsule. This halo effect is eliminated in sections stained with special fungal stains, all of which stain the entire cell wall. Poorly formed pseudohyphae consisting of a few cells attached to each other, and germ tubes are occasionally seen in active lesions that contain abundant budding cells. Rare hyphae and large irregularly shaped yeast forms have been described on or near the surface of valvular vegetations in histoplasma endocarditis.[118] The yeast stains strongly with the GMS stain, often seen with narrow-based budding, in the center of the granulomas (Fig. 10.8). Nonviable yeast forms within necrotic debris are also strongly positive. In fact, within the central area of old coin lesions, the cell walls of the yeast are often visible with GMS stain.

The host response to *H. capsulatum* var. *capsulatum* varies with the inoculum dose and the age and immunologic status of the host.[82,96] When many conidia have been inhaled, pulmonary lesions may be rapidly progressive, even in a nonimmunocompromised host and may contain large numbers of proliferating intraalveolar and interstitial yeast forms. In contrast, yeast cells are relatively sparse in mild, self-limited, chronic, progressive pulmonary infections. In the very young and in persons who are immunosuppressed, the host response is predominantly histiocytic and characterized by intracellular multiplication of the fungi. The dispersed infiltrates of yeast-laden histiocytes often efface the normal architecture of an organ, and widespread involvement of the mononuclear phagocyte system can occur (Fig. 10.9). In patients with profound cell-mediated immunodeficiency, for example in AIDS, the yeast forms multiply profusely and sometimes form extracellular "yeast lakes" associated with bland necrosis. In immunocompetent hosts and patients with the adult form of disseminated disease, the fungus usually elicits an epithelioid and giant cell granulomatous response with or without caseation (Fig. 10.8). Slowly evolving permanent granulomas may be followed by cavitation, fibrosis, emphysema, and calcifications (Fig. 10.7).

A histoplasmoma consists of a large central zone of caseous necrosis surrounded by a thick fibrotic capsule that contains lymphoid aggregates and rare epithelioid and multinucleated giant cells (Fig. 10.5).[94,105] The central caseous material may be calcified and may undergo osseous and myeloid metaplasia. When present, yeast cells are scattered in the caseous material, but are usually impossible to detect in H&E-stained sections. With GMS stain they are often poorly stained (so called ghost forms), distorted, fragmented, and most abundant in the caseous

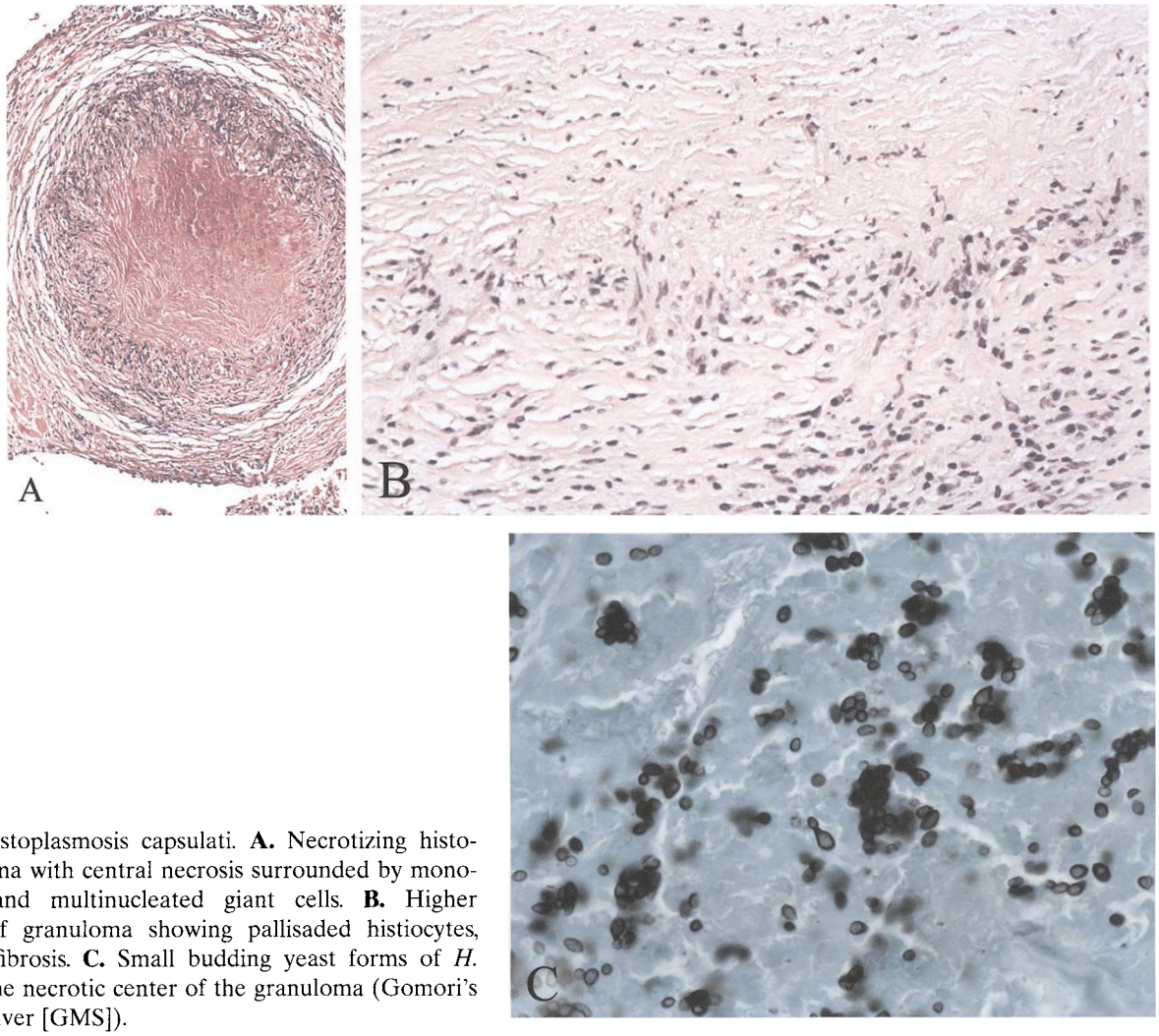

FIGURE 10.8. Histoplasmosis capsulati. **A.** Necrotizing histoplasma granuloma with central necrosis surrounded by mononuclear cells and multinucleated giant cells. **B.** Higher magnification of granuloma showing pallisaded histiocytes, giant cell and fibrosis. **C.** Small budding yeast forms of *H. capsulatum* in the necrotic center of the granuloma (Gomori's methenamine silver [GMS]).

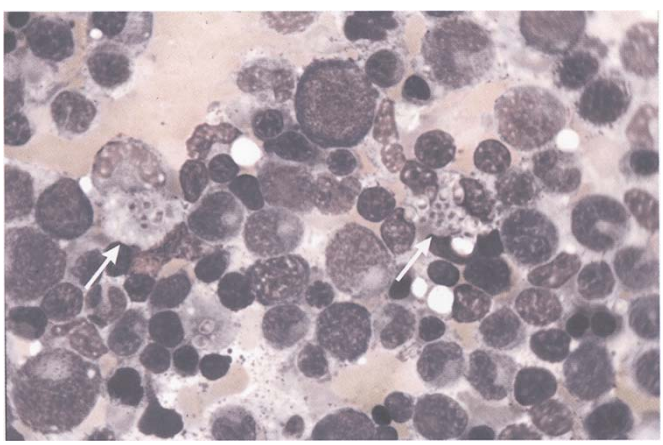

FIGURE 10.9. Disseminated histoplasmosis. Phagocytosed histoplasma yeast forms in histiocytes (arrows) are present in a bone marrow aspirate (Wright–Giemsa stain).

center of the nodule. In suspected cases that are initially negative on GMS stain, repeated staining, with extended time in the silver solution is sometimes successful in demonstrating degenerated organisms. Attempts to culture the fungus from these residual lesions are usually unsuccessful.

Differential Diagnosis

The differential diagnosis includes *Candida glabrata* and *Penicillium marneffei*. C. *glabrata* is of similar size but is amphophilic and stains entirely with the H&E stain without the halo effect. *P. marneffei* is also of similar size and shape but it does not bud to form blastoconidia as in *H. capsulatum*.[119] This dimorphic fungus, endemic in parts of Asia, divides by fission through the central portion of the yeast cell and can form short hypha-like or allantoid

forms that are up to 20μm (see below). The differential diagnosis also includes poorly encapsulated cryptococci; however, these organisms are round, have their blastoconidia attached to the parent cell by a narrow point, are pleomorphic in size, ranging from 2 to 20μm, and stain faintly for mucin by mucicarmine stain. Microforms of *B. dermitidis* are multinucleated with thick double-contoured walls and broad-based budding. *Leishmania* amastigotes may also be included in the differential if the patient has a history of travel to parts of the globe such as the Mediterranean, the Orient, or Africa. However, *Leishmania* organisms have dot-like intracellular basal bodies or kinetoplasts, and do not stain with GMS. *Toxoplasma gondii* may be confused with *H. capsulatum*, but these organisms stain entirely with H&E, do not bud, and like *Leishmania* spp. do not stain with GMS (see Chapter 14).

Diagnosis

Definitive diagnosis of histoplasmosis capsulati depends on the isolation of the etiologic agent from clinical specimens, or its demonstration in smears and tissue sections. Examination of Wright-stained peripheral blood smears may show yeast cells in monocytes and occasionally neutrophils in up to 50% of patients with severe disseminated infection.[120,121] In a study to optimize the diagnosis of histoplasmosis in tissue sections, sections of spleen from infected mice were examined by H&E, Grocott stain, Calmette-Guerin antibody immunostain, Fungiqual A fluorochrome stain, and a nested PCR assay. The nested PCR was the most sensitive method, but not significantly more sensitive than the Grocott stain, and the Grocott stain and fluorochrome stains did not differ significantly in detecting the fungus.[122] Ant-histoplasma antibodies may be detected in the serum of 90% of patients with histoplasmosis, resulting from a previous infection.[123,124] However, a single high antibody titer has no diagnostic or prognostic significance. Chemiluminescent probe assays and antigen detection in serum and urine are available for rapid diagnosis of *H. capsulatum*.[124–127] The immunodiffusion test, which is less sensitive than complement fixation (CF), identifies H and M precipitin bands to *H. capsulatum*. About 90% of patients with histoplasmosis have positive results by CF. Polysaccharide antigen by enzyme-linked immunoassay (EIA) can be detected in bronchoalveolar lavage (BAL) fluid of patients with diffuse pulmonary infiltrates, urine, serum, and cerebrospinal fluid (CSF). Levels of antigen in the BAL fluid can be considerably higher than in blood or urine.[124] Although the histoplasmin skin test has been useful for epidemiologic investigations of acute histoplasmosis capsulati or for mapping of epidemics, histoplasmin skin test reagents are no longer available.

Treatment

The acute pulmonary form of histoplasmosis capsulati is usually a benign, self-limited illness that heals without antifungal therapy. However, progressive pulmonary and disseminated infections require prompt treatment, and immunocompromised hosts may require prolonged courses of antifungal therapy.[128–131] Options for therapy include amphotericin B or one of its lipid formulations, and ketoconazole, itraconazole, or fluconazole.[132] Newer antifungal agents, particularly a new triazole, posaconazole (SCH56592), appears promising. Generally, amphotericin B or one of the lipid formulations is recommended for patients with more extensive disease, and itraconazole for those with milder disease, or for maintenance therapy after response to amphotericin B.[132] High-dose fluconazole can be used for mild disease; however, it is less effective than itraconazole and there is a concern of resistant isolates developing.[133] Treatment is indicated in all patients with chronic pulmonary histoplasmosis, because it can result in progressive loss of pulmonary function and death. Mediastinal granuloma and fibrosing mediastinitis also respond to antifungal therapy and corticosteroids. Options for therapy are the same as those listed above: amphotericin B or one of its lipid formulations, and ketoconazole, itraconazole, or fluconazole.[132] Long-term therapy with antifungal itraconazole may be helpful, and posaconazole also appears promising.[132] Surgical intervention in fibrosing mediastinitis is often associated with a high mortality. Surgical resection of cavities, however, is a valuable adjunct to antifungal therapy in patients with chronic cavitary pulmonary lesions.[83]

Histoplasmosis Duboisii (African Histoplasmosis)

African histoplasmosis is an important deep mycosis endemic in Central and West Africa and the island of Madagascar.[134,135] Infection is characterized by granulomatous lesions in the skin, subcutaneous tissues, bones, and rarely lungs and other organs, caused by a large-cell form (the *duboisii* variety) of *H. capsulatum*, first described and validated as a separate entity by Vanbreuseghem.[136] Diseases caused by both varieties of *H. capsulatum* occur in Africa, but they are discussed separately because each is clinically and pathologically distinct.[137]

Microbiology

It is believed that inhalation of microconidia, 2 to 4μm in diameter, produced by the saprophytic mycelial form of fungus in soil results in infection by this dimorphic fungus.

In culture, the mycelial and yeast form of *H. capsulatum* var. *duboisii* are indistinguishable from those of the

classic, small yeast-celled variety *H. capsulatum*.[31] The two varieties can be distinguished only by observing the differences in the size of their yeast forms in tissue, growth at 37°C, or DNA sequence data.

Clinical Features

With the exception of few cases, initial infection with *H. capsulatum* var. *duboisii* is known to occur naturally only in humans, bats, and nonhuman primates of the African continent.[138–141] Occasionally, *H. duboisii* infection may be seen in the United States and elsewhere in individuals who previously lived in or traveled to Africa.[142–144] Unlike classic histoplasmosis capsulati, pulmonary lesions in histoplasmosis duboisii are uncommon, and when detected are often subtle.[140,141] Few cases of pulmonary involvement have been reported, with mediastinal lymphadenopathy in one case.[145] The chest radiographs may show diffuse infiltrates in both lung fields and multiple granulomas with caseous centers at autopsy. Lanceley et al.[146] reported miliary opacities throughout both lung fields and enlarged hilar shadows in the x-ray of an east African child with histoplasmosis duboisii. This was the first reported case that was culture proven and the first with radiologic evidence of pulmonary involvement in which the typical cells of *H. capsulatum* var. *duboisii* were demonstrated in disseminated lesions. Since then, a few hundred cases have been reported.[147] The fungus is known to occur naturally in soil mixed with bat guano in bat caves.[148]

There are two clinical forms of histoplasmosis duboisii: localized and disseminated. Patients with the localized form usually have lymphadenopathy, mucocutaneous ulcers, and osteolytic lesions, particularly involving the cranium, ribs, sternum, scapula, vertebrae, and long bones.[137,149,150] Multiple skin ulcers frequently communicate with subcutaneous abscesses that are secondary to osteitis and osteomyelitis. Osteoarthritis, fistulas, and draining sinuses may also occur. Hematogenous or lymphatic dissemination from pulmonary or localized foci is often fatal and can involve any organ, particularly the spleen, liver, lung, and intestine.[151] Patients often have hepatosplenomegaly and prominent lymphadenopathy.

Pathology

The lesions are microscopically similar in all organs. Typically there is disseminated granulomatous inflammation, in which large numbers of yeast cells, measuring 8 to 15 μm in diameter are seen within histiocytes and large multinucleated giant cells of both foreign body and Langhans' type. Irregular foci of caseous necrosis containing yeast forms may also be present. In the lung, slowly evolving granulomas may be accompanied by fibrosis, cavitation, and rarely calcification. Involvement of thoracic lymph nodes, pleura, and adjacent bone may also occur. Organisms of *H. capsulatum* var. *duboisii* are uninucleate, have thick walls, and bud by a relatively narrow base to create a "double cell" form when daughter cells enlarge until they are equal in size to the parent cells, to which they remain attached, also referred to as "hourglass" cells (Fig. 10.10). The yeast cells are usually abundant and easily demonstrated in smears of exudative lesions. Tissue forms of *H. capsulatum* var. *duboisii* may be mistaken for *B. dermatitidis*, however, *B. dermatitidis* is multinucleated and its blastoconidia are attached to the parent cells by a broader base. *H. capsulatum* var. *duboisii* may also resemble the capsule-deficient form of cryptococcus. Positive staining of cryptococcus with Fontana-Masson stain may be helpful in this situation. Tissue forms of *H. capsulatum* var *capsulatum* are much smaller (2 to 4 μm) than those of *H. capsulatum* var *duboisii*.

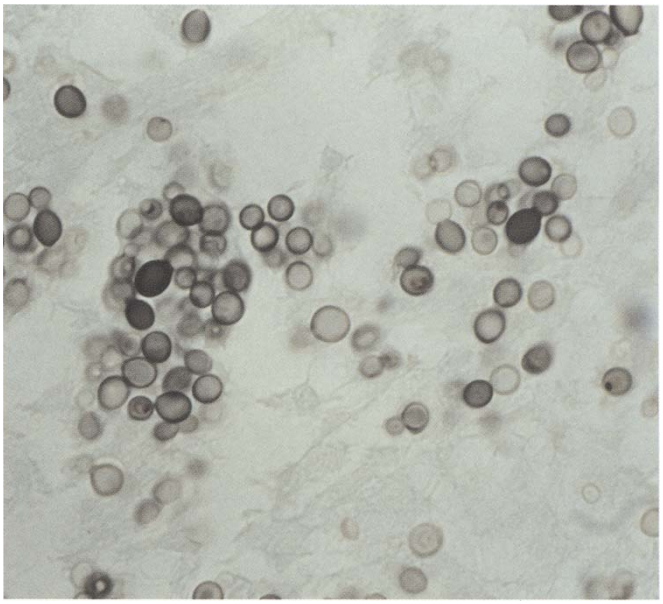

FIGURE 10.10. Gomori's methenamine silver stain of *Histoplasma duboisii* shows uninucleate, thick-walled yeast and narrow-based budding. A few "hourglass" forms are also seen.

Treatment

Amphotericin and azoles, ketoconazole, itraconazole, and fluconazole have been successfully used for treatment, although long-term treatment lasting 12 months or more is often necessary.[135,150,152]

Blastomycosis

Blastomycosis is a systemic infection caused by the dimorphic fungus *Blastomyces dermatitidis*. The major endemic areas in the United States include the southeastern, south central, and midwestern states, especially the

Ohio and Mississippi river valleys, but the endemic area may extend as far north as the Great Lakes region.[153–156] The natural habitat and precise ecologic niche of *B. dermatitidis* are not completely understood, but available evidence indicates that the fungus exists in nature as a wood saprophyte.[157]

In spontaneous infections, airborne conidia of the mycelial form enter the body via the respiratory tract and establish a primary focus of infection in the lungs. Rarely, primary cutaneous infection results from the accidental inoculation of the fungus into the skin and subcutaneous tissues. Person-to-person transmission of the disease is exceptionally rare, but has occurred by the transmission of the yeast in prostate fluid.

Sporadic cases have been reported in Africa, the Middle East, Asia, and Europe.[158–160] Blastomycosis is a sporadic disease, but small epidemics have been reported. Blastomycosis is reported in immunosuppressed patients, and in those with AIDS.[161–164] In a study of 143 patients with confirmed diagnosis in Canada, an increased incidence of blastomycosis was found in the Aboriginal population, with 93% presenting with respiratory infection.[165] Another study from Mississippi reported a predominance of black patients with blastomycosis.[166]

Although any age group can be affected, sporadic blastomycosis occurs most frequently in persons 30 to 50 years of age, and males are affected four times as frequently as females. The disease is most often seen in those who spend much of their time outdoors; however, in reported epidemics, blastomycosis is predominantly a disease of young persons and both sexes are affected with equal frequency.[156,167] Infection can be acquired with laboratory exposure.[168]

Microbiology

B. dermatitidis is a dimorphic fungus that develops in human tissues and in cultures at 37°C as a budding yeast 8 to 15 μm in diameter with thick cell walls and solitary blastoconidia attached by broad-based septum. When grown at room temperature, a mycelial-form develops that is white to tan, downy to fluffy, bearing round, smooth-walled conidia that are 3 to 5 μm in diameter along the hyphae on short simple conidiophores.[158,169]

Clinical Presentation

Blastomycosis is a great masquerader, presenting as either acute or chronic infection, and involving one or several organs.[153] Patients may have a variety of signs and symptoms, depending on the organs involved and the size of the fungal inoculum. Clinically apparent disease can be either systemic or cutaneous. Both forms have a pulmonary inception, but the clinical presentation, cause, and prognosis differ. Systemic blastomycosis is basically a pulmonary infection that initially remains confined to the lung and then disseminates via the bloodstream to other organs. The skin, bones, joints, lymph nodes, mucosal surfaces, male genital urinary tract, heart, adrenal glands, and central nervous system may be affected.[167,169,170] Untreated systemic blastomycosis is a severe, progressive, often fatal disease, with up to 90% mortality.[167]

The cutaneous form of blastomycosis presents as papular, pustular, or indolent ulcerative-nodular and verrucous skin lesions with elevated serpiginous borders, usually on exposed body surfaces.[167,169,170] In the cutaneous form of infection, pulmonary lesions are inapparent, systemic symptoms are absent or mild, and the general health of the patient is not impaired. Unless treated, the clinical course can run from months to years, with remissions and exacerbations and progressive increase in the size of cutaneous lesions.

The incubation period for both clinical forms of blastomycosis is unknown.

Acute Pulmonary Blastomycosis

This disease is usually either an asymptomatic or symptomatic but self-limited illness, even though the chest radiographs may be abnormal.[167,171,172] Hematogenous dissemination probably does not occur as frequently as in patients with acute histoplasmosis capsulati.[167] Following inhalation of infectious conidia, radiographs usually reveal patchy areas of primary consolidation that are often bilateral; pleural effusion and cavitation are uncommon.[171–173] The posterior segments of the lower lobes are most often involved, but the middle and upper lobes may also be affected. Often a wide variety of radiographic findings are seen, ranging from lobar consolidation to miliary infiltrates to large masses.[153]

Symptoms are nonspecific and range from those of a mild influenza-like illness to an acute pneumonia with high fever, productive cough, headache, myalgia, and chest pain that is often pleuritic. Symptoms usually persist from a few days to two weeks, but radiographic abnormalities may not resolve for several months. Some patients with self-limited pulmonary blastomycosis present with lesions at different sites, especially the skin and prostate, even up to 3 years after resolution of the pulmonary infection.[174] Thus, patients with self-limited primary infections should be followed for several years, and treated if they later show evidence of activity. Rarely, acute pulmonary blastomycosis has a more rapid clinical course, with widespread suppuration, necrosis, and cavitation of both lungs.[170,171,175] Overwhelming pulmonary infection may present with adult respiratory distress syndrome (ARDS).[176] In a study of 123 patients from Mississippi, which has the highest prevalence of blastomycosis in the United States, 8.4% developed ARDS with 78% mortality.[177] Extrapulmonary extension of infection may result in chronic fibrosing mediastinitis.[178]

Endobronchial spread of infection may be accelerated by ulcerative bronchitis, which is found in about one third of all cases. Respiratory failure and hematogenous dissemination to distant sites results in fulminating disease and death, often in spite of aggressive therapy.

Chronic Blastomycosis

Most patients with clinically apparent blastomycosis present with chronic respiratory symptoms of insidious onset that have persisted for weeks to months.[167,169,170] Symptoms include chronic cough, low-grade fever, dizziness, chest pain, anorexia, weight loss, and night sweats.[179] Chest radiographs often reveal mediastinal lymphadenopathy, linear pulmonary infiltrates, and fibronodular densities with cavitations that are indistinguishable from those seen in chronic active tuberculosis.[172,173] Cavities are usually thin-walled and calcification is extremely rare. Sometimes perihilar masses often mistaken for bronchogenic carcinoma may be seen.[180] Pleural involvement is common, and when severe is associated with an unfavorable prognosis.[181] Severe pulmonary infection in childhood resulted in abnormal pulmonary function tests in two of 17 children followed for up to 8 years following treatment.[182]

In addition to chronic pulmonary disease, about two thirds of patients have skin lesions, one third have bone lesions, and one fifth have lesions involving the genital urinary tract.[167,169,170] Involvement of one or more of these organs along with positive radiographic findings is an important clue to the diagnosis. Blastomycosis is reported as an opportunistic infection in patients with AIDS or other severe T-cell deficiency.[161–164] It is not, however, an AIDS-defining illness in these patients. The clinical presentation of primary pulmonary blastomycosis in immunocompromised patients is no different from that in immunocompetent individuals, unlike coccidioidomycosis and histoplasmosis capsulati infections.[167] Although primary cutaneous blastomycosis is usually a localized, self-limited infection, a few instances of widespread dissemination following accidental percutaneous inoculation have been reported in immunocompromised patients.[183] Patients with diabetes mellitus have an approximately sixfold increase in the risk of developing clinically apparent blastomycosis when compared to nondiabetics.[167]

Pathology

Infection with *B. dermatitidis* usually follows accidental inhalation of conidia from woody plant material. Transformation from the mold form to the yeast form occurs after deposition in the distal airways. Lesions in the lungs or elsewhere in the body are often nodular, and may mimic localized tumors, or may be scattered in a miliary pattern (Fig. 10.11). Histologically, the yeast may be seen both within and outside of phagocytic cells such as alveolar macrophages or neutrophils. The initial reaction of the tissues to blastomyces is by neutrophils, which are later replaced by monocytes and macrophages. Histologically, the lesions are characterized by abscess-like neutrophilic collections that are rimmed by epithelioid or palisaded macrophages or multinucleated giant cells (Fig. 10.12).[184] Yeast cells are usually numerous in the edge of the abscess. Around these foci may be nonspecific chronic inflammation. In contrast to tuberculosis and histoplasmosis, caseous necrosis and calcification are not usually seen. Chronic pulmonary lesions show fibrosis and hyalinization of the nodules, similar to that seen in other

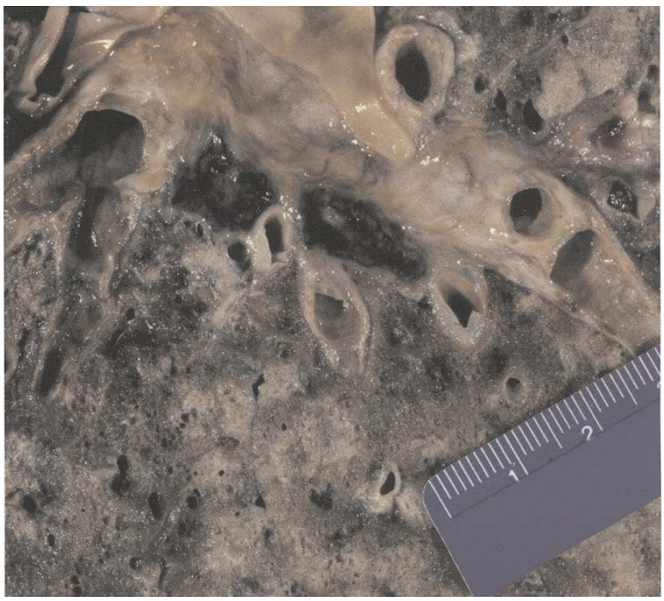

FIGURE 10.11. Acute blastomycosis. Lung section with blastomycosis has multiple tan nodules, some adjacent to airways.

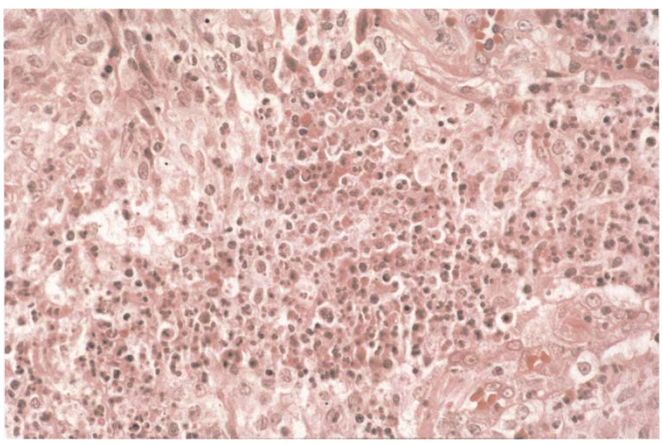

FIGURE 10.12. Blastomycosis. Dimorphic granuloma with central abscess-like aggregate of neutrophils.

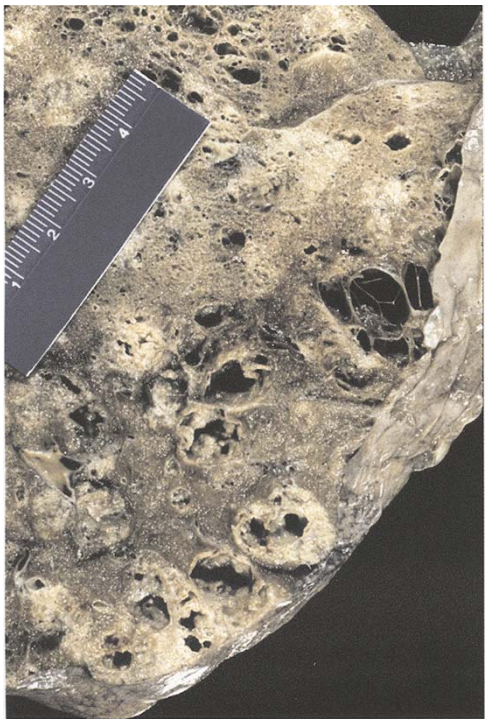

FIGURE 10.13. Chronic blastomycosis. Lung section shows fibrocaseous nodules, cavitary lesions, and pleural fibrosis.

chronic granulomatous diseases, and may be accompanied by cavitation (Fig. 10.13). The host response in early pulmonary and disseminated lesions is often exuberant, with distention of the alveolar spaces sometimes with an enormous number of proliferating yeast cells that elicit minimal host response, resembling the paucireactive picture often seen in cryptococcosis (Fig. 10.14).[185] Within days to weeks of inhalation, a granulomatous response develops around the fungi with epithelioid cells, lymphocytes, plasma cells, and multinucleated giant cells of both foreign-body type and Langhans' type surrounding the foci of suppurative necrosis and caseation (Fig. 10.12). A primary pulmonary–lymph node complex is sometimes found, but is much less frequent than seen in histoplasmosis capsulati.[185] In chronic pulmonary blastomycosis, fibrosis is common and is often accompanied by cavitation. Solitary residual fibrocaseous nodules (coin lesions) are rare, and calcification of such lesions is even rarer.[167,185] Massive proliferation of yeasts in the pulmonary parenchyma is seen typically in patients with ARDS.[166]

Diagnosis

To make a definitive diagnosis, *B. dermatitidis* must be demonstrated in a smear or tissue section, or isolated in culture. *B. dermatitidis* is easily found in both suppurative and granulomatous foci (Fig. 10.14) as intra- and extracellular, round to oval, multinucleated yeast cells, 8 to 15 μm in diameter with thick, refractile double contoured walls, and single broad based buds (Fig. 10.14). The broad-based attachment of the blastoconidia to their parent cells is diagnostic of *B. dermatitidis* and helps in differentiating it from yeast forms of similar size especially those of *H. capsulatum* var. *duboisii*. Occasionally, very small 2- to 4-μm but morphologically typical cells of *B. dermatitidis* are found in tissues as shown in Figure 10.15.[186] These can be confused with *H. capsulatum* var. *capsulatum*. These so-called microforms are almost always present as part of a continuous series of sizes ranging from the very small to the larger yeast forms typical of this fungus. Pseudohyphae and hyphae are rarely formed in tissues.[185,187] Occasionally, macroforms of *Blastomyces* may be present within abscesses, where the yeast form presents as a giant 20- to 40-μm form. Special stains are usually not needed to see *B. dermatitidis* yeast cells, however; GF, GMS, and PAS stains may help to locate the

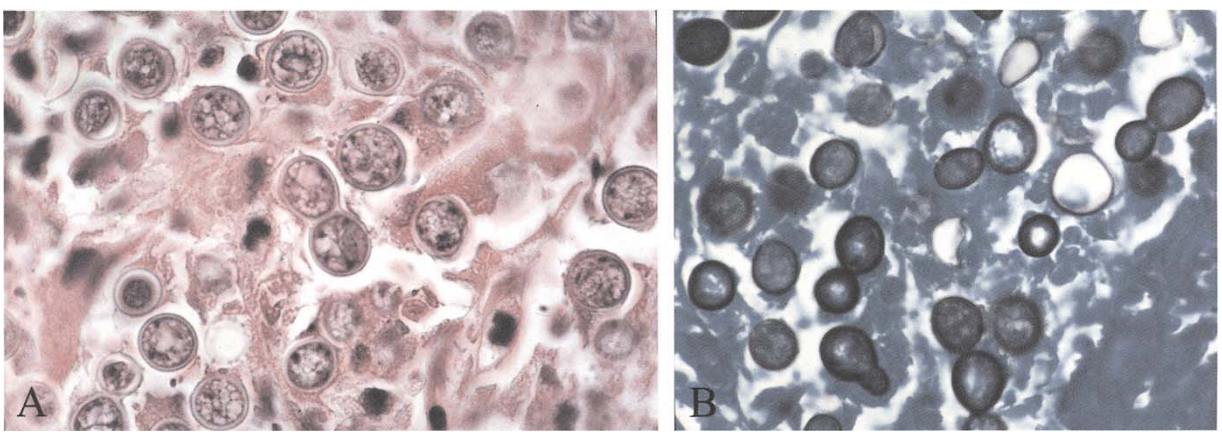

FIGURE 10.14. *Blastomyces dermatitidis.* **A.** Yeast forms with thick, doubly refractile cell wall and prominent nuclear content exhibit broad-based budding. **B.** Gomori's methenamine silver stain showing large yeast forms with broad-based budding.

