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7

Lung Infections

Ann E. McCullough, MD, and Kevin O. Leslie, MD

Lower respiratory tract infections are a leading cause of morbidity and death worldwide.[1,2] A relatively small percentage of these infections come to the attention of the surgical pathologist because most are diagnosed in the microbiology laboratory. The biopsied pulmonary infection typically has eluded standard microbiologic techniques, has not responded to empirical therapy, or requires morphologic analysis for clarification of a critical aspect of the differential diagnosis. In these situations, the diagnostic pathologist is indispensable,[3,4] if not for providing an immediate report intraoperatively (by frozen section or cytologic smears; Box 7.1), then for dramatically improving diagnosis turnaround time with the use of newer rapid tissue-processing systems (Table 7.1).[5]

Diagnostic Tools and Strategies

The histories of pathology and microbiology are intertwined.[6] Pathologists should add their diagnostic techniques to those of microbiology for the best diagnostic yield (Table 7.2).[7] Unfortunately the diagnostic work-up and reporting of findings in anatomic pathology and microbiology typically run along nonintersecting paths, often without one group knowing (or acknowledging) the findings of the other. An interdisciplinary approach based on mutual understanding and communication is the ideal scenario for clinical management.[8] Our concept of an integrated morphologic and microbiologic approach is presented schematically in Fig. 7.1 and with greater detail for specific situations in which bacterial (Fig. 7.2), mycobacterial (Fig. 7.3), fungal (Fig. 7.4), or viral (Fig. 7.5) pathogens are suspected. Specific species diagnoses are typically not possible from most pathology specimens, and attempts at pure morphologic diagnosis can be misleading. Pathologic findings should always be correlated with microbiologic findings. Accordingly, foresight is required on the part of the intraoperative pathologist in obtaining and properly handling tissues for culture.[9] The pathologic report should correlate the relevant microbiologic findings.

Box 7.1 Role of the Diagnostic Pathologist

Rapid diagnosis: frozen section; cytologic smears; rapid tissue process
Identify unculturable pathogens
Establish diagnosis when culture results are negative
Evaluate pathogenic significance of culture isolate
Define "new" infectious diseases
Exclude infection as etiologic disorder; detect comorbid process
Intraoperative triage of limited biopsy tissue
Clinicopathologic-microbiologic correlation

Modified from Watst J, Chandler F. The surgical pathologist's role in the diagnosis of infectious disease. *J Histotechnol.* 1995;18:191–193.

Table 7.1 Diagnostic Tools of the Pathologist

Activity	Objective
Pre-/intra-/postoperative consultation	Information exchange and strategies
Gross examination	Tissue handling and triage
Histopathologic examination	Organism morphology; cytopathic effect; host response
Histochemical stains	Detection and morphologic detail
Immunohistochemical stains	Detection of organisms; confirmation of genus/species
Electron microscopy	Selective use for virus, fungi, parasites, and bacteria
Molecular techniques: in situ hybridization, polymerase chain reaction	Sensitive and specific detection/identification of nonculturable organisms; stain-negative cases
Report	Clinicopathologic and microbiologic correlation

Table 7.2 Diagnostic Tools of the Microbiologist

Activity	Objective
Pre-/intra-/postoperative consultation	Information exchange and strategies
Direct visualization (smears and imprints)	Rapid detection
Culture	Identification of genus and species; susceptibility studies
Antigen detection	Rapid identification
Serologic testing	Specific antibody response
Molecular techniques	Sensitive and specific detection/identification
Report	Traditional versus interpretive format

Knowledge of the Clinical Setting

Identification of the patient's risk factors and immune status is important because these parameters typically influence the spectrum of histopathologic changes, the types of etiologic agents, and number of organisms.[10-15] The degree of immunosuppression can influence the burden of organisms making the etiologic organisms more difficult to demonstrate histopathologically. For example, organisms are less often found in lung tissues from patients with normal or near-normal immunity. In this setting, cultures, serologic studies, and epidemiologic data must be relied on to provide the diagnosis.[16] Contrast the tedious search for rare acid-fast organisms in reactivation tuberculosis granulomas with *Mycobacterium avium* infection in acquired immunodeficiency syndrome (AIDS) patients. In the AIDS patient, *M. avium* infection typically manifests poorly formed granulomas or simply histiocytic infiltrates, with an overabundance of organisms identified by tissue acid-fast stains.

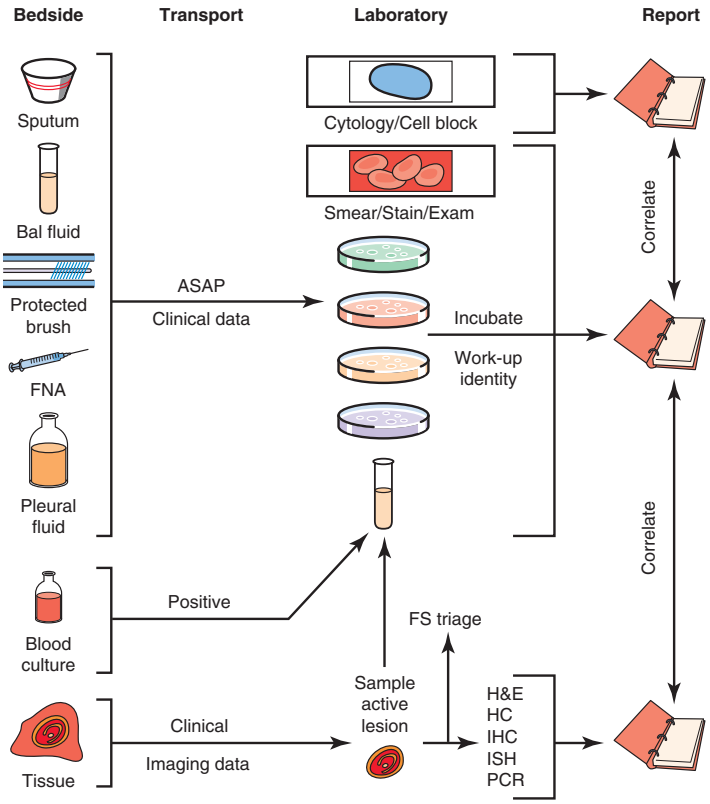


Figure 7.1 Schematic for the work-up of a respiratory specimen for suspected infection. *BAL*, Bronchoalveolar lavage; *FNA*, fine-needle aspiration; *FS*, frozen section; *HC*, histochemistry; *H&E*, hematoxylin and eosin (stain); *IHC*, immunohistochemistry studies; *ISH*, in situ hybridization; *PCR*, polymerase chain reaction (assay).

Similarly, *Pneumocystis* organisms may be easily identified in patients with AIDS, but when immunosuppression is less severe (such as that produced by corticosteroids therapy for arthritis), the organism is rare. The relationship between the level of immunity, burden of organisms, and patterns of disease is illustrated for cryptococcosis in Fig. 7.6.

In the immunocompromised patient, there is always a broader differential diagnosis.[17] In addition to infection, other disorders come into consideration, such as pulmonary involvement by preexisting disease, drug-induced and treatment-related injury, noninfectious interstitial pneumonias, malignancy, and new pulmonary diseases unrelated to the patient's immunocompromised state, such as aspiration, heart failure, and pulmonary embolism. When immunosuppression is intentional, as in transplant recipients, unique additional challenges come into play, such as transplant rejection, graft-versus-host disease, and Epstein–Barr virus (EBV)–associated lymphoproliferative disorders. Immunosuppressed persons are at risk for multiple simultaneous infections, so when one organism is found, a careful search for others is always warranted (Fig. 7.7).

A number of well-characterized genetic disorders of immunity and cellular function are known to predispose affected persons to lung infection.[18-21] Cystic fibrosis bears special recognition in this context because it is associated with reproducible patterns of lung disease and susceptibility to a wide spectrum of infectious organisms. This genetic disease of autosomal recessive inheritance involves mutation of the *CFTR* gene, which affects the ability of epithelial cells to effectively transport chloride and water across cell membranes. As a result, many organs, including the lungs, develop excessively viscous mucous secretions that cannot be cleared from the airways effectively. In the

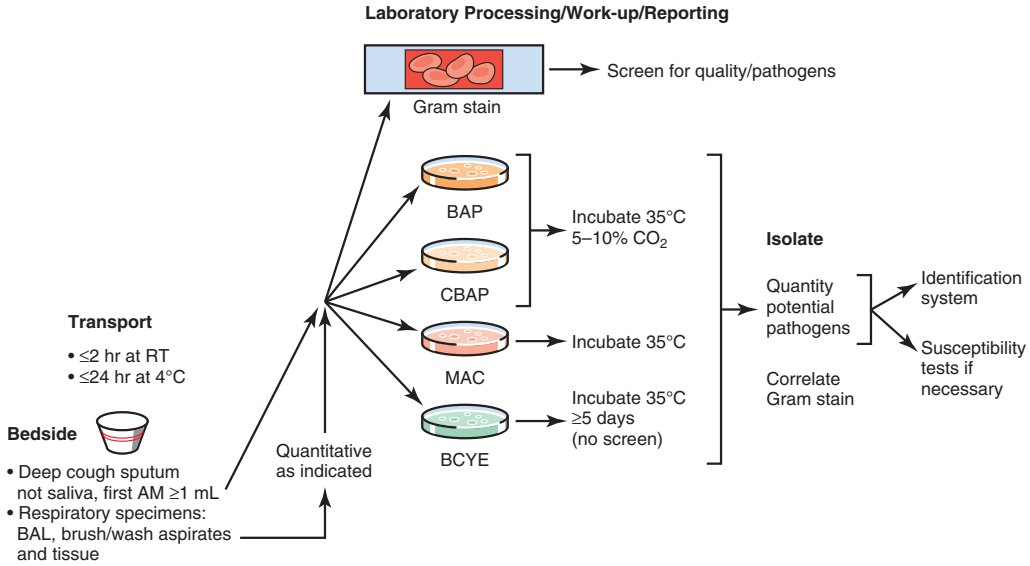


Figure 7.2 Integrated morphologic and microbiologic approach to laboratory diagnosis of bacterial infection. *BAL*, Bronchoalveolar lavage; *BAP*, blood agar plate; *BCYE*, buffered charcoal yeast extract; *CBAP*, chocolate blood agar plate; *MAC*, MacConkey agar; *RT*, room temperature.

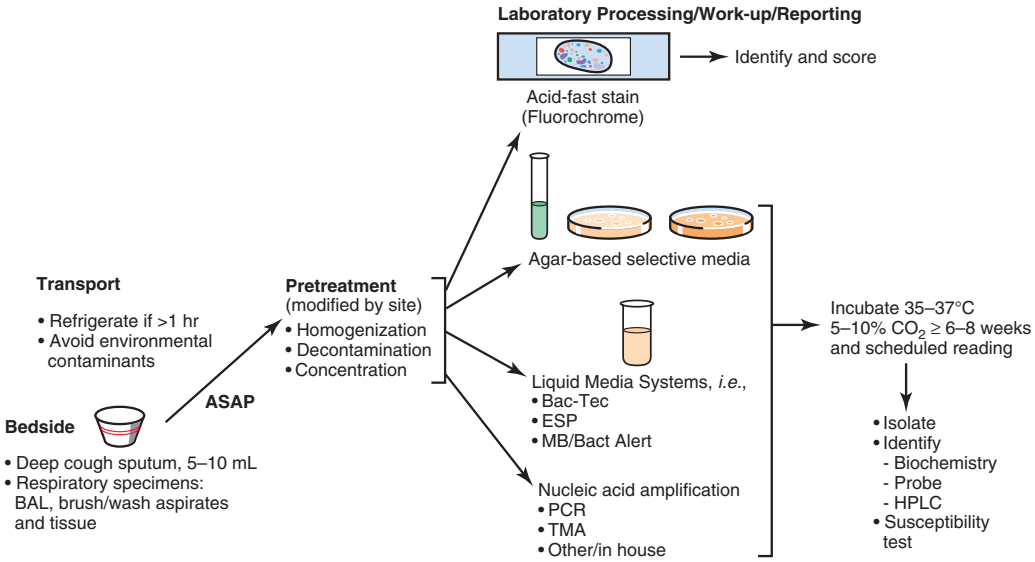


Figure 7.3 Integrated morphologic and microbiologic approach to the laboratory diagnosis of mycobacterial infection. *Bac-Tec*, BD BACTEC Instrumented Mycobaterial Growth Systems; *BAL*, bronchoalveolar lavage; *ESP*, ESP Culture System; *HPLC*, high-performance liquid chromatography; *MB/Bact Alert*, Biomerieux Bact/alert 3D; *PCR*, polymerase chain reaction (assay); *TMA*, transcription-mediated amplification.

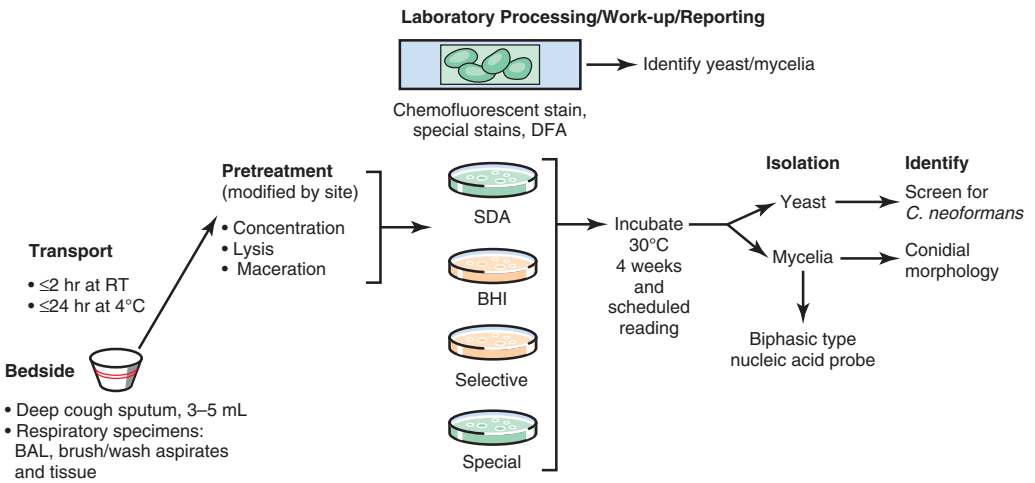


Figure 7.4 Integrated morphologic and microbiologic approach to laboratory diagnosis of fungal infection. *BAL*, Bronchoalveolar lavage; *BHI*, brain-heart infusion; *DFA*, direct immunofluorescence assay; *RT*, room temperature; *SDA*, Sabouraud dextrose agar.

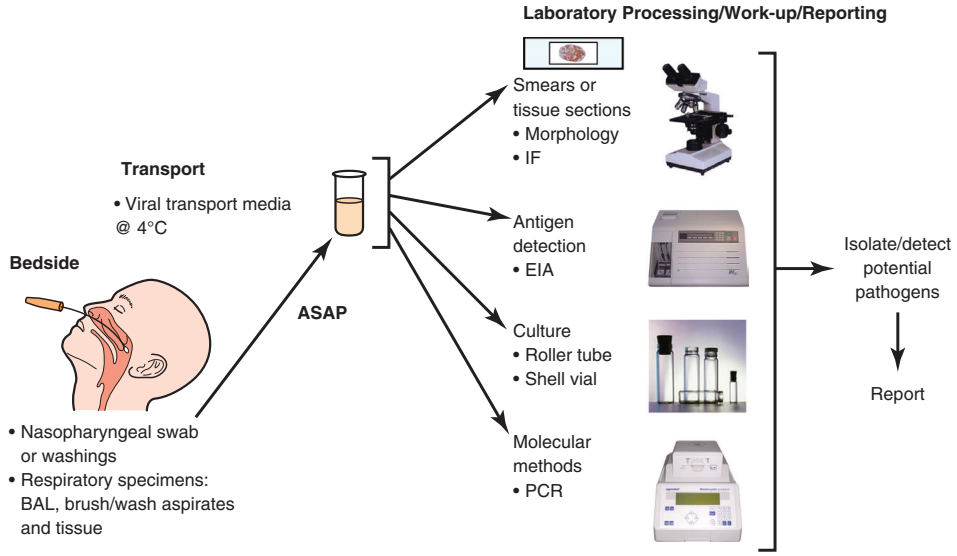


Figure 7.5 Integrated morphologic and microbiologic approach to laboratory diagnosis of viral infection. *BAL*, Bronchoalveolar lavage; *EIA*, enzyme immunoassay; *IF*, immunofluorescence; *PCR*, polymerase chain reaction assay.

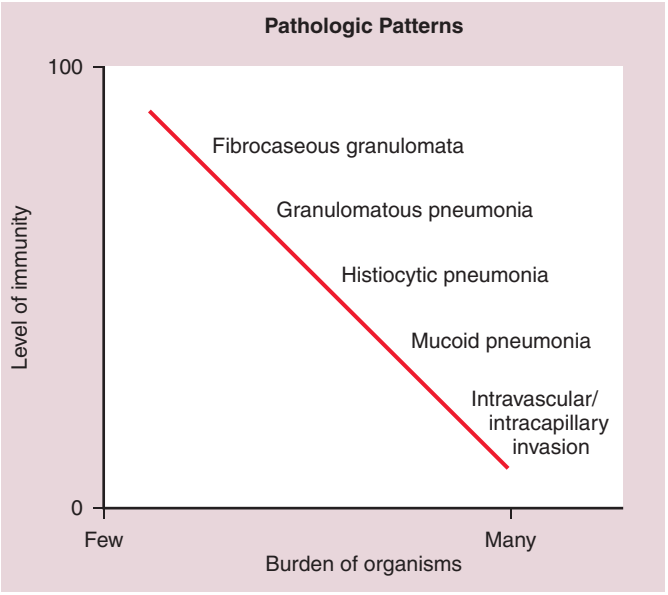


Figure 7.6 Cryptococcosis: Correlation of pathologic patterns with immunity level and organism burden. With cryptococcal pneumonia in patients with normal or near-normal immunity, granuloma formation with few organisms is characteristic. In immunocompromised patients, typical findings include histiocytic infiltrates or mucoid pneumonia with little or no inflammatory reaction and many organisms. (Data from Mark EJ. Case records of the Massachusetts General Hospital. *N Engl J Med.* 2002;347:518–524.)

lung, retention of such secretions leads to progressive and widespread bronchiectasis with airway obstruction, which in turn paves the way for recurrent infection (Fig. 7.8). Bacterial organisms commonly isolated include *Pseudomonasaeruginosa* (both mucoid and nonmucoid strains), *Haemophilus influenzae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*.[22] Polymicrobial infections are not uncommon, and some of these pathogens, especially certain subspecies within the *B. cepacia* complex, are linked to an adverse prognosis.[23] Cystic fibrosis is also a risk factor for nontuberculous mycobacterial infection and allergic bronchopulmonary fungal disease, and the condition is potentially exacerbated by superimposed viral infections.[24-27]

Pattern Recognition

Knowledge of the radiologic pattern of infectious lung disease in a given patient often helps to narrow the scope of the differential diagnosis.[28,29] Patterns of lung infection seen on high-resolution computed tomography (HRCT) are typically dominated by increased attenuation (opacity). Such opacities may occur as one or more localized densities

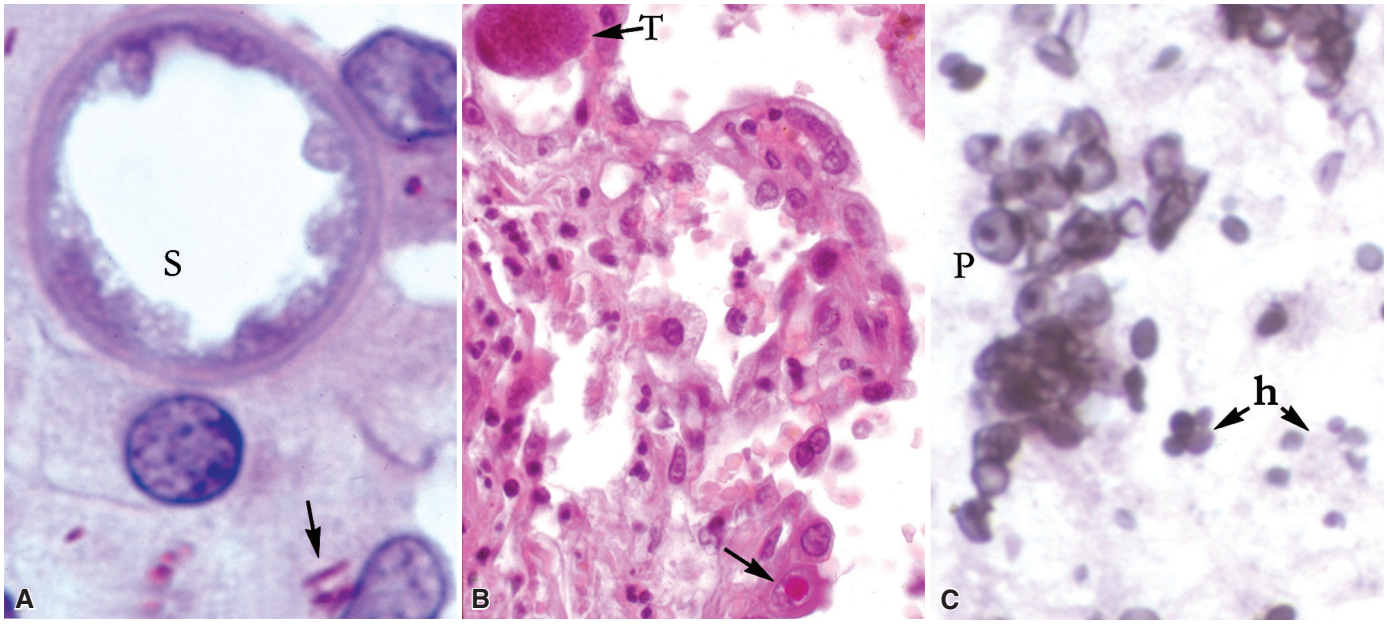


Figure 7.7 Co-infection with dual pulmonary pathogens. (A) Spherule of *Coccidioides (S)* and *Mycobacterium avium* complex acid-fast bacilli (Ziehl–Neelsen/Hematoxylin and eosin stains). (B) *Toxoplasma* pseudocysts *(T)* and cytomegalovirus-infected alveolar lining cell *(arrow)*. (C) Clusters of *Pneumocystis* cysts *(P)* in the midst of *H. capsulatum* yeast cells *(h)* (Grocott methenamine silver stain).

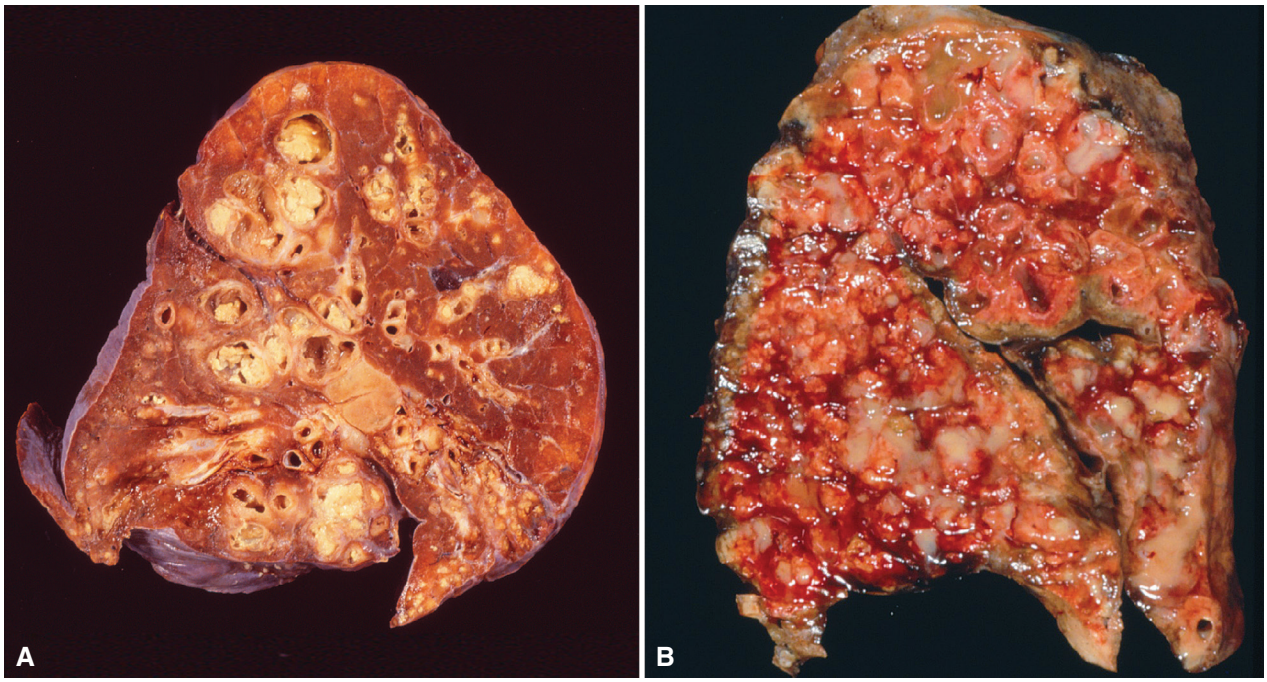


Figure 7.8 Changes of cystic fibrosis in the lung. (A) Explant from a 13-year-old patient. (B) Advanced disease at autopsy.

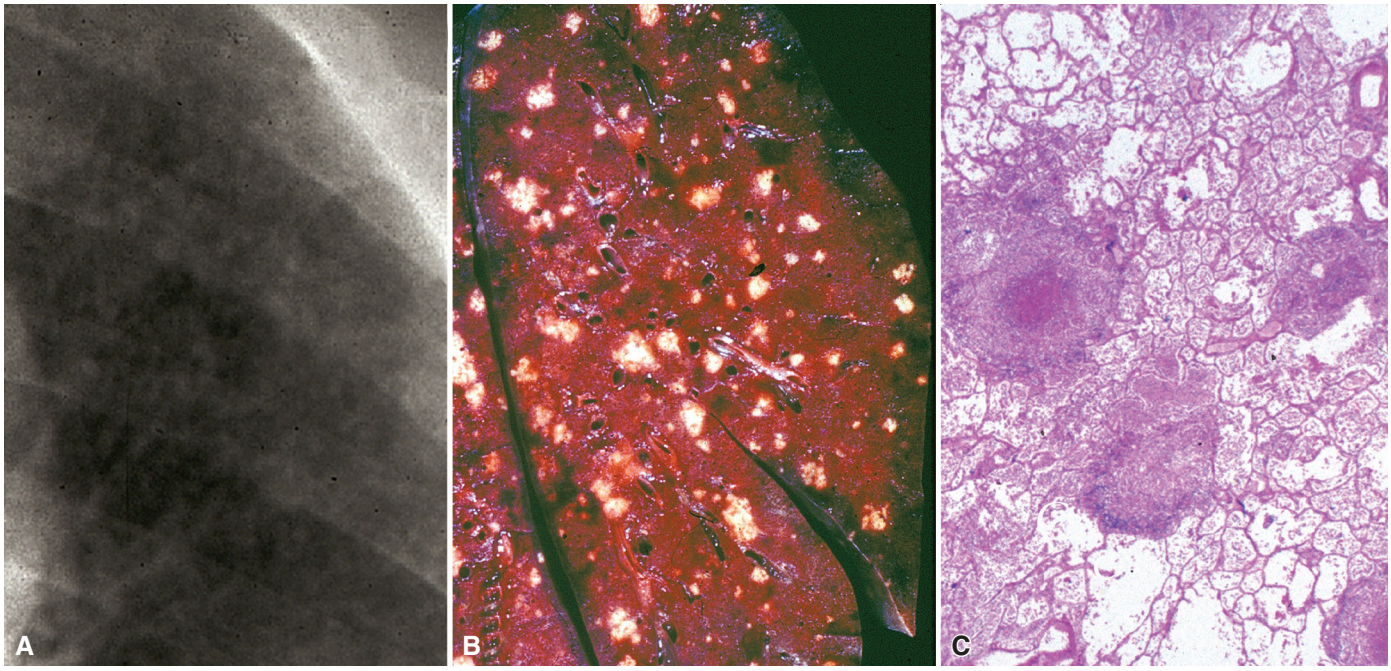


Figure 7.9 Miliary pattern of tuberculosis. (A) Chest film, closeup view of miliary infiltrate. (B) Gross cut surface of pulmonary parenchyma with miliary nodules. (C) Histopathologic features of miliary necrotizing granulomas.

Table 7.3 Histopathologic Patterns and Most Agents of Pulmonary Infection

Pattern	Most Common Agent(s)	Pattern	Most Common Agent(s)
Airway disease	—	Interstitial pneumonia	—
Bronchitis/bronchiolitis	Virus; bacteria; *Mycoplasma*	Perivascular lymphoid	Virus; atypical agents
Bronchiectasis	Bacteria; mycobacteria	Eosinophilic	Parasite
Acute exudative pneumonia	—	Granulomatous	Mycobacteria
Purulent (neutrophilic)	Bacteria	Nodules	—
Lobular (bronchopneumonia	Bacteria	Large	—
Confluent (lobar pneumonia)	Bacteria	Necrotizing	Fungi; mycobacteria
With granules	Agents of botryomycosis (*Staphylococcus aureus*), actinomycosis (*Actinomyces israelii*)	Granulomatous	Fungi; mycobacteria
		Fibrocaseous	Fungi; mycobacteria
Eosinophilic	Parasites	Calcified	Fungi; mycobacteria
Foamy alveolar cast	*Pneumocystis*	Miliary	—
Acute diffuse/localized alveolar damage	Virus; polymicrobial	Necrotizing	Viral; mycobacteria; fungi
Chronic pneumonia	—	Granulomatous	Fungi
Fibroinflammatory	Bacteria	Cavities and cysts	Fungi; mycobacteria
Organizing diffuse/localized alveolar damage	Virus	Intravascular/infarct	Fungi
Eosinophilic	Parasite	Spindle cell pseudotumor	Mycobacteria
Histiocytic	Mycobacteria	Minimal ("id") reaction	Polymicrobial

(nodule, mass, or infiltrate), as ground-glass opacities (attenuation that allows underlying lung structures to be visible), or consolidation (attenuation that overshadows underlying structures).[30] Review of the chest imaging studies and the pace of the disease (acute, subacute, and chronic) can be very helpful in arriving at a clinically relevant diagnosis. (Fig. 7.9). Fortunately the recognized histopathologic patterns of lung infection are fairly limited (airway disease, acute lung injury, cellular infiltrates, alveolar filling, and nodules), and these typically correlate with a particular group of organisms (Table 7.3).

Useful Tissue Stains in Lung Infection

Some pathologists have an aversion to the use of special stains for identifying organisms in tissue sections based on less than optimal specificity and sensitivity and the technical difficulty of performing some of these (especially silver impregnation methods, such as the Dieterle, Steiner, and Warthin-Starry stains). Nevertheless, several tissue section staining techniques are quite useful in detecting bacteria, mycobacteria, and fungi in tissue sections. A list of these is presented

Box 7.2 Useful Tissue Stains in Lung Infection

- Gram stain
  - Brown and Brenn
  - Brown and Hopps
- Silver stains
  - Warthin-Starry
  - Steiner
  - Dieterle
- Fungal stains
  - Grocott methenamine silver (GMS)
  - Periodic acid–Schiff reagent (PAS)
- Mycobacterial stains
  - Ziehl–Neelsen (heat)
  - Kinyon (cold)
  - Auramine O (fluorochrome)
  - Fite-Faraco (peanut or mineral oil)
- Other tissue stains
  - Giemsa; Diff-Quik
  - Mucicarmine
  - Modified trichrome (Weber)
  - Fontana–Masson
  - Chemofluorescent (optical brighteners)
  - Immunofluorescent antibodies
  - Immunohistochemical

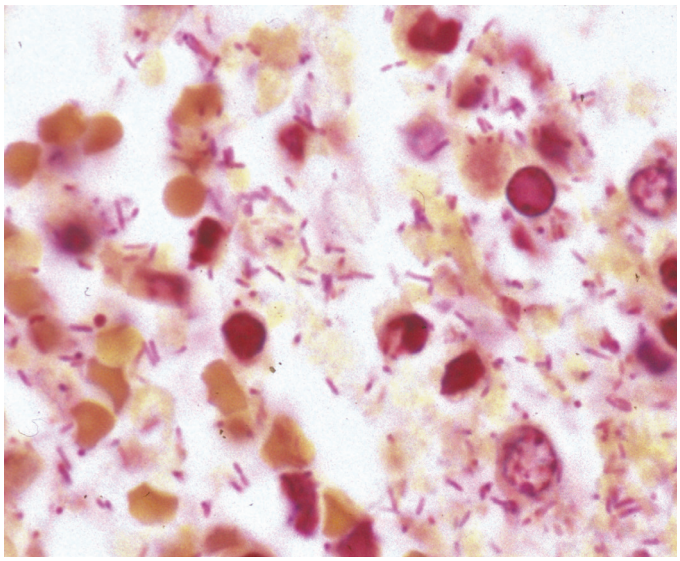


Figure 7.10 Gram-negative bacilli *(Escherichia coli)* in alveolar exudate (Brown and Hopps stain).

in Box 7.2. These stains should be applied as part of an algorithmic strategy for acute lung injury, especially in the immunocompromised patient.[31] For example, when bacteria are being sought, some pathologists would prefer to begin with the tissue Gram stain (e.g., Brown and Hopps, Brown and Brenn; Figs. 7.10 and 7.11), but silver impregnation techniques (e.g., Warthin-Starry) are actually more sensitive and a good starting point for approaching a suspected bacterial infection. By coating the bacteria with metallic silver, the bacterial silhouettes are enhanced (Fig. 7.12) and become more visible.[31] Other stains (e.g., Giemsa) will sometimes detect bacteria that do not stain well with more conventional stains (Fig. 7.13). The Grocott methenamine silver (GMS) stain (Fig. 7.14) is the best stain for most fungi in tissue; it also stains *Actinomycetes*, *Nocardia*, *Pneumocystis* (cysts), free-living soil amebae, algal cells, the spores of certain microsporidia, and the cytoplasmic inclusions of cytomegalovirus (CMV).[7]

Most mycobacteria stain well with the Ziehl–Neelsen procedure (Fig. 7.15), but the auramine-rhodamine fluorescent procedure is superior in terms of sensitivity (Fig. 7.16). *Nocardia* organisms, *Legionella micdadei*, and *Rhodococcus equi* are weakly or partially acid-fast, and the use of modified acid-fast stains such the Fite-Faraco technique is more satisfactory for the identification of these organisms. Some mycobacterial species, such as *M. avium* complex (MAC), are also periodic acid–Schiff reagent (PAS)-positive, GMS-positive, and weakly gram-positive.