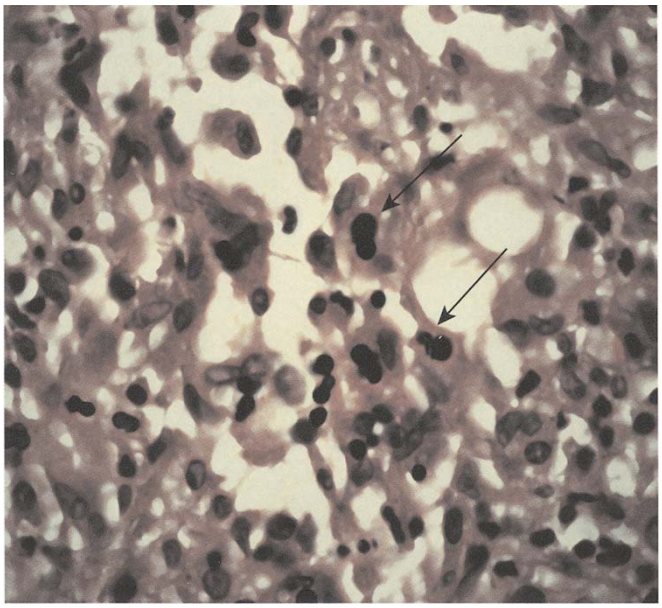

FIGURE 10.15. Small atypical form of *B. dermatitidis* shows round to oval budding yeast (arrows) (GMS with hematoxylin and eosin [H&E] counterstain).

organism in the lesions when they are sparse (Fig. 10.15). However, these stains may obscure the double contour wall as seen in the H&E stain.

In a retrospective study of 119 patients, culture of respiratory specimens obtained by noninvasive means, including sputum, tracheal secretions, and gastric washings, yielded a diagnosis of blastomycosis in 86% of patients.[188] Culture of bronchial secretions obtained during bronchoscopy yielded a diagnosis in 100% of 22 patients, while culture of BAL fluid was positive in only 67% of patients.[188] The yeast form of *B. dermatitidis* can be isolated from clinical specimens on standard mycologic media at 25°C, but growth may be slow, often requiring 2 to 4 weeks. Once isolated, the identity of the isolate as *B. dermatitidis* can be confirmed with DNA-based methods. This is necessary because fungi such as the *Chrysosporium* species are morphologically similar to *B. dermatitidis* in culture. Because *B. dermatitidis* and the other dimorphic fungi are not sensitive to cycloheximide, cultured media containing this antifungal agent can be used. More rapid diagnosis is achieved by demonstrating classic yeast cells with broad-based buds in clinical specimens from patients with either acute or chronic infection. Diagnosis of blastomycosis by direct microscopic examination of Papanicolaou stained smears of respiratory secretions is well documented; the fungi are seen as large pink or red, thick-walled yeasts.[153,189,190] When fungal elements are atypical or sparse, direct immunofluorescence using a specific conjugate directed against fungal cell wall polysaccharide antigens is valuable for identifying *B. dermatitidis* in smears or tissue sections.[184] Serologic tests using the purified *B. dermatitidis* (A) antigen are rapid, specific, and provide presumptive evidence of infection.[179,191]

The differential diagnosis of *B. dermatitidis* includes *H. capsulatum* var. *duboisii*, which is of similar size and shape; however, *H. capsulatum* var. *duboisii* has narrow-based budding. Also included in the differential diagnosis is *Cryptococcus*, from which *Blastomyces* can be distinguished by being negative with mucicarmine stain. *H. capsulatum* is much smaller and lacks the broad-based budding and double contoured cell wall. Blastomyces can be mistaken for *Coccidioides immitis*, especially in KOH preparations of tissue.[192] The presence of a coccidioidal spherule distinguishes these two fungi. Ancillary tests that may help in tissue sections include direct immunofluorescence antibody studies. Molecular methods are not contributory toward the pathologic diagnosis in tissue sections at this time, but PCR shows promise.

Treatment

Because acute primary blastomycosis is often mild and self-limited, antifungal therapy is not always required.[167] However, long-term follow-up is necessary since patients may present with extrapulmonary lesions months to years after resolution of the primary infection.[193] For patients with noncavitary pulmonary disease or disseminated lesions confined to the skin, itraconazole has been shown to be the drug of choice for both infections, except in cases of life-threatening infection, when amphotericin B should be used.[193–195]

Cryptococcosis

Cryptococcosis is a systemic infection caused by the yeast *Cryptococcus neoformans*, which is found worldwide, being especially abundant in avian, particularly pigeon, excreta.[196–198] The respiratory tract serves as the portal of entry for aerosolized cryptococci in almost all human infections.[197,199,200] There is a marked predilection for cerebral and meningeal dissemination from the primary pulmonary focus, which may not be clinically apparent.[198,200,201] Primary cutaneous infection is rare and results from direct percutaneous inoculation.

Although cryptococcosis is a cosmopolitan disease, the prevalence of clinically apparent infection appears to be highest in the United States and Australia.[196,202] Epidemiologic studies have shown that most infections are sporadic and occur in the young or middle-aged adults. Clustered outbreaks seldom occur; however, a recent outbreak has been reported in 59 mostly immunocompetent individuals from Vancouver Island in Canada.[203] The outbreak was caused by a rare subspecies, var. *gatti*. Although *C. neoformans* is pathogenic in apparently healthy individuals, it is more often encountered as an

opportunistic infection.[204–208] Disseminated cryptococcosis is almost never seen in the immunocompetent host, and between 40% and 85% of patients with disseminated cryptococcosis have defective cellular immunity or severe underlying disease.[200,209–212] Factors that predispose to opportunistic cryptococcosis include hematologic malignancies (especially Hodgkin disease), long-term corticosteroid therapy, sarcoidosis, diabetes mellitus, AIDS, and other conditions that are known to impair cell mediated immunity.[210,211,213]

Microbiology

Unlike most invasive yeast-like fungi, *C. neoformans* is not dimorphic. On Sabouraud glucose agar, isolates grow rapidly at either 30° or 37°C to form moist, smooth, mucoid, convex, and white to pale yellow colonies.[117] The colonies of capsule-deficient cryptococci are smaller, more convex, drier, and wrinkled. Microscopically, the colonies are composed of round to oval, thin-walled, yeast cells that are 2 to 20 μm in diameter, encapsulated, and have a single daughter cell attached to the parent cells by narrow necks. Chains of budding cells or rudimentary pseudohyphae are occasionally seen. The capsule varies in thickness from isolate to isolate, and is best demonstrated by using India ink to create the illusion of dark field microscopy, or by mucin stain such as mucicarmine or Alcian blue. Although *C. neoformans* is the organism that usually causes infection, two other species, *C. albidus* and *C. laurentii,* have occasionally been implicated in human infections.[214–216] The colonies and cells of *C. neoformans* cannot be distinguished morphologically from other nonpathogenic species. Specific identification is based on appropriate biochemical tests, immunofluorescence, and molecular studies.[197,200,217]

Clinical Features

Clinically, two forms of cryptococcosis are seen: pulmonary and cerebromeningeal.[200,201,212] Other organs less commonly involved by dissemination from a primary pulmonary focus include the skin, bones and joints, lymph nodes, kidneys, prostate, spleen, liver, and other internal organs. Skin lesions are seen in 10% to 20% of cases, and osteolytic lesions, particularly of the pelvis, ribs, vertebrae, and long bones occur in about 10% of cases of disseminated cryptococcosis.[201,218–221]

The spectrum of pulmonary involvement includes (1) transient, asymptomatic colonization of the tracheal bronchial tree without tissue invasion; (2) self-limited or progressive pulmonary disease with or without extrapulmonary dissemination; and (3) the residual pulmonary nodule or "cryptococcoma."[200,222]

Saprophytic colonization of the respiratory tract by *C. neoformans* has been reported to occur in about 1% of patients with preexisting bronchial pulmonary disease such as tuberculosis, chronic bronchitis, asthma, neoplasms, and allergic bronchopulmonary aspergillosis.[222,223] Thus, a positive sputum culture alone cannot be considered diagnostic of cryptococcosis, but it is highly suggestive of infection. These patients are at little or no risk of developing invasive infection, and antifungal chemotherapy is not indicated.

The majority of immunocompetent, apparently healthy patients with cryptococcosis are thought to have asymptomatic or mildly symptomatic but self–limited pulmonary infection for which antifungal therapy is not usually required. These localized infections either resolve spontaneously or encapsulate, to be detected months to years later in chest radiographs or incidentally at autopsy. Residual fibrocaseous nodules (cryptococcomas), are usually subpleural, discrete, rounded, 0.2 to 7.0 cm in diameter, and noncalcified.[200,224,225] A primary pulmonary lymph node complex develops in about 1% of patients with primary infection of cryptococcosis.[226]

Progressive pulmonary cryptococcosis usually has a subacute or chronic course and may be associated with concomitant extrapulmonary infection. About one third of patients with progressive pulmonary infections are asymptomatic.[200,212] The remainder usually present with chronic cough, low-grade fever, pleuritic or nonpleuritic chest pain, mucoid sputum, malaise, and weight loss. Chest radiographs may reveal alveolar interstitial infiltrates, single or multiple nodules that resemble neoplasms, segmental or lobular consolidation, and, less commonly, hilar adenopathy, pleural effusion, and empyema.[200,214,225,227–229] The upper lobes are reported to be more frequently involved. Fibrosis and calcification are uncommon and cavitation occurs in less than 10% of cases.[228] Diffuse interstitial, peribronchial, or miliary pneumonic infiltrates develop in profoundly immunodeficient patients who are exposed to a large inoculum.[228,230] The macroscopic features of miliary and chronic progressive pulmonary cryptococcosis are respectively seen in Figures 10.16 and 10.17. Chest radiographs often show a diffuse interstitial or perivascular pattern that suggests hematogenous dissemination. Massive pulmonary infection with rapid clinical deterioration, cerebromeningeal dissemination, and death may occur. In one series, 24 of 25 immunosuppressed patients with progressive pulmonary cryptococcosis developed cerebromeningeal infection, 2 to 20 weeks after radiographic documentation of the pulmonary infection.[212]

The most common clinical presentation of cryptococcosis is cerebromeningeal. For reasons that are poorly understood, *C. neoformans* is extremely neurotropic, involving the central nervous system via hematogenous spread from a primary focus that may or may not be clinically apparent. The leptomeninges are involved most often and infection may extend into adjacent brain parenchyma to form cryptococcal "mucoid cysts" and intra-

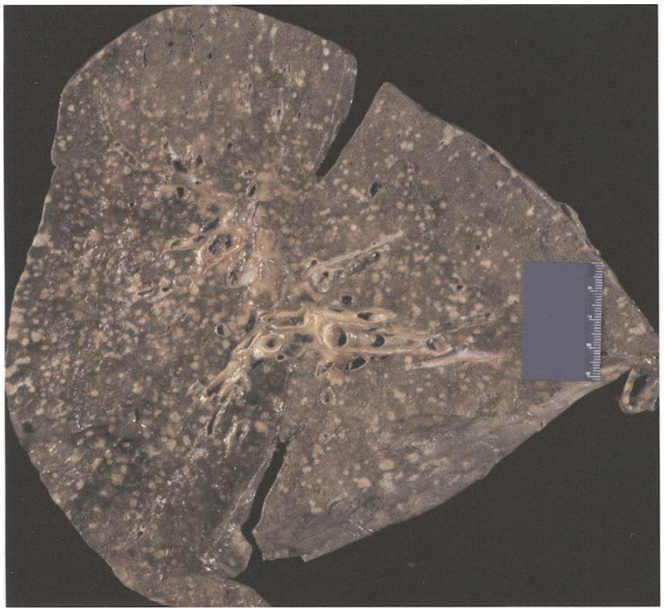

FIGURE 10.16. Lung section from a patient with AIDS shows a miliary pattern of cryptococcosis with uniform tan-colored nodules present throughout the lung.

cerebral mass lesions. The onset of symptoms is usually insidious, and the clinical course may vary from a few days to 20 years or more. In most patients, however, the course is fulminant and is almost always fatal unless promptly treated.[201] Presenting symptoms of cerebromeningeal cryptococcosis include fever, headache, altered consciousness, nausea, and vomiting. About 25% of patients undergo exploratory craniotomy because they have symptoms of an expanding intracranial lesion that mimics those of a neoplasm.

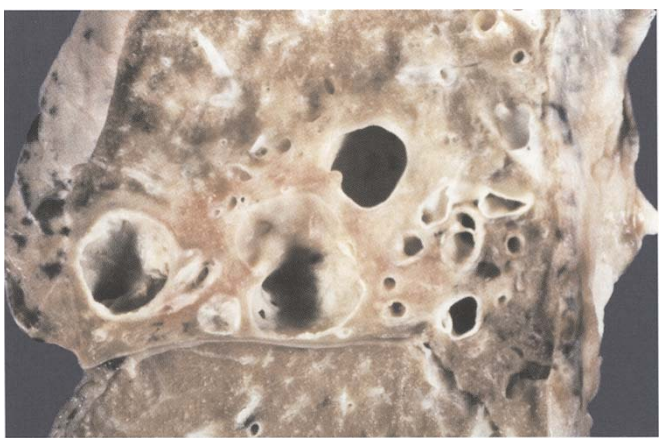

FIGURE 10.17. Chronic progressive pulmonary cryptococcosis with thin-walled abscess cavities and surrounding fibrous consolidation.

Pathology

The severe infections seen in AIDS patient may present as diffuse miliary lesions or as areas of patchy consolidation, which have a mucoid texture and appearance in freshly sectioned lungs. In H&E-stained tissue sections, typical cryptococci appear as pleomorphic, lightly eosinophilic or amphophilic, uninucleate, thin-walled, round yeasts that are 2 to 20 μm in diameter, surrounded by a wide, clear, and polysaccharide capsule (Fig. 10.18A).[226,231] Some yeast cells often appear as if one side has collapsed, resulting in an elliptical-shaped cell. Single blastoconidia are attached to parent cells by narrow necks (Fig. 10.18B). Active lesions contain large numbers of rapidly dividing cryptococci; however, short chains of budding cells may be rarely seen. *C. neoformans* cells are easily demonstrated with special fungus stains. The capsules react positively with mucin stains such as mucicarmine and Alcian blue; a histologic diagnosis is established because cryptococcus species are the only common pathogenic fungi that produce capsular material (Figs. 10.18C and 10.19). *Rhodotorula* species may have a capsule, but they are exceptionally rare pathogens. The mucin-positive capsule often has a spinous appearance because of irregular shrinkage due to tissue processing.

The host response to *C. neoformans* is variable and depends on the degree of cell-mediated immunodeficiency, the severity of underlying disease, and whether or not the fungus is encapsulated.[232,233] T-cell–activated macrophages probably play a major role in preventing progressive infection.[234] In those patients who are profoundly immunodeficient, progressive pulmonary or disseminated cryptococcosis develops and there is often a paucireactive pattern with little or no inflammation regardless of the organ involved. Cryptococci multiply profusely, displacing normal tissues, and form "cystic" lesions composed of densely packed, heavily encapsulated organisms that elicit little surrounding reaction, and contribute to cellular destruction by mechanical means (Fig. 10.20).[226] The organisms stain magenta red with PAS stain and are mucicarmine positive (Fig. 10.18C). In AIDS patients, an overwhelming cryptococcal infection may produce large "lakes" of organisms (Fig. 10.20) that stain deep blue with Alcian blue stain. The cryptococci may fill the alveolar spaces, and individual and clustered organisms may be seen also in thickened alveolar septa and within the lumina of septal capillaries (Fig. 10.19). This may be accompanied by a lymphocytic and histiocytic infiltration.

The initial lesion in individuals who do not have underlying immunodeficiency or other predisposing conditions, consists of an intense, focal inflammatory reaction with suppuration and necrosis of tissue[200] (Fig. 10.21). The lesion usually remains localized and either resolves or becomes granulomatous, and in time

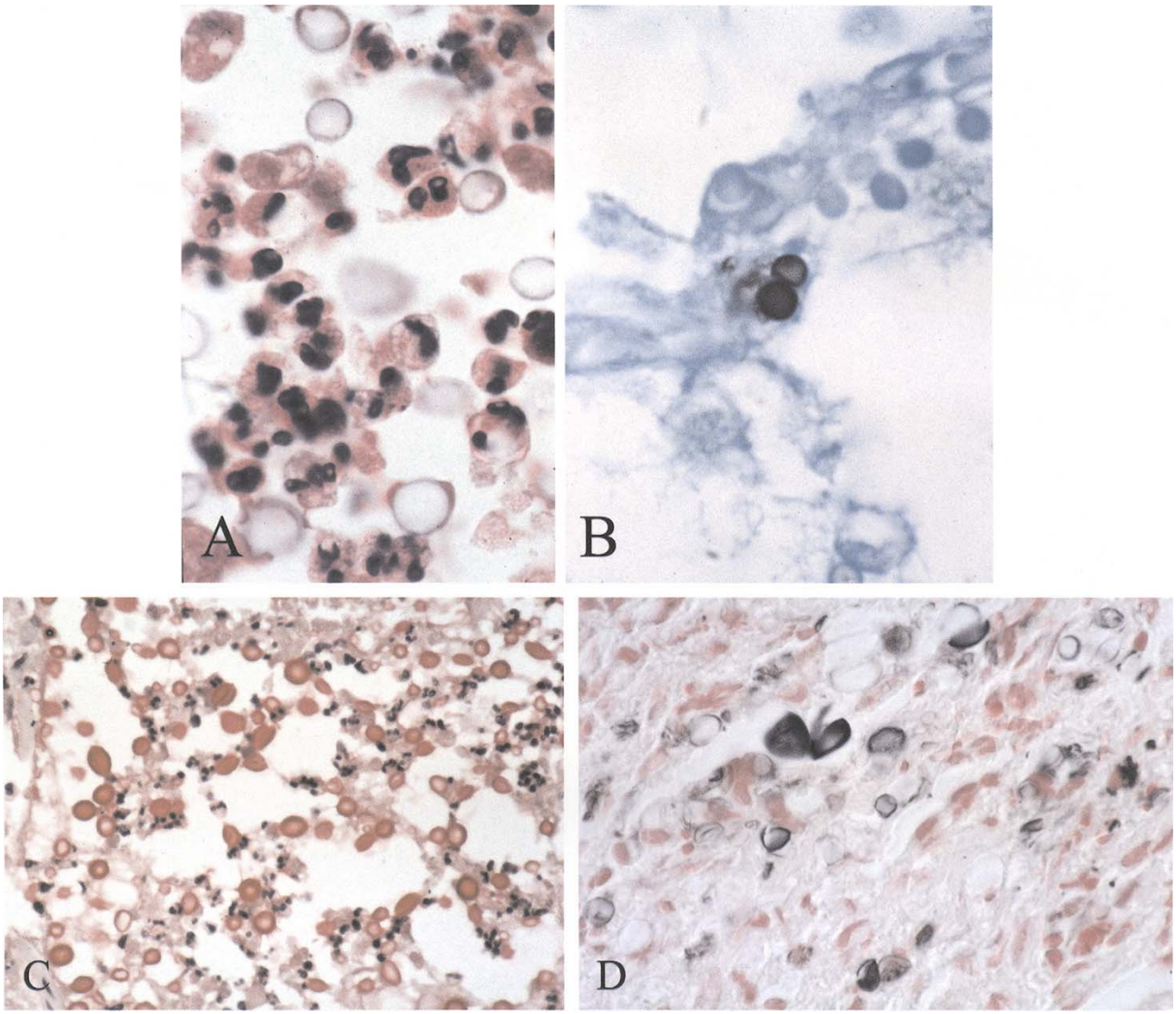

FIGURE 10.18. Cryptococcosis. **A.** Yeast-like organisms with peripheral halo are visible but unstained with H&E. **B.** Gomori's methenamine silver stain showing typical narrow-necked budding. **C.** Mucicarmine stain shows spiculated mucoid capsule. **D.** Fontana–Masson stain highlights cell wall, including that of capsule deficient forms.

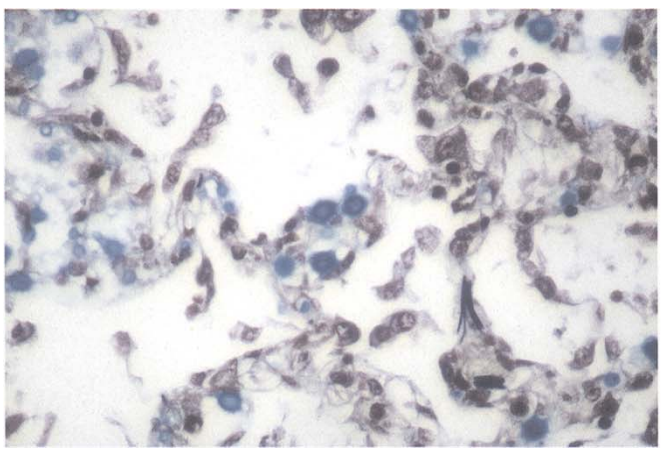

FIGURE 10.19. Alcian blue stain shows cryptococcal yeast forms in alveolar capillaries in severely immunocompromised patient. Alcian blue stains the mucoid capsule, a staining characteristic unique to cryptococcus.

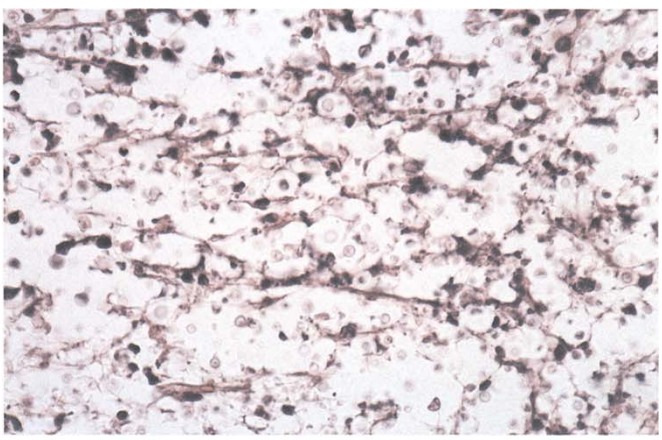

FIGURE 10.20. Paucicellular cryptococcal lesion in a hilar lymph node of a patient with AIDS. Myriad yeast forms and gelatinous capsular material have replaced the nodal tissue.

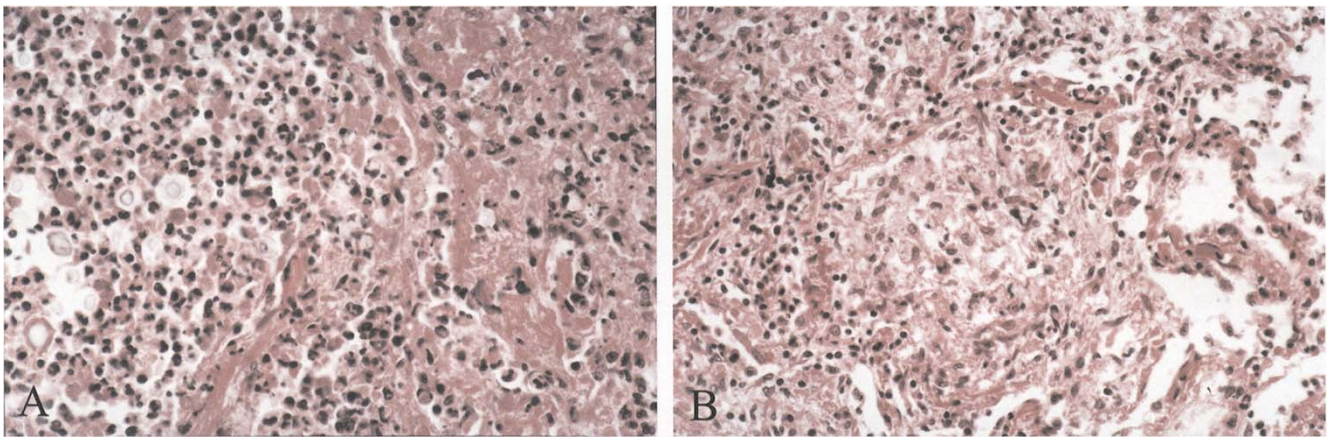

FIGURE 10.21. Cryptococcosis. **A.** Central area of cryptococcal lesion. Neutrophils surround encapsulated cryptococcal organisms. **B.** Periphery of lesion has a granulomatous appearance.

nodular and fibrocaseous. These firm grayish-white fibrotic nodular cryptococcomas with central necrosis and cavitation are similar to the lesions that develop in residual pulmonary histoplasmosis capsulati and coccidioidomycosis, but they are rarely calcified (Fig. 10.22).[200] Smaller satellite nodules may also be present but they are usually not connected to the bronchial tree. Cryptococci are not seen easily in these lesions with H&E stain; however, with GMS, varying numbers of organisms can be demonstrated within the central caseous material and at the nodule's margin, often within epithelioid histiocytes and multinucleated giant cells. The cryptococci

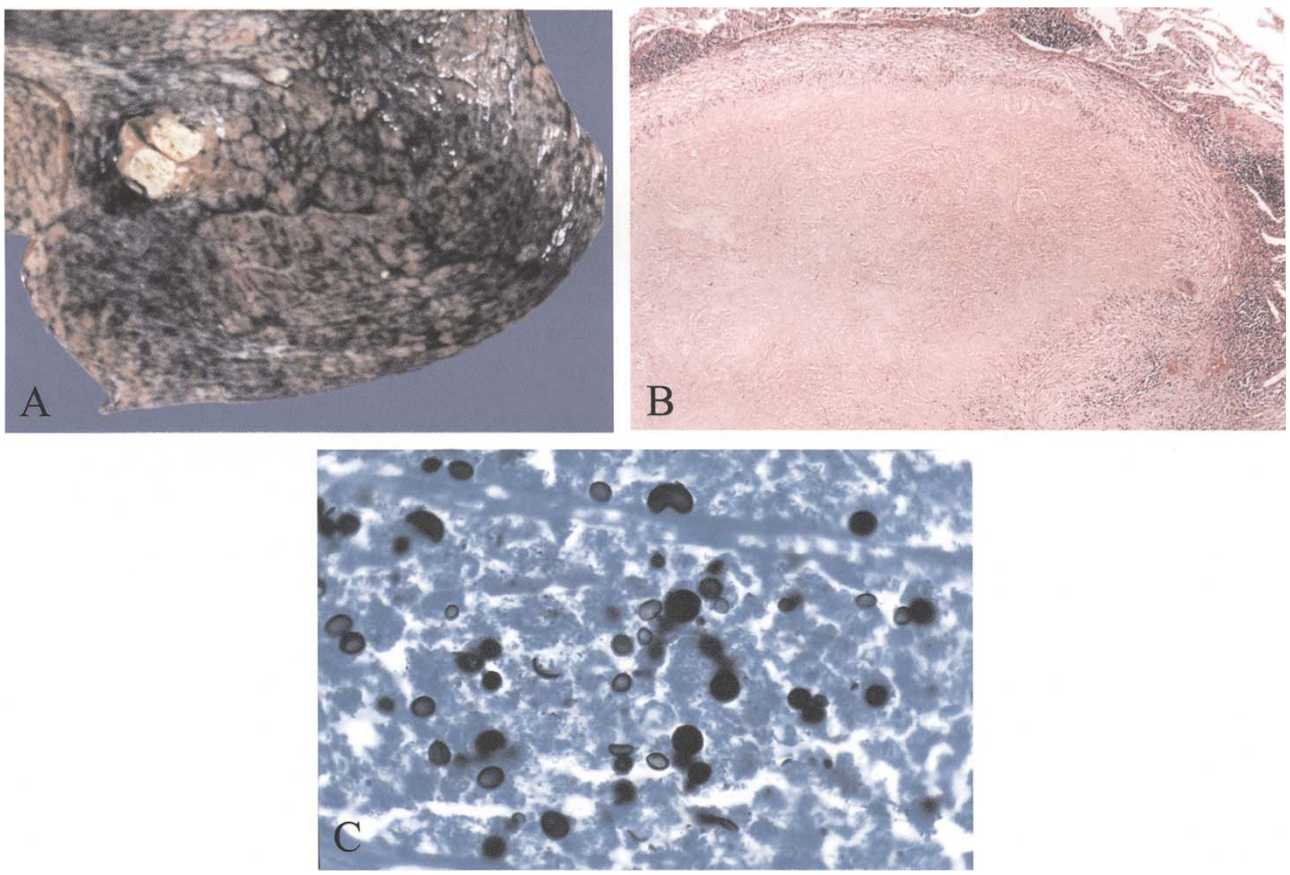

FIGURE 10.22. Cryptococcoma. **A.** A bisected, discrete, subpleural fibrocaseous lesion. **B.** Necrotizing granuloma. **C.** Degenerated and pleomorphic cryptococcal yeast forms within the necrotic zone.

in these caseous areas are usually capsule deficient, distorted, fragmented, stain unevenly, and are small 2 to 4μm in diameter (Fig. 10.22C). In this setting, they are easily confused with *H. capsulatum* var. *capsulatum*.[235] Attempts to culture the fungus from residual nodules are often unsuccessful, but a presumptive histologic diagnosis can be confirmed by direct immunofluorescence.[236,237]

Pulmonary infections caused by capsule deficient strains of cryptococci almost always occur in immunocompetent, apparently healthy subjects, and often are confined to the lungs.[233,238] The host response to poorly encapsulated cryptococci is characterized by a dispersed granulomatous inflammatory reaction with tissue suppuration, caseation, and fibrosis. Varying numbers of small, pleomorphic yeast forms can be seen within the cytoplasm of epithelioid and multinucleated giant cells. The majority of cryptococci in these lesions lack a capsular material with mucin stains, but a few fungal cells with attenuated and faintly mucin positive capsules can usually be demonstrated. A modified Fontana-Masson stain can be used to identify poorly encapsulated cryptococci, because this positive reaction does not depend on the presence of capsular material,[217] but rather on the presence of melanin pigments within the cell wall of *C. neoformans* (Fig. 10.18D). The histologic diagnosis is best confirmed by isolating and identifying *C. neoformans* in culture.