Finally, for the identification of most protozoa and helminths, as well as viral inclusions, a good-quality hematoxylin and eosin (H&E)–stained section suffices; in fact, a well-prepared H&E section alone is diagnostic for many infectious diseases. This stain can often detect and even distinguish between bacterial cocci and bacilli when the burden of organisms is high (Fig. 7.17).

Immunologic and Molecular Techniques

The application of ancillary studies—such as immunohistochemistry, in situ hybridization (Fig. 7.18),[32] or nucleic acid amplification technology—can provide a specific etiologic diagnosis in certain cases. These techniques have the best chance of diagnosing infections caused by fastidious species that are difficult or impossible to culture from fresh samples; they are also useful in situations where only formalin-fixed, paraffin-embedded tissues are available. Immunohistochemical reagents for microbiologic detection are becoming increasingly available and provide added power to determining specific diagnoses on formalin-fixed paraffin-embedded tissue (Fig. 7.19).[33] Although these techniques provide the diagnostic equivalence of culture confirmation, they are not without limitations and diagnostic pitfalls. The polymerase chain reaction (PCR) method first introduced in the 1980s has undergone a number of modifications. Non-PCR DNA amplification methods and methods based not on the amplification of the DNA target per se but on amplification of the signal or probe have also been introduced.[34] Among the more recently available technologies is the rapid-cycle real-time PCR assay, representing an especially powerful advance in that it is significantly more sensitive than culture. The adaptation of various amplification methods to real-time and multiplex formats enables laboratories to detect a wide range of respiratory pathogens. Furthermore the transition from traditional and analyte-specific methods to more global technologies such as PCR arrays, liquid bead arrays, microarrays, and high-throughput DNA sequencing is under way. Over time, these methods will find a place in laboratories of all sizes and dramatically impact the speed and accuracy of microbiologic testing practice for all types of microorganisms.[35-39]

Limiting Factors in Diagnosis

Needless to say, the diagnostic tools employed by both pathologists and microbiologists have their limitations in terms of sensitivity and specificity.[7] Some common tools are listed in Box 7.3. Culture alone cannot distinguish contamination from colonization, or in the case of viruses, asymptomatic shedding from true infection. Molecular tests may require specialized, often costly equipment and are susceptible to false-positive and false-negative results.[37] If a surgical biopsy is available, correlation of the histopathologic features can help assign an etiologic role to an agent recovered in culture or help establish if the microbiologically discovered organism has caused any microscopically visible lesion. The host inflammatory pattern and morphologic features of an organism can be characteristic for certain types of infections, but often the organism's morphology alone is not sufficient for a diagnosis at the genus or species level. Furthermore, the classic histopathologic findings for a given infection may be incomplete or lacking, making specific

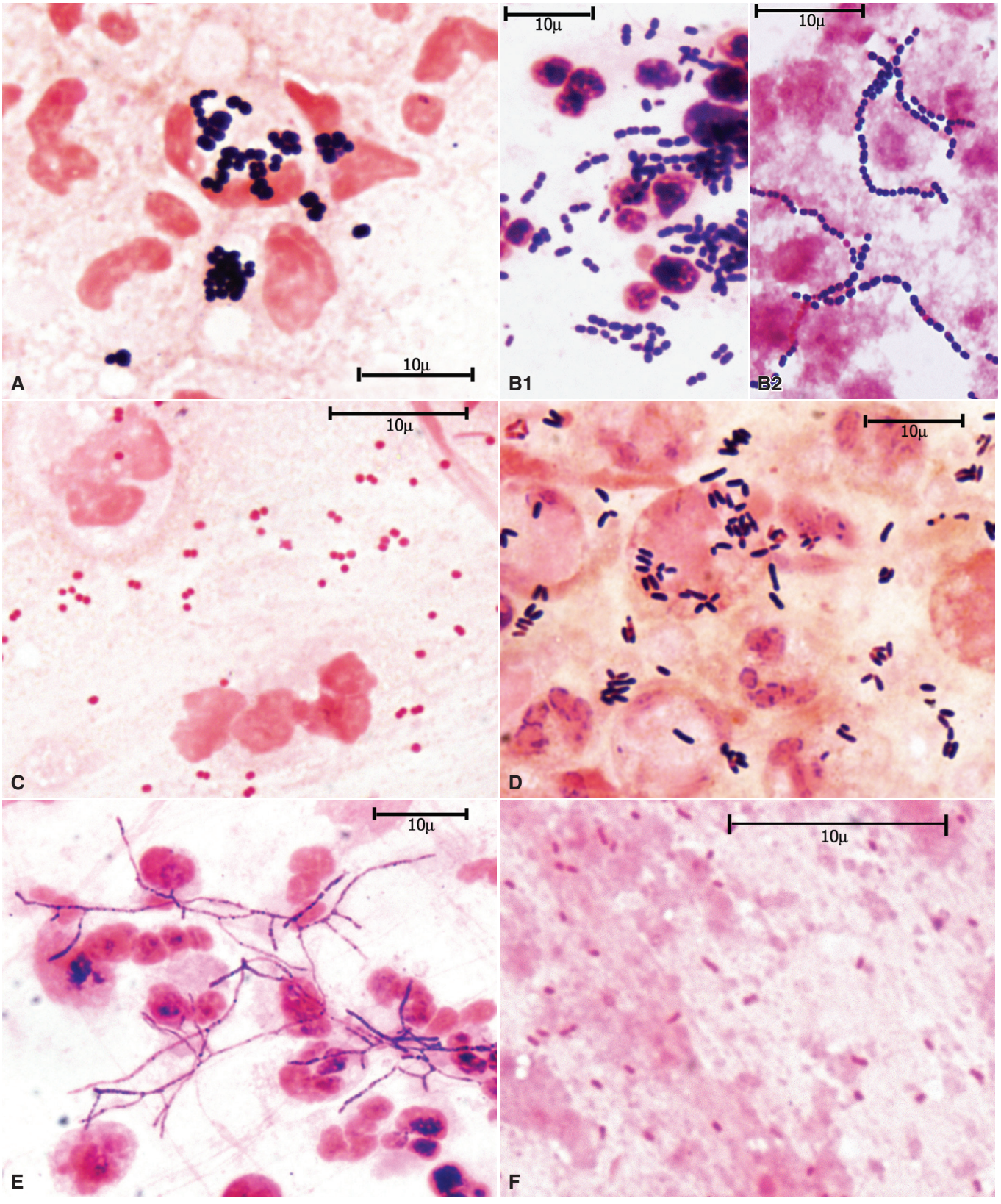
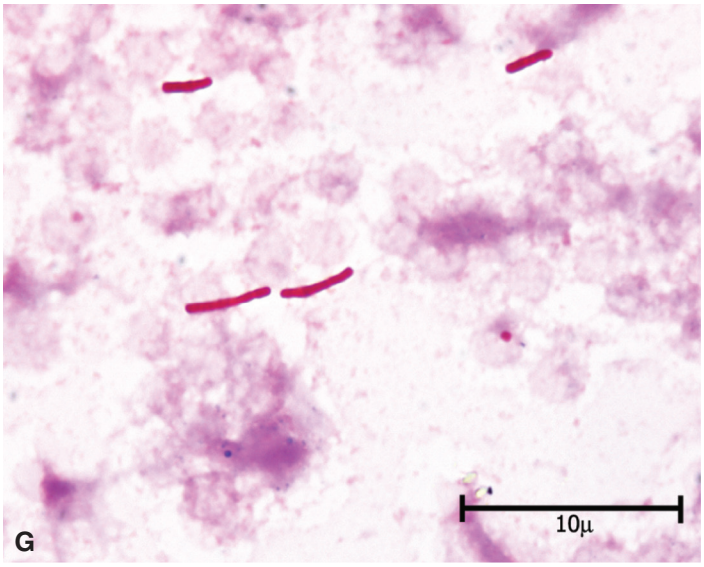
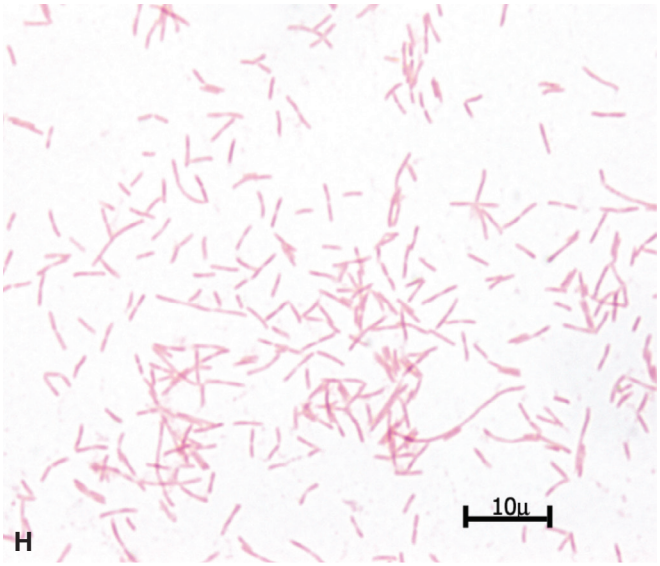


Figure 7.11 (A) Gram-positive cocci in clusters: *Staphylococcus aureus*. (B) Gram-positive cocci in pairs/chains: 1: *Streptococcus pneumoniae*. 2: *Streptococcus pyogenes*. (C) Gram-negative diplococci: *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Moraxella catarrhalis*.*(D) Short gram-positive bacilli/coccobacilli: *Corynebacterium jeikeium*, *Listeria monocytogenes*. (E) Filamentous gram-positive bacilli: *Nocardia* spp., *Actinomyces* spp., *Rhodococcus equi*, *Bartonella henselae*.†(F) Gram-negative coccobacilli: *Haemophilus influenzae*, *Acinetobacter baumannii*.





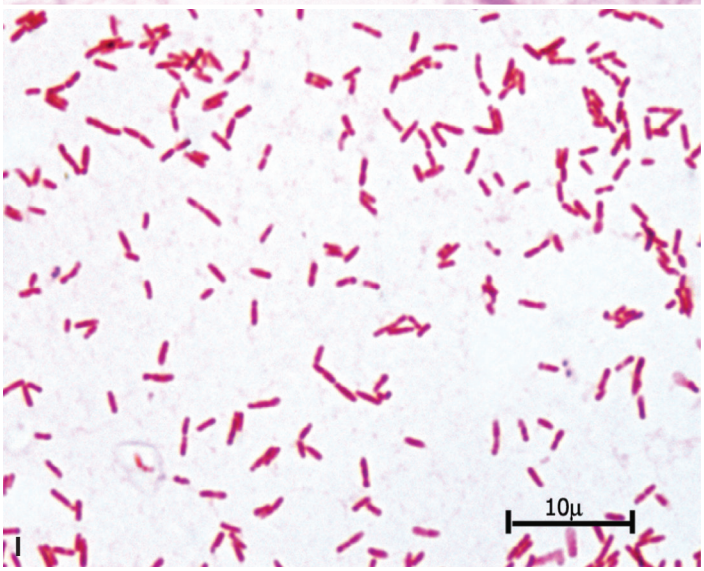


Figure 7.11, cont'd (G) Large gram-negative bacilli: *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*, *Salmonella typhi*, *Yersinia pestis*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter* spp., *Salmonella* spp., *Yersinia enterocolitica*. (H) Faintly staining gram-negative bacilli: *Legionella* spp, *Francisella tularensis*, *Brucella* spp, *Bordetella* spp. (I) Long slender gram-negative bacilli: *Pseudomonas aeruginosa*, *Burkholderia pseudomallei*, *Burkholderia cepacia*. Note: *Technically *Moraxella catarrhalis* has been placed in a bacillary genus, although this organism does have coccal morphology and responds as a coccus in the penicillin test. †Sometimes filamentous. (Bacterial Gram stain montage courtesy Drs. A.E. McCullough, S. Stewart, and L. Burdeaux, Mayo Clinic Hospital Microbiology Laboratory, Scottsdale, Arizona; From Tomashefksi JF, et al. *Dail & Hammer's Pulmonary Pathology*, 3rd ed. 2008:246. with permission of Springer.)

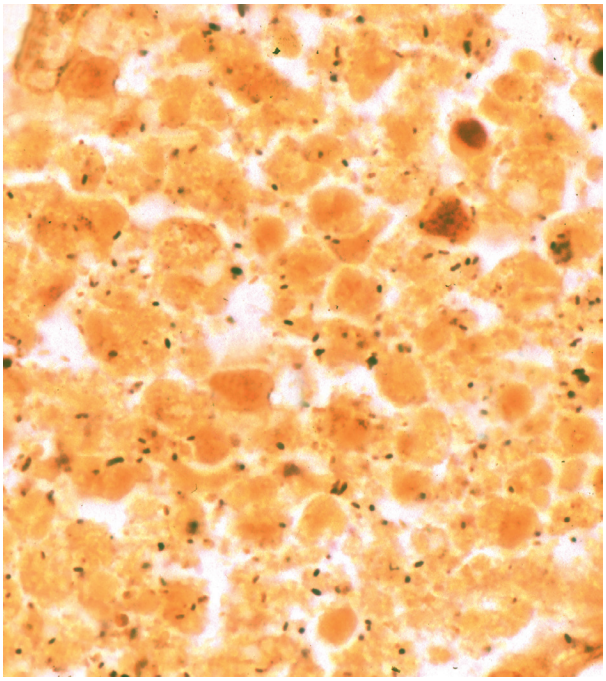


Figure 7.12 Black (silver-coated) bacilli *(Legionella pneumophila)* in alveolar exudate (Dieterle stain).

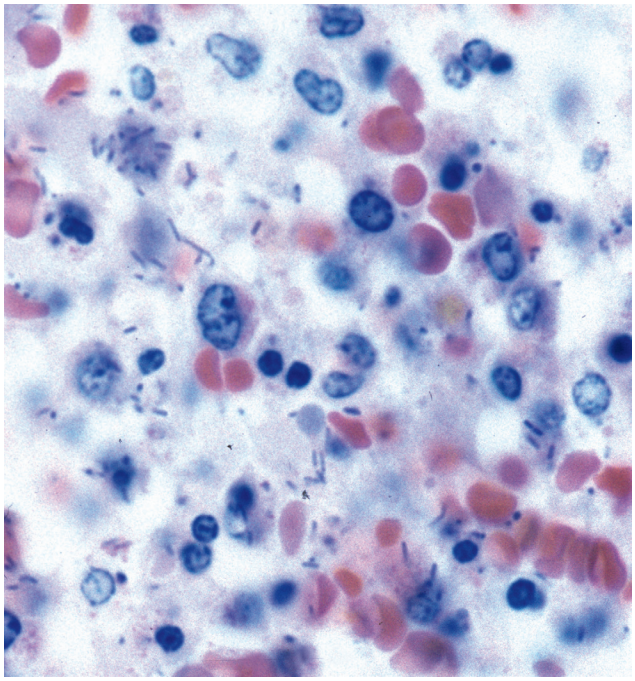


Figure 7.13 Bacillary organisms in alveolar exudates (Giemsa stain).

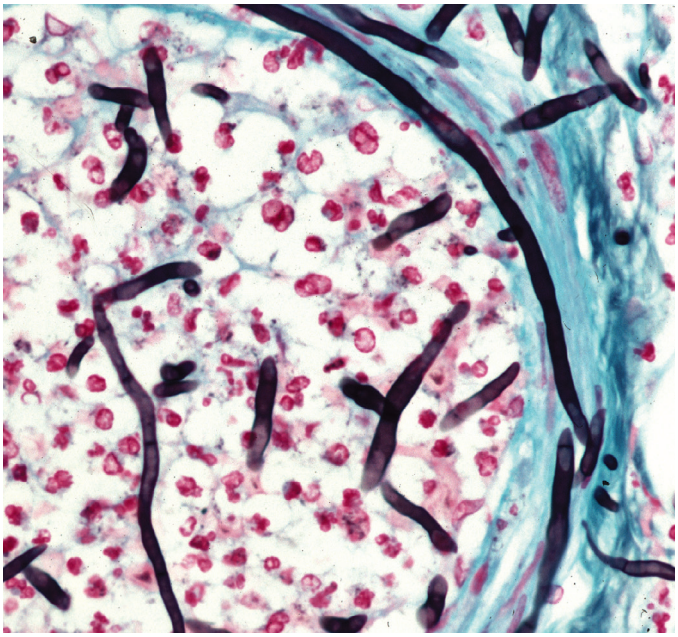


Figure 7.14 Angioinvasive *Aspergillus* species (Grocott methenamine silver stain). (Courtesy Dr. Francis Chandler, Augusta, Georgia.)

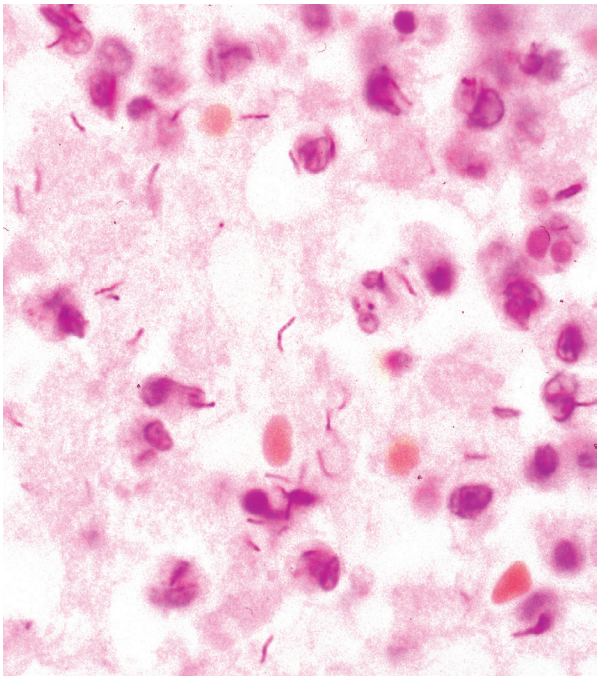


Figure 7.15 Acid-fast bacilli: *Mycobacterium tuberculosis* (Ziehl–Neelsen stain).

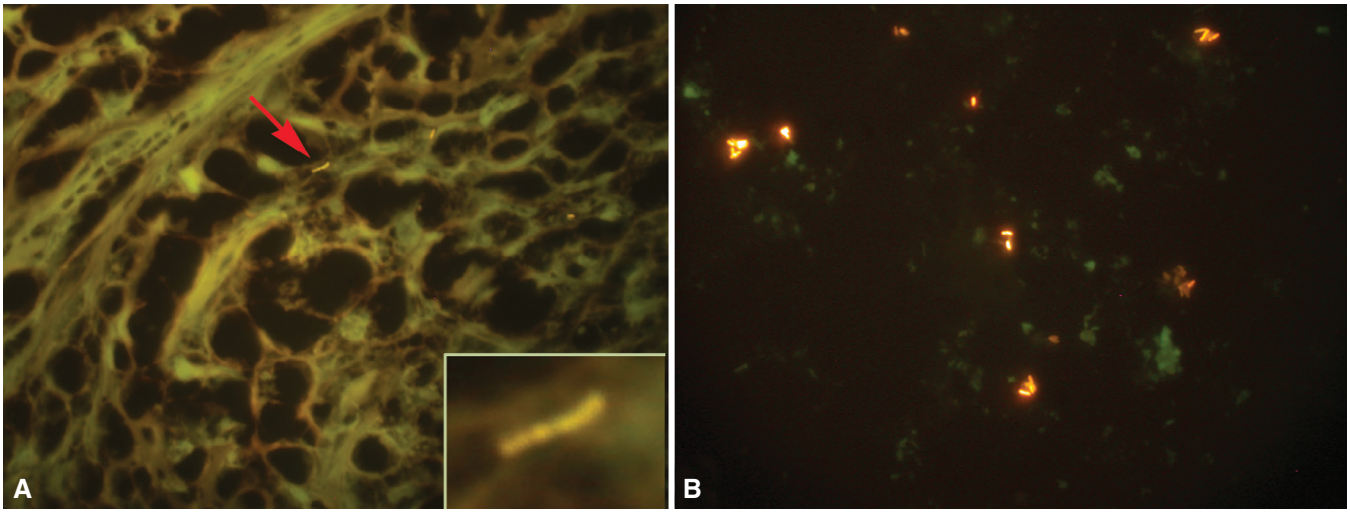


Figure 7.16 Fluorescent bacillary organisms: *Mycobacterium tuberculosis*. (A) Tissue section with two bacilli. Note beaded character in closeup view *(inset)*. (Auramine-rhodamine stain.) (B) Low-power view.

morphologic diagnosis possible for relatively few organisms. For example, the etiologic diagnosis is straightforward when large spherules with endospores characteristic of *Coccidioides* species are present, when the small budding yeasts of *Histoplasma capsulatum* are seen, or when yeasts with the large mucoid capsules of *Cryptococcus neoformans* are identified. However, atypical forms of these organisms can be confusing.[40] Similarly, hyphal morphology is helpful when it is characteristic of a specific genus or group, but the many look-alikes (Fig. 7.20) require separation by searching for subtle differences under high magnification (or oil immersion) or by relying on special techniques and culture.[41]

Certain viruses may have characteristic inclusions in tissue, but there are notable pitfalls. For example, the eosinophilic intranuclear inclusions of adenovirus may resemble the early inclusions in herpes simplex virus (HSV) or CMV, especially when the typical smudged cellular forms of adenovirus are absent. Also, simulators of viral cytopathic effect (CPE), such as macronucleoli, optically clear nuclei, and intranuclear cytoplasmic invaginations, can occur in a number of conditions and need to be recognized (Fig. 7.21).

Pseudomicrobe artifacts also have been recognized on routine and special stains for the identification of bacteria and fungi. Such potential artifacts include fragmented reticulin fibers, pigments, calcium deposits, Hamazaki-Wesenberg (yellow-brown) yeast-like bodies (Fig. 7.22), pollen grains, and even lymphoglandular bodies.[42] For all of these reasons, the pathologist must maintain a high threshold for diagnosing organisms on morphologic grounds. If any question remains, it is best to repeat special stains liberally on deeper levels or in different tissue blocks.

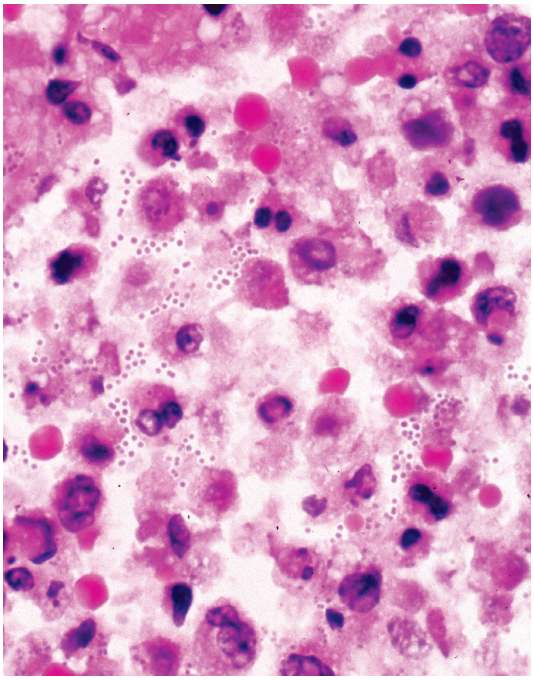


Figure 7.17 Streptococci in necrotizing pneumonia.

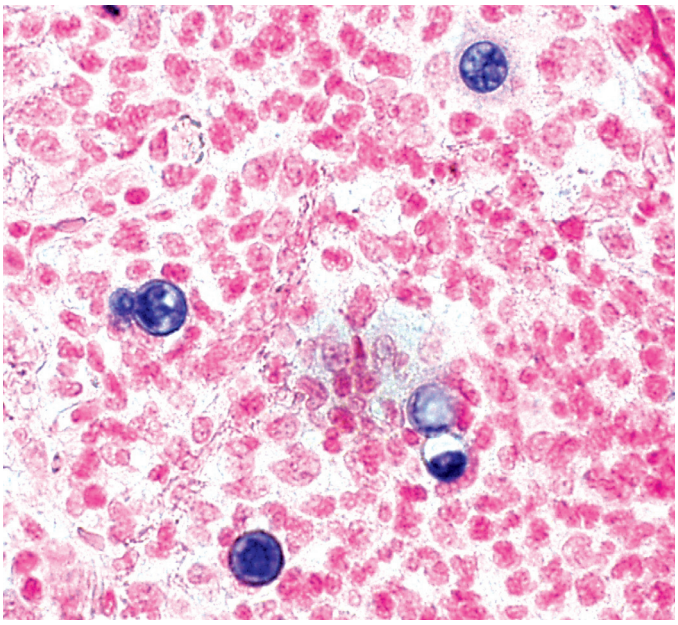


Figure 7.18 *Blastomyces dermatitidis*. In situ hybridization. (Courtesy Ricardo Lloyd, MD, Rochester, Minnesota.)

Role of Cytopathologic Examination in Diagnosis of Lung Infection

A wide variety of infectious diseases of the lung—including bacterial, mycobacterial, fungal, viral, and parasitic—can be diagnosed through exfoliative or fine-needle aspiration cytologic techniques.[43-46] Fine-needle aspiration is an especially powerful tool compared with the exfoliative cytology study of respiratory secretions: sputum, bronchial washings/brushings, and bronchoalveolar lavage (BAL) fluid. The usefulness of exfoliative cytology examination is often limited owing to the difficulty of distinguishing colonizing/contaminant organisms in the airways from true pathogens. Nonetheless both diagnostic techniques are complementary and have been used in recent years to evaluate pneumonias and pulmonary nodules in both immunocompetent and immunocompromised patients.

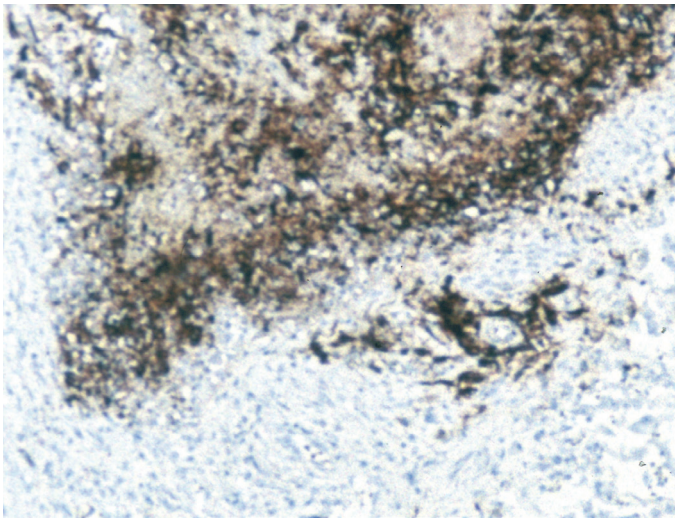


Figure 7.19 Herpes simplex virus necrotizing pneumonitis (immunohistochemical stain).

Box 7.3 Limitations of Diagnostic Tools

Morphology

Histopathologic examination: Inflammatory changes nonspecific, atypical, or absent; organisms not visualized or nonspecific morphology (e.g., "*Aspergillus*-like"); unexpected or unfamiliar site

Special stains, immunohistochemical/molecular techniques: Sensitivity and specificity issues; misinterpretation (e.g., aberrant forms, artifacts, nonmicrobial mimics); limited reagents, false-negative and false-positive results

Cytopathologic analysis: Limitations similar to those with histopathologic examination

Microbiology

Direct visualization: Sensitivity and specificity

Culture/identification: Normal flora versus pathogens; colonization or asymptomatic shedding versus invasion; difficult, dangerous, or slow to grow; treated; fixed, contaminated tissue; too small or nonrepresentative sample

Serologic studies: Single sample; no early response or lack of response; nondiagnostic for highly prevalent/persistent microbe; cross reaction; acute versus chronic; false-positive result on IgM tests

Mass-like infiltrates are often the target of aspiration biopsy needles when suspicion or exclusion of an infectious process ranks high in the differential diagnosis. Besides the morphologic features of the microorganism, important cytologic clues to the diagnosis include the accompanying cellular response and the presence and character of any necrotic debris, as outlined in Table 7.4. Although nonspecific, such features can suggest certain possibilities to the cytopathologist and assist the microbiology laboratory in triaging the specimen.[47] To this end, the presence of a cytopathologist, microscope, and staining setup during the aspiration process can be useful. The cytopathologist can correlate the clinical setting, radiologic features, and clues from the gross character of the aspirate (color, consistency, odor, and so on), thereby assisting in narrowing the diagnostic possibilities and avoiding false-positive and false-negative diagnoses.[48] Also, immediate evaluation of smears by rapid stain procedures allows the cytopathologist to either make or suggest a specific diagnosis, as with the preparation and evaluation of a frozen section during intraoperative consultation. Smears

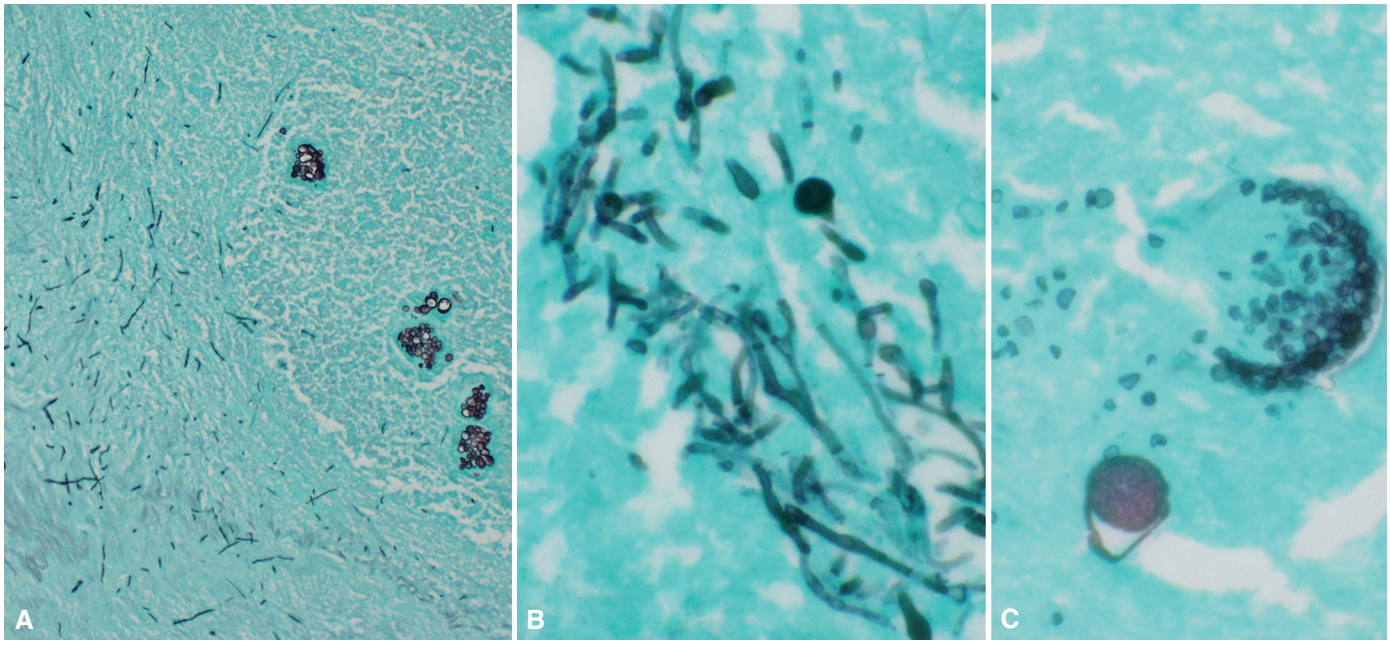


Figure 7.20 *Coccidioides immitis* demonstrating biphasic features versus those of other organisms. Culture grew *C. immitis* and *Fusarium* species. (A) Spherules and mycelia; (B) mycelia; (C) ruptured spherules with endospores (Grocott methenamine silver stain).