Diagnosis

The diagnosis of cerebromeningeal cryptococcosis is made by isolating and identifying the fungus in culture, by demonstrating typical fungal cells in an India ink preparation of CSF, or by detecting capsular polysaccharide antigen in CSF by the latex agglutination test.[200,232,239]

Because the symptoms and radiographic findings in pulmonary cryptococcosis are not specific, the diagnosis must be based on the microscopic demonstration of *C. neoformans* in sputum, bronchial washings and brushings, or lung biopsies. Direct immunofluorescence can be used to specifically identify the fungus in smears and conventional tissue sections, but whenever possible, the diagnosis should be confirmed by isolating and identifying *C. neoformans* in culture.[53,236,237] Capsule-deficient C. *neoformans* have to be differentiated from *H. capsulatum* var. *capsulatum* and *B. dermatitidis* microforms, S. *schenkii*, C. *glabrata*, blastoconidia of *Candida* spp., and immature spherules of C. *immitis*. Fontana Masson stain is helpful, since capsule-deficient cryptococci stain black or brown; however, S. *schenckii* and C. *immitis* spherules may also stain positive.[40] DNA-based probes with PCR technique and Gen-Probes may be helpful.[58,59,236,237] Partial-genome microarrays for delineating regulatory cascades that contribute to microbial pathogenesis may be used for identification.[240] The latex agglutination test for capsular polysaccharide antigen is also available; however, sera with high titers of rheumatoid factor can give a false-positive reaction unless they are pretreated with dithiothreitol to inactivate immunoglobulin M (IgM).[200]

In addition to CSF, blood, urine, and sputum should be cultured for *C. neoformans*. Only about 50% of patients with culture proven cerebromeningeal cryptococcosis have a positive India-ink preparation. Yeast cells can be detected in CSF by this method up to several years after clinical symptoms have disappeared.

Treatment

The combination of amphotericin B and 5-fluorocytosine is synergistic against *C. neoformans* in vitro, and this is the treatment of choice for the cerebromeningeal and progressive pulmonary forms of cryptococcosis. Fluconazole plus 5-fluorocytosine is better than fluconazole alone in acute invasive disease when amphotericin B requires an alternative.[241–243] Maintenance suppressive therapy with fluconazole should be used. Itraconazole can be substituted for fluconazole, but it is less effective. Surgical excision of chronic, localized, pulmonary lesions may be a valuable adjunct to antifungal chemotherapy.[244]

Paracoccidioidomycosis

Paracoccidioidomycosis (South American blastomycosis) is a chronic progressive fungal infection that is largely confined to Latin America. The disease occurs predominantly in males after the age of puberty.[245–247] This clinical feature is related to the glucans in the cell wall of the fungus that are influenced by female hormones. The disease is caused by a single species, *Paracoccidioides brasiliensis*. Primary infection begins in the lungs; dissemination occurs eventually in most patients and may involve the mucosa of the oral cavity and upper respiratory tract, and the skin, lymph nodes, liver, spleen, adrenal glands, intestines, and other organs.[248] Although some cases occur in Mexico and much of Central America, paracoccidioidomycosis is found primarily in South America, particularly in tropical and subtropical regions of Brazil, Columbia, and Venezuela.[245–247] Cases diagnosed in the United States have been acquired in Latin America, and most of the cases diagnosed in the United States represent reactivation of quiescent pulmonary disease following long latent periods of up to 3 to 20 years.[249–252] The disease occurs almost exclusively in adult males over the age of 30 years, most of whom are rural dwellers and have occupational contact with the soil and plants.[253] The natural habitat of *P. brasiliensis* remains largely undefined, although the fungus has been isolated from thorns,

wood fragments, and soil.[253,254] An article by Brumer and colleagues[255] provides an overview of the disease.

Microbiology

P. brasiliensis is a dimorphic fungus. In culture, the mycelial forms grow slowly at 25° to 30°C to produce a white mold, whereas the yeast forms grow as cerebriform colonies after 5 to 10 days of incubation at 35° to 37°C.[256] The yeast form consists of round or oval yeast-like cells that may vary in size from 3 to 30 μm or more in diameter. A yeast cell forms several blastoconidia (buds) attached to the parent cell by narrow necks. Subsequently, while still attached to the parent cell, the daughter cells form their own daughter blastoconidia, 1 to 3 μm in diameter, attached by narrow necks. Collectively, the mass of blastoconidia resembles a mariner's wheel (Fig. 10.23). Such budding is often referred to as multiple budding and is considered characteristic of *P. brasiliensis* in culture and in tissue.[10,257]

Clinical Features

Three clinical forms of paracoccidioidomycosis have been described.[247,254,257] The acute or subacute disseminated form is uncommon and occurs almost exclusively in young patients, in whom initial pulmonary infection is followed rapidly by dissemination to lymph nodes, liver and spleen, and in some cases detected as fungemia.[257] The chronic progressive form occurs predominantly in older patients, following the initial infection by a latent period of many years.[247,254,258] This is the most common clinical form of infection, accounting for 90% of cases in some series. The disease remains clinically confined to the lungs in about 40% of these patients, whereas limited or widespread dissemination, most commonly to the oropharyngeal mucous membranes, occurs in the remaining 60%. The third, inactive or residual, form follows successful treatment or natural resolution of the disease. Clinically overt pulmonary disease is present during the course of infection in 85% or more of all patients.[254,259]

Paracoccidioidomycosis is not considered to be an opportunistic infection, although reactivation of the quiescent disease with delayed response to therapy may occur following immunosuppression, and is seen in patients with AIDS.[260,261] Patients usually present with symptoms referable to the respiratory tract that include cough, dyspnea, and fever. Hemoptysis occurs in about 25% of patients, and constitutional symptoms of fatigue, malaise, and weight loss in 40% to 50%. Pleuritic chest and pleural effusions are uncommon. Lesions of mucous membranes, found in half of the patients, consist of painful ulcers involving the gingiva, palate, tongue, tonsils, nasal cavity, nasopharynx, and larynx. Often regional lymphadenopathy is present.[260,262] About 10% of patients present with lesions clinically restricted to the mucous membranes and less than 5% of patients present with disseminated lymphadenopathy, clinically resembling malignant lymphoma.[263] Adrenocortical involvement has been reported.[264]

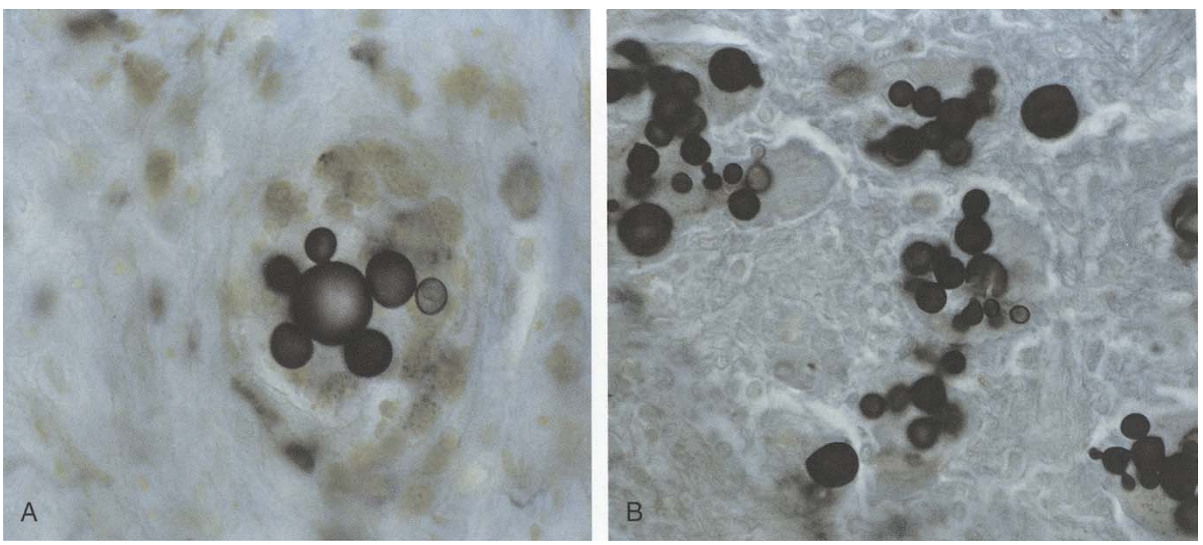

FIGURE 10.23. Paracoccidioidomycosis. **A.** Gomori's methenamine silver stain shows yeast cell with multiple blastoconidia attached to the parent cell by narrow necks creating the "teardrop" budding, and a "mariner's wheel" pattern. **B.** Morphologically diverse yeast cells of varying sizes and with different cell wall thickness within multinucleated giant cells. Yeast forms have single or multiple blastoconidia. A fractured cell wall is also seen (GMS stain).

Chest radiograph abnormalities in paracoccidioidomycosis are nonspecific, and may include micronodular infiltrates, consolidation, cavities, residual nodules, calcifications, or fibrosis, depending on the clinical presentation.[247,259,262] Nodular radiographic patterns can be confused with tumor masses or metastatic carcinoma, mediastinal adenopathy in young patients with lymphoma, consolidation with central cavitation with bacterial lung abscess, and diffuse bilateral interstitial pattern with idiopathic interstitial fibrosis. Radiographic changes may be confused with tuberculosis, which may coexist in as many as 30% of patients with paracoccidioidomycosis.[256,258,262,265]

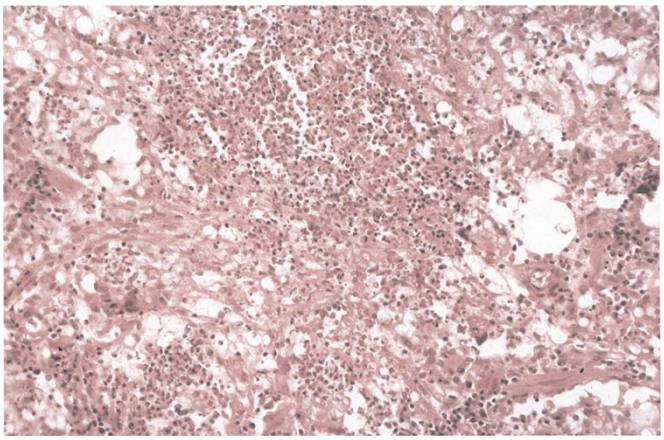

FIGURE 10.24. Paracoccidioidomycosis. Loosely formed dimorphic granuloma with histiocytic lining and central area of neutrophilic abscess. Organisms are barely discernible at this magnification.

Pathology

In infected patients, pulmonary lesions are found at autopsy in 94% to 100% of cases.[256,265] Most of these patients have had chronic progressive pulmonary disease of many years' duration, with a cobblestone appearance of the lungs resulting from advanced fibrosis and emphysema. Sectioned surfaces of the lungs show a variety of changes that correlate with the patterns observed in chest radiographs. In the interstitial form, linear streaks of fibrosis radiate peripherally from the hilum, accompanied by emphysema.

Microscopically, fibrosis of interalveolar and interlobular septa, and remnant granulomas or multinucleated giant cells may be found in areas of fibrosis. Pulmonary blood vessels show marked intimal proliferation that is often associated with right ventricular enlargement, and cor pulmonale; found at autopsy in 70% of patients in one series.[256,265]

Nodular lesions consist of miliary, interstitial, tuberculoid granulomas, or large granulomas with central caseous or suppurative necrosis, and peripheral fibrosis. Cavitary lesions consist of large centrally necrotic granulomas. An acute bronchopneumonic form may be found in patients with an acute or subacute clinical course, most often juveniles or patients treated with corticosteroids (Fig. 10.24). The residual lesion consists of a solitary, circumscribed granuloma. This lesion, rarely encountered at autopsy, is similar to the residual pulmonary lesion of histoplasmosis, except that calcification is uncommon.[256] Hilar and mediastinal lymphadenopathy is found at autopsy in about 70% of patients who have pulmonary lesions.[265]

Extrathoracic lesions, either granulomatous or suppurative, commonly a manifestation of hematogenous or lymphatic dissemination, are found in majority of patients at autopsy. Lesions are typically found in the lymph nodes, spleen, liver, oropharyngeal mucosa, adrenal glands, skin (often contiguous with mucosal lesions), larynx, trachea, intestines, and kidneys.[256,264,265]

Diagnosis

The yeast cells of *P. brasiliensis* are optimally identified in histologic section with a silver stain (GMS), although they can often be seen in H&E-stained sections. The cells vary in diameter from 3 to 30 μm, and occasionally reach a diameter as great as 60 μm. The larger cells have walls up to 1 μm thick. Most of the yeast-like cells in pulmonary or extrathoracic lesions have single to multiple blastoconidia. However, unless confirmed by direct immunofluorescence, the specific histologic diagnosis of paracoccidioidomycosis is warranted only when typical multiple budding cells are identified (Fig. 10.23). Two patterns of budding are found: the large teardrop blastoconidia (attached to the parent cell by narrow necks), and smaller oval or tubular blastoconidia (Fig. 10.23). Hyphae and pseudohyphae are rarely produced. Yeast cells with fractured walls, so-called mosaic forms, are almost constantly present in chronic pulmonary lesions. Although characteristic, they are not specific for this disease (Fig. 10.23B). Small yeast cells 2 to 4 μm in diameter are occasionally predominant in the lesions and can be mistaken for the cells of *H. capsulatum* var. *capsulatum*.[266] Confusion with *H. capsulatum* and other yeast in tissue sections can be resolved by direct immunofluorescence, or by the identification of typical multiple budding cells and wide variation in cell size in the lesions of paracoccidioidomycosis. In active granulomatous lesions, the cells of *P. brasiliensis* are found within the cytoplasm of histiocytes and multinucleated giant cells. In necrotic granulomas, the yeast cells are found within necrotic material and are concentrated peripherally at the interface between necrosis and granuloma.

The clinical diagnosis of paracoccidioidomycosis in endemic areas is strongly suggested by the combination of chronic pulmonary symptoms, chest radiographic abnormalities, and mucosal lesions of the oral cavity and upper respiratory tract.[247,259] The clinical diagnosis can be confirmed serologically. *P. brasiliensis* produces 43- and 70-kDa glycoproteins that are the main antigenic compounds of the fungus.[267,268] The 43-kDa glycoprotein, identified as a concanavalin A–binding glycoprotein, is the predominant IgG reactive antigen recognized in sera from all patients with overt disease.[267] Direct microscopy, cytology, and culture of respiratory secretions yield a positive diagnosis in up to 95% of patients.[269] The diagnosis is also confirmed by culture or biopsy of accessible lesions such as those of the oral mucosa, skin, and lymph nodes.

Treatment

Therapy of paracoccidioidomycosis includes sulfonamides, amphotericin B, and the azole derivatives ketoconazole, itraconazole, and fluconazole.[263,270,271] Therapy with itraconazole may consist of long-term administration for most patients with paracoccidioidomycosis. Amphotericin B is also effective. Sulfonamides can be used in less severe cases, but relapse may be seen in as many as 40% of patients.[272]

Sporotrichosis

Sporotrichosis is a chronic, localized, or rarely disseminated infection caused by the dimorphic fungus *Sporothrix schenckii*.[273–275] This organism is found in nature growing as a saprophyte on plants, trees, wood timbers, sphagnum moss, and other plant materials.[276] Sporotrichosis is worldwide in temperate as well as tropical areas, but most documented cases have originated from the United States, South Africa, Mexico, and South America. Most infections are nonpulmonary, and result from accidental cutaneous inoculation of the fungus growing on plant materials such as thorns. The mycosis is considered to be an occupational disease, occurring most often in farmers, gardeners, forestry workers, florists, and others who are frequently exposed to plants.[277] Primary pulmonary infections are rare, and result from inhalation of conidia.[278,279] Cutaneous and rarely primary pulmonary infections can be the source of the fungus that disseminates to the bones, joints, lungs, meninges, and other internal organs.[280,281] Patients who are profoundly immunosuppressed, alcoholics, or those with AIDS are at a greater risk of disseminated disease.[282–284] The possible sequelae of exposure to *S. schenckii* are shown in Figure 10.25. Disseminated sporotrichosis was recently reported to be associated with treatment with immunosuppressants and with tumor necrosis factor-α antagonists.[285]

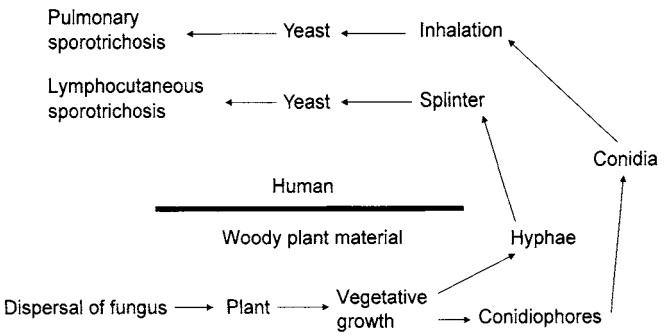

FIGURE 10.25. Sequelae of exposure to *Sporothrix schenckii*.

Sporotrichosis is not contagious, but infections can result from contamination of broken skin with lesional exudates from humans or animals having the disease.[286] Care should be taken when handling infectious material to prevent accidental infection.

Microbiology

S. schenckii is a dimorphic fungus, and grows as a yeast in culture at 37°C and in the tissues of a living host; it grows in a mycelial form when cultivated at 30°C. Yeast colonies at 37°C are moist, creamy, white, and composed of round, oval to elongated, single celled yeasts that are 2 to 6 μm or larger in diameter.[273,287] In culture, the mycelial form develops as a rapidly growing, whitish, later brownish-black mold that has a wrinkled or folded membranous surface. Microscopically, the mycelium is composed of narrow, branched, septate, hyaline hyphae and abundant conidia formed on delicate conidiophores along the hyphae. The conidia develop on delicate sterigmata along the hyphae and terminally on the conidiophores. Lateral one-celled conidia that are black contribute to the dark color of the colony.

Clinical Features

The classical clinical form of sporotrichosis is lymphocutaneous, and consists of a series of chronic subcutaneous nodules along the course of lymphatic drainage from a primary nodular-ulcerative skin lesion.[273,288] These lesions may develop within 7 to 90 days or longer after a penetrating injury. In time, the lymphatic nodules ulcerate and discharge pus, but regional lymphadenopathy is usually absent.

The true prevalence of primary pulmonary sporotrichosis in the U.S. is unknown. Dixon et al.[289] reported the isolation and characterization of *S. schenckii* in a large epidemic of sporotrichosis, including the environmental sources. Two reports from Peru examine the prevalence of sporotrichosis in the endemic areas; 238 cases of culture-proven sporotrichosis were studied in a relatively

remote area of the south central highlands of Peru, collected during 1995 to 1997.[274,277] Children had an incidence three times higher than adults and were more likely to have lesions on the face and neck. Risk factors included owning a cat, playing in crop fields, working outdoors, and conditions associated with lower socioeconomic status. An association with cats has been observed in several patients throughout the world. Histologic findings of primary pulmonary sporotrichosis were described in eight cases identified from the files of the Armed Forces Institute of Pathology (AFIP).[290]

Pulmonary lesions seen in sporotrichosis may develop during the course of dissemination from a primary cutaneous infection, where articular, osseous, and widespread cutaneous lesions predominate. The lungs are involved secondarily in less than 20% of these cases. Primary pulmonary sporotrichosis does occur.[278,280,281,290] Lesions are insidious but progressive if left untreated; even when treated the prognosis is poor. Clinical findings in patients with pulmonary involvement are indistinguishable from those of other pulmonary infections resulting in chronic progressive granulomatous and cavitating lesions.[291–294] Radiographic findings are nonspecific, and include linear streaks, patchy and fibronodular infiltrates, cavitary lesions, and rarely pleural effusions.

Primary pulmonary sporotrichosis is usually a bilateral, apical, cavitary, progressive, destructive, and debilitating infection that most often occurs in middle-aged men with a history of chronic obstructive pulmonary disease or alcoholism.[290] Clinically, radiographically, and pathologically, the pulmonary lesions closely resemble tuberculosis and histoplasmosis capsulati. Patients usually present with nonspecific symptoms including fever, chills, chest pain, dyspnea, hemoptysis, cough, malaise, and weight loss. In the AFIP series, pulmonary lesions consisted of large, often confluent, necrotizing and nonnecrotizing granulomas that contained scattered or clustered yeast cells of *S. schenckii*. Granulomas were sometimes fibrotic, and some patients had solitary peripheral, necrotizing, permanent nodules similar to those seen in residual pulmonary histoplasmosis capsulati. Unlike the histoplasmosis lesions, calcification is not seen.[290]

In a few patients with disseminated infection, a primary cutaneous or pulmonary lesion may not be evident, and the predominant manifestations are those of a suppurative arthritis, osteomyelitis, periosteitis, or tenosynovitis often involving the elbows and knees.[290,293,295] Without preexisting cutaneous involvement, these lesions may result from hematogenous spread of inapparent pulmonary infection.

It has been speculated that many asymptomatic, immunocompetent individuals who have high antibody titers to *S. schenckii* may have had previous pulmonary exposure with infection limited to hilar lymph nodes, similar to that seen in asymptomatic histoplasmosis capsulati. In 1969, Ajello and Kaplan[296] described a new variant of *S. schenckii* var. *luriei*, isolated from a cutaneous nodule of a South African. Since then other cases have been described.[297,298] In culture, the *luriei* variant is indistinguishable from the classical form, but in tissue, the *luriei* variant not only forms the typical budding yeast cells, but also distinctive large (15 to 20 μm) spherical, thick-walled cells that divide by either budding or septation, producing the appearance of a pair of eyeglasses.[297,299]

Pathology

The patterns of host response in localized and disseminated sporotrichosis are similar.[273,276,288] Skin lesions may develop pseudoepitheliomatous hyperplasia and epidermal ulceration, and a mixed suppurative and granulomatous inflammatory reaction in the dermis and subcutaneous tissue. In the lung, caseating granulomas may develop peripheral fibrosis and subsequently cavitate, but calcification has not been reported.

Because of their scarcity, *S. schenckii* cells are difficult to detect in H&E-stained tissue sections. However, round or oval yeast forms can be demonstrated with silver stain (GMS) or PAS. Some blastoconidia may be elongated and appear cigar-shaped. The yeast cells and blastoconidia are 2 to 6 μm or greater in diameter (Fig. 10.26). The yeast-like cells may be coated with an eosinophilic, refractile, radially oriented Splendore-Hoeppli material to form asteroid bodies that are usually located in microabscesses or suppurative centers of granulomas (Fig. 10.26).[276,288] Asteroid bodies are not often found in the lesions of sporotrichosis, and when present are not pathognomonic for this disease. Splendore-Hoeppli material is also not pathognomonic, and may be seen surrounding other structures such as parasite ova, foreign objects such as silk sutures, other fungi, and actinomycotic or botryomycotic granules.[300] *S. schenckii* rarely forms hyphae in tissues, and intracavitary pulmonary fungus balls are also unusual. Hamazaki-Wesenberg bodies (yellow-brown bodies) seen in lymph nodes with sarcoidosis and other granulomas may be mistaken for the yeast cells of *S. schenckii* (see Chapter 18).[301]

Diagnosis

Diagnosis is established by isolating the fungus from clinical specimens or by direct immunofluorescence of *S. schenckii* in smears and tissue sections.[53,302] Serology is also a useful adjunct to diagnosis. The tube agglutination and latex agglutination tests are considered to be the most reliable, particularly for the diagnosis of extracutaneous infections; however, low titers do not exclude invasive infection.

Treatment

The prognosis of disseminated infection has been poor even when treated; in one series 11 of 37 patients with

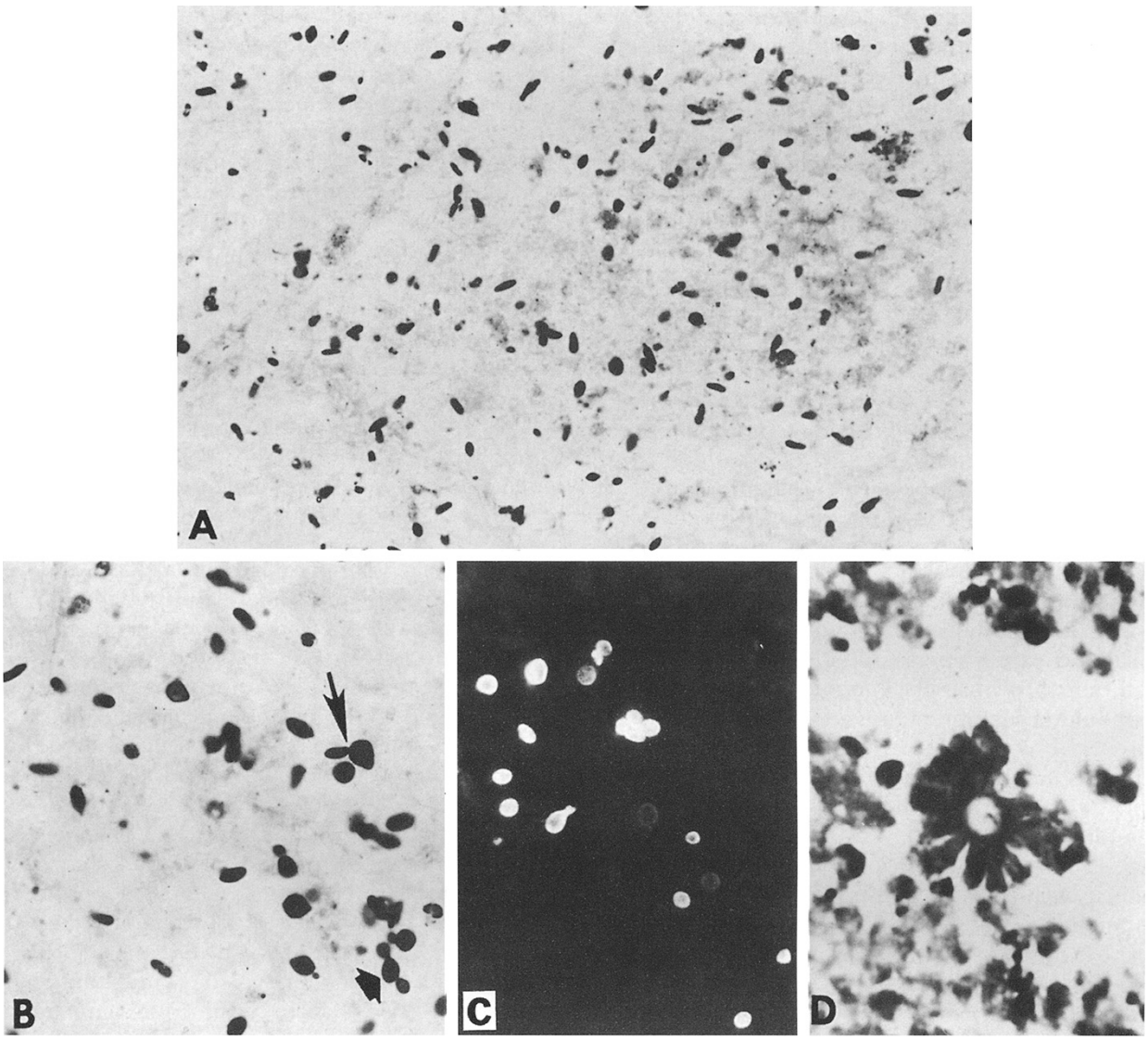

FIGURE 10.26. Pulmonary sporotrichosis. **A.** Spherical, oval, and elongated (cigar-shaped) yeast-like cells in caseated granuloma. GMS, × 480. **B.** *Sporothrix schenckii* cells with elongated (arrow) and multiple (blunt arrow) buds attached to parent cells by narrow bases. GMS, × 760. **C.** Individual and clustered yeast forms in fibrotic granuloma. Direct immunofluorescence, × 680. **D.** Asteroid body in suppurative center of granuloma. × 700. (Courtesy of Drs. F.W. Chandler and J.C. Watts.)

disseminated sporotrichosis died despite treatment.[293] Recent developments in the management of invasive fungal infections, however, have improved the outlook for patients with disseminated sporotrichosis.[303–305] Amphotericin B and itraconazole are most effective for disseminated infection.[305] Chronic cavitary pulmonary sporotrichosis is usually refractory to antifungal chemotherapy, but can be cured when chemotherapy is combined with surgical resection.[306]

Candidiasis

Candidiasis comprises a group of superficial, mucocutaneous, and systemic opportunistic mycoses caused by yeast-like fungi of the genus *Candida.* Deep-seated candidosis is a major problem in critically ill patients. *Candida* septicemia has a high mortality rate.[307] Candidiasis is the most frequently encountered human opportunistic infection, accounting for approximately 50% of fungal infections among immunocompromised patients, and up to 75% or more of infections in patients with acute leukemia, lymphoma, or solid tumors.[308–313] Although the genus *Candida* contains more than 100 different species, only 10 have been isolated from human tissues or fluids with sufficient frequency to be considered pathogenic.[314] By far the most common isolate is *Candida albicans*, causing 70% to 80% of cases of systemic candidiasis. Other species of *Candida* that are important include *C. glabrata, C. dubliniensis,*

C. tropicalis, C. viswanathii, C. parapsilosis, C. krusei, C. zeylanoides, C. lipolytica, and *C. famata.*

Controversy had surrounded the taxonomic classification of torulopsis, leading to confusion in the literature.[315,316] In 1978, it was proposed that this yeast should be incorporated into the genus *Candida*[315]; however, this change was not validated until recently.

While *C. albicans* remains the most common pathogen in the U.S., non-*albicans* Candida species are being increasingly isolated.[307] Of particular concern is the increased occurrence of triazole-resistant isolates of *C. glabrata* and *C. krusei.*[307,313,317–319] A recent report from Italy on candidosis in intensive care units also found an increase in non-*Candida* species from the 1980s to 2000.[320] A 1-year survey of candidemia in Belgium in 2002 found that *C. albicans* was the cause of infection in 55%, *C. glabrata* in 22%, and *C. parapsilosis* in 13%.[321] A similar trend was found in a retrospective study of hospitalized patients in a teaching hospital in the United Kingdom.[322] *Candida* species account for 98% of all yeasts isolated from clinical specimens of cancer patients.

Normal skin and mucosal surfaces are an effective barrier against invasive candidiasis, and leukocytes, particularly neutrophils are the most important line of defense once mucosal penetration has occurred.[323,324] Humoral factors play a less important role in host defense, although IgG and complement components enhance phagocytosis of *Candida* species. Defective cell-mediated immunity may result in severe, progressive, but localized lymphocutaneous infection, as seen in chronic mucocutaneous candidiasis, or in invasive bronchopulmonary candidiasis, as seen in some patients with AIDS and with bone marrow transplant.[325–327]

Factors that impair host defense mechanisms predispose to invasive candidiasis. Thus, breaks in the barriers such as trauma, burns, peritoneal dialysis, gastrointestinal surgery, mucosal ulcers, and indwelling venous catheters permit mucosal invasion or provide direct access to the vascular system.[318,319,328,329] Neutropenia, induced by acute leukemia and chemotherapy, and defective leukocyte function caused by corticosteroid therapy impair phagocytosis and killing of the *Candida* species.[330–332] Broad-spectrum antibiotic therapy promotes local overgrowth and mucosal colonization by *C. albicans*. Broad-spectrum antibiotics, corticosteroids, and neutropenia are a potent predisposing combination and account for the high incidence of candidiasis in patients treated for acute leukemia and lymphoma. Other predisposing factors include parenteral hyperalimentation, prematurity, diabetes mellitus, and associated bacterial infections.[328,333] In a study of 325 premature infants, 8.6% developed *C. albicans* or *C. parapsilosis* infection.[334] The presence of premature rupture of membranes and duration of ventilation were significant risk factors. Pulmonary candidiasis occurs rarely in patients who have no recognized underlying illness or predisposing factors.[335] Community-acquired *Candida* pneumonia is also on the rise, especially in patients with chronic parenchymal lung damage, for example those with smoking related emphysema.[336]

Microbiology

C. albicans constitutes part of the normal microflora of the mouth and oropharynx, upper respiratory tract, digestive tract, and vagina, but is seldom isolated from environmental sources.[337] *C. albicans* is therefore considered a true endogenous pathogen. The other *Candida* species have been isolated from hospital personnel, soil, food, and occasionally air.[338] *Candida* species grow rapidly on standard mycologic media at either 30° or 37°C, producing smooth or wrinkled, creamy white, pasty colonies. Although *C. albicans* forms germ tubes and chlamydospores under certain conditions of growth, the other *Candida* species do not.[339] However, occasional strains of *C. stellatoidea* and *C. tropicalis* also produce chlamydoconidia. Based on recent taxonomic proposals regarding what has been traditionally identified as *C. parapsilosis*, this species is actually a complex of several species. *C. albicans* grows as round to oval yeast cells 5 to 7 μm in diameter, with pseudohyphae consisting of chains of elongated yeast cells, septate hyphae 3 to 5 μm in width, or any combination of these forms. *Candida* species can be further identified by their patterns of carbohydrate fermentation and assimilation.