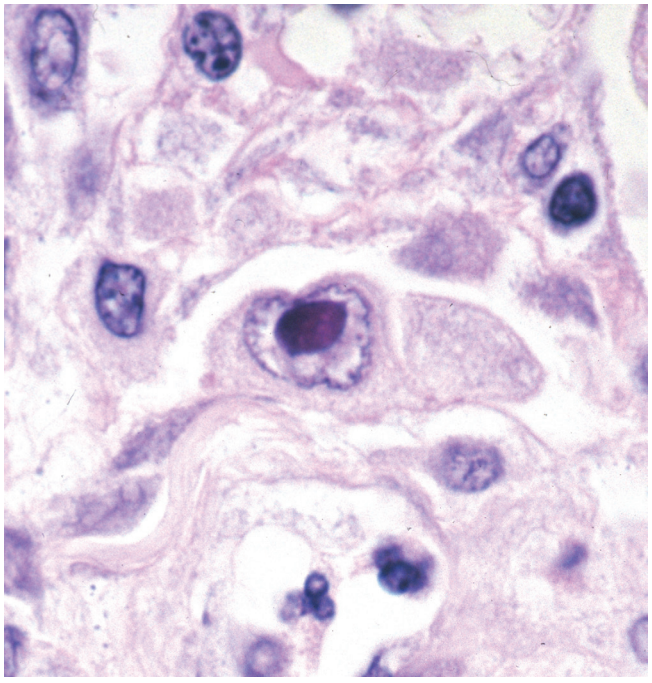


Figure 7.21 Macronucleolus mimicking a viral inclusion in an alveolar lining cell.

Table 7.4 Fine-Needle Aspiration Patterns of Pulmonary Infectious Diseases

Pattern	Possible Etiologic Agent(s)
Acute purulent inflammation/ abscess	Bacteria, fungi
Granuloma pattern (epithelioid cells with or without necrosis): Caseous/necrotizing; suppurative epithelial mixed	Mycobacteria, bacteria, parasites, fungi
Foamy alveolar cast pattern	*Pneumocystis jirovecii*
Histiocytic	Mycobacteria, bacteria, fungi
Chronic inflammation (lymphocyte and plasma cell)	Virus, other, agent not otherwise specified
Null ("id") reaction	Virus, any, other

can be prepared for special stains, needle rinses can be performed for culture and other ancillary studies, and additional aspirations may be encouraged for these purposes.[49] Special stains for bacteria, mycobacteria, and fungi should be used whenever the character of the aspirate and the clinical setting (e.g., compromised immune status) indicate that such studies may be useful.

Some interventionists prefer to provide only a needle core biopsy in lieu of an aspirate for a variety of reasons. These two techniques can be viewed as complementary; whereas needle core biopsies work well for neoplasms and many granulomas, the aspirate is often superior for diagnosing many types of infections, especially bacterial abscesses. Sometimes a rapid and specific etiologic diagnosis is possible at the bedside, based on the microscopic features of the organism itself. However, when the organism is not readily apparent or its features are inconclusive, the microbiology laboratory can be invaluable for its role in isolation and identification.[49] BAL, typically performed in the evaluation of infection in an immunocompromised host, provides a standard panel of microbiology results, which should always be correlated with the cytology findings.[50,51]

Summary

The successful treatment of pulmonary infections depends on accurate identification of the pathogen involved. In turn, this requires collecting the best specimens, transporting them to the anatomic and microbiology sections of the laboratory under optimal conditions, and processing them with techniques appropriate for the spectrum of possible etiologic disorders. An interdisciplinary approach enhances this process. It is best that pathologists, clinicians, and microbiologists communicate frequently and recognize the strengths and weaknesses of their respective disciplines. Joint strategies can be developed for the approach to certain

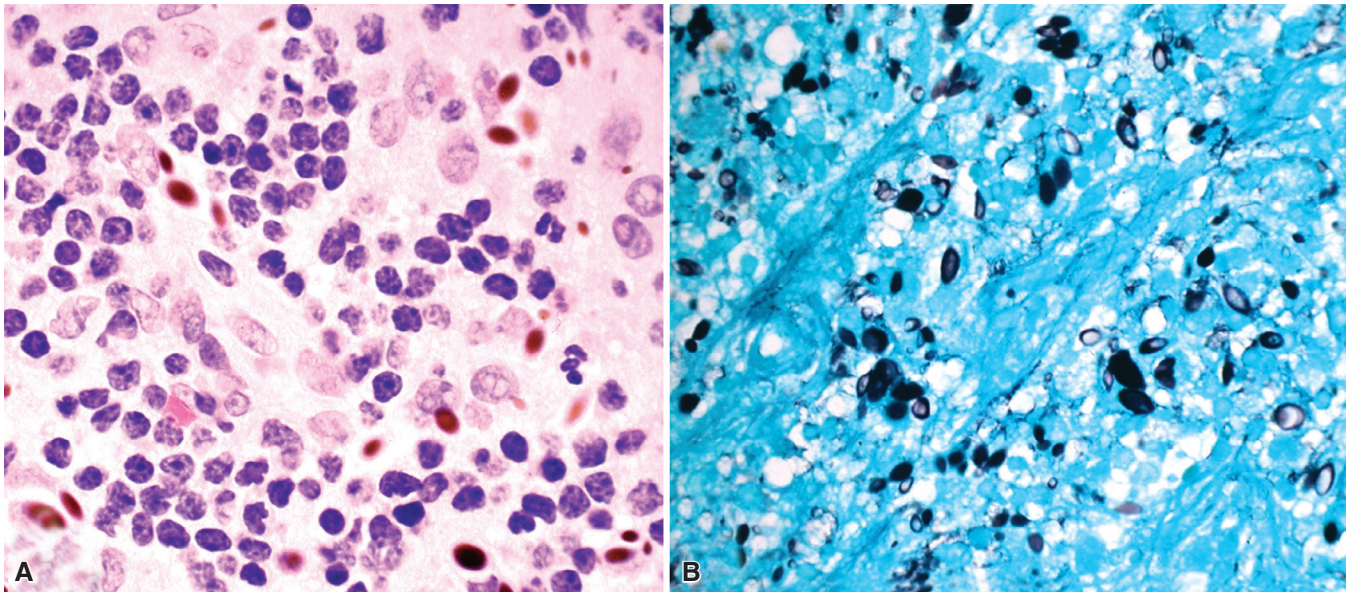


Figure 7.22 Yellow-brown Hamazaki-Wesenberg bodies (A) Hematoxylin and eosin; (B) Grocott methenamine silver stain.

Box 7.4 Work-up of Pulmonary Infections

Pre/Intraoperative Consultation

Inquiry regarding
History
Risk factors; immune status
Radiographic pattern
Advise regarding
How and what to collect
What cultures and tests to order
Devices, media, and containers for obtaining and transporting specimens
Fixatives for morphologic study

Written Protocol

Handling tissue for cultures
Special stains and ancillary tests
Logistics
Requisition—designed to communicate

Morphologic Examination

Inflammatory pattern
Persistence and repeat studies
Oil immersion studies if necessary
Strict criteria for positive
Consider multiple pathogens

Report

Presumptive versus definitive diagnosis; correlate with results of culture, other studies
Comment
Clinicopathologic-microbiologic correlation
Differential diagnosis
Ancillary tests
Suggestions for further work-up

types of suspected infections, helping to foster the development of laboratory foresight in surgical colleagues and medical consultants. It is good practice to look up the microbiologic and culture results in interpreting the biopsy. Communication and consideration of the histologic and microbiologic methods of diagnosis should be symbiotic. An example of such collaboration is presented in Box 7.4.

Bacterial Pneumonias

The surgical pathologist rarely receives biopsy specimens from patients with community-acquired or nosocomial pneumonias. Most of these infections are suspected clinically by symptoms and physical and radiologic findings; some are confirmed immediately by Gram stains (or later by culture) performed on respiratory secretions in the microbiology laboratory. Serologic studies sometimes prove to be diagnostic. Even when conventional microbiologic approaches are applied, however, approximately 50% of bacterial pneumonias remain undiagnosed.[52-54] Patients with mild disease are often not tested and treated empirically with antibiotic regimens following established guidelines. By contrast, patients with severe disease, whether immunocompromised or not, often become candidates for invasive procedures.

Etiologic Agents

Bacterial pneumonia may be classified according to various parameters including pathogenesis, epidemiology, anatomic pattern, clinical course, and organism type (Box 7.5).[55] Using bacterial type as a starting point allows the pathologist to correlate anatomic and histopathologic patterns of lung injury with categories of etiologic agents.

The pyogenic bacteria most commonly associated with community-acquired pneumonias include *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis*.[54] Other pathogens such as *Legionella* species, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* (often referred to as the *atypical group*) are clinically important, but controversy exists with regard to the relative frequency of these organisms as etiologic agents. Although community-acquired pneumonia is considered to be fundamentally different in children and in adults, severe or complicated pneumonias in both of these age groups are of similar etiology.[56] The enteric gram-negative bacilli cause relatively few community-acquired pneumonias, whereas they account for most of the nosocomial pneumonias, along with *Pseudomonas* species, *Acinetobacter* species, *S. aureus*, and anaerobes.[57,58] Most nosocomial pneumonias result from aspiration of these bacterial species that colonize the oropharynx of hospitalized patients, and such pneumonias can be polymicrobial. Any of the bacterial organisms listed (including mixtures with fungi and viruses) can cause pneumonia in immunocompromised patients.[14,59] Ventilator-associated pneumonia is a special subset of nosocomial pneumonia and an important

Box 7.5 Classification of Bacterial Pneumonia

Pathogenesis
Primary
Exogenous
Endogenous
Secondary

Epidemiology
Community-acquired
Nosocomial

Anatomic Type
Lobular
Lobar

Clinical Course
Acute
Chronic

Bacterial Type
Pyogenic species
Atypical agents
Granule/filamentous group

Box 7.6 Histopathologic Patterns in Bacterial Lung Injury

Bronchitis/bronchiolitis
Acute exudative pneumonia
  Lobular (bronchopneumonia)
  Confluent (lobar pneumonia)
With granules
Fibroinflammatory and/or organizing pneumonia
Interstitial pneumonia
Nodular/necrotizing lesions
  Miliary lesions
  Abscess

cause of morbidity and mortality in the intensive care unit.[60-62] The bacterial etiology in this setting is quite diverse and dependent on such factors as patient characteristics, underlying lung disease, and geographical location.[63] Most recently, an increase in skin and soft tissue staphylococcal infections due to methicillin-resistant strains has led to the recognition of these organisms as an important cause of both community-acquired and nosocomial pneumonia with attendant morbidity and mortality.[64] In rare nosocomial pneumonias, a number of unusual organisms, such as *Salmonella*, *Rhodococcus*, and *Leptospira* species, may be the etiologic agent.[65,66]

The atypical pneumonia agents do not commonly produce lobar consolidation. Although this potentially implicates a wide variety of bacterial, viral, and protozoal pathogens, a selective list by convention includes *M. pneumoniae*, *Legionella* species, and *C. pneumoniae* as the three dominant nonzoonotic pathogens, and *Coxiella burnetii* (the agent of Q fever), *Chlamydia psittaci* (causing psittacosis in people), and *Francisella tularensis* (causing tularemia) as the three more common zoonotic pathogens.[67,68]

The filamentous/granule group refers to those bacteria that form long, thin, branching filaments in tissues, such as *Actinomyces* (anaerobic actinomycetes) or *Nocardia* (aerobic actinomycetes).[69] Botryomycosis is caused by nonfilamentous bacteria, especially *S. aureus*, or gram-negative bacilli, such as *P. aeruginosa* and *Escherichia coli*, which form organized aggregates referred to as *grains* or *granules*.[70]

Histopathology

Bacterial lung injury patterns will vary in accordance with the virulence of the organism and the host response. These patterns are further modulated by therapeutic or immunologic factors. Although some of the patterns presented in Box 7.6 are characteristic, none are diagnostic. Overlap and mixed patterns occur.

Acute Exudative Pneumonia

Acute exudative pneumonia is most often caused by pyogenic bacteria, such as streptococci, which typically produce a neutrophil-rich intra-alveolar exudate (i.e., alveolar filling) with variable amounts of fibrin and red cells. Pathologists recognize this constellation of findings as

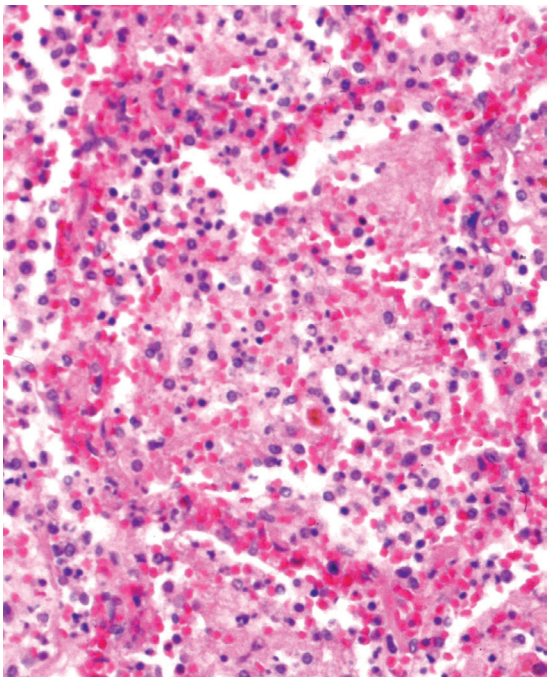


Figure 7.23 Alveoli filled with fibrinopurulent exudate with variable hemorrhage.

acute lobular pneumonia (Fig. 7.23), which usually correlates with patchy segmental infiltrates on the chest film (consolidation pattern on HRCT).[29,71-73]

With increasing organism virulence and disease severity, lobular exudates may become confluent (i.e., lobar pneumonia). In milder cases, the disease may be limited to the airways (bronchitis/bronchiolitis) with a mixed cellular infiltrate of mononuclear cells and neutrophils (Fig. 7.24). One very common manifestation of such airway-limited infection has been designated as *acute exacerbation of chronic obstructive pulmonary disease* (COPD). A majority of these exacerbations are caused by particular bacteria (specifically *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis*) with approximately one third resulting from viral airway infections, typically resulting from rhinovirus, respiratory syncytial virus (RSV), and human metapneumovirus.[74]

Nodular/Necrotizing Lesions

Nodular inflammatory infiltrates with or without necrotizing features (Fig. 7.25) are characteristic of infection by certain species, such as *R. equi* (Fig. 7.26).[75] Necrotizing pneumonias may also be produced by pyogenic bacteria such as *S. aureus*, *Streptococcus pyogenes*, and the gram-negative bacilli—*Klebsiella*, *Acinetobacter*, *Pseudomonas*, and *Burkholderia* species.

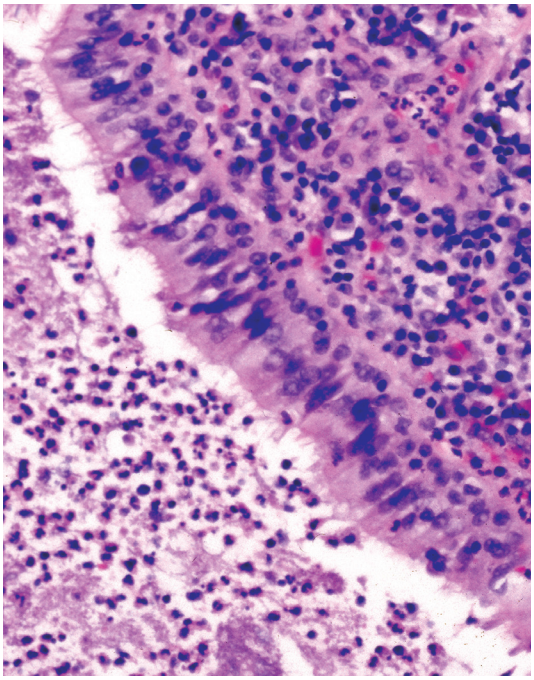


Figure 7.24 Bronchiolitis with intraluminal exudate.

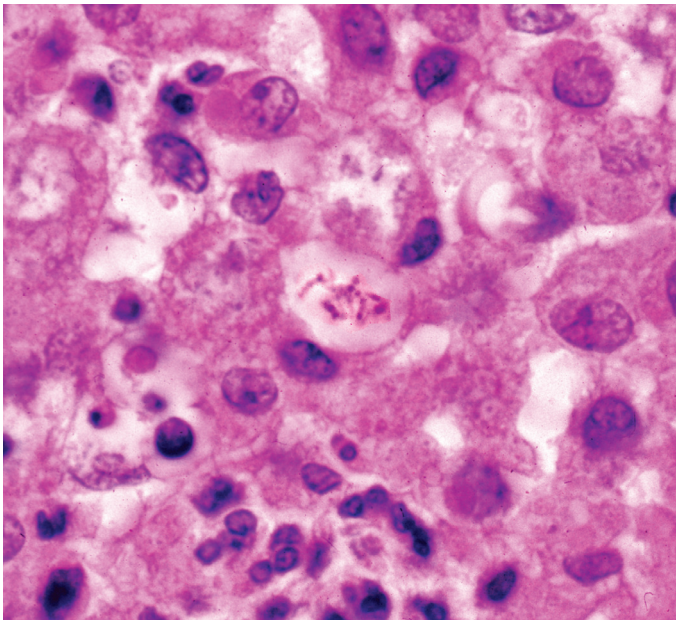


Figure 7.26 *Rhodococcus equi* bacilli in macrophage.