Clinical Features

The clinical features of pulmonary candidiasis are nonspecific and resemble those of other opportunistic pulmonary infections. Typically, patients who are being treated for the underlying diseases mentioned previously develop persistent fever unresponsive to broad-spectrum antibiotic therapy, and exhibit new or changing pulmonary infiltrates on chest radiographs, associated with cough and dyspnea.[310,318,327] Radiographic abnormalities are correspondingly nonspecific and can often be attributed to concurrent infection, hemorrhage, or underlying disease and its treatment.[311,340] Patterns of radiographic abnormality correlate with the route of pulmonary infection. Thus, patients with endobronchial pulmonary candidiasis develop patchy or diffuse bilateral areas of air-space consolidation that are indistinguishable from bronchopneumonia resulting from other causes. Patients with hematogenous pulmonary candidiasis develop bilateral miliary nodules several millimeters to 1.0 cm in size.[311,340] Embolic pulmonary candidiasis, virtually restricted to children, produces a pattern consistent with pulmonary infarction.[341] The presence of preterm premature rupture of membranes and the duration of ventilation are significant risk factors for development of *Candida* pneumonia in premature infants with a birth weight of less than

1250 g.[334] Abnormalities that can almost always be attributed to concurrent disease rather than to candidiasis include hilar or mediastinal lymphadenopathy, pleural effusion, large mass-like opacities, infarcts, and cavities.[311,341,342] Patients with pulmonary candidiasis often have concurrent bacterial or fungal infections, pulmonary edema, hemorrhage, infarcts, aspiration pneumonia, or diffuse alveolar damage, which could also account for many of the observed radiographic abnormalities.[311,341,343] Almost 50% of patients with histologically documented pulmonary candidiasis do not have any demonstrable radiographic abnormalities. This is most likely related to either agranulocytosis, small size of the lesion, or technically inferior films.[311,318,340,343] The radiographic diagnosis of pulmonary candidiasis, therefore, is neither specific nor sensitive.

Pathology

The gross features of pulmonary candidiasis are largely determined by the route of infection.[344,345] *Candida* ulcers of the respiratory tract have sharp margins and a shaggy base covered with exudates. Endobronchial infection from aspiration of *Candida* species from a focus of infection in the oropharynx or upper respiratory tract produces patchy, asymmetric areas of consolidation with predilection for lower lobes. Extensive pulmonary hemorrhage is associated with this form of infection in about 50% of cases, and associated with small yellow abscesses (Fig. 10.27).[345] When aspiration occurs as a preterminal event, the lesions are grossly inconspicuous and clinically insignificant.

FIGURE 10.27. Lung section of *Candida* pneumonia shows small yellow abscesses within pale consolidated areas. This pattern is typical of endobronchial spread.

FIGURE 10.28. Disseminated candidiasis. Pale miliary nodular foci, best seen at apex, are set against a background of hemorrhagic diffuse alveolar damage.

Hematogenous seeding of the lungs produces random, bilateral, more or less symmetrically distributed miliary or nodular lesions (Fig. 10.28). This form of infection is frequently associated with extrapulmonary candidal lesions in the kidneys, liver, spleen, and myocardium; the gastrointestinal tract and indwelling venous catheters are the usual portals of entry. These hematogenous nodules termed "target lesions" are round and well circumscribed, 2 to 4 mm in diameter, and have yellow or gray granular centers with peripheral hemorrhagic margins.[344,346] Occasionally abscesses, several centimeters in diameter may also be found (see also Fig. 7.41 in Chapter 7).

Gross embolic spread to the lungs in about 50% of infants with fatal pulmonary candidiasis was reported in one series.[341] In this form of infection, the pulmonary arterial tree is seeded with emboli originating in central or peripheral veins or the right atrium and is almost always associated with indwelling venous catheters. Embolic pulmonary candidiasis produces peripheral hemorrhagic infarcts that may undergo liquefaction and cavitation. Pulmonary infarcts are distinctly unusual in adult patients with disseminated candidiasis, whereas these are the hallmark of pulmonary infections caused by *Aspergillus* spp. and zygomycetes.

Microscopically, the lesions of invasive and disseminated candidiasis show pale blue- or lilac-colored oval

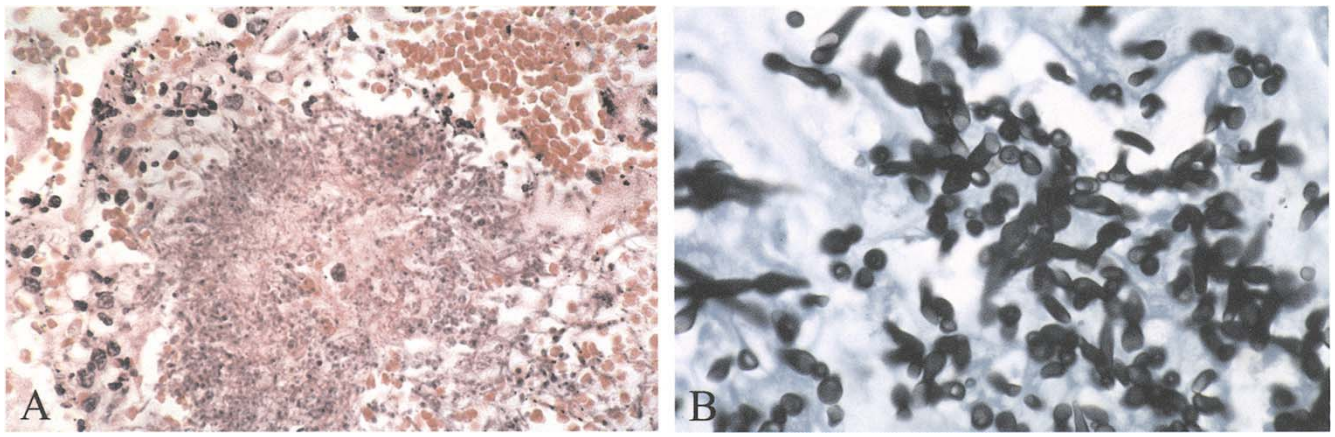

FIGURE 10.29. **A.** Necrotizing candidal pneumonia. Weakly basophilic yeast and pseudohyphae are surrounded by sparse degenerating neutrophils and hemorrhage in necrotic parenchyma. Bone marrow transplant patient with disseminated candidiasis. **B.** Gomori's methenamine silver stain shows budding yeast and pseudohyphae.

yeast cells or blastoconidia measuring 3 to 5 μm in diameter. Pseudohyphae are also 3 to 5 μm wide and have periodic constrictions at the point where budding yeast cells are joined end to end (Fig. 10.29). Occasionally true hyphae are present. All pathogenic *Candida* species have a similar appearance in histologic sections, and therefore cannot be speciated by their morphology. A predominance of well-developed pseudohyphae is suggestive of *C. albicans, C. tropicalis*, or *C. krusei*. Small yeast cells without pseudohyphae suggest members of the *C. parapsilosis* complex and *C. glabrata*.

Pseudohyphae can be distinguished from true hyphae by the presence in pseudohyphae of constrictions at the septa, septa at the union of branches from the main filaments, septa that are curved rather than straight, and small buds at the tip of pseudohyphae (Fig. 10.29B). True hyphae have parallel walls without constrictions at the septa, the first septum of a branch is a short distance from the main filament, and the tip of the hypha is rounded or squared-off. Some hyphae and pseudohyphae may contain swollen cells called vesicles. The tissue forms of the *Candida* species are weakly basophilic and often visible in sections stained with H&E, but best demonstrated with special stains such as GMS or PAS (Fig. 10.29).

The cellular reaction of invasive infection by the *Candida* species in the nongranulocytopenic host is characteristically neutrophilic (Fig. 10.29). Yeast cells and pseudohyphae or hyphae, if present, may be diffusely distributed throughout areas of acute suppurative inflammation, or may form compact radiating microcolonies similar to those seen in invasive pulmonary aspergillosis. In the granulocytopenic host, cellular reaction is minimal, and the lesions are mainly characterized by bland coagulative necrosis and hemorrhage.[344,345,347,348] A component of granulomatous inflammation may be seen in patients with chronic, indolent infections, or in those who have been treated with antifungal chemotherapy. Chronic eosinophilic pneumonia has been reported, with sensitization to *Candida* demonstrated by the presence of specific serum antibodies to *C. albicans*.[349–351]

The microscopic pattern and distribution of pulmonary lesions are largely determined by the route of infection. With endobronchial spread, yeast cells and pseudohyphae proliferate within the conducting airways and extend from the bronchiolar lumen through the wall of the bronchi into the peripheral and peribronchial alveolar spaces. Foreign material in these lesions, particularly food particles, provides evidence of aspiration. Since endobronchial candidiasis is frequently a preterminal infection, invasion of small veins and lymphatics by pseudohyphae is unusual. Occasionally however, such lesions may serve as a primary site for hematogenous dissemination, in which case microscopic vascular invasion is conspicuous.

In case of infection via the hematogenous route, pulmonary candidiasis is associated with the production of "target lesions," angiocentric rather than bronchocentric lesions, composed of a central core of necrotic pulmonary parenchyma with yeast cells, surrounded by an intermediate zone of neutrophils and a peripheral zone of parenchymal hemorrhage. A necrotic arteriole or small artery can usually be found within or at the edge of such lesions. With time, these lesions may enlarge and coalesce to produce necrotic abscesses, which may then be indistinguishable from the lesions produced by endobronchial infection.

In infants, the embolic lesions are characterized by thromboembolic occlusion of medium sized or small pulmonary arteries, and hemorrhagic infarction of distal pulmonary parenchyma. The thromboemboli contain yeast cells and pseudohyphae that penetrate through the vas-

cular walls into the infarcts and adjacent alveolar spaces. Acute suppurative inflammation may then produce liquefaction with cavitation of the infarcts. As a rule, *Candida* species do not invade large arteries or veins in the adult patients.

Differential Diagnosis

Candida species as a group can easily be distinguished from most other pathogens in histologic sections if both the budding yeast cells of appropriate size and shape and filamentous elements (pseudohyphae and hyphae) are present. The differential diagnosis includes other fungi, such as *B. dermatitidis, C. neoformans, H. capsulatum,* and *S. schenckii*, the tissue forms of all of which consist of yeast. However, none of these fungi typically produce pseudohyphae or hyphae in tissues. The other differential diagnosis includes fungi that produce mycelial forms—*Aspergillus, Fusarium,* and *Pseudallescheria*—when their hyphae are moniliform in shape, thus resembling pseudohyphae. These fungi do not produce yeast in tissues. *Trichosporon* species may be difficult to distinguish from some *Candida* species, because both may produce yeast cells, pseudohyphae, and hyphae. However, the yeast forms of *Trichosporon* species are slightly larger and more pleomorphic than those of *Candida.* The presence of arthroconidia in *Trichosporon* and their absence in *Candida* helps to distinguish these two fungi. Poorly pigmented cell walls of the dematiaceous fungi that cause phaeohyphomycosis can also be mistaken for *Candida* species. Therefore, it is imperative to submit fresh tissue, if available, for cultural confirmation of the histologic diagnosis.

Diagnosis

The laboratory diagnosis of pulmonary and disseminated candidiasis can be very difficult, since *Candida* spp. constitute part of the normal human flora and readily colonize mucosal surfaces. Positive cultures of sputum, bronchoscopy specimens, urine, and feces are usually diagnostically inconclusive. Blood cultures, widely considered to be diagnostically insensitive, are negative in 50% to 60% of patients with disseminated candidiasis. However, a positive blood culture may indicate only transient candidemia and does not prove that an actually invasive infection is present. Immunohistochemical studies suggest that invasive *Candida* pneumonia can be distinguished from noninvasive aspiration, colonization, or specimen contamination by *C. albicans* on the basis of the character, extent, and distribution of *Candida* antigen in histologic sections.[345] At present, the demonstration of typical budding yeast cells and pseudohyphae in a lung biopsy specimen from an immunocompromised patient is evidence of candidiasis.

Although conclusive evidence of pulmonary candidiasis often requires histologic demonstration of pulmonary parenchymal invasion or isolation in culture of a *Candida* species from material obtained by transthoracic needle aspiration of a pulmonary lesion, molecular techniques are now available for definitive diagnosis. *Candida* species can also be diagnosed generically in deparaffinized sections of formalin-fixed tissue with direct immunofluorescence or by immunoperoxidase staining.[345,352]

Many special techniques including autofluorescence, whitening agents such as Calcofluor white, direct immunofluorescence (IF), immunohistochemistry using polyclonal or monoclonal antibodies, DNA or RNA probes, and PCR techniques are now available.[353–356] Molecular analysis using Southern blotting with Ca3 probe hybridization is available for definitive diagnosis of different strains of *Candida*.[322] Specific serum IgE and IgG against *C. albicans* can be used for diagnosis on serum and BAL fluid.[349] A new commercially available 20-minute test, GLABRATA RTT, can be used for rapid identification of *C. glabrata*.[357]

Treatment

Treatment of systemic or pulmonary candidiasis includes azole compounds (miconazole, ketoconazole, fluconazole, and itraconazole).[358] Amphotericin B in lipid vehicles and other azole compounds are under development.[358,359] Fluconazole alone or in combination with another antifungal agent is the treatment of choice in most cases.[321] However, resistance to azoles is becoming common; in particular, *C. glabrata* is often resistant to fluconazole.[307,321] Reports of *C. glabrata* endocarditis successfully treated with intravenous liposomal amphotericin B and intravenous caspofungin could increase the medical options of treatment.[360,361]

Aspergillosis

Aspergillosis remains a major cause of morbidity and mortality in immunosuppressed patients, and has become an increasingly serious problem with the use of corticosteroid, immunosuppressive, and antineoplastic drugs, and for those with organ transplants.[362–364] Aspergillosis, however, is not a common infection associated with AIDS. The cause of this paradox may be related to the relative preservation of neutrophil and macrophage function in AIDS.[363,365] In a large retrospective study of patients with aspergillosis, the most common predisposing factors were hematologic disease, particularly those with bone marrow transplantation, leukemia, and lymphoma, in 61% of patients.[366] In later studies, the majority of fungal infections were caused by *Aspergillus* species, with an incidence of 4% to 10%.[367–369]

Aspergillus species are common molds, ubiquitous within the environment in a worldwide distribution. They can be isolated from soil, decaying vegetation, and organic debris. Their conidia are ubiquitous in ambient air, and constantly being inhaled.[370] Although numerous *Aspergillus* species are recognized, only a few are important agents of human disease. Of these, *A. fumigatus, A. flavus, A. niger,* and *A. terreus* are the most commonly isolated pathogens. Other much less common pathogenic species include *A. oryzae, A. nidulans, A. ustus, A. versicolor*, and *A. ochraceus*.[371,372] *Aspergillus fumigatus* is by far the most common agent of invasive pulmonary aspergillosis and together with *A. niger* accounts for most cases of intracavitary aspergilloma.

Microbiology

The pathogenic aspergilli are thermal tolerant and grow rapidly on standard mycologic media free of cycloheximide, usually within 1 to 3 days. Species identification is based on colony morphology, pigmentation, and morphology of the conidial heads and conidia.[371,373] The common pathogenic species can be accurately identified in most clinical laboratories.

Clinical Features

The spectrum of pulmonary aspergillosis includes several clinical presentations: (1) allergic aspergillosis, a reaction in hypersensitive hosts; (2) colonization of preexisting cavities in patients with normal immunity, with formation of "aspergilloma"; (3) chronic necrotizing bronchial aspergillosis (CNBA), a noninvasive or superficially invasive chronic necrotizing tracheobronchitis; (4) chronic necrotizing pulmonary aspergillosis (CNPA), a progressive and destructive pulmonary infection in mildly compromised patients; and (5) invasive pulmonary aspergillosis (IPA), a rapidly progressive infection in severely immunosuppressed patients. The clinical spectrum of pulmonary aspergillosis is shown in Figure 10.30.

Although the different forms of aspergillosis are often discrete clinicopathologic entities, some degree of overlap often exists among them. Pulmonary aspergillosis manifests in various forms depending on the dose of infection, the presence of underlying lung disease, and host immunity.[374,375]

Allergic Aspergillosis

Allergic aspergillosis is a consequence of hypersensitivity to aspergillus antigens. Pathologic manifestations of allergic bronchopulmonary aspergillosis, typically seen in patients with bronchial asthma, include eosinophilic pneumonia, eosinophilic bronchiolitis, mucoid impaction of proximal bronchi, and bronchocentric granulomatosis (see Chapter 15). Microgranulomatous hypersensitivity pneumonitis, a form of extrinsic allergic alveolitis, has been associated with the inhalation of spores of *A. clavatus* (malt worker's lung) (see Chapter 17).

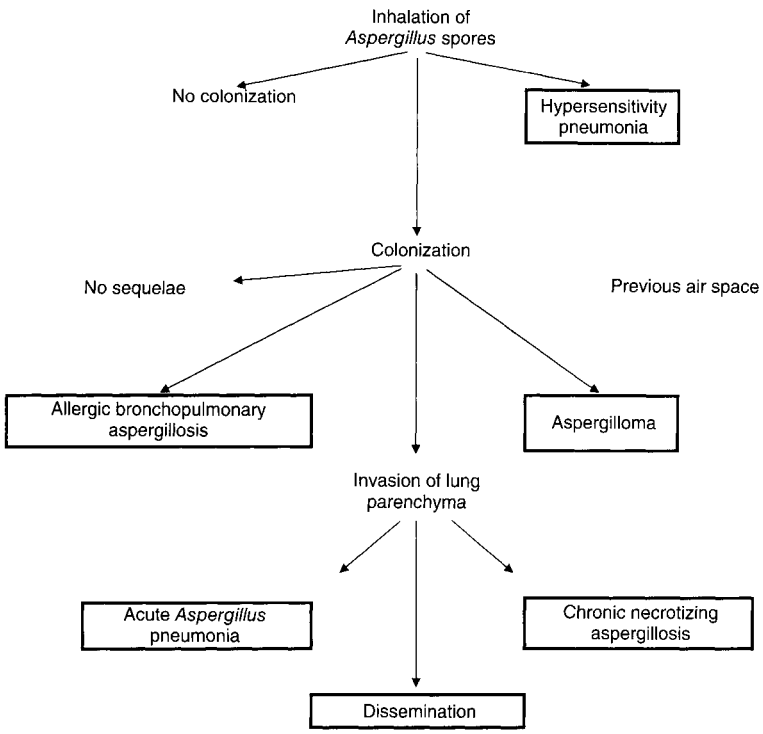

FIGURE 10.30. Clinical spectrum of aspergillus infection.

Colonization of Preexisting Cavities

The aspergilli readily colonize obstructed bronchi, where they proliferate as saprophytes. Colonization of obstructed bronchi typically occurs in patients with cystic fibrosis, bronchial asthma, chronic bronchitis, bronchiectasis, or neoplasms.[376] Colonization produces no symptoms, and patients are recognized by incidental isolation of *Aspergillus* species from sputum cultures. Antifungal therapy is unnecessary in these cases. These patients may rarely develop specific antibodies and symptoms of allergic bronchopulmonary aspergillosis (see above and Chapter 15). Upper airway aspergillosis resulting in sinusitis is not uncommon, and has an incidence of approximately 3%.[377–379] Aspergillus tracheitis, bronchitis, and laryngitis are uncommon.[363,380]

Aspergilloma (fungus ball) develops when aspergilli colonize a preformed pulmonary cavity, often secondary to tuberculosis or sarcoidosis, or as a result of other fibrocavitary diseases such as histoplasmosis, asbestosis, lung abscess, pulmonary infarct, bronchial cyst, bronchiectasis, bullous emphysema, or necrotic neoplasms.[381–383] An aspergilloma may also develop secondary to invasive aspergillosis and chronic necrotizing pulmonary aspergillosis (see below). Although asymptomatic for years, approximately 75% of patients with aspergilloma eventually develop hemoptysis, and 5% of the patients may die of uncontrollable hemorrhage.[376,384,385]

The clinical diagnosis of aspergilloma may be suspected by the triad of hemoptysis, positive serology, and radiographic demonstration of an intracavitary mass. Serum precipitins are present in 90% to 100% of patients, but culture of respiratory secretions yields an *Aspergillus* species in only 50% of cases.[376,385] The radiographs typically show a thick-walled cavity 3 to 5 cm in diameter, usually in the upper lobe or apex that contains an opaque rounded mass surrounded by a crescent of air (Monod's sign).[385–387] The adjacent pleura is usually thickened, and positional movement of the fungus ball can be demonstrated with decubitus films. If there is severe or recurrent hemoptysis, surgical resection is indicated. Other modes of treatment involve direct instillation of amphotericin B into the cavity in symptomatic patients who are not surgical candidates, although this form of therapy may not be effective. Approximately 10% of fungal balls disappear spontaneously.

Chronic Necrotizing Bronchial Aspergillosis

Chronic necrotizing bronchial aspergillosis (CNBA) occupies an intermediate position in the spectrum between colonizing and invasive forms of aspergillosis. It occurs in mildly compromised patient and is characterized by limited invasiveness.[388] Bronchial aspergillosis develops in patients with mild leukopenia, some of whom may have been treated with corticosteroids or antineoplastic drugs.[375,389] Symptoms include dyspnea, wheezing, and nonproductive cough. Extensive colonization of the bronchial tree may cause mucosal erosions, ulceration, and formation of pseudomembranes, with plugging of the bronchial lumen by casts composed of mucus and compacted mycelium.[390] Sputum cultures are rarely positive.[375] Computed tomograms show irregular or nodular bronchial wall thickening and focal bronchial narrowing involving a lobar or segmental bronchus, often associated with atelectasis.[391]

Chronic Necrotizing Pulmonary Aspergillosis

Chronic necrotizing pulmonary aspergillosis (CNPA) is a progressive locally destructive form of aspergillosis that occurs in mildly compromised patients, most of whom have underlying noncavitary lung disease. This entity was described in detail by Binder et al.[392] in 1982. Underlying disease or conditions associated with chronic necrotizing pulmonary aspergillosis include chronic obstructive pulmonary disease, inactive mycobacterial infection, sarcoidosis, pneumoconiosis, rheumatoid arthritis, ankylosing spondylitis, postradiation fibrosis, diabetes mellitus, alcoholism, anergy, and previous pulmonary resection. Approximately 25% of patients have no recognized predisposing disease. Most patients are middle aged, and approximately 25% have a history of being treated with low-dose corticosteroids. Predominant symptoms are fever, productive cough, weight loss, and malaise. Over 90% of patients have serum precipitins against *Aspergillus* antigens, a positive culture of respiratory secretions for *Aspergillus* species, and a normal leukocyte count. Chest radiographs may show pulmonary infiltrates and thick-walled cavities involving the upper lobes or superior segment of the lower lobes, often associated with pleural thickening. Approximately 40% of patients develop fungus balls in these newly formed cavities. Treatment consists of surgical resection or antifungal therapy with external drainage of the cavity. The disease duration may range from several months to several years. Survival is almost 80%, although many patients live with residual fibrocavitary disease. Systemic dissemination has not been reported in this form of aspergillosis.

Invasive Pulmonary Aspergillosis

Invasive pulmonary aspergillosis (IPA) is a fulminant and highly lethal opportunistic infection of severely compromised patients. Profound granulocytopenia (less than 500 neutrophils per square millimeter), and treatment for hematologic malignancy, particularly acute leukemia, are major risk factors for the development of IPA.[374,393–397] Other factors that may predispose to development of IPA include corticosteroid therapy, cytotoxic chemotherapy, broad-spectrum antibiotic therapy, concurrent or recent bacterial infection, immunosuppression following organ

transplantation, and exposure to large doses of aerosolized conidia.[366,370,392,398–401] Invasive pulmonary aspergillosis has been infrequently reported in patients with AIDS. One study reported an incidence of 0.69% among 2611 patients with AIDS, with poor survival despite treatment.[364,383] In another series of patients with AIDS, clinical symptoms, risk factors, and radiographic findings in this group were found to be similar to those in non-AIDS patients.[362] Interestingly, patients with AIDS, however, were found to sustain the whole spectrum of *Aspergillus*-related lesions, a feature not seen in other risk groups. It was also found that *Aspergillus* infections occurred predominantly in patients with an advanced stage of AIDS.[363]

Mononuclear phagocytic cells are the single most important line of defense against invasive aspergillosis; these cells ingest and kill the conidia of *Aspergillus,* whereas neutrophils damage the hyphal forms, probably by nonphagocytic microbicidal mechanisms.[402] Therefore, persistent granulocytopenia was found to be the only independent risk factor predisposing to IPA in patients with acute leukemia.[375] Corticosteroid treatment is another predisposing factor.[375,394–397] Humoral and cellular immune mechanisms appear to play a minor role in host defense against invasive aspergillosis.[375,402]

The symptoms of IPA, like those of many other opportunistic pneumonia, are nonspecific. Patients typically develop fever unresponsive to broad-spectrum antibiotics and new or changing pulmonary infiltrates in the setting of granulocytopenia and corticosteroid therapy. Nonproductive cough and dyspnea occur less commonly. Sputum cultures are positive in only one third of cases, and false-positive culture results are common.[376,394] Nevertheless, isolation of an *Aspergillus* species from respiratory secretions must be regarded as strong presumptive evidence of IPA in the proper clinical setting, and confirmation by other means should be aggressively sought. Blood cultures are almost always negative, and serodiagnosis is notoriously unreliable.[394,403]

Chest radiographs show a variety of abnormalities including patchy, multifocal or diffuse bilateral areas of consolidation; nodules; peripheral wedge-shaped, pleural-based infiltrates in an infarct pattern; and rarely bilateral miliary nodules.[394–396] The nodules and infarcts may cavitate following recovery from granulocytopenia, and later develop fungus balls within the cavities.[372,394] Up to one third of patients have a negative chest radiograph. In a pathoradiologic correlative study, approximately 50% of patients had concurrent pulmonary infection that obscured or mimicked the radiographic lesions of IPA.[395]

Pathology

The histologic diagnosis of *Aspergillus* infection depends on the identification of *Aspergillus* hyphae with their characteristic appearance. The hyphae are 3 to 6 μm in width, uniform in shape, regularly septate, and hyaline, with parallel walls. Branches arise at acute angles from the parent hypha, and the pattern of branching is progressive and dichotomous (of the same size as the parent hyphae) (Fig. 10.31; see also Figs. 10.42C and 10.53B, below). The viable hyphae are basophilic, whereas necrotic hyphae are hyaline or eosinophilic. Although visible with H&E, hyphal morphology is best demonstrated with special stains such as GMS. The hyphae may exhibit atypical or degenerative features under certain circumstances. For example, in an aspergilloma (Fig. 10.32) the hyphae may appear bizarre, swollen, varicose, or globose, up to 15 μm in diameter with irregular contours, inconspicuous septa, and abortive branches (Fig. 10.32B). Calcium oxalate crystals, which are recognizable by polarization, are occasionally deposited within aspergillomas or invasive disease, particularly that associated with *A. niger* (Fig. 10.33; see also Fig. 23.4 in Chapter 23).[404,405] The association of aspergillus infection with calcium oxalate crystals was first demonstrated by Nime and Hutchins.[406] It is postulated that *A. niger* produces oxalic acid via citrate in the tricarboxylic acid cycle, followed by combination of oxalate with tissue or blood calcium resulting in precipitation of calcium oxalate. Invasive *A. niger* infection with oxalosis has been associated with a false-positive cytoplasmic-staining antineutrophil cytoplasmic antibody (C-ANCA) level.[407]

In fungus balls or chronic granulomatous lesions, the hyphae may be surrounded by a radiating, eosinophilic corona of proteinaceous material, referred to as the

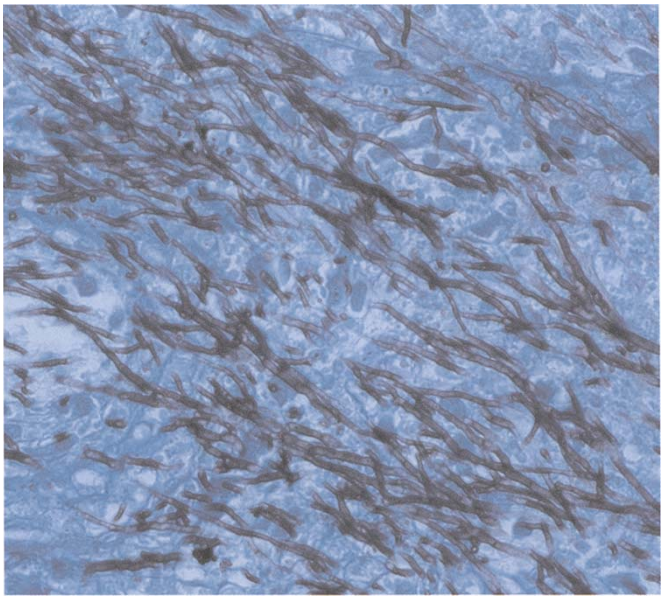

FIGURE 10.31. *Aspergillus.* Septate hyphae have parallel margins and dichotomous branching pattern (GMS).

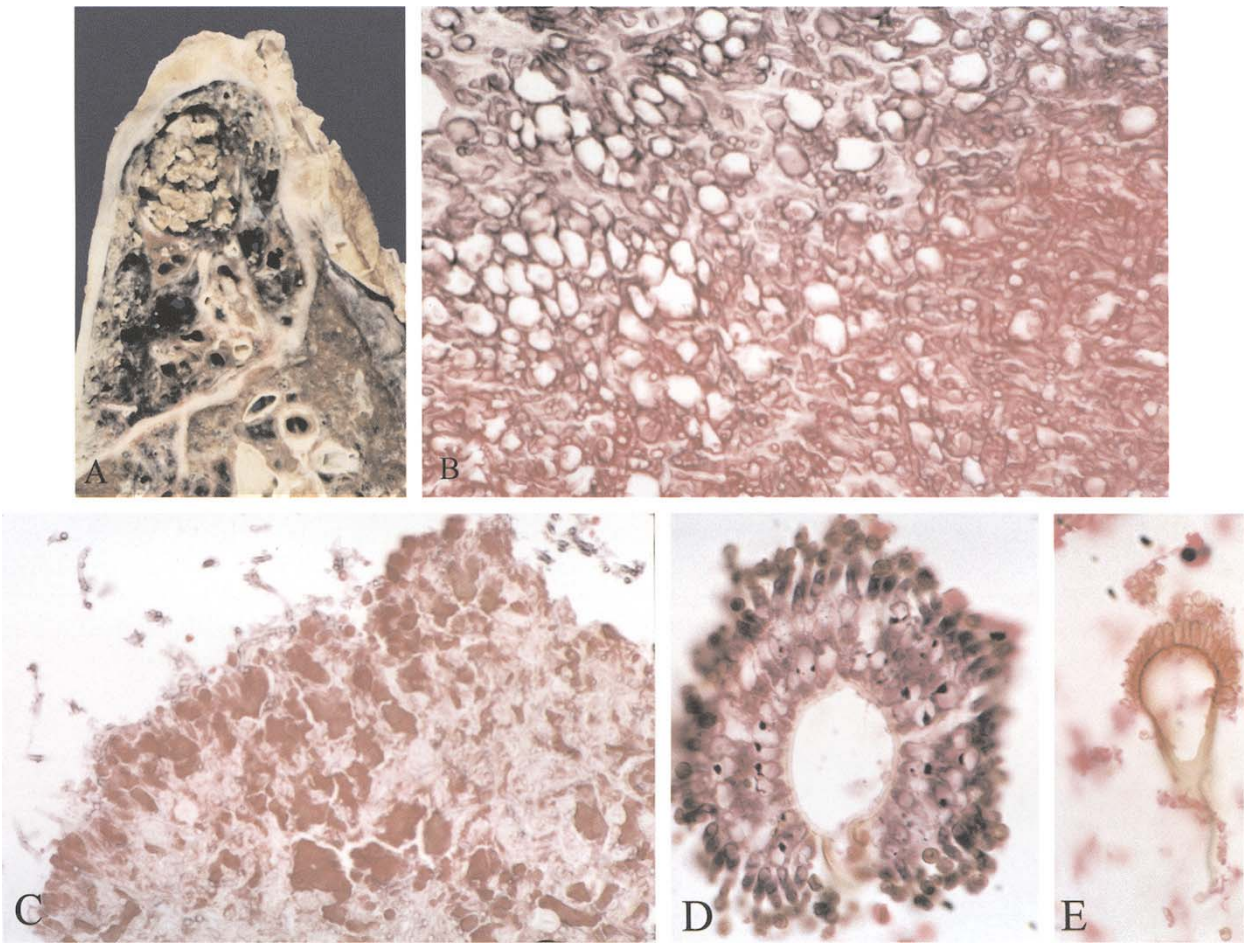

FIGURE 10.32. Aspergilloma. **A.** Fragmented, yellow-tan, friable fungus ball fills an apical cavity in end-stage sarcoidosis. There is marked pleural thickening and adhesions. **B.** Degenerated globose hyphae. **C.** Deeply eosinophilic Splendore-Hoeppli phenomenon coating degenerated hyphae. **D.** Conidiophore of *A. niger* with circumferential double-layer of phialides covered with brown spores (H&E, × 40). **E.** Conidiophore of *A. fumigatus* in which phialides project from the upper part of the vesicle (H&E, × 100).

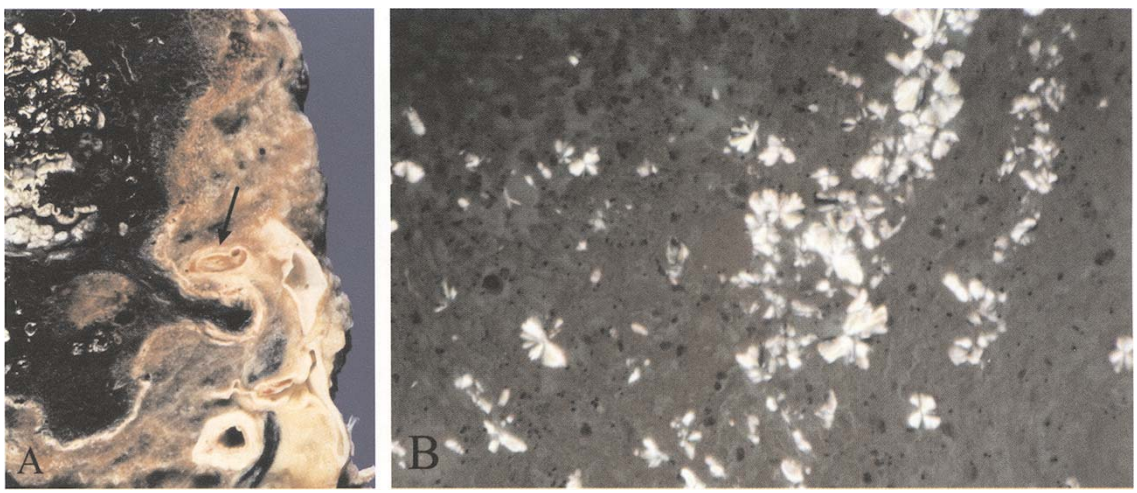

FIGURE 10.33. Aspergillosis niger. **A.** Irregular black necrotic area probably represents a superinfected infarct. A thrombosed pulmonary artery (arrow) is proximal to the area of necrosis. Whitish areas within the necrotic zone probably represent non-pigmented mycelia. **B.** Abundant birefringent calcium oxalate crystals are present in an area of necrosis (partially polarized light).

Splendore-Hoeppli phenomenon (Fig. 10.32C). *Aspergillus terreus* may produce pyriform or globose aleurioconidia, 3 to 6 μm in diameter, on short conidiophores that arise laterally from the hyphae.[408] These can be confused with lateral blastoconidia arising from *C. albicans* hyphae. Occasionally conidial heads produced by *Aspergillus* species in cavitary lesions exposed to ambient air may be found in aspergillomas and in the lesions of necrotizing tracheobronchitis (Fig. 10.32D,E). The conidial heads, specialized for asexual reproduction, are structures that arise directly from vegetative mycelium. The conidial heads are composed of a vesicle, which is the terminal bulbous dilatation of the conidiophore, upon which are borne one or two layers of phialides. Conidia arise in chains from the distal ends of the phialides. A definitive histologic diagnosis of aspergillosis can be made when conidial heads are present, and their distinct morphology often may suggest which species is present (Fig. 10.32D,E). Because the hyphae of some other opportunistic pathogens resemble those of the aspergilli, a specific histologic diagnosis of aspergillosis solely on the basis of hyphal morphology is not justified unless confirmed by culture or direct immunofluorescence.

The intracavitary aspergilloma, sometimes erroneously referred to as a mycetoma, is a compact, round conglomerate of hyphae that develops within a preformed cavity (Fig. 10.32A).[409,410] Most such cavities are round or oval and sharply circumscribed, 1 to 7 cm or greater in diameter, and communicate with the bronchial tree. Aspergillomas may occasionally be multiple or bilateral. The walls of the cavities, 1 to 5 mm thick, are grayish-white and fibrous with smooth or shaggy inner surfaces. If close to the surface, the adjacent pleura is thickened and fibrotic. Microscopically the walls of the cavities are composed of vascularized fibrous connective tissue infiltrated by lymphocytes, plasma cells, histiocytes, and occasionally neutrophils and eosinophils. Granulomas may be found occasionally, particularly in the walls of tuberculous cavities. The internal surfaces may be lined by respiratory or metaplastic epithelium that is often extensively eroded, accounting for the frequency of hemoptysis. Bronchial artery branches are usually prominent around cavities and richly vascular granulation tissue is prominent adjacent to the lumen.

The fungus ball, which may fill most of the cavity, but is usually unattached to the wall, is smooth but lobulated, yellowish-brown, and friable (Fig. 10.32A). Microscopically, the fungus ball is composed of concentric or convoluted layers of radially arranged and intertwined hyphae. Variation in the density of hyphae in adjacent layers, produced by alternation of rapid and slow phases of hyphal growth, sometimes resembles the zonation that may be observed in colonies cultured on solid media.[409] In the center of the fungus ball, the hyphae are often nonviable and eosinophilic, whereas those at the periphery are basophilic. The morphology of degenerated hyphae can be quite atypical, and these may be mistaken for other pathogens, such as the zygomycetes. Conidial heads, produced in some cases, emerge from the surface of the fungus ball and cavity wall, and are shed into the cavity. Although hyphae can be found along the surface and within the fibrous wall of the cavity, invasion into the adjacent lung parenchyma does not occur unless the host is immunocompromised. Most examples of "invasive aspergilloma" previously reported in the literature actually represent aspergilloma developing in IPA or CNPA. Fungus balls that develop within the lesions of IPA following restoration of bone marrow activity differ in their histogenesis from those that develop in preformed cavities, since the fungus balls are in fact autoamputated spheres of necrotic lung tissue ("lung balls") that contain invasive hyphae (Fig. 10.34).[399,411] The margins of these hyphae and of lung tissue forming the cavity walls contain an unusually large number of degenerated neutrophils; these neutrophils are believed to produce the cavitary lesion and fungus ball by enzymatic digestion of the necrotic lung.

Chronic necrotizing bronchial aspergillosis (CNBA) accounts for 6% to 9% of cases of bronchopulmonary aspergillosis in some series.[394,395] Bronchial aspergillosis

FIGURE 10.34. Invasive cavitary aspergillosis. A homogeneous necrotic sequestrum of lung ("lung ball") has retracted to form a cavitary lesion. (Compare with the friable appearing fungus ball in Figure 10.32A).

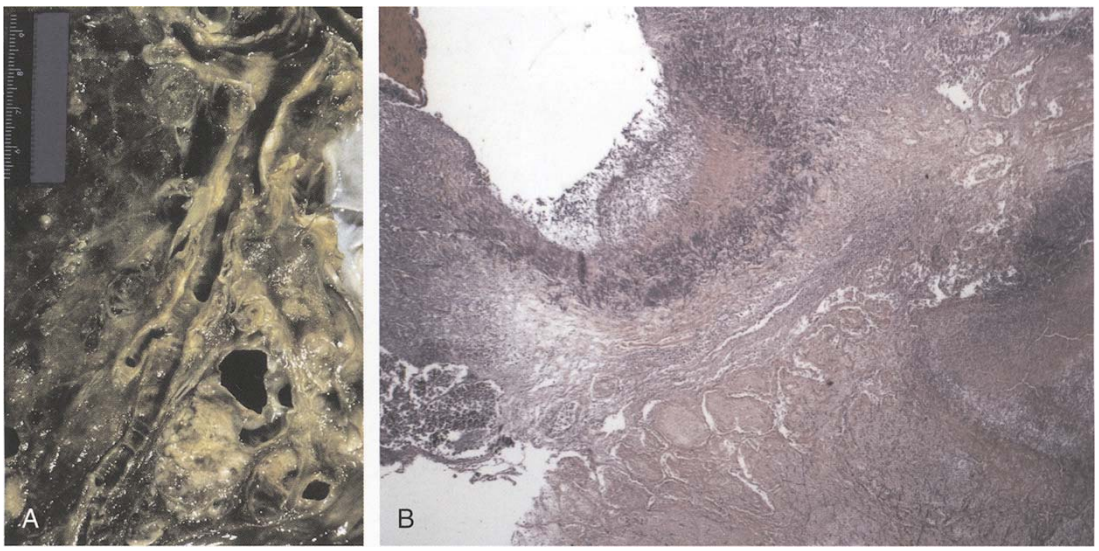

FIGURE 10.35. Chronic necrotizing bronchial aspergillosis (CNBA). **A.** Lung section shows a mildly dilated bronchus with yellowish shaggy lining that is involved by superficially invasive aspergillosis, a feature of CNBA. A tan-yellow area of consolidation adjacent to the bronchus is also present. **B.** Bronchial wall section from the lung seen in **A** shows a thick layer of aspergillus hyphae infiltrating the bronchial wall. The underlying mucosa is ulcerated and congested, and the surrounding lung is infarcted (H&E).

may involve much of the tracheobronchial tree or remain confined to localized segments.[390,409] Patients in whom the infection is confined to the airways are less severely immunocompromised than those with IPA.[375] The respiratory mucosa is eroded and replaced by a granular, brown apparent pseudomembrane composed of inflammatory exudate, mucus, and hyphae that may occlude the subsegmental bronchi (Fig. 10.35; see also Fig. 23.5 in Chapter 23). In about 40% of cases conidial heads may be found within air spaces.[390,394] Although the infection is often confined to airways, limited invasion of hyphae into peribronchial lung tissue sometimes occurs, and bronchial localization of hyphae may rarely be a dominant pattern in IPA (Fig. 10.35B). Microscopically, invasion and destruction of bronchial walls with extension of hyphae into the peribronchial blood vessels and parenchyma may be seen in these cases.

The pathologic findings in CNPA incorporate some pathologic features of both aspergilloma and IPA. The major findings include large cavities that contain amorphous mycelial aggregates of well-formed fungus balls with some degree of invasion and destruction of surrounding lung tissue.[392,412] The parenchyma adjacent to the cavities usually shows chronic inflammation and fibrosis, which seems often to result from preexisting lung disease, but abscesses that contain hyphae may also be found.[392,413] Yousem[414] observed three histologic features in CNPA: necrotizing granulomatous pneumonia, granulomatous bronchiectatic cavity with fungus ball, and bronchocentric granulomatosis-like appearance. The pathologic diagnosis of CNPA requires demonstration of invasion and destruction of noncavitary lung tissue in an appropriate clinical and radiologic setting. CNPA is distinguished from IPA by the limited extent of parenchymal invasion, as well as the absence of vascular invasion and infarction. Fibrocaseous granulomas that contain aspergillus hyphae are often included in the category of CNPA (Fig. 10.36).

Invasive pulmonary aspergillosis is initiated by colonization of the tracheal bronchial tree with an *Aspergillus* species, followed by endobronchial mycelial proliferation, necrotizing bronchial aspergillosis, and then transbronchial invasion.[376] Invasion that occurs distally in the bronchial tree into adjacent small pulmonary blood vessels produces focal parenchymal lesions, whereas more proximal invasion into lobar and segmental blood vessels produces large hemorrhagic infarcts (Fig. 10.37).[376,395] Hyphal invasion of arteries and veins is the pathologic hallmark of IPA, and accounts for most of the observed parenchymal lesions. Occasionally secondary invasion by *Aspergillus* of the necrotic lesions produced by other pathogens such as *Pseudomonas aeruginosa* or a *Candida* species may account for some of the lesions of IPA.[375,394,395] Hematogenous dissemination of *Aspergillus* to the lungs from an extrapulmonary focus seldom occurs.

The characteristic parenchymal lesion of IPA is a nodular pulmonary infarct (target lesion) that results

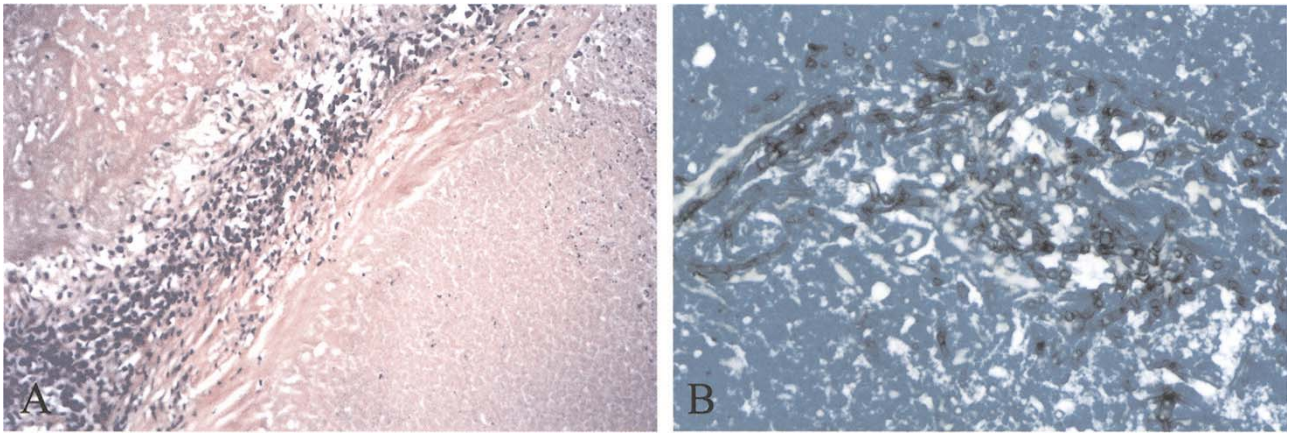

FIGURE 10.36. Chronic necrotizing pulmonary aspergillosis (CNPA). **A.** Necrotizing fibrocaseous granuloma found in a 68-year-old man with bullous emphysema. **B.** Aspergillus hyphae in the necrotic center of the granuloma (GMS).

from hyphal invasion of a small peripheral pulmonary artery. The target lesions may be several millimeters to 3 cm or greater in diameter, often appearing as yellowish-gray, necrotic nodules surrounded by hemorrhagic rims (Fig. 10.37A). Microscopically these lesions are composed of a central zone of ischemic necrosis, an intermediate zone of fibrinous exudate that may contain degenerated neutrophils, and a peripheral zone of parenchymal hemorrhage. An occluded necrotic artery can often be identified within or at the edge of this lesion. Hyphae often extend through the vascular wall and invade by radial growth throughout the surrounding necrotic parenchyma (Fig. 10.37B). Target lesions are usually multiple and at least 50% of the larger nodules undergo cavitation. Other descriptive terms used for the pathologic lesions seen in IPA are patchy necrotizing bronchopneumonia, focal pulmonary necrosis, nodular consolidation, and rounded bronchopneumonia. The target lesion is not specific for IPA, and may be seen with other opportunistic angioinvasive mycotic infections such as candidiasis and mucormycosis.

Large, wedge-shaped, pleural-based hemorrhagic infarcts, often involving most of a lobe, are invariably associated with thrombosis of a major pulmonary arterial branch caused by hyphal invasion from an adjacent bronchus. Such infarcts, which are often multiple and bilateral, are found in about one third of cases of pulmonary aspergillosis.[375,394,395] Bronchoarterial fistulas may be a cause of

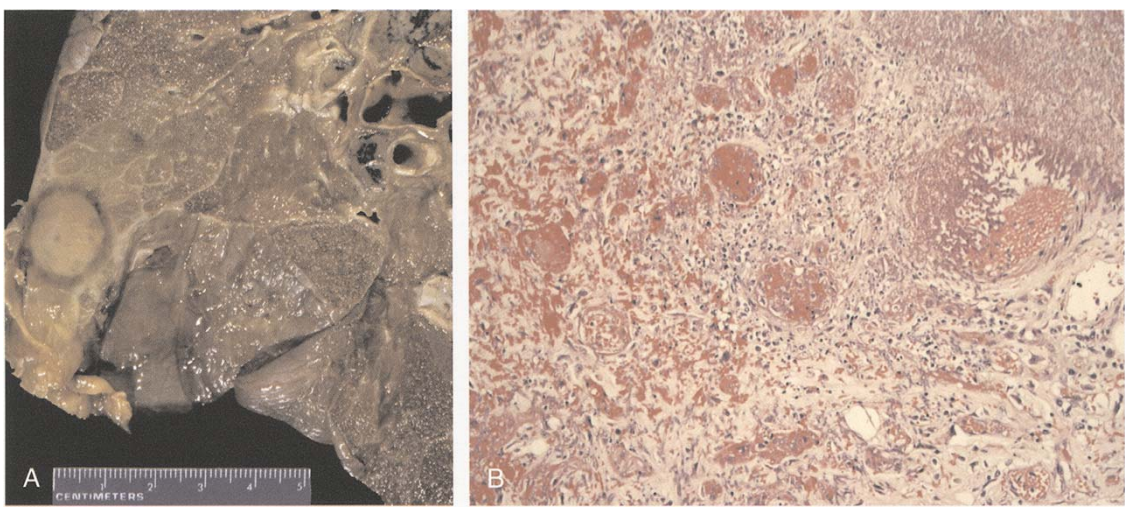

FIGURE 10.37. Invasive pulmonary aspergillosis (IPA). **A.** Lung section shows invasive aspergillosis, with a circular hemorrhagic infarct in the lower lobe ("target lesion"), surrounded by a consolidated area. **B.** Lung with early infarction. A small pulmonary vessel is invaded by aspergillus hyphae.

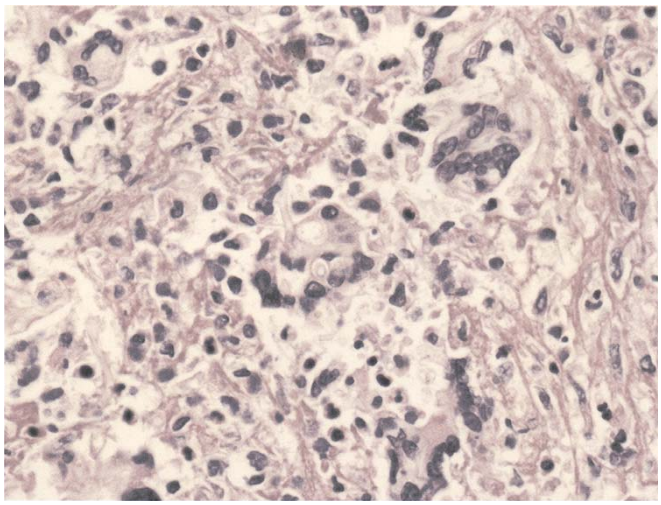

FIGURE 10.38. Margin of pulmonary infarct has a mixed inflammatory infiltrate and multinucleated giant cells with intracytoplasmic aspergillus hyphae (seen in cross section).

sudden massive hemoptysis. Suppurative bronchopneumonia may occur in nongranulocytopenic patients, and necrotizing bronchial aspergillosis without extensive vascular or parenchymal invasion may be found in about 10% of IPA cases. In approximately 1% to 6% of patients with IPA, fungus balls develop within the necrotic lesions, more commonly in those diagnosed early and treated aggressively (see Fig. 10.34).[375,394,399,415]

Typical *Aspergillus* hyphae are abundantly found in the bronchial, vascular, and parenchymal lesions of IPA. The hyphae often radiate outward from the center of the target lesions, and extend through tissue planes in parallel waves (Fig. 10.31). A granulomatous inflammatory reaction may be seen at the periphery of the infarct in nonimmunocompromised patients, and in patients treated with antifungal therapy (Fig. 10.38).[392,395,410] Hematogenous dissemination may occur in 25% to 35% of severely immunocompromised patients. The respiratory tract is almost always the portal of entry, and pulmonary lesions can be demonstrated in 90% to 97% of patients who have hematogenous dissemination. Other organs frequently involved in systemic aspergillosis include the brain, heart, kidneys, gastrointestinal tract, liver, spleen, and thyroid gland.[410] The pleura may be involved, resulting in a necrotizing fibrinous or occasionally a granulomatous pleuritis or aspergillus empyema (Fig. 10.39).

The differential histologic diagnosis of *Aspergillus* hyphae includes *Fusarium, Pseudallescheria,* and zygomycetes (*Absidia* and *Rhizopus*). *Fusarium* and *Pseudallescheria* both form branched, septate hyphae that closely resemble those of *Aspergillus* and cause invasive pulmonary infection and intracavitary fungus balls (see below). *Aspergillus* hyphae in aspergillomas and in some chronic granulomatous lesions are difficult to distinguish from the hyphae of zygomycetes. *A. terreus* has pyriform and globose aleuroconidia on short conidiophores that arise laterally from the hyphae, and may cause difficulty in diagnosis. The hyphal forms of *Candida* and *Trichosporon* species are easily distinguished from aspergilli, since they are accompanied by yeast cells and pseudohyphae, and hyphae with arthroconidia, respectively. Fluorescent antibodies and immunoperoxidase conjugates can help confirm a histologic diagnosis of aspergillosis in difficult cases.[48,50]

Diagnosis

The clinical diagnosis of IPA is difficult because of the nonspecific nature of the symptoms, the frequency of

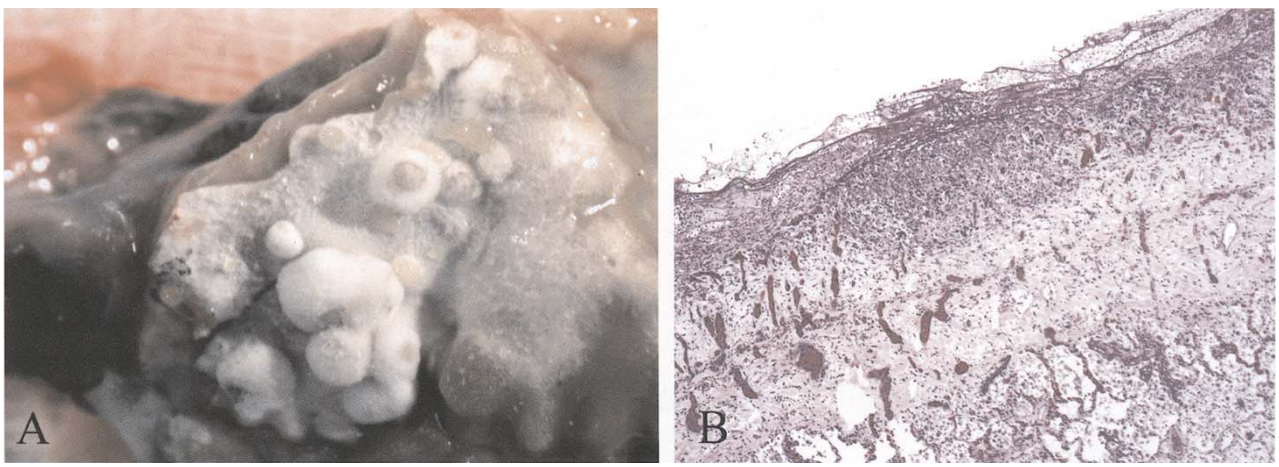

FIGURE 10.39. *Aspergillus* pleuritis and empyema. **A.** Exuberant fungal growth fills the thoracic cavity and obscures the underlying lung in this autopsy specimen. (Courtesy of Dr. Imran Sharief, University of Arizona.) **B.** Pleural invasion by aspergillus hyphae can occur in association with a pleura-based infarct, resulting in necrotizing pleuritis.

concurrent infections, and the lack of reliable microbiologic and radiographic findings. The infection has a high mortality, with less than 30% survival.[370] Early detection of circulating galactomannan by sandwich ELISA is associated with high sensitivity and specificity.[416–418] Fungal DNA can be examined from BAL fluid or serum using PCR, which, although very sensitive, can give false-positive results.[63,419–421] Computed tomography of the chest in high-risk patients is very helpful in the diagnosis of aspergillosis.[367] Survival is crucially dependent on early diagnosis, aggressive therapy, and remission of underlying disease.

Treatment

Traditionally, amphotericin B has been the drug of choice; however, it has severe side effects and a high death rate.[376] More favorable responses are seen with sequential amphotericin B followed by itraconazole, posaconazole, and voriconazole, and the less toxic lipid formulations of amphotericin are also effective against the aspergilli.[422,423] The echinocandin caspofungin is effective, especially for salvage therapy.[303,304] Surgical excision is reserved for localized infection and for aspergillomas, with survival rate of up to 84% at 5 years and 74% at 10 years in a study of 84 patients.[424]

Zygomycosis (Mucormycosis)

The term *zygomycosis* refers to a variety of opportunistic infections caused by fungi classified in the class Zygomycetes (formerly Phycomycetes). The more generic term *mucormycosis* is often used synonymously.[31,425] Zygomycosis includes localized nonopportunistic infections of subcutaneous tissues and rhinofacial structures, prevalent in the tropics, that are caused by zygomycetes in the order Entomophthorales.[426] These two forms of zygomycosis are clinically and pathologically distinct. Zygomycosis has a wide spectrum of clinicopathologic presentations. The rhinocerebral form is an invasive infection of the nasal cavity, paranasal sinuses, palate, face, and orbit that extends to the central nervous system. This form of infection occurs most often in diabetics, and rarely in patients with leukemia.[427,428] Pulmonary and disseminated zygomycosis are particularly prone to occur in patients with acute leukemia or lymphoma.[429–432] There is at least one report of pulmonary zygomycosis caused by *Cunninghamella bertholletiae* in a nonimmunocompromised patient.[433]

Zygomycosis is the third most common opportunistic mycosis among patients with neoplastic diseases.[434,435] The incidence of zygomycosis appears to be increasing, largely because of advances in the treatment of hematologic malignancy.[436] Pulmonary zygomycosis occurs most frequently in patients with acute leukemia or lymphoma, and is associated with leukopenia, corticosteroids and chemotherapy, antibiotic therapy, concurrent bacterial infection, and relapse or lack of sustained remission of the underlying disease.[437–439] Other underlying diseases that can predispose to pulmonary zygomycosis include poorly controlled diabetes mellitus, renal failure with acidosis, severe burns, and therapy for nonhematologic neoplasms and AIDS.[440–443] Lung transplant patients are also at high risk for invasive fungus infection.[444] Pulmonary infection is rare in patients without an underlying identifiable predisposing illness.

Microbiology

The agents of zygomycosis include species within several genera including *Rhizopus, Absidia, Mucor, Rhizomucor, Saksenaea, Cunninghamella, Mortierella, Syncephalastrum*, and *Apophysomyces*. The *Rhizopus* and *Absidia* species are the most frequently implicated agents of human infection. Most of the pathogenic zygomycetes grow rapidly in culture on enriched media, producing cottony mold-like colonies composed of nonseptate mycelia. Asexual reproduction occurs by the formation of sporangiospores, or rarely chlamydoconidia, and sexual reproduction by formation of zygospores. Some genera produce rhizoids or anchoring rootlets in culture. Species identification is complex, difficult, and based on the morphology of asexual structures, physiologic characteristics such as thermotolerance, mating behavior, and morphology of the sexual zygospores.[427] The zygomycetes are widely distributed in nature and can be isolated from soil and decaying organic material.

Clinical Features

The clinical features of pulmonary zygomycosis are similar to those of invasive aspergillosis. The patients typically have persistent fever and new or progressive pulmonary infiltrates that are unresponsive to antibacterial therapy.[431,445] Signs and symptoms of pulmonary infection may develop because of propensity of the fungi to invade the pulmonary vascular tree and cause pulmonary arterial thrombosis. Chest radiographic abnormalities include patchy or nonhomogeneous infiltrates, and solitary or multiple areas of consolidation.[446,447] Cavitation and pleural effusion are infrequent, and occasionally no abnormalities can be detected. The sequence of radiographic changes beginning with "rounded pneumonia," which progresses to a pulmonary infarction pattern or to large areas with the appearance of bronchopneumonia, is considered by some to be highly indicative of an opportunistic pulmonary fungal infection in the appropriate clinical setting.[448] A miliary or nodular pattern may be found in patients with hematogenous pulmonary dissemination from another primary site.

Pathology

Pulmonary zygomycosis results from germination of inhaled sporangiospores, or aspiration of hyphae from a focus of infection in the upper respiratory tract, followed by proliferation of the mycelia within the proximal bronchial tree.[449] The hyphae are aggressively invasive and penetrate through the bronchial wall and grow into the adjacent blood vessels, particularly the arteries, resulting in thrombosis. This results in pulmonary infarction, which is usually hemorrhagic and often parahilar as well as peripheral in location. Proximal infarcts are rounded or irregular in configuration, whereas peripheral infarcts are more often typically wedge shaped and accompanied by pleural invasion (Fig. 10.40). Acute exudative bronchopneumonia results from the spread of hyphae into adjacent parenchyma in the nongranulocytopenic host. Cavities may develop in some of the infarcts and may contain necrotic tissue fragments mixed with hyphae. However, abscesses are uncommon and often signify secondary bacterial infection. Complications of infection include rupture of pulmonary arteries secondary to hyphal invasion resulting in massive hemoptysis, and granulomatous mediastinitis from extension of infection to the mediastinum.[425,450]

The frequently involved organs in patients who die of disseminated zygomycosis include pulmonary (in 80% to 100% of patients), followed by central nervous system, kidneys, spleen, liver, heart, and gastrointestinal tract.[31,441,451] Microscopically, the lesions of pulmonary zygomycosis consist of hemorrhagic infarcts, nodular infarcts, and suppurative pneumonitis (Fig. 10.41). Zygomyces are neurotropic fungi, and invade the perineural sheaths to track along the nerve fibers.[452,453] As a result, complete surgical excision of the infection becomes difficult, particularly in sinofascial infections. Chronic indolent and partially treated infections may have a granulomatous component.[425,428] The hyphae are distributed haphazardly throughout these lesions, and are conspicuous within the walls of blood vessels and in thrombi. The hyphae are pleomorphic, broad (10 to 25 μm or greater in width), with delicate thin walls, and rare septa. The hyphae are also often twisted, folded or wrinkled owing to their large size. Variation in the hyphal caliber produces uneven contours in longitudinally sectioned hyphae. The branching pattern is also irregular, and branches are often oriented at right angles to the parent hyphae (Fig. 10.42A). The hyphae are demonstrated well with H&E stains as well as silver stains (GMS), although the intensity of silver staining is often less than that of *Aspergillus* hyphae (Fig. 10.42B,C). Occasionally thick-walled, densely stained, round or ovoid chlamydoconidia and sporangia may be found with the invasive hyphae (Fig. 10.42D).[454]

The differential diagnosis of zygomycete hyphae include *Aspergillus* species in tissue sections; however, *Aspergillus* hyphae are narrower, more uniform, regularly septate, and have an orderly progressive, dichotomous pattern of branching (Fig. 10.42B,C). The hyphae of *Rhizopus, Absidia,* and other zygomycete species can be

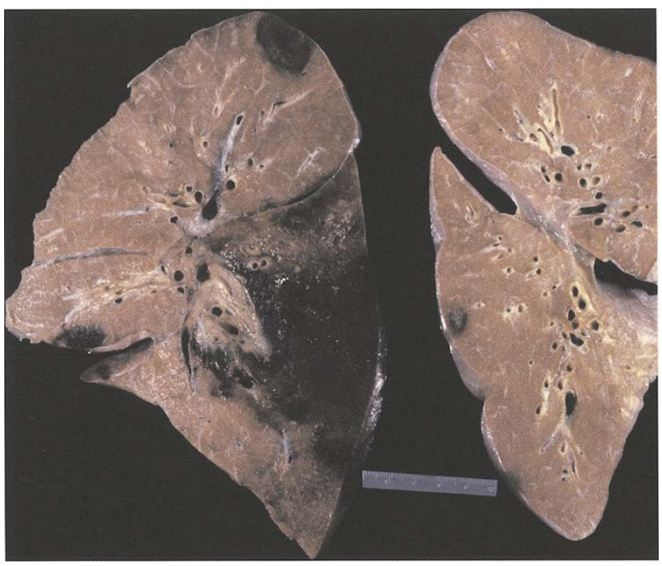

FIGURE 10.40. Zygomycosis. Hemorrhagic infarcts, including wedge-shaped and round lesions, are seen in this lung specimen of invasive zygomycosis.