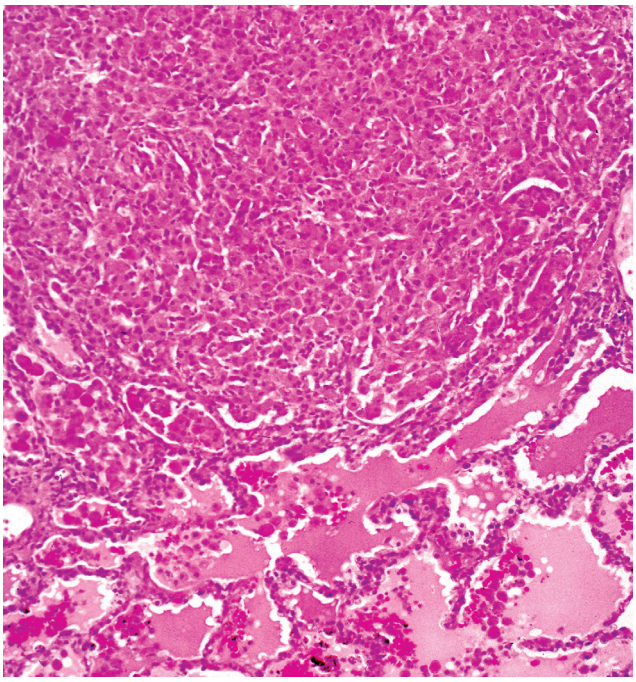


Figure 7.25 Nodular histiocytic infiltrate in rhodococcal pneumonia.

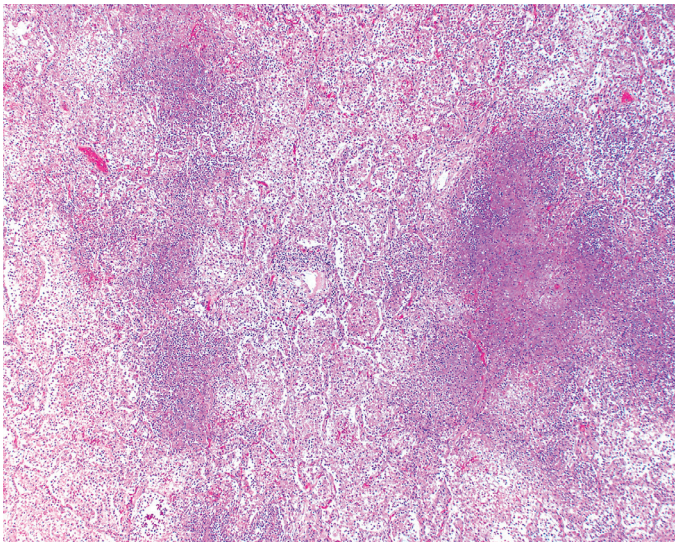


Figure 7.27 Necrotizing pneumonia, miliary pattern.

Miliary Lesions

A subset of the nodular histopathologic pattern, miliary infection (Fig. 7.27), strongly implies pneumonia secondary to the hematogenous spread of bacteria (septicemia). This pattern of infection can be seen with other organisms, such as *Nocardia* and the anaerobic Actinomycetes. In these settings, histopathologic examination may show a combination of both nodular disease and alveolar filling.

Aspiration Pneumonia and Lung Abscess

There are multiple scenarios for aspiration pneumonia, including cases caused by chemical pneumonitis (so-called Mendelson syndrome), airway obstruction, exogenous lipoid pneumonia, chronic interstitial fibrosis, diffuse bronchiolar disease, bacterial pneumonia, and lung abscess.[76,77] *Aspiration pneumonia* refers specifically to the aspiration of bacteria in oropharyngeal secretions, with the bacterial species depending on whether the aspiration event occurs in the community or hospital setting. Recognition of food particles (so-called pulses) is important in diagnosis. These may or may not be invested by giant cells but are usually found in purulent exudate or granulomatous foci. In the organizing phase of the pneumonia, food particles may be found within polyps of organizing pneumonia in the alveolar ducts and alveoli. Lobular pneumonia, lipoid pneumonia, organizing pneumonia, and bronchiolitis, alone or in combination, may also be seen.[73,78] The pathogens in lung abscess (Fig. 7.28) usually encompass a polymicrobial mixture of aerobic and anaerobic bacteria,[79] and formation of such abscesses most often is secondary to aspiration (Fig. 7.29). Infections due to *Actinomyces* species (Fig. 7.30) and *Nocardia* species may also

manifest this pattern, as can those infections caused by certain pyogenic bacteria, such as *S. aureus* and the other organisms listed previously for necrotizing pneumonias. Granulomatous inflammation with foreign bodies may be present if aspiration is the cause (Fig. 7.31).

Chronic Bacterial Pneumonias

Chronic bacterial infections (Fig. 7.32) that are slow to resolve as a result of inappropriate initial therapy, involvement with certain microbial species, a noninfectious comorbid process, or an inadequate host response can produce a nonspecific fibroinflammatory pattern, with lymphoplasmacytic infiltrates, macrophages, or organization with polyps of immature fibroblasts in alveolar ducts and alveolar spaces.[80-83] If not resorbed, polyps of airspace organization may become polyps of intra-alveolar fibrosis, which sometimes ossify (dendriform ossification). Such scarring in chronic pneumonia is often associated with localized interlobular septal and pleural thickening (Fig. 7.33), producing a jigsaw puzzle pattern of scarring best seen at scanning magnification.

Diffuse alveolar damage is the histopathologic correlate of the acute respiratory distress syndrome (ARDS), and today lung infection is the leading cause of diffuse alveolar damage and ARDS in the United States.[84] Diffuse alveolar damage may coexist with any of the necroinflammatory patterns described earlier. The initial exudative phase of this ARDS is accompanied by hyaline membranes (Fig. 7.34), the later organizing phase by airspace and interstitial fibroplasia. In clinical practice, diffuse alveolar damage accompanied by tissue necrosis is nearly always a manifestation of lung infection.

The atypical pneumonias include the well-described cases due to *Legionella* species and the less well-described cases caused by other

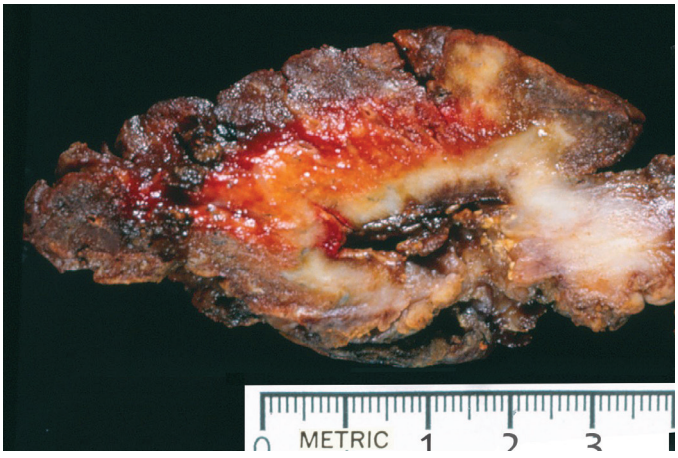


Figure 7.28 Lung abscess showing gross evidence of chronicity with fibrosis in surrounding parenchyma.

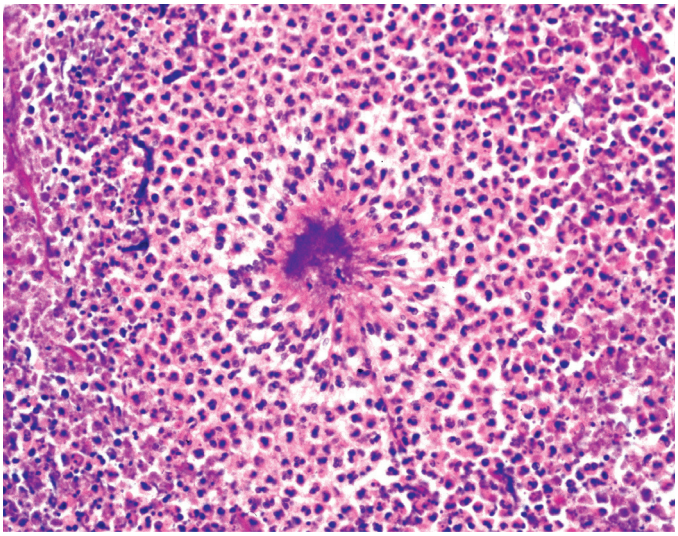


Figure 7.30 Lung abscess with sulfur granule of actinomycosis in purulent exudate.

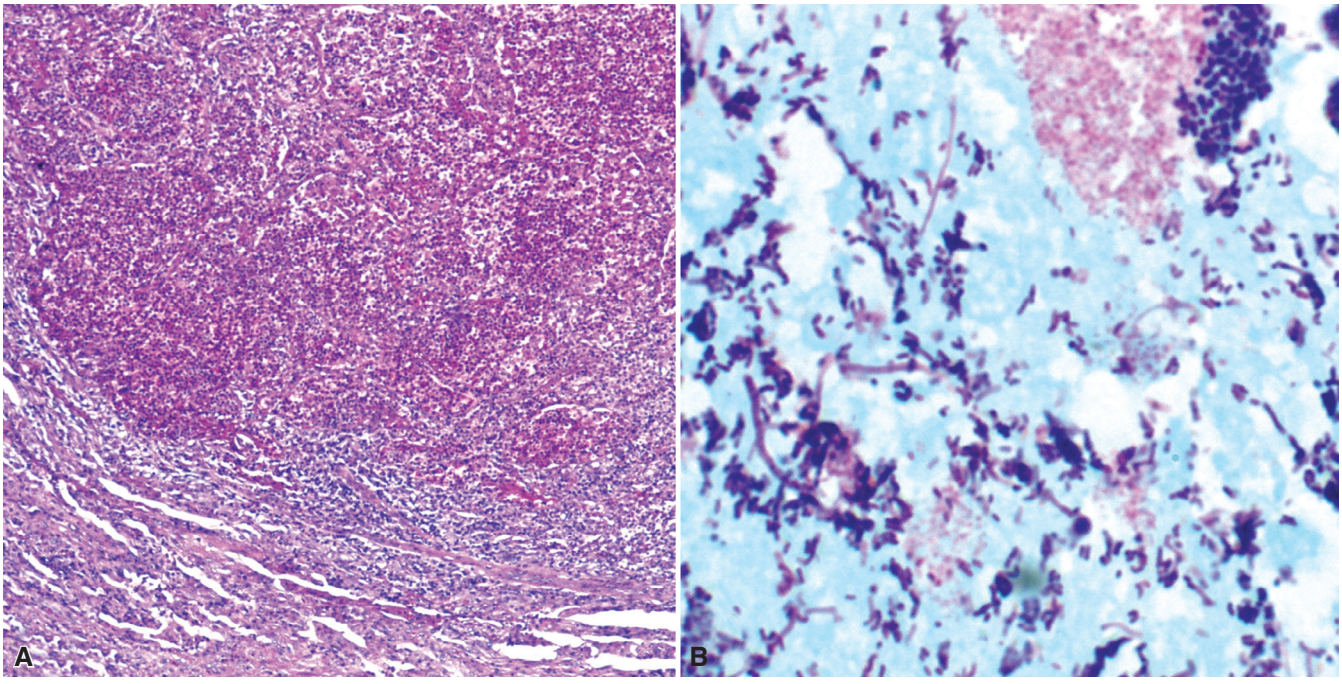


Figure 7.29 (A) and (B) Lung abscess with polymicrobial bacterial population (Gram stain).

organisms comprising the atypical group. *Legionella* infection typically results in an intensely neutrophilic acute fibrinopurulent lobular pneumonia (Fig. 7.35A).[3,5,71] *Legionella* bacilli can be identified in silver impregnation-stained sections (Fig. 7.35B) or recovered in culture, but newer diagnostic methods, such as real-time PCR and in situ hybridization (Fig. 7.36), can also be applied when standard approaches fail.[85] The histopathologic patterns associated with the other members of the atypical group (i.e., *Chlamydia*, *Mycoplasma*) are not well characterized, mainly because investigation of these pneumonias rarely includes biopsy. The few well-documented cases of *Mycoplasma*, *Chlamydia*, and *Coxiella* infections resemble viral bronchitis or bronchiolitis, with mixed inflammatory infiltrates in airway walls and in the adjacent interstitium (Fig. 7.37).[86,87] Relative sparing of the peribronchiolar alveolar spaces has been described, although patchy organized fibrinous exudates are seen in some cases and complications may superimpose additional findings.

The grains and granules formed by the *Actinomycetes* and bacteria of botryomycosis may have a uniform tinctorial hue on routine H&E–stained sections, but sometimes these bacterial aggregations display a distinctive body with a hematoxylinophilic core and an outer investment of eosinophilic material; formation of this array is referred to as the *Splendore-Hoeppli phenomenon* (Fig. 7.38). *Actinomycetes* species tend to form similar-appearing granules, and both they and the bacteria of botryomycosis are typically found in the midst of purulent exudates.[69,88-90] *Nocardia* species may aggregate in colonies simulating granules, but

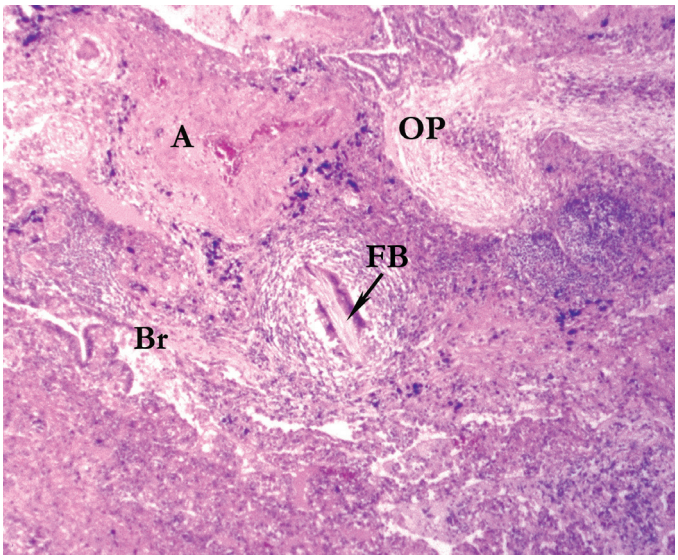


Figure 7.31 Aspiration pneumonia. Giant cells surround vegetable matter *(FB)* in purulent exudates, organizing pneumonia *(OP)*, bronchiolitis *(BR)*, artery *(A)*.

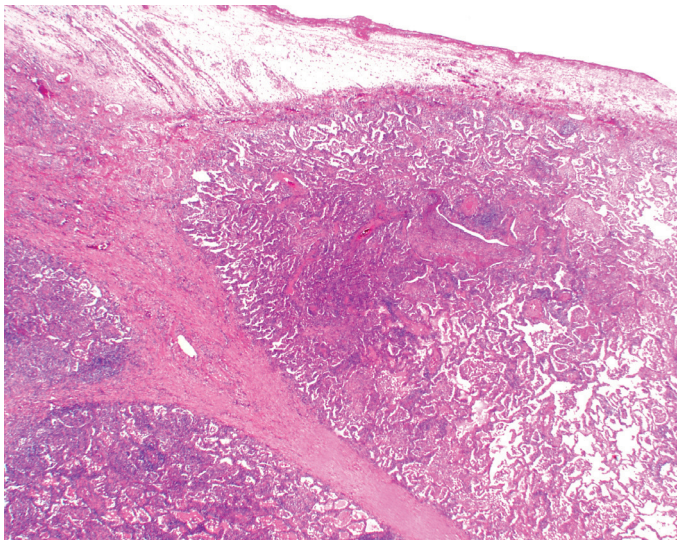


Figure 7.33 Chronic pneumonia with thickened interlobular septum.