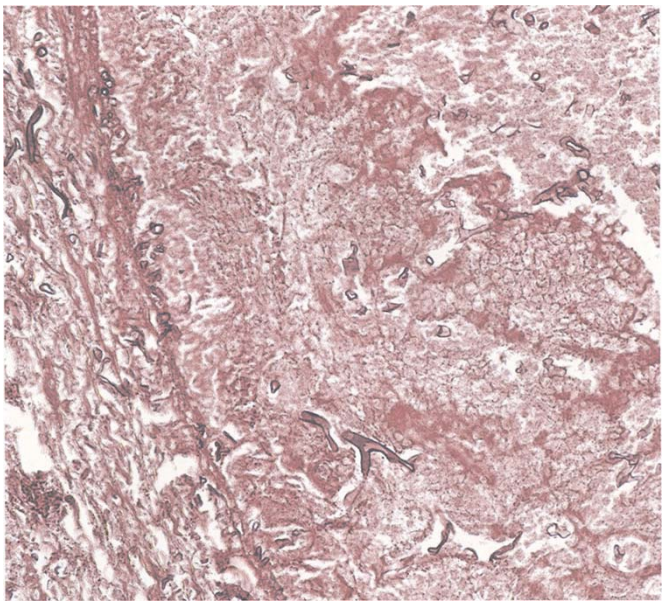

FIGURE 10.41. Zygomycosis. Photomicrograph of an infarct shows coagulative necrosis of lung parenchyma. Fungal hyphae are well seen, even at low magnification, in this H&E-stained section.

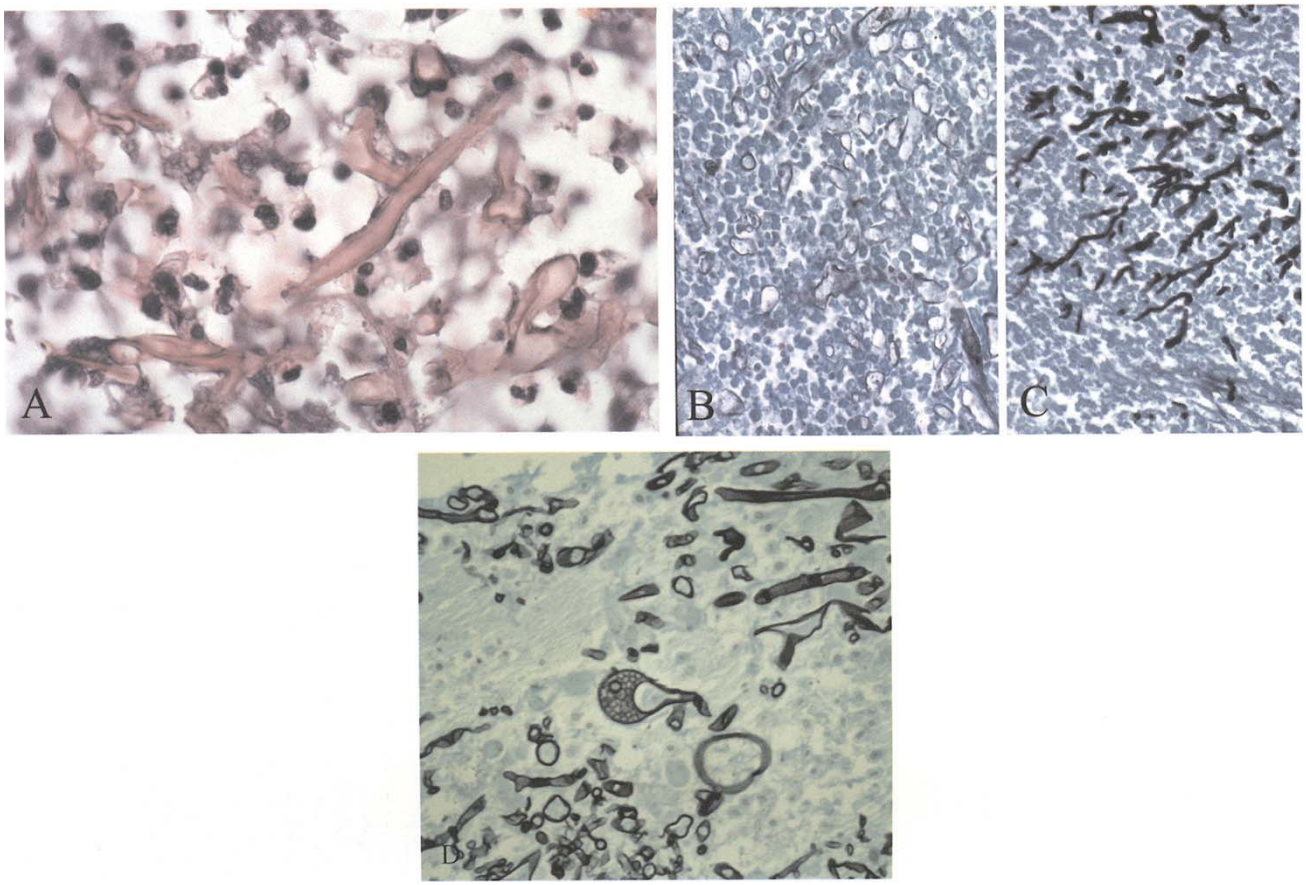

FIGURE 10.42. Zygomycetes. **A.** Large nonseptate hyphae with hollow appearance and right angle branching (H&E). **B.** Relatively weak staining of zygomycete hyphae with GMS. **C.** Gomori's methenamine silver–stained section of *Aspergillus* for comparison. There is intense staining of smaller caliber hyphae showing dichotomous acute angle branching. **D.** *Absidia corymbifera* sporangia and hyphae (GMS).

identified in deparaffinized sections of formalin-fixed tissue by direct immunofluorescence with a screening conjugate.

Diagnosis

The diagnosis of pulmonary zygomycosis is often difficult to establish. Cultures of material from the respiratory tract or other sites are usually negative in up to 60% of patients, and no reliable serologic test to confirm the diagnosis is available. Therefore, definitive diagnosis usually depends on identification of the hyphae in biopsy specimens obtained from the respiratory tract.[455]

Molecular techniques such as PCR, DNA sequencing of the PCR product, and homology search with nucleotide basic local alignment search are now available for identification of fungi causing zygomycosis.[456]

Treatment

The successful treatment of zygomycosis involving superficial tissues involves aggressive debridement or resection of localized foci of infection and adjunct antifungal therapy.[427,447,457,458] Pulmonary zygomycosis, however, is an almost uniformly fatal infection for several reasons: the patients are debilitated, the underlying disease is most often hematologic malignancy, the clinical diagnosis may be difficult to establish, and invasive diagnostic procedures may be delayed until the infection has progressed beyond a localized stage. The antifungal agent of choice is amphotericin B, but its effectiveness may be limited due to poor penetration of infarcted tissue. Patients with the best chance of survival with pulmonary zygomycosis are those with a localized focus of infection amenable to segmental resection or lobectomy, or those who have a controllable underlying disease such as diabetes mellitus.[455,457,459,460]

Coccidioidomycosis

Coccidioidomycosis is an increasingly important pulmonary fungal infection that is endemic in the Western Hemisphere. Coccidioidomycosis has been rediscovered

as an emerging disease because of the migration of Americans to the rapidly developing Sun Belt states where *Coccidioides immitis* is intensely endemic.[22,461–464] As the endemic population has expanded, a growing segment of susceptible population has evolved. Increased travel to endemic areas has contributed to an increased incidence of outbreaks in nonendemic areas.[465–467] Corticosteroid therapy, chemotherapy, organ transplantation, and the AIDS epidemic have contributed to the increased incidence of coccidioidomycosis.[468–475] The majority of cases are clinically inapparent and resolve spontaneously. Pregnant women, however, have a high risk of developing disseminated disease, with a rate 40 to 100 times that of general population. The rate is higher in dark-skinned races and the mortality rate is also higher at 20% to 60%.[476] Symptomatic primary infection usually resolves as well but may lead to benign residual nodules of cavities, persistent pneumonia, or chronic progressive pneumonia. Extrapulmonary dissemination is rare except in certain high-risk groups. Approximately 100,000 new cases occur annually in the United States, and about 70 cases per year are fatal.

The disease is caused by two dimorphic species: *C. immitis* and *C. posadasii*. Because the two species are morphologically identical and only differ by a few sequences in their genomes, distinguishing these two species does not appear to be necessary.[477] We have elected in this chapter to consider *C. immitis* as the sole etiologic agent of coccidioidomycosis.

Coccidioides immitis is widely distributed throughout the lower Sonoran life zone, which is characterized by a semi-arid climate with a short, intense rainy season. Coccidioidomycosis is highly endemic within parts of Southern California, Arizona, New Mexico, Nevada, Utah, and Texas.[478,479] The infection also occurs in patients living in northern and central Mexico, Baja California, Guatemala, Honduras, Nicaragua, Venezuela, Argentina, Columbia, Bolivia, and Paraguay.[480]

Microbiology

Coccidioides immitis exists in nature and grows in culture at 25°C. Its saprobic mold form is composed of septate branched hyphae, 2 to 4μm wide. Arthroconidia, often barrel-shaped and alternating with empty cells (disjunctor cells) are produced from aerial mycelium. Although barrel-shaped arthroconidia typify the mycelium of *C. immitis* in culture, definitive identification requires that a suspected isolate be confirmed with an exoantigen test or DNA probe. Because the airborne arthroconidia are highly infectious, mycelia from cultures of *C. immitis* must be handled with extreme caution in a biosafety level (BSL)-3 laboratory.[481]

Infection is acquired by inhalation of a single cell (arthroconidium), approximately 3 to 5μm in size that results from disruption of mycelia in soil. The arthroconidium changes from a barrel-shaped cell to a spherical structure in the lung, and then enlarges to the spherule form, which may become 30 to 100μm in diameter. The enlarging spherules develop internal septations, and within each of the resulting subcompartments, endospores evolve. After several days, mature spherules rupture, releasing endospores into the infected tissues. Each endospore can potentially develop into another spherule, and repeat the cycle.[479,480] Under certain circumstances, endospores can germinate within host tissues to produce hyphae and arthroconidia; these develop into spherules.[482,483] The sequence of inhalation of the spores and the life cycle of *C. immitis* is illustrated in Figure 10.43.

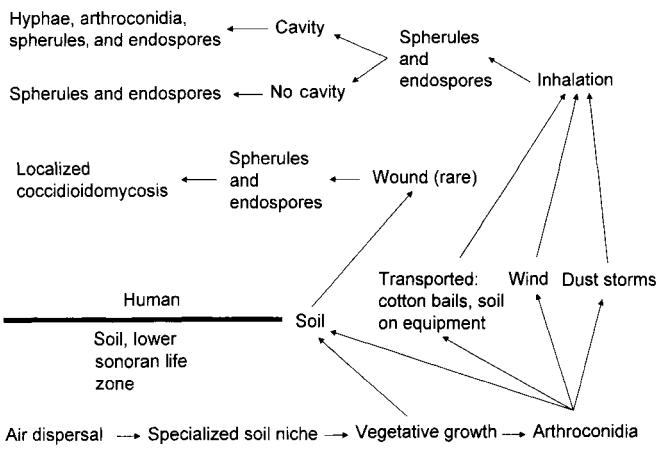

FIGURE 10.43. Sequelae of coccidioides spore inhalation and life cycle.

Clinical Features

There are several clinical forms of coccidioidomycosis, including acute infection with and without hypersensitivity reactions, miliary coccidioidomycosis, chronic progressive pneumonia, coccidioidoma, fibrocavitary disease, and disseminated disease.[484–486]

Acute Infection

Approximately 60% cases of primary pulmonary coccidioidomycosis are clinically inapparent. These asymptomatic individuals can be recognized by a positive skin test reaction to coccidioidin. The remaining 40% of patients develop a spectrum of symptoms ranging from a flu-like illness to frank pneumonia following an incubation period of 1 to 4 weeks. Symptoms most often include cough, fever, headache, chest pain, dyspnea, and malaise. About 10% of symptomatic patients develop allergic manifestations such as erythema nodosum, erythema multiforme, and arthralgias, which signify the development of hypersensitivity to *C. immitis*; these usually have a benign

clinical course. Chest radiographs are normal in up to 20% of symptomatic patients or show predominantly soft, hazy, patchy, or segmental pneumonic infiltrates, and less commonly solitary or multiple nodules, cavitary lesions, hilar lymphadenopathy, and pleural effusion.[487,488]

Chronic Progressive Coccidioidal Pneumonia

Chronic progressive coccidioidal pneumonia (CPCP) develops in less than 1% of patients hospitalized for primary pulmonary coccidioidomycosis. This chronic infection mimics chronic tuberculosis both clinically and radiographically. Symptoms persist for several years and sputum cultures are positive for *C. immitis*, complement fixation titers are often high, and skin test reactivity is usually negative, signifying loss of delayed type hypersensitivity to the fungus. Conversion of a positive skin test to a negative one indicates a poor prognosis.[486] Chest radiographs usually show biapical fibronodular lesions and multiple cavities with retraction of pulmonary parenchyma. Since host defenses in CPCP cannot successfully overcome the infection, patients must be treated with amphotericin B.

Miliary Pulmonary Coccidioidomycosis

This is another serious form of the disease, seen in about 4% of patients hospitalized for primary coccidioidomycosis. Miliary pulmonary spread signifies hematogenous dissemination, and occurs early in the course of primary pulmonary infection in high-risk patients.[486,489] Its clinical evolution is rapid and explosive, and mortality is high unless an early diagnosis is made and therapy with amphotericin B rapidly initiated.

Coccidioidoma

Benign residual pulmonary lesions may follow primary pulmonary infection in a few cases.[485] Coccidioidal nodules (coccidioidomas) appear radiographically as solitary coin lesions, 1 to 4 cm in diameter, in the upper lobes and middle lung fields, and they can be mistaken for carcinoma. Sputum cultures are positive for *C. immitis* in less than 15% of cases, but the skin test is usually positive and the complement fixation test is positive with low titer in about 70% of patients. Coccidioidomas may cavitate or calcify.

Fibrocavitary Coccidioidomycosis

About 2% to 8% of patients with symptomatic primary pulmonary coccidioidomycosis develop cavitary lesions, another form of residual disease. Thin-walled cavities develop within parenchymal infiltrates and may result from necrotizing bronchitis.[479] Thick-walled cavities result from necrosis and drainage of residual nodules. About 90% of cavities are solitary, and 70% are located in upper lung fields. The cavities vary in size from 2 to 14 cm, but most are in the range of 2 to 4 cm. Cavities usually remain stable for long periods but at least half of these spontaneously close. Complications of coccidioidal cavities include recurrent hemoptysis, rarely massive; progressive expansion with parenchymal compression; secondary bacterial or fungal infection, usually by an *Aspergillus* species; and rupture with the development of pyopneumothorax or bronchopleural fistula. All these complications are relative indications for surgical resection.

Disseminated Disease

Less than 1% of patients with primary pulmonary coccidioidomycosis develop extrapulmonary dissemination. Risk of dissemination depends on host factors such as race, sex, age, pregnancy, and immunosuppression.[479,490] Dissemination occurs more frequently in African-Americans, Filipinos, Mexicans, and American Indians than in Caucasians; however, the reason for this racial susceptibility is unknown. Men are 1.5 to 6 times more susceptible to disseminated infection than nonpregnant women. Dissemination is likely to occur at the extremes of age, and pregnant women are more susceptible, especially during the second and third trimesters. Immunocompromised patients, particularly those treated for hematologic malignancy or organ transplantation with corticosteroids and cytotoxic drugs, are at high risk for dissemination.[490,491] Common sites of dissemination include the meninges, skin and subcutaneous tissues, bones and joints, liver, spleen, lymph nodes, genital urinary tract, and adrenal glands.[479] The gastrointestinal tract is invariably spared. Coccidioidemia develops in a small proportion of patients and has a grave prognosis.

Pathology

The pulmonary lesions in coccidioidomycosis can be broadly classified as pneumonic, cavitary, nodulocaseous, and bronchiectatic.[492] The pathologic features of primary pulmonary coccidioidomycosis are not well delineated, since patients with the primary infection generally do not die or undergo surgical resection.[493] Pneumonic infiltrates correspond to areas of suppurative or mixed suppurative and granulomatous inflammation (Figs. 10.44 and 10.45). Tissue eosinophilia similar to allergic bronchopulmonary aspergillosis has been reported with coccidioidomycosis (see Fig. 15.14 in Chapter 15).[494,495] Chronic fibrocavitary coccidioidomycosis macroscopically resembles chronic histoplasmosis or tuberculosis with fibrosis, caseation, cavity formation, and calcification, often in an upper lobe distribution (Fig. 10.46). Coccidioidal cavities have fibrous walls that contain a cellular infiltrate, necrotic debris, and granulomatous inflammation. While some of the bronchiectatic cavities evolve from necrotizing bronchitis, other

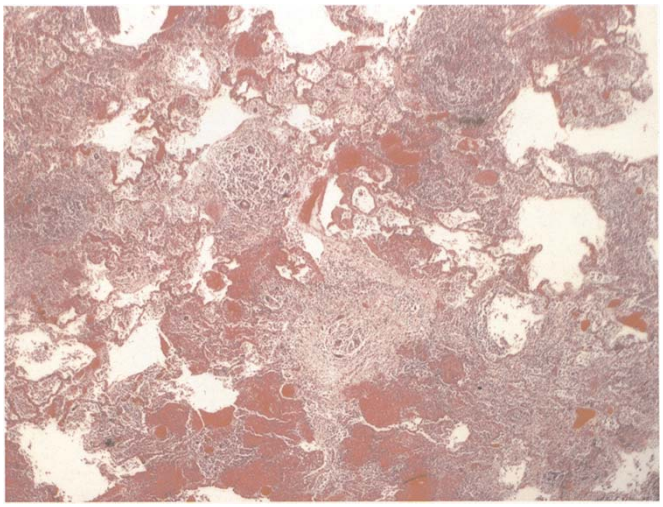

FIGURE 10.44. Low magnification photomicrograph shows granulomas and hemorrhage.

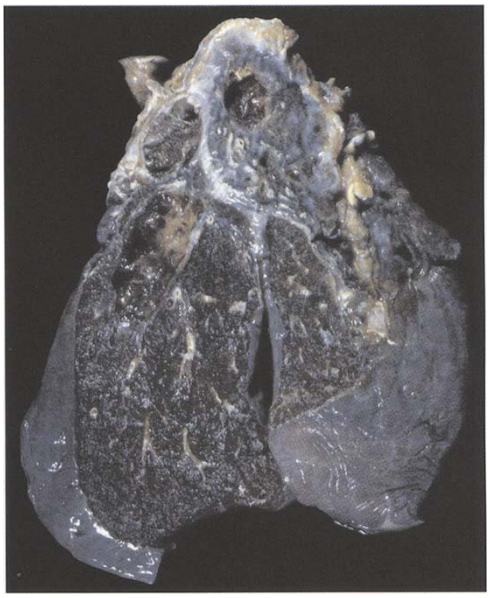

FIGURE 10.46. Chronic progressive coccidioidomycosis. There is marked apical fibrosis of lung surrounding a cavity, associated with pleural fibrosis. The lower lobe has focal pneumonic lesions.

cavities may develop from within acute suppurative foci or evacuated fibrocaseous nodules. Cavities that contain coccidioidal fungus balls, erroneously called mycetomas, are usually resected because of recurrent or severe hemoptysis.[496,497] The fungus ball is usually soft, grayish-brown, friable or pultaceous, and consists of collections of mycelium of *C. immitis* (Fig. 10.47). Because many of the cavities communicate with the bronchial tree, person-to-person transmission of infection of exhaled or expectorated arthroconidia is theoretically possible, but has never been documented.

The pathology of surgically resected residual pulmonary coccidioidomycosis and coccidioidomas consist of discrete encapsulated fibrocaseous nodules, 0.5 to 3.5 cm in diameter that are often subpleural.[498] Most are located in the upper lobes, 25% are cavitated, and 50% communicate with the bronchial tree. Microscopically, fibrocaseous nodules are seen with peripheral granulomatous inflammation surrounded by a mixed cellular infiltrate. Diagnostic sporulating spherules can be found in only half of these nodules, and mycelia with arthroconidia in about 15%.

Spherules are generally abundant in active pulmonary and disseminated lesions (Fig. 10.48), but they may be

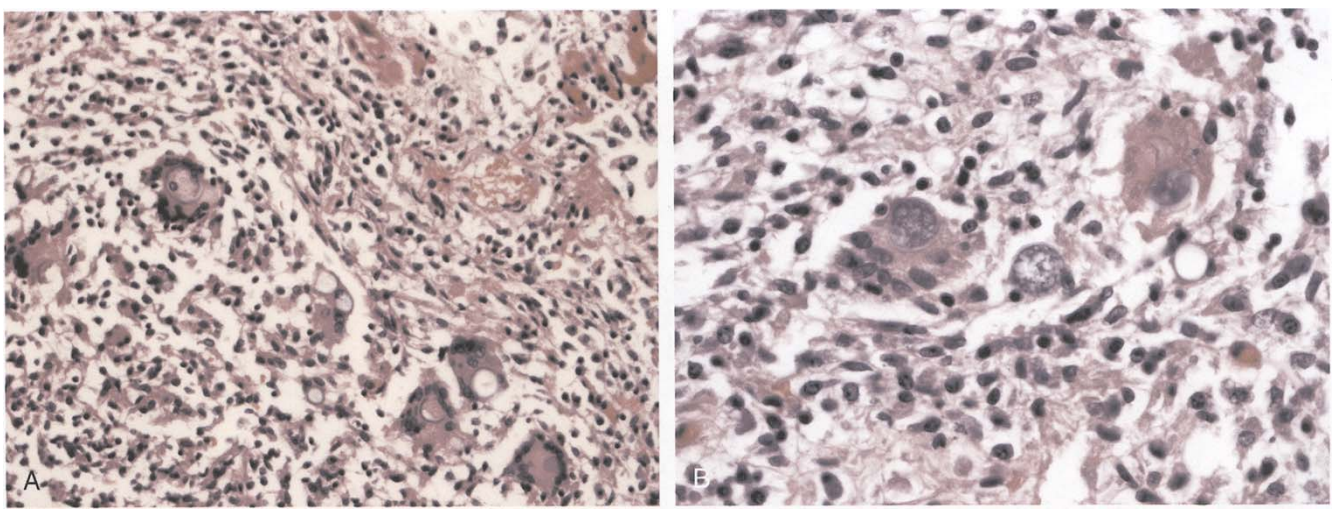

FIGURE 10.45. **A,B.** Higher magnification shows multiple giant cells with sporulating spherules.

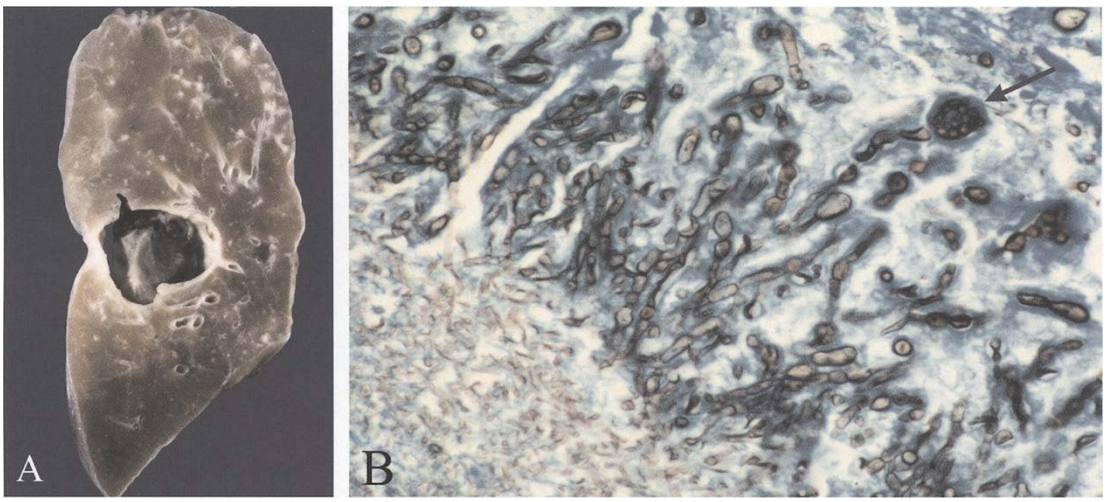

FIGURE 10.47. Coccidioidal fungus ball. **A.** Upper lobe thin-walled cavity contains gelatinous appearing fungus ball. Note also multiple small fibrocaseous granulomas in apical segment. **B.** Hyphae, arthroconidia, and spherule (arrow) within the fungus ball (GMS).

difficult to find in the inactive residual lesions. Miliary coccidioidomycosis is characterized by myriad spherules with little inflammatory reaction, frequently in a background of diffuse alveolar damage (Fig. 10.49). When present in sufficient numbers, the spherules of *C. immitis* are readily visualized with H&E, but their morphology is best demonstrated with the GMS stain (Fig. 10.48). The spherules may be surrounded by a radiating corona of eosinophilic Splendore-Hoeppli material. Hyphae and arthroconidia are seldom produced in the tissues. When sporulating spherules of the appropriate size and morphology are present in the lesions, a definitive histopathologic diagnosis of coccidioidomycosis can be rendered with confidence. Figure 10.48B shows the different stages of maturation of spherules. If only immature spherules or groups of endospores associated with fragments of the spherule wall are found, a specific histologic diagnosis is less certain and should be confirmed by culture, serology, or immunofluorescence.

The distinctive spherules of *C. immitis* are unlikely to be confused with other pathogens. *Rhinosporidium seeberi* also replicates by endosporulation, but its endospores are distributed in a distinct zonal pattern within the sporangia, contain globular inclusions, and have carminophilic walls. The sporangia are so large that they can be seen with the unaided eye in tissue sections.

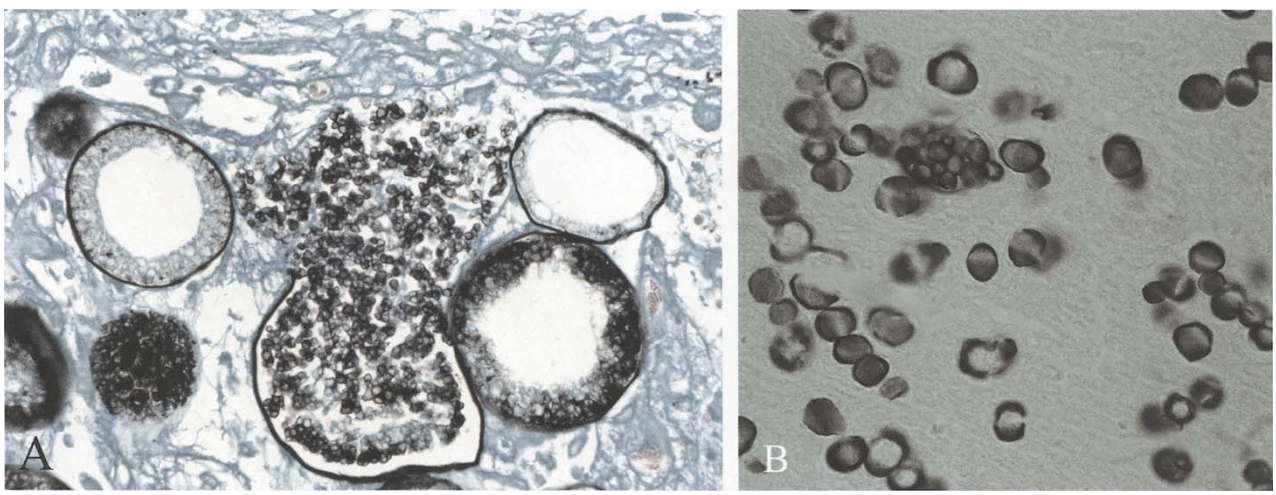

FIGURE 10.48. Mature and immature spherules. **A.** Rupture of spherule with release of endospores (GMS). **B.** Intact mature spherule is seen in association with immature spherules at different stages of development (GMS).

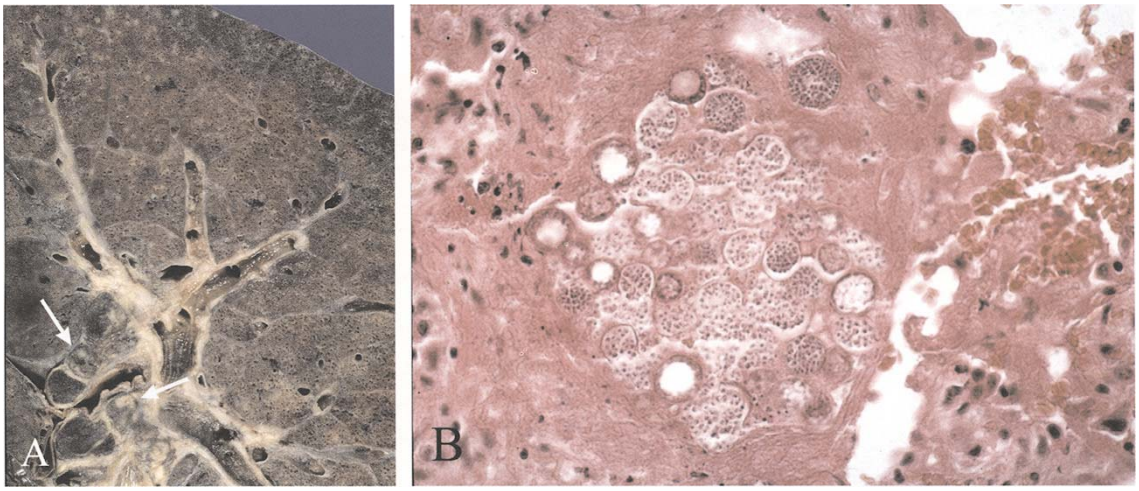

FIGURE 10.49. Miliary coccidioidomycosis. **A.** Pale miliary nodules are present in a background of diffuse alveolar damage in a patient with hematologic malignancy. Larger necrotic lesions are present in juxtabronchial lymph nodes (arrows). **B.** Pneumonic lesions contain numerous spherules with little inflammation.

The spores are in zones of maturity—larger mature spores near the pore and small immature spores opposite the pore. The parent bodies and spherules of myospherulosis, a pseudomycosis of the peripheral soft tissues, upper respiratory tract, and middle ear, closely resemble the spherules of *C. immitis* in both size and morphology. However, these can be easily distinguished by their inherent brown pigment and lack of reactivity with GMS and PAS stains.[499–503] Another differential diagnostic problem may arise when the typical spherules of *C. immitis* are not present and immature spherules of *C. immitis* are present that resemble the budding yeast cells of *B. dermatitidis*. The observation of spherule wall material and the presence of broad-based blastoconidia, bud pores, and multiple nuclei in *B. dermatitidis* differentiate these two fungi at this stage of development. Another differential diagnostic problem can be caused by *H. capsulatum* var. *capsulatum;* however, the endospores of *C. immitis* are much larger in size than the yeast of *H. capsulatum*. In addition, the endospores of *C. immitis* do not bud as the yeast form of *H. capsulatum* does in tissue. Even though groups of yeast cells of *H. capsulatum* occur within phagocytic cells, there is no cell wall structure that encloses them. Endospores of *C. immitis* are enclosed by a thick spherule wall during their development.