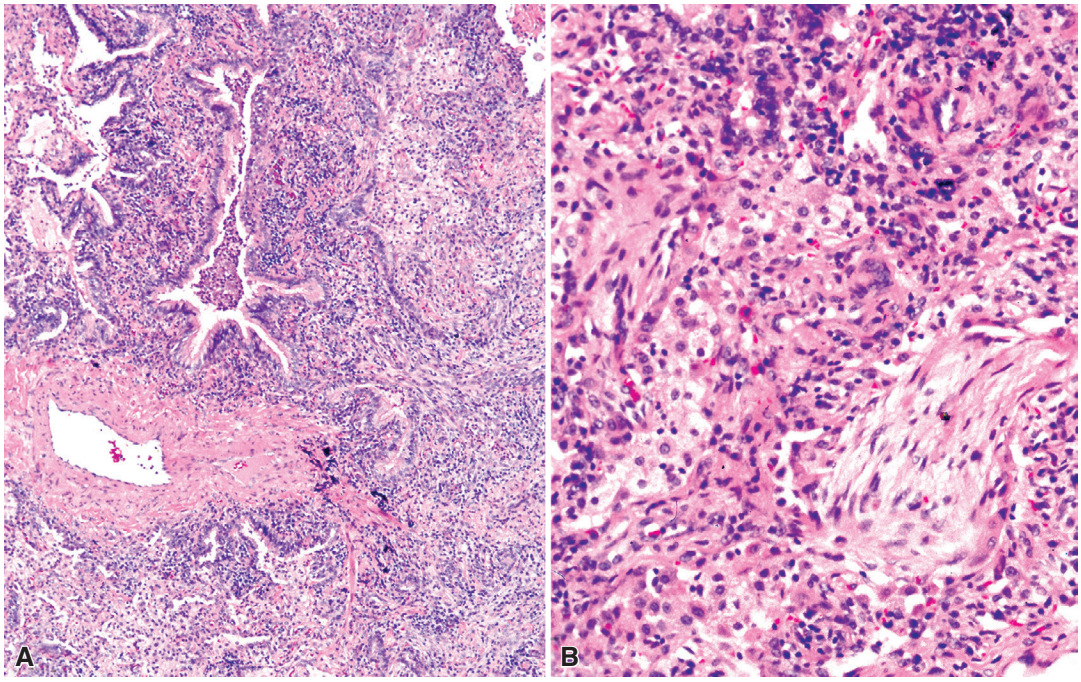


Figure 7.32 Chronic pneumonia. (A) Lymphoplasmacytic infiltrate. (B) Fascicles of fibroblasts in alveolar ducts and spaces.

with a much looser texture (Fig. 7.39) and more monochromatic tinctorial properties.[91] Rarely, these colonies may be identical in appearance to the grains or granules of botryomycosis or actinomycosis in H&E sections.

Bacterial Agents of Bioterrorism

The potential for use of microbial pathogens as agents of bioterrorism requires that clinicians be alert to this possibility when community-acquired pneumonias are found to be caused by these agents. In turn, pathologists must become familiar with the histopathologic features these agents can produce.[92] Respiratory disease caused by the inhalation of *Bacillus anthracis*, *Yersinia pestis*, and *F. tularensis* is especially pertinent in this context and is discussed next.[93]

Bacillus anthracis

In 1877, Robert Koch's conclusive demonstration that *B. anthracis* was the etiologic agent of anthrax revolutionized medicine by linking microbial cause and effect.[6] Inhalational anthrax causes a severe hemorrhagic mediastinitis.[94-98] This pathologic process in combination with the toxemia (*B. anthracis* produces an exotoxin with three potent components—protective antigen, lethal factor, and edema factor) from

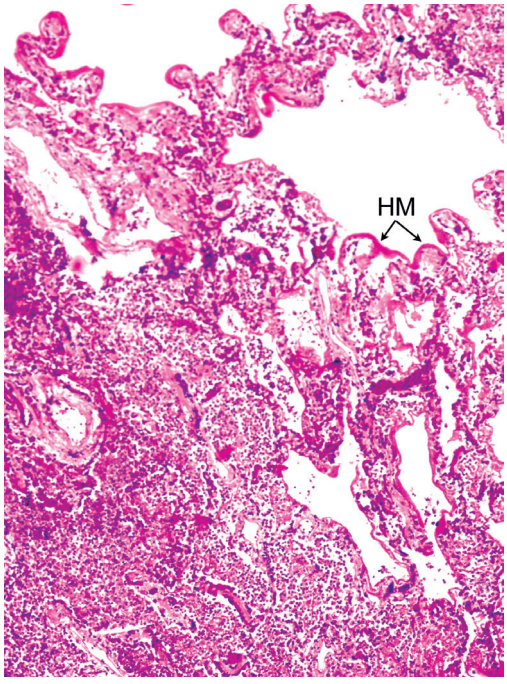


Figure 7.34 Bacterial pneumonia with hyaline membranes *(HM)* at periphery.

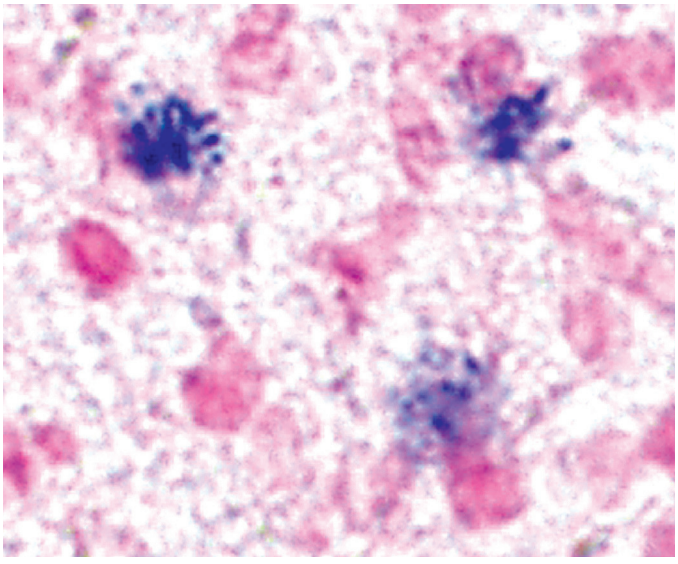


Figure 7.36 Legionnaire's disease. Detection of organisms by in situ DNA hybridization. (Courtesy R.V. Lloyd, MD, Rochester, Minnesota.)

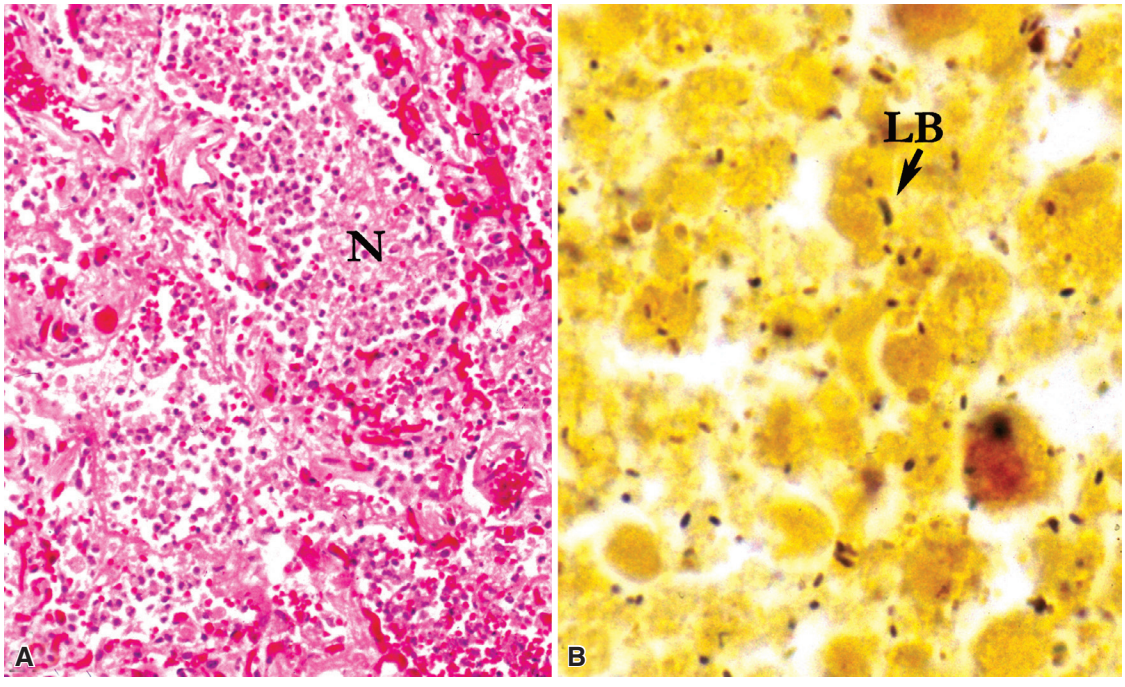


Figure 7.35 (A) Legionnaire's disease with intraalveolar necroinflammatory exudates *(N)* and hemorrhage. (B) Enhanced silhouette of *Legionella* bacilli *(LB)* in alveolar exudate with silver impregnation (Dieterle stain).

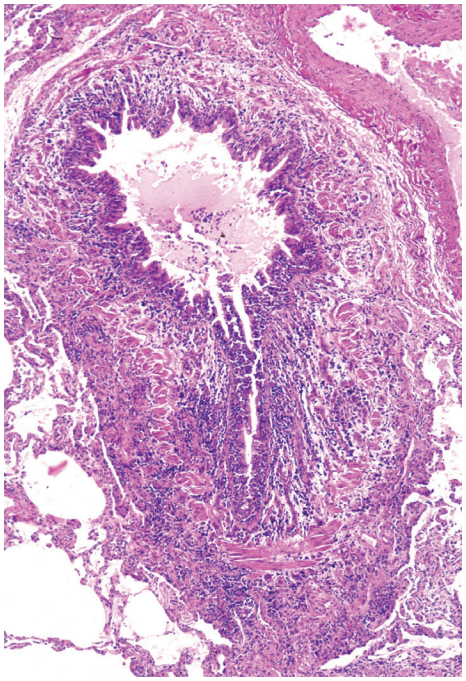


Figure 7.37 *Mycoplasma* pneumonia. Bronchiolitis with patchy infiltrates in peribronchial interstitium.

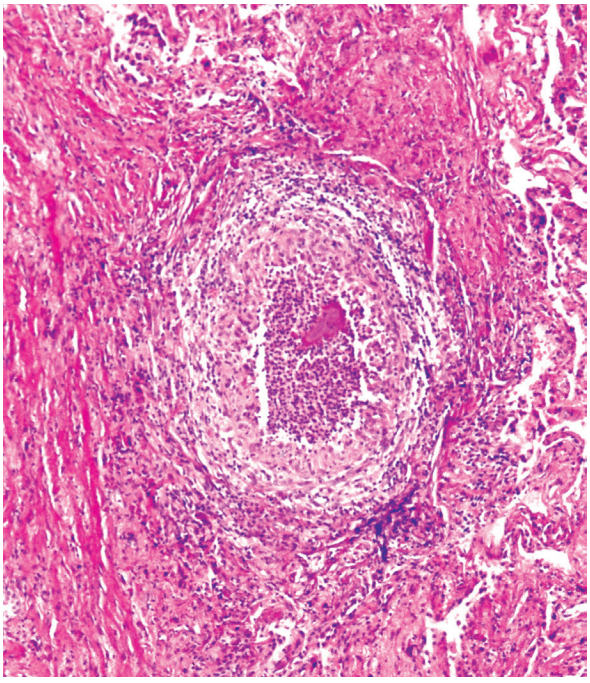


Figure 7.38 Botryomycosis granule with hematoxylinophilic core and eosinophilic investment known as the *Splendore-Hoeppli effect*.

the ensuing massive bacteremia severely compromises pulmonary function, leading to death in 40% or more of the cases. Pleural effusion may be present, but pneumonia generally is minor and secondary. In those patients in whom pulmonary parenchymal changes are found, the alveolar spaces contain a serosanguineous fluid with minimal fibrin deposits and some mononuclear cells but few if any neutrophils.[97] Large gram-positive bacilli (some may appear partially gram-negative) without spores pervade the alveolar septal vessels, with a few in the alveolar

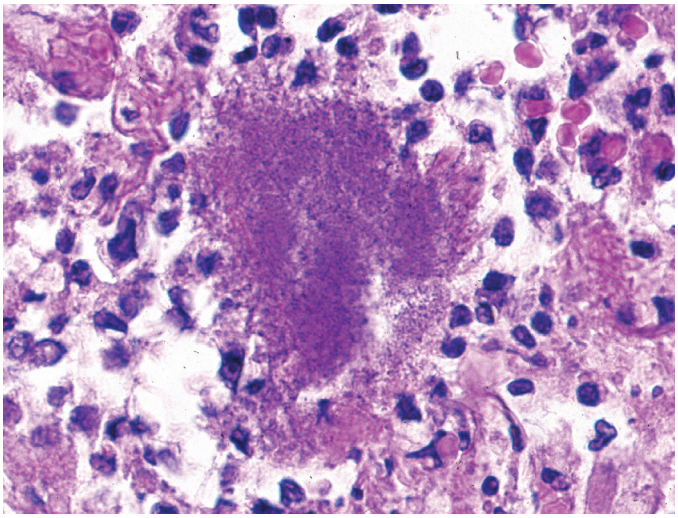


Figure 7.39 Loose-textured aggregate of *Nocardia* filamentous bacteria surrounded by neutrophils.

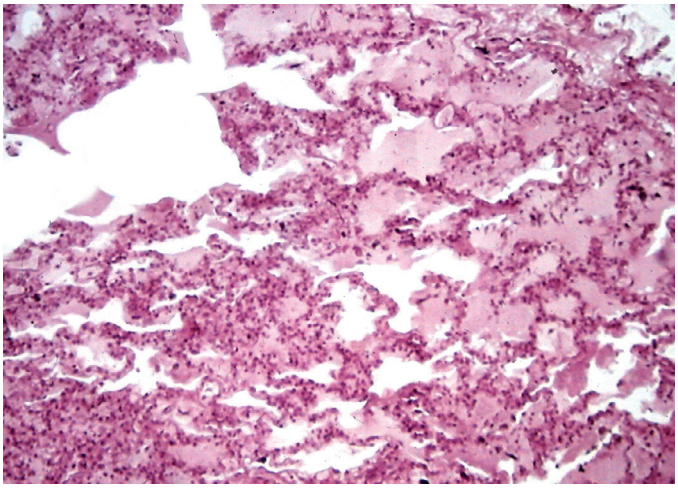


Figure 7.40 Plague pneumonia, early phase. Edema, fibrin, and sparse inflammatory cells are evident.

spaces. This distribution suggests hematogenous rather than airway acquisition. Hemorrhagic mediastinitis in a previously healthy adult is essentially pathognomonic for inhalational anthrax. The lymph node parenchyma generally is teeming with intact and fragmented gram-positive bacilli, which can be identified as *B. anthracis* by immunohistochemical studies.[96,97] Cultures of blood and pleural fluid, if available, are likely to yield the earliest positive diagnostic results.[98] Sputum studies are much less useful in this regard. Specific guidelines for pathology and microbiology specimens for anthrax diagnosis (as well as other potential agents of bioterrorism) are current and available on the Centers for Disease Control and Prevention (CDC) website.[99]

Yersinia pestis

Primary pneumonic plague follows inhalation of *Y. pestis* bacilli in a potential bioterrorism scenario.[100,101] The infection begins as bronchiolitis and alveolitis that progress to a lobular and eventual lobar consolidation.[102] The histopathologic features evolve over time, beginning with a serosanguineous intraalveolar fluid accumulation with variable fibrin deposits (Fig. 7.40), progressing through a fibrinopurulent phase, and culminating in a necrotizing lesion.[103] The presence of myriad bacilli

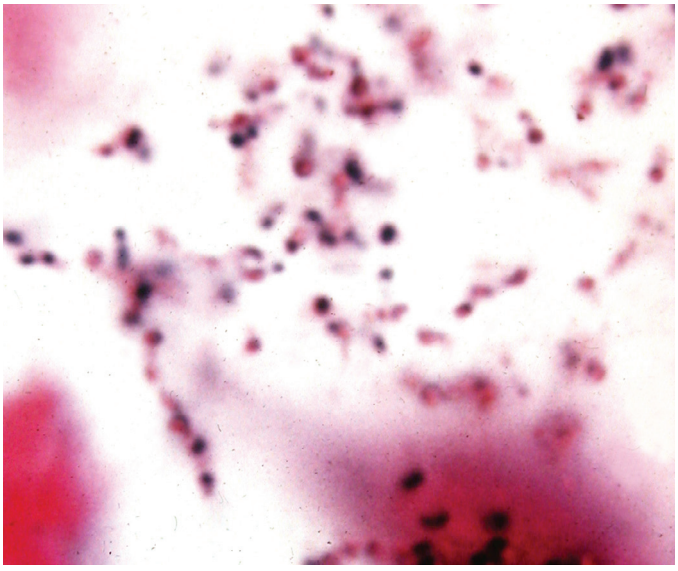


Figure 7.41 *Yersinia pestis* bacilli in alveolar space.