Diagnosis

Histopathologic examination may be the only way to establish the diagnosis in patients with inactive lesions, if serology and cultures of respiratory secretions or tissue specimens are negative.[53,504] A clinical diagnosis of disseminated coccidioidomycosis can often be confirmed by a needle biopsy of the liver, which is involved in 45% to 60% of cases.[485,492] Biopsy of skin lesions and direct examination and culture of synovial fluids can also be useful in confirming the diagnosis, particularly in patients who have atypical clinical features.[505]

Direct fluorescent antibody conjugate can be used to identify both the immature spherules and endospores in deparaffinized sections of formalin-fixed tissue.[53] The walls of mature spherules are not consistently reactive with this conjugate. A similar staining pattern is also seen with the PAS stain where the endospores and immature spherules are positive, and the wall of mature spherules is negative.

The diagnosis can be also confirmed by isolation of *C. immitis* from respiratory secretions, skin test conversion from negative to positive, or positive serology such as immunodiffusion, enzyme immunoassay, or latex particle agglutination, which permits the detection of the major antibody responses-coccidioidal IgM in early coccidioidomycosis, and complement fixing IgG antibodies later.[506] The IgG antibodies are more persistent, and their quantitation is useful for both prognosis and diagnosis.[507–510] The presence of complement-fixing and tube precipitin anticoccidioidal antibodies in serum are specific markers of coccidioidomycosis.[511] In general, a positive serologic test is clinically relevant. Most patients lose serologic reactivity within a few months, unless there is active infection. An ELISA to detect antibodies against a 33-kDa cell wall antigen purified from developing spherules of *C. immitis* can be useful as a screening test.[511,512] A skin test to coccidioidal antigens becomes positive soon after primary infection and remains so for life, except in those with immunosuppression.

Treatment

In most patients the illness is self-limited, and symptoms generally resolve within a few weeks, without treatment. Persistent coccidioidal pneumonia is diagnosed when symptoms or radiographic abnormalities persistent beyond 6 to 8 weeks. These patients then should be treated with amphotericin B, since some will develop progressive pneumonia or disseminated infection if untreated. Amphotericin B lipid-formulation may be advantageous. Azole therapy is generally inferior to amphotericin B in disseminated disease.[504,513] An atypical infection in an AIDS patient was successfully treated with fluconazole.[514]

Pseudallescheriasis

Pseudallescheria boydii is a ubiquitously occurring fungus that rarely causes infection in immunocompetent humans.[31,515] Pulmonary mycosis caused by *P. boydii*, or pseudallescheriasis, comprises two distinct clinicopathologic entities: colonization of cavities in patients with underlying lung disease, and invasive necrotizing pneumonia in immunocompromised patients.[516,517] The fungus is most often isolated from clinical specimens in its asexual form, *Scedosporium apiospermum.* The sexual form of the fungus is *Pseudallescheria* (formerly *Petriellidium boydii* or *Allescheria boydii*).[518] When the fungus is introduced by trauma into the subcutaneous tissues it may cause a mycetoma, which is a localized process characterized by tumefaction, draining sinuses, and sclerotia (grains, granules). The fungus has caused occasional cases of otomycosis, keratitis, endophthalmitis, meningitis, septicemia, and disseminated infection. It is also a common cause of fungal pneumonia in near-drowning, and associated with high mortality.[519]

Microbiology

Pseudallescheria boydii is a cosmopolitan saprophyte that can be isolated from moist soil, polluted water, and sewage. In culture, the asexual form grows rapidly, producing white to smoky gray colonies that consist of hyaline, branched, septate hyphae.[520] Ovoid, hyaline-to-darkly pigmented conidia, 5 to 10 μm in size, are borne terminally or laterally on short conidiophores. Development of the sexual or ascocarpic form occurs by some isolates. The cleistothecia are dark brown fruiting bodies 50 to 150 μm in diameter that contain elliptical brown ascospores measuring 4 × 7 μm.

Clinical Features

Patients with preexisting cavitary or cystic lung disease are predisposed to the colonizing form of pulmonary pseudallescheriasis, which clinically resembles aspergilloma. About one half of the patients have previous residual tuberculous cavities,[521,522] and many are farmers or rural dwellers. Other underlying diseases include bronchiectasis, bronchogenic cysts, and honeycomb lung.[517,523,524] Patients may have cough, hemoptysis, or excessive dyspnea, and chest radiographs show underlying cavitary disease, often with an intracavitary mass suggestive of a fungus ball. The protracted clinical course of this noninvasive form of infection is characterized by massive hemoptysis, which may necessitate resection. Sinusitis and allergic bronchopulmonary mycosis is reported with pseudallescheriasis.[525,526]

Invasive pulmonary pseudallescheriasis is a disease of immunocompromised hosts.[517,524,527,528] Most patients have acute leukemia, and are further exposed to opportunistic infection due to granulocytopenia, cytotoxic chemotherapy, corticosteroid therapy, and antibiotic therapy for undiagnosed fever.[529–531] Other predisposing factors include systemic lupus erythematosus, renal allograft recipients being treated with azathioprine and corticosteroids, and AIDS[532,533]; rarely, an underlying factor cannot be determined. The patients present with fever, pulmonary infiltrates, hemoptysis, and pleuritic chest pain. Chest radiographs show localized or bilateral infiltrates, often with cavitation.[534]

Pathology

In the pulmonary colonizing form of pseudallescheriasis, cavities range from 3 to 8 cm in size and may communicate with the bronchial tree (Fig. 10.50). The walls of these cavities are formed of granulation tissue with suppurative or granulomatous inflammation. Bronchiectatic cavities and bronchogenic cysts may be lined partially by respiratory epithelium with squamous metaplasia. The intracavitary mycelium consists of either soft, amorphous hyaline aggregates of hyphae, or true fungus balls having concentric rings of compact tangled hyphae. The hyphae are not invasive unless the patient is otherwise immunosuppressed.[516,535,536]

Invasive pulmonary pseudallescheriasis is a destructive, necrotizing pneumonia with abscess formation, mycelial vascular invasion, and hemorrhagic infarction (Fig. 10.51).[517,530,536] The hyphae in this form of disease are scattered throughout the areas of pneumonia or infarction. The pathogenesis of invasive pseudallescheriasis is presumed to be similar to that of invasive aspergillosis. In some cases of invasive disease cavitation with intracavitary fungus balls may evolve.[535]

Diagnosis

In tissue sections, the hyphae of *P. boydii* are septate, branched, and narrow, 2 to 5 μm wide (Fig. 10.51). Although the pattern of branching is neither progressive nor dichotomous as in aspergillosis, it may be exceedingly

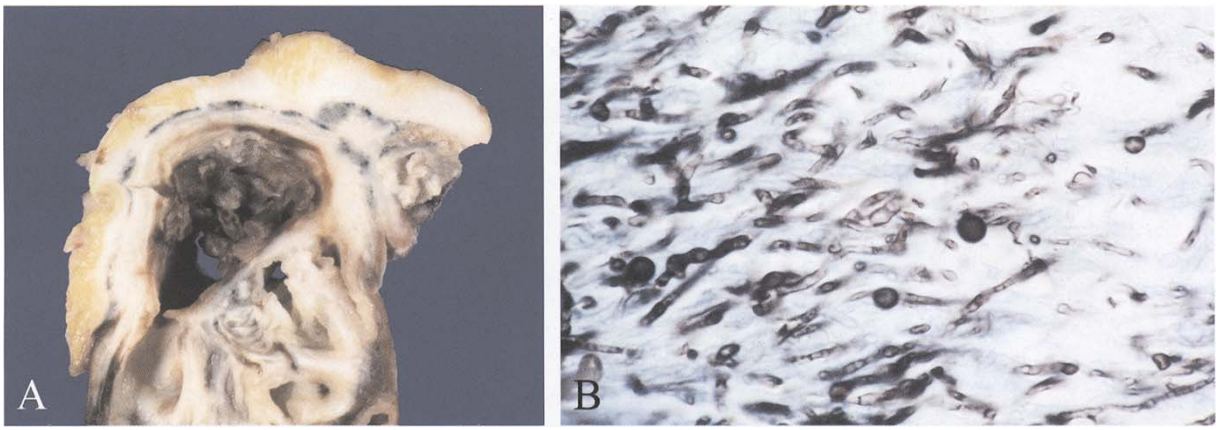

FIGURE 10.50. Pseudallescheria boydii mycetoma. **A.** Brown friable amorphous fungus ball is adherent to wall of apical cavity. There is marked overlying pleural fibrosis and extensive bronchiectasis below the cavity. **B.** Hyphae with more intensely staining ovoid terminal spores (GMS).

difficult to distinguish hyphae of pseudallescheria from those of *Aspergillus* species. The hyphae of *P. boydii* may produce thin-walled vesicles.[517,530,537] and terminal conidia[535] of the *Scedosporium* type; only the latter are helpful in differentiating *P. boydii* from an *Aspergillus* sp. The hyphae of *P. boydii* tend to be more irregular in shape and size than those of *Aspergillus*. However, morphology is often unreliable in differentiating these fungi, and a definitive diagnosis requires isolation and identification of the fungus in culture, or direct immunofluorescence, particularly if fresh tissue is not available for culture.

Although *P. boydii* can be isolated from sputum in up to 75% of patients with pulmonary pseudallescheriasis, most isolates from the respiratory tract are environmental contaminants or colonizers of no clinical significance.

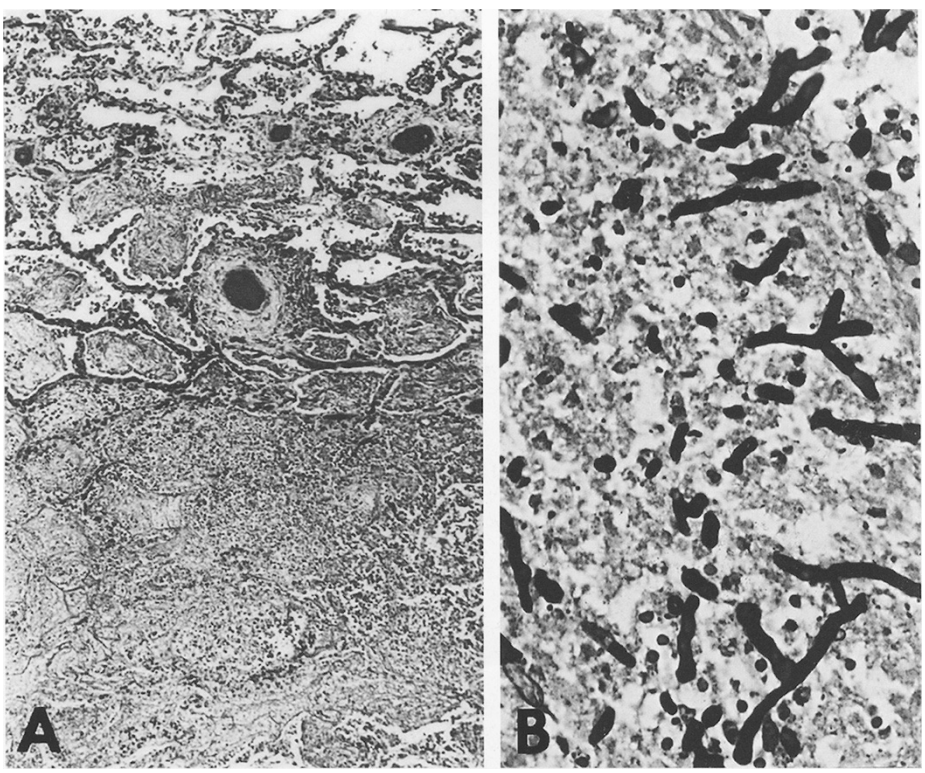

FIGURE 10.51. Invasive pulmonary pseudallescheriasis. **A.** Margin of nodular pulmonary infarct (×50). **B.** Branched, septate hyphae of *Pseudallescheria boydii* (GMS). (Courtesy of Drs. F.W. Chandler and J.C. Watts.)

The diagnosis may be suspected on finding the characteristic fungi.[524]

Treatment

Surgical resection is the treatment of choice for noninvasive pseudallescheria and is usually reserved for patients with recurrent or uncontrolled hemoptysis. *P. boydii* is generally resistant to amphotericin B and flucytosine, but may be susceptible to azoles such as miconazole and ketoconazole.[521,531,537–539]

Fusariosis

Fusariosis is one of the emerging infectious diseases in immunocompromised patients.[540] *Fusarium* species can cause a variety of infections including mycotic keratitis, endophthalmitis, onychomycosis, mycetoma, and cutaneous or disseminated infections in immunocompromised and burn patients.[541,542]

Localized and disseminated infections caused by *Fusarium* have been reported in burns, malignant lymphoma and acute leukemia, bone marrow transplantation, renal transplantation, and aplastic anemia.[543–549] Disruption of a cutaneous or mucosal barrier appears to be a major factor in the pathogenesis of invasive fusariosis. Patients usually have coexisting infections with bacteria, viruses or other fungi, most often *Candida* or *Aspergillus* species. Other factors predisposing to opportunistic infection among these patients include neutropenia and therapy with cytotoxic drugs, corticosteroids, and multiple antibiotics.

Microbiology

Three of the major human pathogens within the genus *Fusarium* are *F. oxysporum*, *F. moniliforme*, and *F. solani*. These species are widely distributed in nature as soil saprophytes and plant pathogens.[542] They grow rapidly in culture at 25° and 37°C, producing septate mycelium, macro- and microconidia, and often intercalary or terminal chlamydoconidia. Their characteristic septate macroconidia are fusoid or sickle shaped. The identification of *Fusarium* species is becoming complex because many of the phylogenetic-based species possess the same morphology. Thus, classical species such as *F. oxysporum* and *F. solani* are often thought of as complexes of several phylogenetic species.

Clinical Features

Some immunosuppressed patients develop painful erythematous cutaneous nodules that progress to necrotic ulcers, the biopsy and culture of which help to establish the diagnosis. Chest radiographic abnormalities include nodular or fluffy densities of progressive pulmonary infiltrates.[541,542,550] However, some of these patients have coexisting pulmonary infection with herpes simplex virus or *Candida* species that could have produced or contributed to the radiographic findings.

Pathology

Invasive and disseminated lesions produced by *Fusarium* consist of abscesses, infarcts secondary to vascular invasion and thrombosis, and granulomas (Fig. 10.52).[541,551] The hyaline septate hyphae of *Fusarium* measure 3 to 7 μm in diameter and are sparsely branched; the branches often arise perpendicular to parent hyphae (Fig. 10.53). Intercalated vesicles are occasionally found, but the characteristic conidia are not produced in tissue. Sporodochia having blankets of conidia do occur on thermal wounds. Hyphal vascular invasion is typical of disseminated infection with these fungi. In histologic sections, the hyphae of *Fusarium* are most frequently mistaken for those of *Aspergillus* species and *P. boydii* (Fig. 10.53). While *Fusarium* species do not show the regular pattern of progressive dichotomous branching characteristic of the aspergilli, definitive diagnosis requires isolation and identification of the fungus in culture. Immunohistologic techniques are not widely available for identification of *Fusarium* species.

Treatment

The drugs of choice for the treatment of invasive fusariosis are ketoconazole, liposomal amphotericin B, and fluconazole. Unfortunately, in vitro resistance to these drugs has been reported. Most *Fusarium* isolates are resistant to flucytosine.[552]

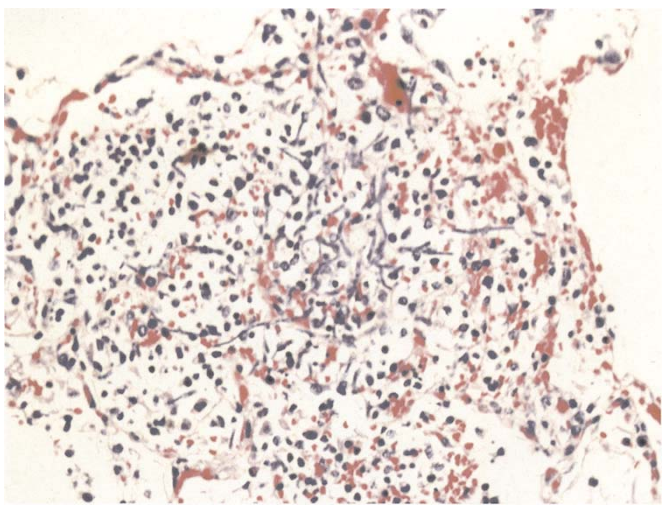

FIGURE 10.52. Pulmonary fusariosis. Acute neutrophilic exudate in which hyphae are randomly distributed.

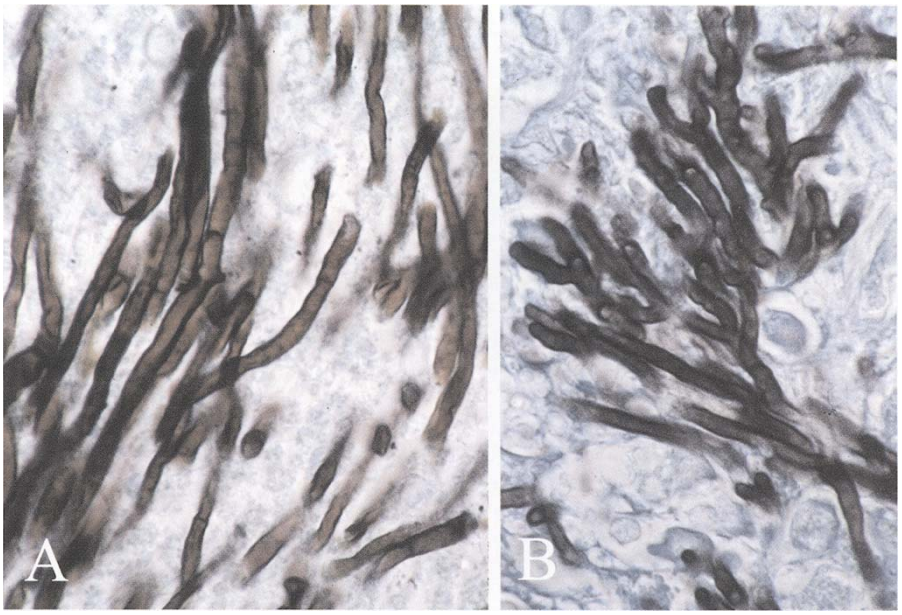

FIGURE 10.53. Comparison of *Fusarium* **(A)** and *Aspergillus* **(B)** hyphae. Although hyphae have similar caliber and septate morphology, *Aspergillus* hyphae demonstrate a dichotomous branching pattern (GMS, × 100).

Trichosporonosis

Trichosporonosis is a disseminated opportunistic fungal infection caused by *Trichosporon* species, a yeast-like fungus within the family Cryptococcaceae. There are a number of *Trichosporon* species associated with human infection. *Trichosporon beigelii*, previously called *T. cutaneum*, has been considered the main species of *Trichosporon* isolated in humans. However, the genus has recently been revised, and six different species are now considered to be causative agents of human disease: *T. asahii, T. asteroides, T. beigelii, T. inkin, T. mucoides* and *T. ovoides*.[553] *Trichosporon asahii* and *T. mucoides* are the most common species that cause disseminated trichosporonosis. The portal of entry of infection in immunosuppressed patients is unknown, although respiratory, cutaneous, and enteric routes have been proposed.

Microbiology

Trichosporon spp. are soil saprophytes, widely distributed in nature, form a minor component of normal skin flora, and are occasionally isolated as contaminants from urine and throat cultures.[554–556] *Trichosporon* spp. grow rapidly in culture and produce off-white to tan colonies that consist of hyaline yeast cells, pseudohyphae, mycelia, and arthroconidia, the last being characteristic of the genus.

Clinical Features

Most reported cases of disseminated trichosporonosis have been published only after 1980.[555,557,558] This recently recognized disease occurs mainly in patients treated for acute leukemia or lymphoma, renal transplant recipients, bone transplant recipients, patients with chronic active hepatitis C, following mitral valve replacement, and in heroin addicts with mycotic endocarditis.[554,559–561] All of these patients were immunosuppressed, and about 50% were neutropenic, or received chemotherapy, corticosteroids, or multiple broad-spectrum antibiotics. Clinical findings in these patients are those of sepsis, and about one third of the patients have purpuric papular or nodular skin lesions with vesiculation or ulceration.[556,562,563] Chest radiographies show localized or bilateral pulmonary infiltrates. Many patients have positive blood cultures.

Pathology

Pulmonary lesions are hemorrhagic, necrotizing bronchopneumonia, with nodular infarcts containing radiating fungal colonies with little inflammatory response, and widespread mycotic emboli.[564] Mycotic vascular invasion was found in more than 50% of patients in a review of 17 autopsied patients.[31,555] Extrapulmonary lesions usually consist of abscesses, and occasionally of necrotic granulomas. In tissue sections, *T. beigelii* is seen in the form of pleomorphic hyaline yeast-like cells 3 to 8μm in diameter, with septate hyphae and arthroconidia (Fig. 10.54). The arthroconidia are produced by fragmentation of hyphae. The arthroconidia may be inconspicuous in some cases, in which case, the fungus may be mistaken for *Candida*. *Trichosporon* budding yeast cells and hyphae are typically

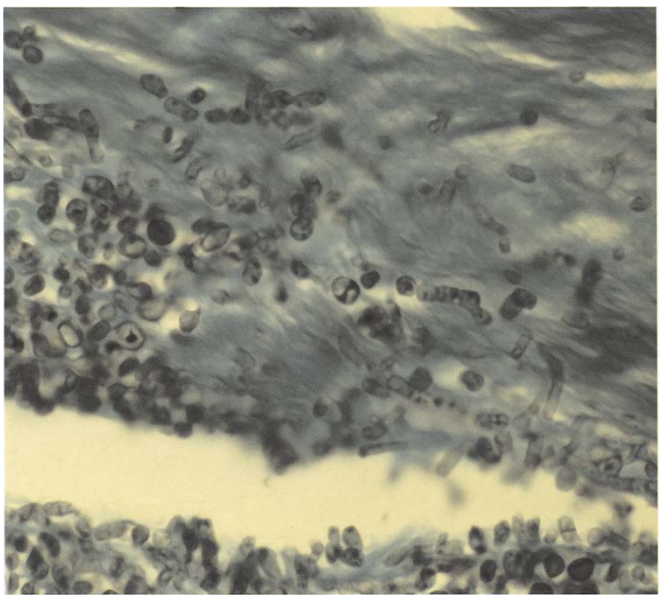

FIGURE 10.54. Trichosporonosis. Pleomorphic, hyaline yeast with septate hyphae and arthroconidia (GMS).

more pleomorphic than those of *Candida* species; however, definitive diagnosis requires confirmation by culture.

Diagnosis

Immunoperoxidase methods are available for diagnosis of the fungus in tissue sections.[51] The PCR technique to distinguish between deep and superficial isolates of *T. beigelii* using isoenzymes, and restriction fragment length polymorphisms of rDNA are available also.[565] More recently, glucuronoxylomannan-like polysaccharide antigen from isolates of *T. beigelii* has been used to determine pathogenicity.[566]

Treatment

Most isolates of *Trichosporon* are sensitive to amphotericin B, and some to the azoles and flucytosine.[564,567]

Geotrichosis

Geotrichosis is a rare opportunistic fungal infection caused by *Geotrichum candidum*, a ubiquitous saprophyte found in soil, decomposing organic matter, and contaminated foods such as oranges, vegetables, and especially dairy products.[568] It is also a transient commensal of the oropharynx, tracheobronchial tree, and gastrointestinal tract, sites that have been implicated as potential endogenous sources of infection. *G. candidum* is not an aggressive opportunist and appears to be introduced following trauma, inhalation, ingestion, or catheter insertions.[569]

Microbiology

Geotrichum candidum is a filamentous mold and not yeast. It also produces arthroconidia. On Sabouraud glucose agar incubated at 25°C, colonies are soft, yeast-like and well formed within 2 to 4 days. The colonies are initially white but become cream colored and develop groups that radiate from a dense central core.

Clinical Features

The clinical spectrum of geotrichosis includes transient fungemia, colonization of bronchi, cavitary lung lesions, and rarely oropharyngeal, gastrointestinal, cutaneous, and invasive disseminated infection.[568,570,571] Infections almost always occur in debilitated patients with serious underlying disease, or as a complication of immunosuppressive therapy, hyperalimentation therapy, and prolonged treatment with broad-spectrum antibiotics.[572] Although other species of geotrichum exist, only *G. candidum* has been documented to cause human infection.

Transient or prolonged colonization of the respiratory tract, especially the bronchial tree and preexisting pulmonary cavities, is the most common clinical form of geotrichosis. Important predisposing factors are tuberculosis, chronic obstructive lung disease, chronic bronchitis, bronchogenic carcinoma, and other mycoses. Clinical signs include persistent cough, thick grayish mucoid or purulent and rarely blood tinged sputum, low-grade fever, and medium to coarse rales in the lungs. Occasional patients with endobronchial geotrichosis may also have symptoms of severe asthma. Chest radiographs may be either normal or show peribronchial thickening and patchy infiltrates with cavitation, especially in apical and hilar regions. Bronchoscopy may show white, thrush-like patches on the bronchial mucosa, and abundant fungal elements can be demonstrated in or isolated from the sputum. If predisposing factors are not eliminated, endobronchial and intracavitary infections persist, and patients may develop fulminant fatal infections, particularly those patients who are profoundly immunosuppressed, neutropenic, or have malignant hemopoietic neoplasia.[568,573,574] In one series of 257 patients with cancer, 12 catheters were colonized with geotrichum.[575] Geotrichosis is still quite rare; oropharyngeal and cutaneous lesions must be differentiated from candidiasis.

Pathology

In tissue sections, *G. candidum* appears as hyaline, septate, infrequently branching hyphae, 3 to 6 μm wide with nonparallel contours and usually acute angle branching. Rectangular to oval arthroconidia that are 4 to 10 μm wide with rounded or squared ends are produced by disarticulation of the hyphal segments (Fig. 10.55). Occasionally, thick-walled spherical cells, 12 μm or less in

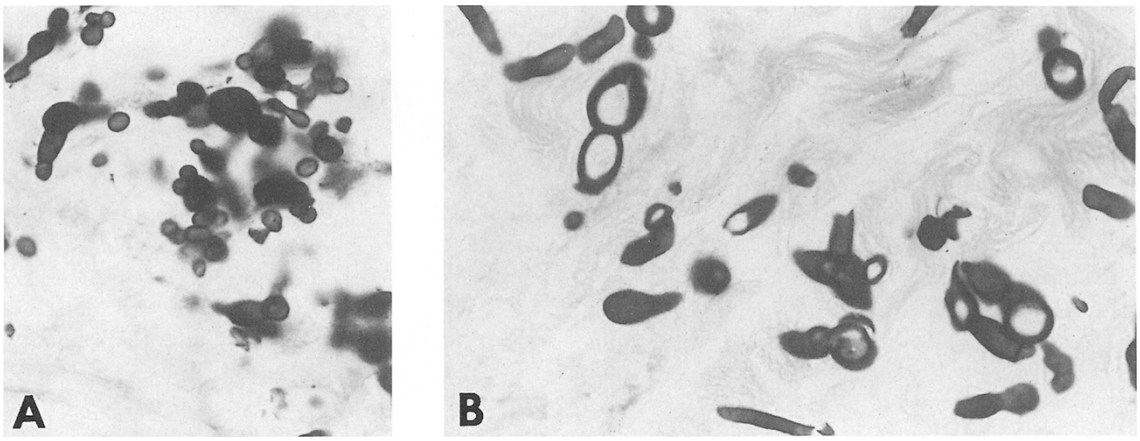

FIGURE 10.55. Geotrichosis. **A.** Short hyphae and spherical to elongated yeast-like cells of *Geotrichum candidum* within area of suppurative and necrotizing pneumonia (GMS). **B.** Hyphae and arthroconidia with rounded or squared ends in necrotizing skin lesion of renal transplant recipient (GMS, × 480). (Courtesy of Drs. F.W. Chandler and J.C. Watts.)

diameter, and germ tubes may be present, causing confusion with *Candida* spp. and *Trichosporon* spp. The absence of blastoconidia (budding cells) distinguishes *G. candidum* from these two other fungi. Germ tubes from the arthroconidia can be confused with blastoconidia. In tissue sections, the fungus is poorly stained with H&E, and best demonstrated with GMS.

Diagnosis

The diagnosis of geotrichosis is made by obtaining multiple positive cultures of *G. candidum* from blood, sputum, or biopsy specimens, and by demonstrating the fungus in tissue sections or respiratory secretions. Isolation of the fungus from the sputum of an asymptomatic individual has no diagnostic significance. An indirect immunofluorescence test can be used to identify *G. candidum* in tissue sections.[576] Serologic tests are not available for routine diagnosis.

Treatment

Treatment of the bronchopulmonary form of geotrichosis includes liposomal amphotericin B and the azoles. Cutaneous geotrichosis has been successfully treated with isoconazole.[577] Elimination of predisposing factors is important for cure.

Penicilliosis marneffei

Penicillium marneffei is a dimorphic fungus recognized as an AIDS-related infection.[578,579] *Penicillium* species are ubiquitous environmental saprophytes that rarely cause invasive infection in humans. Only *P. marneffei* is known to cause invasive pulmonary disease that frequently disseminates to other tissues in the immunocompromised patient.[5119,579–581] Penicilliosis marneffei is a chronic, progressive, and disseminated fungal infection first reported in 1973.[582] *P. marneffei* is endemic in Thailand, Hong Kong, Vietnam, Indonesia, and the Guangxi Province of China. Infections have also been reported in the U.S., U.K., and other European countries in immunodeficient patients who had traveled to endemic areas.[581,583] Although before 1987 most infections were in non-AIDS patients, since then, most patients with penicilliosis were HIV infected or had AIDS. One study of 400 patients with AIDS found 35% with *P. marneffei* infection. In Southeast Asia, *P. marneffei* infection has become an early indicator of HIV infection.[584,585]

The fungus has been isolated from rodents (*Rhizomys sinensis, R. pruinosus,* and *Cannomys badius*) and from soil in the burrows of infected bamboo rats.[119,579] The fungus enters the host by inhalation of infectious conidia from the environment. It has been postulated that some individuals may have a self-limited pulmonary form of penicilliosis similar to that which occurs in histoplasmosis capsulati.

Microbiology

On Sabouraud glucose agar, the fungus grows rapidly to form downy, grayish-pink mycelia with a red diffusible pigment that goes into the agar and the underside of the colony. As conidiophores develop and produce conidia, the surface of the colony becomes grayish-green. The conidia are ellipsoidal, smooth walled, 2 to 3 μm in diameter, and borne in chains on the tips of bottle-shaped phialides that occur as a penicillus. At 37°C, colonies are yeast-like, white to tan, soft or convoluted.

Clinical Features

AIDS patients with disseminated penicilliosis marneffei often have a history of prolonged intermittent fever, weight loss, generalized lymphadenopathy, chronic

productive cough, mucoid sputum, chest pain, septicemia, and hepatosplenomegaly.[119,579] Pericarditis with effusion has also been described, as well as papules and draining ulcers of skin, multiple subcutaneous abscesses, and osteolytic lesions. Chest radiographs may show pulmonary congestion, pleural effusion, and patchy infiltrates that are often bilateral and confined to the lower lobes. Once infected, the course of disease may be progressive and range from months to years. Pulmonary lesions have been described as random abscesses, and pleural involvement has been found in four or five autopsy patients. Organisms are especially numerous in the enlarged hilar lymph nodes, where sheets of yeast-laden histiocytes efface the normal nodal architecture.

Pathology

Three inflammatory patterns are described in penicilliosis: granulomatous, suppurative, and anergic or necrotizing. The first two are seen in patients with intact immunity, and the third in patients who are immunocompromised.[119] In H&E- and GMS-stained tissue sections, the yeast cells within the histiocytes measure 2.5 to 5 μm in diameter and resemble the yeast cells of *H. capsulatum* var. *capsulatum* (Fig. 10.56). In fact, some of the earliest case reports of histoplasmosis in that region were actually found to be penicilliosis upon reexamination. Unlike histoplasma and other pathogenic yeasts, *P. marneffei* divides by fission through the central portion of the intracellular yeast form. It does not reproduce by budding. This fungus produces individual transverse septa that stain more intensely and are wider than the external cell wall. Intracellular yeast forms of *P. marneffei* are more pleomorphic than those of *H. capsulatum* var. *capsulatum*. Short hyphal forms and elongated oval and curved forms, up to 20 μm in length with one or more septa and rounded ends, are occasionally seen in necrotic foci and pulmonary cavities.[586,587] Differential diagnosis includes *H. capsulatum*, which divides by budding, while *P. marneffei* shows septation.

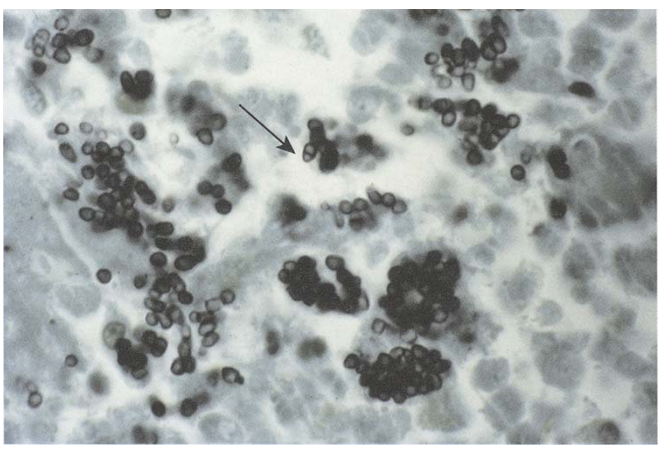

FIGURE 10.56. Penicilliosis marneffei. Gomori's methenamine silver stain shows the small oval yeast forms, 2.5 to 5.0 μm in diameter, usually present within histiocytes. Note thick septation in some yeast forms (arrow).

The host response in early pulmonary lesions is predominantly histiocytic similar to that seen in histoplasmosis capsulati. Large numbers of round to oval *P. marneffei* proliferate within distended histiocytes. As the lesion expands, central necrosis, release of fungal cells, infiltration of neutrophils, and abscess formation occur. Slowly forming and evolving granulomas may lead to fibrosis and cavitation, but calcifications have not been reported.

Diagnosis

The diagnosis of penicilliosis marneffei is made by demonstrating yeast cells with characteristic septa in tissue sections and smears of lesional exudates, and by isolating and identifying the fungus in culture.[588,589]

Immunohistochemical staining using a monoclonal antibody, and an immunofluorescent stain using an indirect immunofluorescent antibody have been developed for diagnosis, although the latter needs validation.[590,591] In addition, specific probes using oligonucleotide primers from the nuclear rDNA are available for PCR identification.[591–593] Serodiagnosis using galactomannan is now available.[594]

Treatment

P. marneffei is usually susceptible to both amphotericin B and the azoles. Treatment with amphotericin B has been successful in the majority of cases. Fluconazole and itraconazole should be considered for mild to moderate cases. Relapse, however, is common approximately 6 months after discontinuation of therapy.[595]

Phaeohyphomycosis

Phaeohyphomycosis comprises a heterogeneous group of subcutaneous and systemic infections caused by a wide variety of dematiaceous (naturally pigmented) opportunistic fungi that develop as black molds in culture and as dark-walled (brown) septate hyphae in tissue.[596–599] More than 80 genera and species of fungi that are common saprophytes of wood, soil, and decaying matter are included in this group. Some of the common fungi in this group include *Alternaria, Anthopsis, Aureobasidium, Bipolaris, Chaetomium, Cladophialophora, Cladosporium, Curvularia, Exophiala, Ochroconis, Phialophora, Scedosporium, Scytalidium*, and *Thermomyces*, to name a few. Because these fungi are ubiquitous in many environments, proof of their etiologic role in disease usually

requires microscopic demonstration of the fungal elements in tissue. A positive culture in the absence of compatible lesions is insufficient for diagnosis.[31] Although infections have been reported in healthy persons, those who are immunocompromised or severely debilitated are most susceptible.[599]

Clinical Features

Two main clinical forms of phaeohyphomycosis are recognized in humans: subcutaneous (phaeomycotic cyst) and systemic (cerebral phaeohyphomycosis).[600] The subcutaneous form is most common.[601–603] The infection occurs when fungi enter the body through a skin wound or by traumatic implantation of a wood splinter or other foreign object that serves as a source of the fungus.[602,604] Patients are typically found to have solitary, firm to fluctuant, painless, subcutaneous abscesses up to 7.0 cm in diameter on exposed parts of the body. Lymphangitis and regional lymphadenopathy are uncommon. The more common etiologic agents of subcutaneous phaeohyphomycosis include *Exophiala jeanselmei*, *Phialophora parasitica*, *Phialophora richardsiae*, and *Wangiella dermatitidis*. Systemic phaeohyphomycosis usually has a pulmonary origin following inhalation of the fungal mycelia in the environment.[605] The most commonly encountered agent is *Cladophialophora bantianum,* which has a marked but unexplained tropism for the brain, particularly the cerebral hemispheres.[598,606] Involvement of other organs is extremely rare.[605–608] The fungus reaches the brain via hematogenous dissemination from the primary pulmonary site, which is usually a clinically inapparent pulmonary lesion. Some patients may have symptoms such as cough, chest pain, dyspnea, and hemoptysis. Symptoms of cerebral phaeohyphomycosis include headache, nausea, vomiting, fever, and nuchal pain and rigidity. Cerebral lesions are solitary or multiple, up to 5.0 cm in diameter, and consist of encapsulated abscesses or generalized inflammatory infiltrates.[609] Phaeohyphomycosis may also present as intrasinus and pulmonary fungus balls, granulomatous pulmonary disease, and allergic bronchopulmonary disease.[599,603,607–611] Granulomatous pulmonary disease has also been reported.[612]

Pathology

Subcutaneous phaeohyphomycosis almost always has an accompanying cystic or dispersed granulomas that are usually solitary, encapsulated by dense collagenous connective tissue, and have liquified centers.[31,600,602] The fungi appear either as individual or small, loose aggregates of irregularly shaped, short to long, septate and branched hyphae 2 to 6 μm wide, budding yeast cells, occasional pseudohyphae, and large thick-walled vesicles in the necrotic centers of the granuloma (Fig. 10.57). The brown cell walls of the hyphal-yeast structures are easily detected in H&E-stained sections, but special fungus stains (GMS and PAS) are usually needed for detailed morphologic studies. The special stains, however, mask the natural brown color of the fungi, which is easily detected in H&E-stained sections. When a dematiaceous fungus is suspected, but the brown color of the cell wall is not evident, the Fontana-Masson stain for melanin can be used to demonstrate the presence of melanin in the fungal cell wall.[31,41]

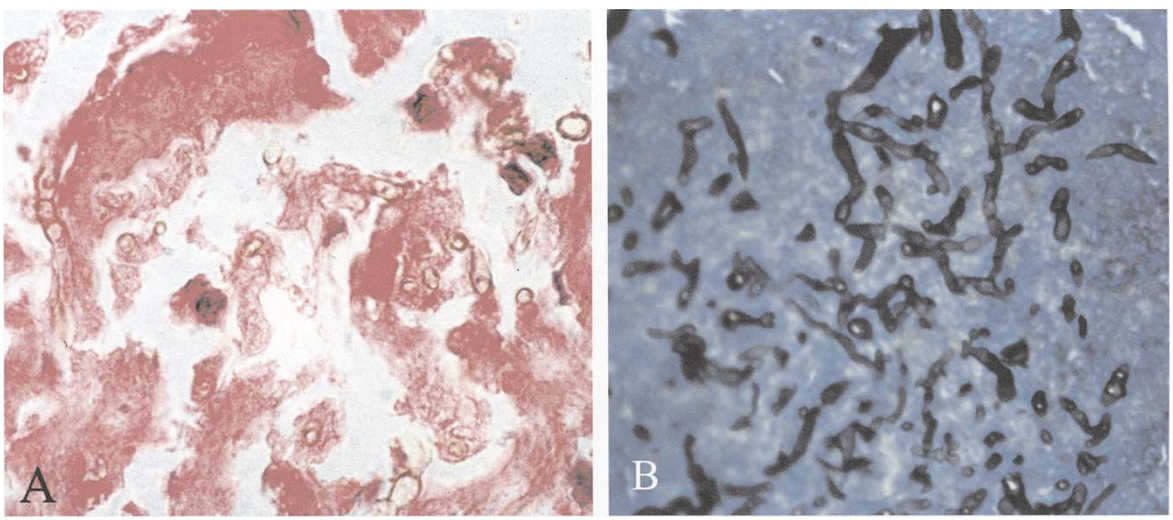

FIGURE 10.57. Phaeohyphomycosis. **A.** Phaeohyphomycotic granuloma. Note the faint natural brown pigment of the hyphal forms in this H&E-stained section. **B.** Gomori's methenamine silver stain of *Bipolaris spicifera* shows the broad hyphae with irregular branching pattern.

Diagnosis

The diagnosis of phaeohyphomycosis can be made based on the natural brown color of the variable fungal elements in clinical or biopsy specimens. The differential diagnosis includes the black grained eumycotic mycetomas caused by pigmented fungi, and chromoblastomycosis. The causative agents of mycetoma form large granules of interwoven mycelia, up to 3.0 mm in diameter, but agents of phaeohyphomycosis never form granules.

In tissue sections, the agents of chromoblastomycosis appear as dark brown muriform cells that characteristically have thick walls and divide by septation into vertical and horizontal planes.[598] At the skin surface, hyphae may be present. Immunologic tests for identification of these fungi are not available at present.

Treatment

Because most phaeohyphomycotic cysts are solitary and encapsulated, surgical excision is usually sufficient for cure. When *E. jeanselmei* is recovered from subcutaneous phaeohyphomycotic lesions, it should be determined if the patient has diabetes as an underlying disease. Excision of localized lesions and the use of azoles like itraconazole and amphotericin B are the treatments of choice in systemic phaeohyphomycosis.

Adiaspiromycosis

Adiaspiromycosis is an extremely rare pulmonary mycosis caused by the filamentous fungus *Emmonsia parvum* (previously *Chrysosporium parvum* var. *crescens*). This soil saprophyte is widely distributed throughout the world in temperate climates and frequently infects rodents and other small mammals. Human infection is uncommon, self-limited, and confined to the lungs.[613,614] Human infection has been reported from France, former Czechoslovakia, Russia, Honduras, Guatemala, Brazil, and Venezuela.[613–615]

Microbiology

E. parvum is a dimorphic fungus that produces white, glabrous-floccose colonies composed of branching, septate mycelium with aleurioconidia, 2 to 4 μm in diameter, in culture at room temperature. When incubated at 40°C or introduced into the lungs, the aleurioconidia progressively enlarge to become thick-walled, spherical cells, 200 to 400 μm or more in diameter, the adiaconidia. In the lungs, the adiaconidia are usually empty, but may contain small eosinophilic, refractile globules along the inner surface of the wall.[614] Adiaconidia do not contain nuclei or other organelles, and do not exhibit evidence of replication.

Clinical Features

Most patients have solitary adiaspiromycotic granulomas that are clinically and radiographically inapparent, and detected incidentally in lung tissue either examined at autopsy or excised surgically for other reasons. Some patients are reported to have localized or diffuse bilateral lung disease that produced a finely nodular pattern in chest radiographs.[613–616] Only those patients who have widespread, severe bilateral disease manifest symptoms, which include cough and slowly progressive dyspnea.[613] Rarely, respiratory failure may occur.[617]

Pathology

The pulmonary lesions consist of confluent fibrotic granulomas 1 to 3 mm in size. Each granuloma contains one or more spherical swollen cells, adiaconidia, that result from the inhalation of a conidium that simply swells, increases in size, and develops an extremely thick cell wall. These cells do not bud or form germ tubes in tissue (Fig. 10.58). The largest cells measure 200 to 300 μm in diameter, some being occasionally up to 500 μm. The cell wall is 20 to 70 μm thick and has an eosinophilic outer layer and a broad hyaline inner layer.[618] The GMS stain defines three wall layers, which correspond to the trilaminar appearance demonstrated by electron microscopy. The interior of the swollen cell adiaconidia is empty or contains small eosinophilic globules 1 to 3 μm in size (Fig. 10.58B). Some cells may contain an internal honeycomb cytoplasmic meshwork of unknown composition.[614,615,619] The cells are incapable of reproduction or dissemination in the human host. Symptoms when present can be attributed to mechanical displacement of tissue by the progressively enlarging swollen cells.

Rhinosporidiosis

Rhinosporidiosis is primarily an infection of mucosal surfaces, caused by *Rhinosporidium seeberi*. The disease is endemic in India and Sri Lanka, but sporadic cases have been reported from all over the world, including the U.S.[371,620] The distribution of *R. seeberi* in nature is unknown, although infection is associated epidemiologically with rural and aquatic environments. Certain animals, particularly dogs and horses, are also reported to develop infection.[621]

Microbiology

The fungus cannot be isolated on synthetic media or transmitted experimentally to animals; however, it will grow in cell culture.[622] Diagnosis depends on identification of the characteristic endosporulating sporangia in smears or tissue sections.

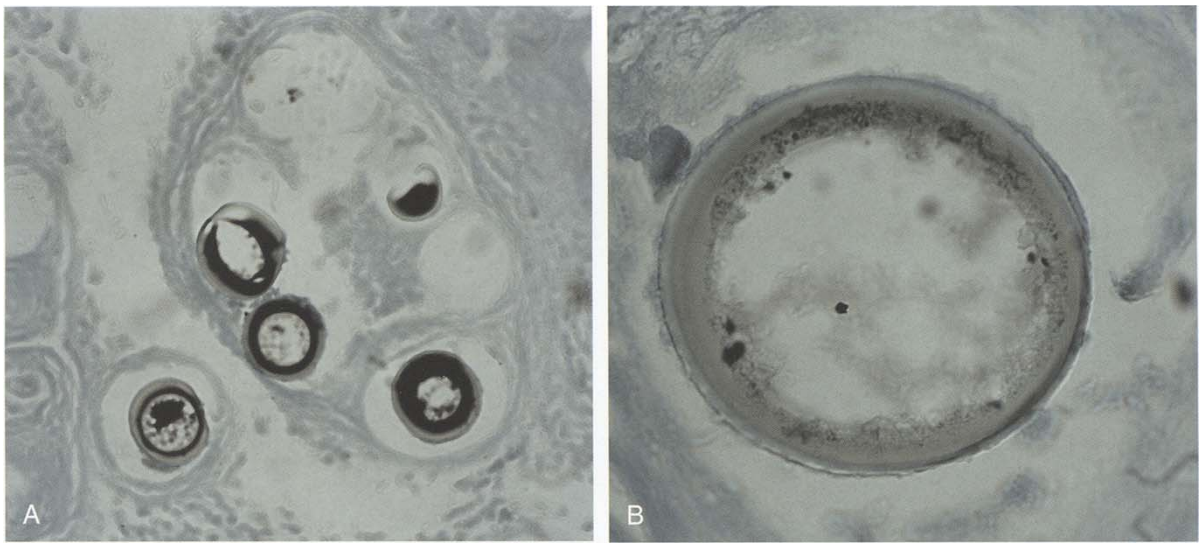

FIGURE 10.58. Adiaspiromycosis. **A.** *Emmonsia parva* is characterized by thick-walled cysts (from monkey lung; GMS). **B.** Higher magnification of the cysts showing trilaminar cell wall and hyaline droplets along the inner cell wall (GMS).

Clinical Features

Clinically, rhinosporidiosis produces bulky, friable mucosal polyps involving the nasal cavity, nasopharynx, palate, and upper respiratory tract. Other mucosal surfaces less commonly involved include the conjunctiva, lacrimal sac, and external genitalia.[620] Cutaneous lesions arise directly from adjacent mucosa or secondarily by autoinoculation. Disseminated infection is rare.[623,624] In one reported case, small granulomas containing the characteristic sporangia of *R. seeberi* were found in both lungs at autopsy.[624]

Pathology

The nasal polyp stroma contains abundant sporangia and trophocytes surrounded by chronic inflammation and granulation tissue. There is often a granulomatous response surrounding the ruptured sporangia. Two developmental forms of *R. seeberi* are recognized in tissue sections.[625–628] The vegetative (non-endosporulating) form or trophocyte is 10 to 100 μm in diameter, and has a single, large, central karyosome (nucleus) with a prominent nucleolus. The cytoplasm is granular or lacy, and the refractile eosinophilic cell wall is 1 to 3 μm thick. The larger trophocytes endosporulate by nuclear division and progressive cytoplasmic cleavage, to produce the mature sporangia. These sporangia are 100 to 300 μm in diameter, and have 2- to 5-μm-thick walls with a thin eosinophilic outer layer and a broad hyaline inner layer (Fig. 10.59). The distinctive morphology and zonation of endospores within the sporangia are characteristic of this fungus. Immature, uninucleate endospores, 1 to 2 μm, are flattened or oval, and are distributed around the periphery of the sporangia in a germinative layer. As they mature the endospores enlarge and move centrally within the sporangia. Fully mature endospores are 8 to 10 μm in diameter and contain multiple eosinophilic globules. Following the rupture of sporangia, the endospores are released into the surrounding tissues

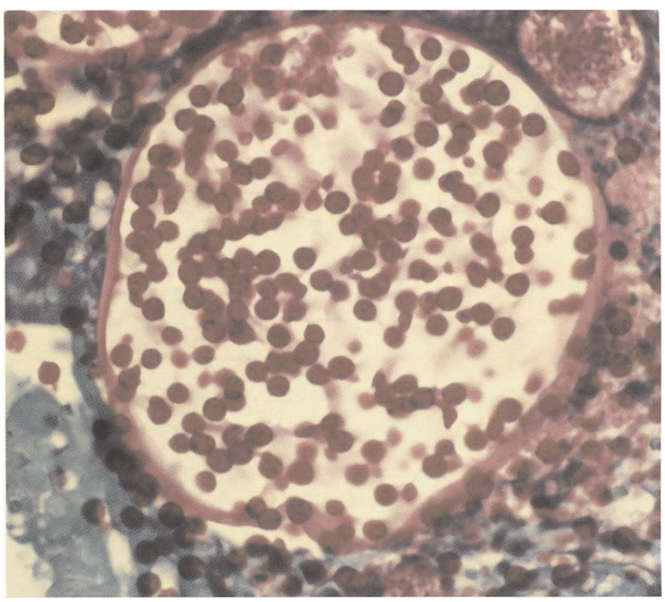

FIGURE 10.59. The large trophocyte of *Rhinosporidium seeberi* has many sporangia and a distinctive zonation with the smaller immature endospores present at the periphery and the larger endospores in the center (periodic acid-Schiff).

and enlarge to become trophocytes, and repeat the cycle. Differential diagnosis includes *C. immitis*, adiaspiromycosis, and Schneiderian papillomas with intraepithelial mucous cysts.[629]

Treatment

Rhinosporidial polyps involving mucosal surfaces are best treated by surgical excision. Recurrence rates are high, if excision is incomplete. Antifungal therapy alone is ineffective in eradicating the infection.

Protothecosis

Protothecosis is an uncommon infectious disease caused by achlorophyllous algae of the genus *Prototheca*. Two of the species in this genus, *P. wickerhamii* and *P. zopfii*, are pathogenic for animals and humans.[630]

Microbiology

Protothecae are widely distributed in nature and can be isolated from soil, sewage, stream water, and other sites.[630,631] In culture at 25° or 37°C, *Prototheca* produces smooth, white to cream-colored yeast-like colonies that are composed of sporangia. The two pathogenic species can be identified in culture by their patterns of sugar and alcohol assimilation and by direct immunofluorescence.[632]

Clinical Features

Cutaneous infection, accounting for almost two thirds of the cases, produces a localized or slowly progressive papular or eczematous dermatitis, usually on an extremity. This infection occurs in patients with underlying debilitating disease such as malnutrition, diabetes mellitus, and alcoholism, and patients with intrinsic defects in cell-mediated immunity or neutrophil function.[633] Olecranon bursitis, the other major form of protothecosis, occurs in otherwise healthy people and is often associated with local injury to the elbow. Protothecotic endocarditis in an HIV-positive patient has been reported.[634,635]

Pathology

Prototheca appears in culture and tissue sections as round to oval white cells (sporangia) that replicate asexually by nuclear division and cytoplasmic cleavage, producing two to 20 uninucleate sporangiospores. The sporangium frequently has a single central sporangiospore, with other sporangiospores radially arranged around its periphery. In tissue sections, the sporangia of *P. wickerhamii* are 2 to 12 μm in diameter, whereas those of *P. zopfii* are 10 to 25 μm or more (Fig. 10.60).[31] With H&E stain the algal cells are either unstained or hematoxylinophilic; the hyaline walls of the sporangia and endospores stain well with GMS, PAS, and Gridley stains, all of which highlight the distinctive morula cells.

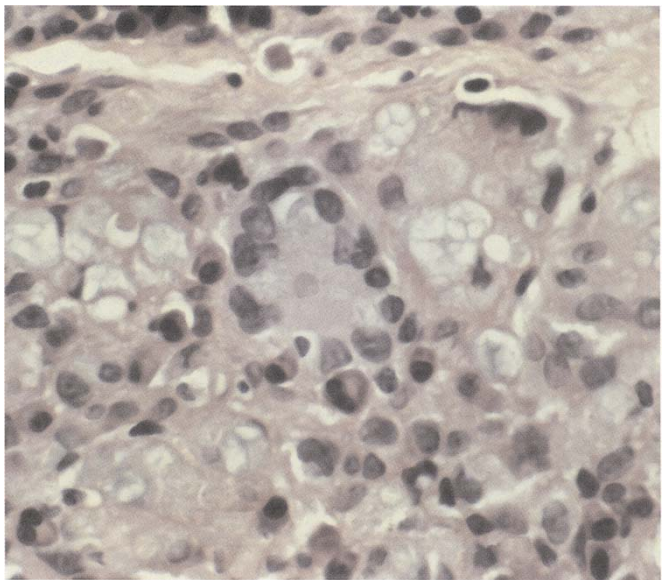

FIGURE 10.60. *Prototheca wickerhamii* sporangia associated with a granulomatous reaction (H&E).

The cutaneous lesions may show either little cellular reaction or a mixed cellular infiltrate with multinucleated giant cells and focal necrosis. The lesions of olecranon bursitis consist of geographic necrotizing or suppurative granulomas that can be confused with rheumatoid nodules.[636] Disseminated lesions, described histopathologically in only one case, were granulomatous.[637]

Although the sporangia of *P. wickerhamii* and *P. zopfii* can often be distinguished from each other in tissue sections on the basis of size, shape, and frequency of morula forms, definitive diagnosis requires confirmation by culture or direct immunofluorescence.

Treatment

Olecranon bursitis is cured by surgical excision. Systemic therapy of cutaneous and disseminated infections using amphotericin B and ketoconazole is reported to be effective.[638,639]

Malassezia

Malassezia is a genus of lipophilic yeasts that are constituents of the normal human skin flora. These organisms are classified into seven species—*M. furfur, M. pachydermatis, M. sympodialis, M. slooffiae, M. obtuse, M. globose,* and *M. restricta*—based on PCR and restriction endonuclease

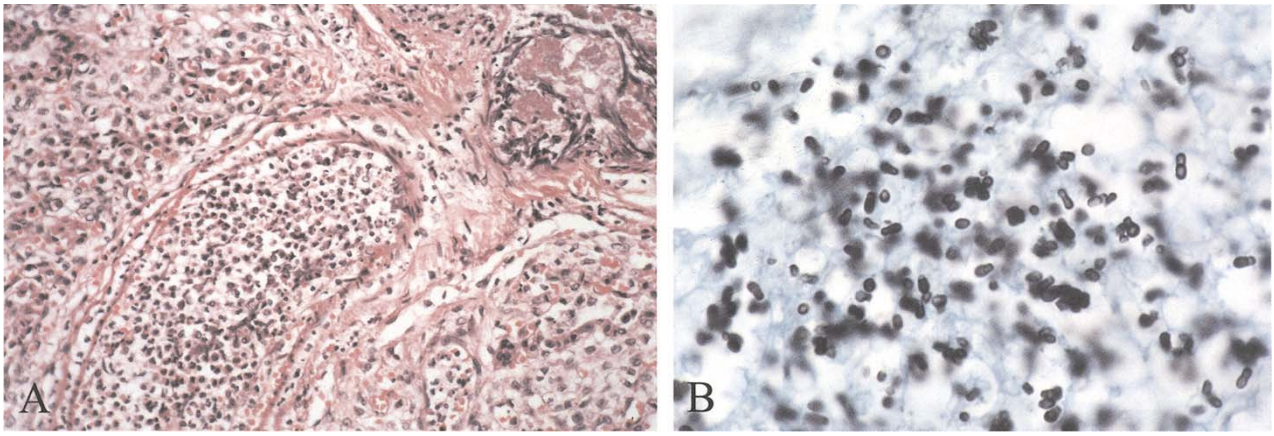

FIGURE 10.61. **A.** Malassezia vasculitis in a patient with bronchopulmonary dysplasia (BPD) on hyperalimentation. Acute inflammation of a muscular pulmonary artery with surrounding necrotizing pneumonia. The vascular lumen is filled with neutrophils and platelet fibrin thrombi (right upper). **B.** Myriad minute yeast-like cells of *Malassezia furfur*, with single buds (phialoconidia), are located within the intravascular exudate (GMS). (Courtesy of Dr. Beverly Dahms, Cleveland, OH.)

analysis.[640] The organisms appear to become part of the normal skin flora by 3 to 6 months of age; 98% of infants in a surgical unit were found to harbor these fungi.[641]

Microbiology

Malassezia requires fatty acids for growth; hence they do not grow on routine laboratory media and may be missed if routine culture methods are used. The organisms grow on Sabouraud dextrose agar at 37°C when covered with a layer of olive oil. Blood cultures processed by lysis-centrifugation onto lipid-enriched media (e.g., addition of olive oil to the fungal culture medium) offer the best chance for recovery of these fungi.

Clinical Features

The most common clinical manifestations of infection are the skin ailment tinea versicolor, and intravascular catheter-related systemic infections. High temperature, humidity, and lipid infusions appear to predispose to catheter colonization and infections.[642] In immunocompromised hosts, these fungi cause both localized and systemic infection.[643] Clinical infections have been reported in bone marrow transplant recipients, patients with underlying hematologic malignancies and other immunodeficiencies, and low birthweight infants receiving lipid infusions.[644,645] Hospital outbreaks of *Malassezia* infection have been described, with simultaneous occurrence of *M. furfur* infection in three intensive care unit patients in neighboring beds.[646]

Malassezia causes tinea (pityriasis) versicolor in normal hosts, producing discolored hypo- or hyperpigmented lesions with minor scaling and without any apparent inflammatory response.[647] Folliculitis may present as red papules and pustules and diagnosed on biopsy.

Bloodstream infections and catheter-associated sepsis, especially in newborns receiving lipid infusions through a central catheter (see Chapter 28), and septic thrombosis of the superior vena cava and peripheral thromboembolism have been described in patients with *Malassezia* fungemia (Fig. 10.61).[648]

Pathology

Budding yeasts may occasionally be seen on a smear of peripheral blood in fungemic patients, and in skin biopsy specimens from patients with folliculitis. The yeast cells are small, oval to bottle-shaped with unipolar budding and without hyphae, well demonstrated with PAS or GMS stains (Fig. 10.61B). Within the lung, angiocentric necrotizing lesions with neutrophilic exudates are seen (Fig. 10.61A).

Diagnosis

The organism has been isolated from skin scrapings, from sterile swabs premoistened with sterile saline, and from premoistened sterile towelettes. The organism has also been recovered from blood and urine cultures using subculture techniques onto lipid-containing media.

Treatment

Catheter-related *Malassezia* infections are treated with catheter removal, discontinuation of the lipid infusion, and administration of antifungal therapy. Antifungal options include amphotericin B for systemic infection, and oral fluconazole or topical azoles and selenium sulfide

for folliculitis.[649,650] Favorable outcomes of *Malassezia* infections in bone marrow transplant recipients, especially with catheter removal and cessation of intravenous lipid infusions, have been reported.[651]

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Glossary

Adioconidium: An aleurioconidium that enlarges in size but does not germinate.

Aerial hyphae: Hyphae above the nutrient agar surface.

Aleurioconidium (pl. aleurioconidia): A conidium that develops as an expanded end of an undifferentiated hypha or on a short pedicle, and is released by rupture of the supporting cell.

Aleuriospore: *See* Aleurioconidium.

Alternate arthroconidium: An arthroconidium separated by disjunctor cells and released by their rupture.

Arthroconidium (pl. arthroconidia): A conidium that is released by fission through a double septum or fragmentation/lysis of a disjunctor cell.

Arthrospore: *See* Arthroconidium.

Aseptate: Lacking septa.

Asexual reproduction: Development by mitosis.

Blastoconidia (sing. blastoconidium): Conidia that are produced by a blowing out process.

Blastospore: *See* Blastoconidia.

Bud: A young conidium. Usually used to denote the young blastoconidia of yeasts.

Capsule: A hyaline gelatinous polysaccharide sheath surrounding a cell.

Chlamydospore: A conidium that becomes rounded and enlarged with an increase in cell wall thickness and protoplasm density. Chlamydospores are released by the disintegration of the surrounding cells.

Conidiophore: A specialized hypha upon which conidia develop.

Conidium (pl. conidia): An asexual, usually deciduous, nonmotile propagule that forms in any manner other than by cytoplasmic cleavage, free-cell formation, or conjugation. Ascospores, basidiospores, sporangiospores, zoospores, and zygospores are not considered to be conidia.

Dimorphic: Having two morphological forms. The term is commonly used to describe those fungi that grow as a mold at room temperature and either a yeast or spherule in tissue, depending on the species of fungus.

Endospore: A spore produced within a spherule. In reality, endospores are sporangiospores. The term is maintained for a specific reproductive propagule in tissue.

Filamentous: Having filaments.

Germ tube: The initial hypha that develops from a conidium or spore.

Hypha: A filament of a fungus.

Moniliform: Beadlike, having swellings.

Mycelium: The mass of hyphae making up the thallus of a fungus.

Mycetoma: An infection caused by a number of different fungi and actinomycetes classically characterized by draining sinuses, granules, and tumefaction.

Phaeohyphomycosis: An infection caused by a number of dematiaceous fungi, characterized by the development of dematiaceous hyphae in the tissue that are short to elongate, distorted or swollen, regular, or irregular in form.

Phialide: A cell that gives rise to conidia in succession at its tip. A ring of cell wall called a collarette may be present at the tip of the phialide.

Propagule: An individual unit that can give rise to another organism.

Pseudohypha (pl. pseudohyphae): A series of blastoconidia that remain attached to each other forming a hyphalike filament.

Pseudomycelium: A large quantity of pseudohyphae.

Septate: Having cross-walls or septa.

Septum (pl. septa): A cross-wall in a conidium, hypha, or spore.

Spherule: A thick-walled saclike cell that contains numerous endospores.

Sporangiospore: An asexual spore produced within a sporangium.

Sporangium (pl. sporangia): A saclike structure in which the entire internal contents are cleaved into asexual spores.

Spore: A propagule derived by sexual or asexual means. If by asexual means, a cleavage process is usually involved.

Vacuole: In a cell, a minute cavity devoid of protoplasm.

Vesicle: A swollen cell; the swollen apex of the conidiophore of an *Aspergillus* sp.

Yeast: In a strict sense, a unicellular budding fungus that reproduces by both sexual and asexual means.

Yeastlike: Having a unicellular budding form that reproduces by asexual means only. In this chapter, yeastlike and yeast are used interchangeably, but are referred to as yeast.