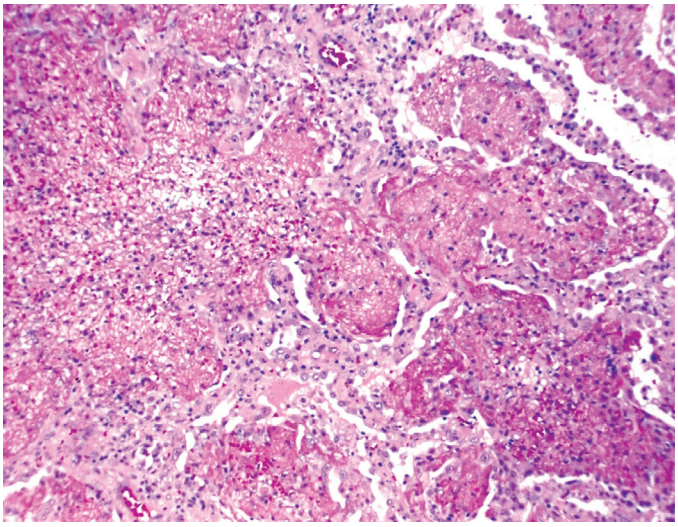


Figure 7.42 Tularemia. Fibrinous lobular pneumonia phase.

in the intraalveolar exudates with significantly fewer organisms in the interstitium (a characteristic of primary pneumonia) is one of several pulmonary and extrapulmonary features used to distinguish primary from secondary pneumonic plague.[104] These bacilli may be obvious in H&E-stained sections (Fig. 7.41) but generally are better visualized with Giemsa rather than Gram stain. Immunohistochemical staining provides a rapid and specific diagnosis.[102] In contrast to inhalational anthrax, sputum Gram stain and culture are useful tests that are likely to yield a positive result at clinical presentation. Also, because sepsis is an integral component of the pneumonia, it is important to collect blood culture specimens.[105]

Francisella tularensis

Inhalation of *F. tularensis* bacilli following a bioterrorism aerosol release is generally expected to result in a slowly progressing pneumonia with a lower case-fatality rate than with either inhalational anthrax or plague.[104,106] Initially a hemorrhagic and ulcerative bronchiolitis is followed by a fibrinous lobular pneumonia with many macrophages but relatively few neutrophils (Fig. 7.42). Necrosis then supervenes and evolves into a granulomatous reaction. The small, gram-negative coccobacillary organisms are difficult to identify in a tissue Gram stain, and the use of silvering techniques (e.g., Steiner, Dieterle, Warthin-Starry) is required to enhance their silhouette.[107] Specific fluorescent antibody testing for formalin-fixed tissue and immunohistochemical studies are available through public health laboratories. In the microbiology laboratory, Gram stain and culture of respiratory secretions are useful for diagnosis, but blood cultures are often negative. Antigen detection and molecular techniques, such as PCR amplification, can also identify *F. tularensis*. Serologic tests are available but probably would not provide timely information in an outbreak situation.[104]

Cytopathology

The stereotypic cellular response to pyogenic bacteria is acute inflammation, characterized by variable numbers of neutrophils. Bacteria may be visualized in various stained preparations made from respiratory tract secretions and washings using the Papanicolaou and Diff-Quik methods.[45] The clinical significance of bacteria in such specimens may be limited owing to potential contamination by oral flora and the problem of distinguishing colonization from infection. However, when the upper respiratory tract can be bypassed by means of either transtracheal or transthoracic needle aspiration, the presence of bacteria becomes much more significant, especially when sheets of neutrophils or necroinflammatory debris are present (Fig. 7.43A), as would be the case with a typical lobar or lobular consolidation, lung abscess, or other complex pneumonia.[53,90,108,109] In this context, transthoracic needle aspiration can establish the etiologic diagnosis of community-acquired and nosocomial pneumonias in both children and adults when coupled with modern microbiologic methods.[49,58,110,111] Proponents consider it an underused technique the potential benefits of which, in experienced hands, outweigh the modest associated risks.

Many types of bacilli and cocci can be seen within and around neutrophils on Diff-Quik–stained smears (Fig. 7.43B). A smear can also be prepared for Gram stain and the aspirate needle rinsed in nonbacteriostatic sterile saline or nutrient broths for culture. The size (length and width) and shape of organisms and the Gram reaction allow rough categorization of organisms into groups, such as enteric-type bacilli, pseudomonads, fusiform anaerobic-type bacilli, tiny coccobacillary types suggestive of the *Haemophilus–Bacteroides* group (Fig. 7.44), or gram-positive cocci.[112] Branching filamentous forms suggest *Actinomycetes* or *Nocardia* species (Fig. 7.45), with the latter distinguished by being partially acid-fast.[113,114]

Although most aspirated cavitary lung lesions with the abscess pattern are the result of bacterial infection, considerations in the differential diagnosis include necrotic neoplasm (particularly squamous cell carcinoma), granulomatosis with polyangiitis, and nonbacterial infections associated with suppurative granulomas such as those due to fungi and mycobacteria.

Microbiology

Microbiology techniques in current use for the laboratory diagnosis of bacterial pneumonia are summarized in Box 7.7.[39,115-117] The traditional morphologic and functional approach to microbiologic diagnosis is gradually shifting to molecular methods, and diagnostic arrays of common respiratory pathogens are marketed by several vendors; they are adjusted for laboratory size, for individual random access testing, or for test batching in larger laboratories.

The work-up of respiratory secretions such as sputum in the microbiology laboratory may or may not be indicated based on the clinical and immunologic status of the patient. The value of microbiologic work-up for community-acquired pneumonias has been questioned

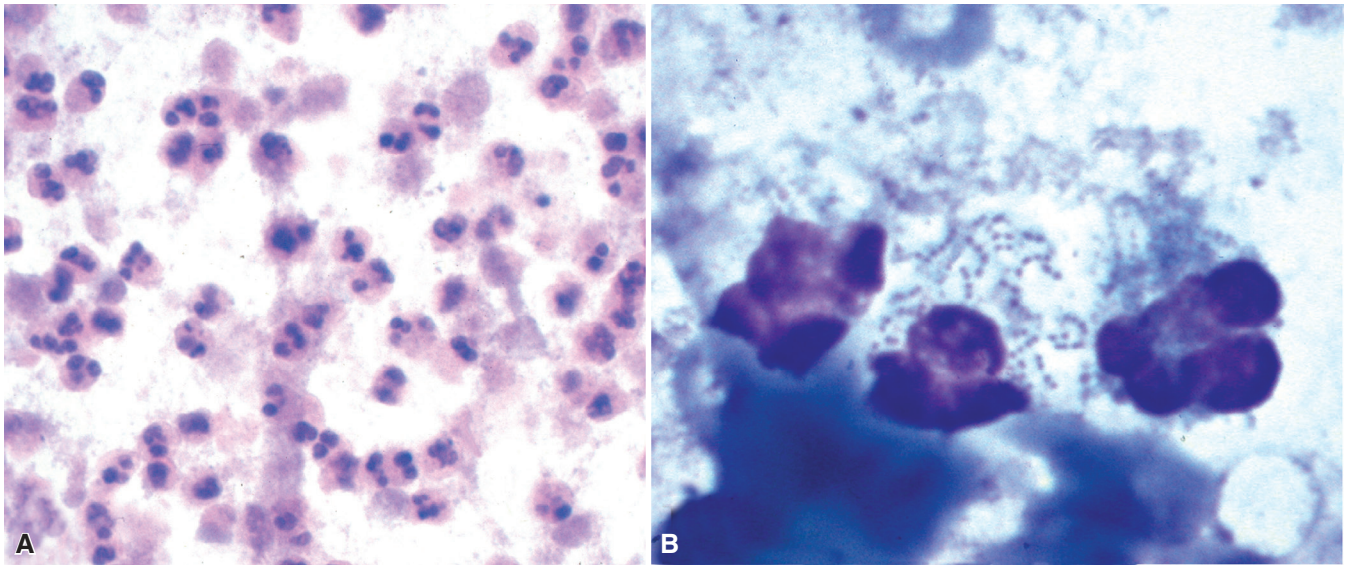


Figure 7.43 (A) Purulent exudate of nodular pulmonary infiltrate in fine-needle aspirate (alcohol-fixed). (B) Streptococci (*viridans* group) in cytoplasm of neutrophil seen in fine-needle aspirate (Diff-Quik preparation).

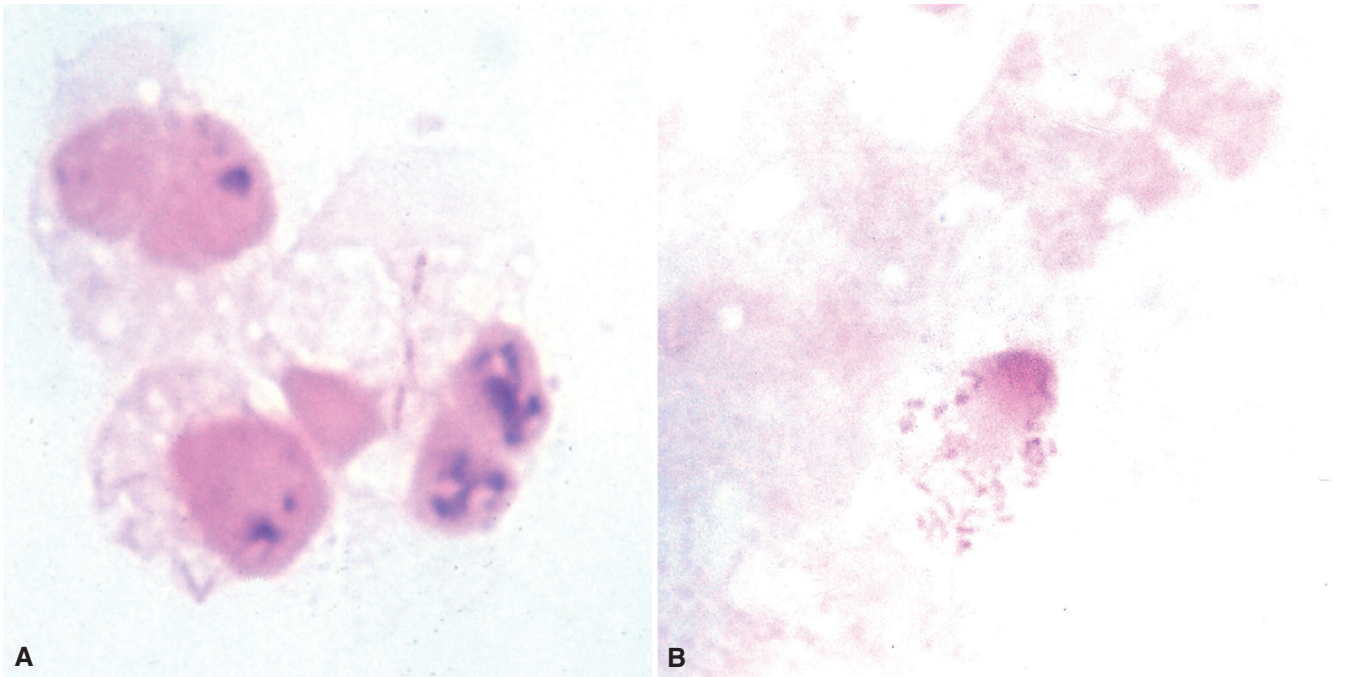


Figure 7.44 (A) Fusiform bacteria (*Fusobacterium* organisms) in cytoplasm of neutrophil in fine-needle aspirate (Gram stain). (B) Coccobacilli *(Haemophilus influenzae)* in cytoplasm of leukocyte in fine-needle aspirate (Gram stain).

for some time, and evolving guidelines from two specialty societies—the American Thoracic Society and the Infectious Disease Society of America—have lately coalesced.[118-121] Despite microbiologic testing, in a retrospective review of 2259 patients with radiographic evidence of pneumonia hospitalized from January 2010–June 2012 in selected US communities, no pathogen was detected in the majority of patients.[122] When a carefully collected specimen reveals one or two predominant bacterial morphotypes on a well-prepared Gram stain (Fig. 7.46), especially in the presence of neutrophils and few or no squamous cells, a presumptive diagnosis can be offered and correlated with whatever grows on culture plates.[123-125] A mixed bacterial population is usually considered nondiagnostic, especially in the absence of inflammation or the presence of many benign oral squamous cells. Pneumonia in the hospitalized or immunocompromised patient requires an aggressive strategy to collect a good sputum sample for Gram stain and culture. If this attempt is unsatisfactory or the findings are nondiagnostic, then the use of invasive techniques beginning with fiberoptic bronchoscopy and BAL with protected catheters should be considered.[60,62,126] Anaerobic pulmonary infections, typically in the form of a lung abscess, can also be approached in this way or with transthoracic needle aspiration.[79]

Gram staining of tissue sections from bronchoscopic or surgical biopsy specimens is notoriously insensitive and nonspecific. As with sputum, the presence of a predominant bacterial morphotype in a distinctive necroinflammatory background carries diagnostic weight,

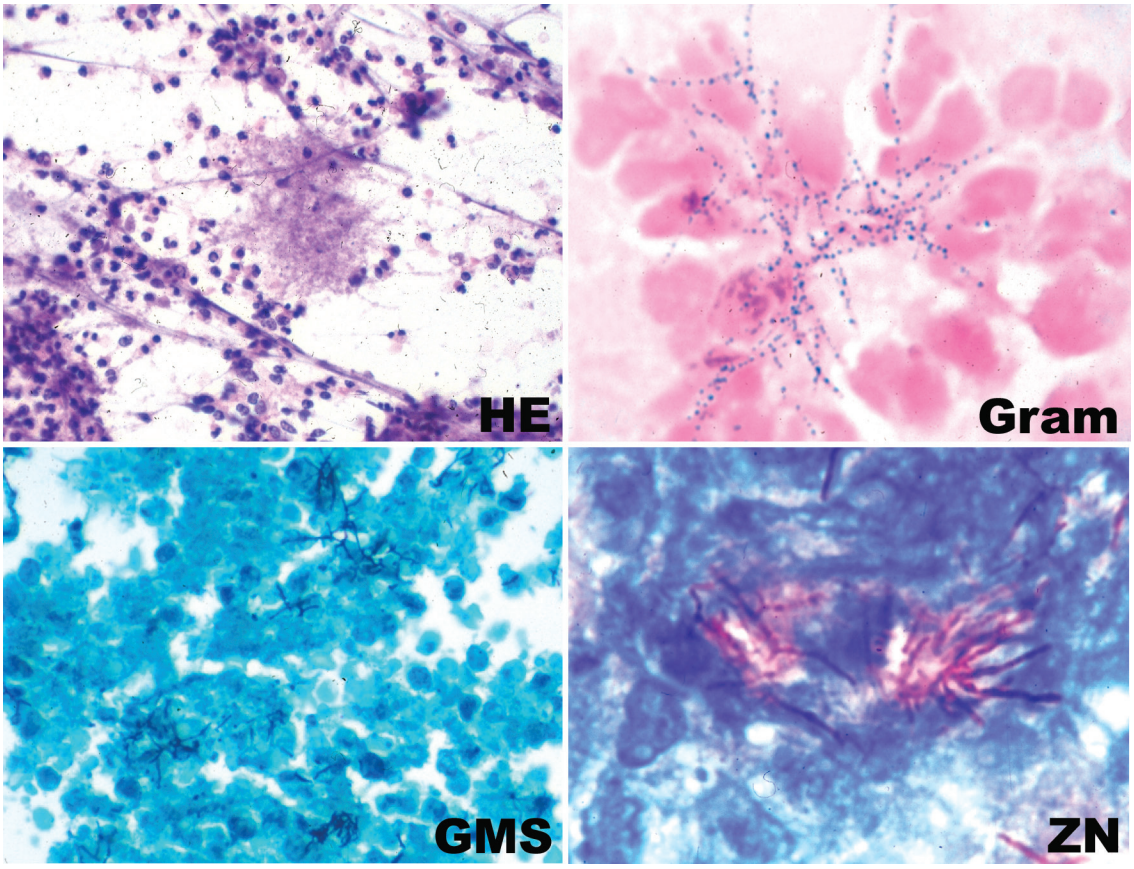


Figure 7.45 Nocardia. Loose, feathery cluster of bacilli in purulent exudate seen in a fine-needle aspirate: alcohol-fixed, Hematoxylin and eosin stain *(HE)*; Gram stain *(Gram)*; Grocott methenamine silver stain *(GMS)*; Ziehl–Neelsen stain *(ZN)*.

Box 7.7 Laboratory Diagnosis of Bacterial Pneumonia

- Direct detection of organisms
  - Gram stain; other stains of respiratory secretions and fluids
  - Direct fluorescent antibody stain
  - Histopathologic/cytopathologic examination
  - Immunohistochemistry
- Antigen detection (with *Legionella pneumophila* [LP1] and *Streptococcus pneumoniae*)
- Culture
  - Conventional media for usual pyogenic bacteria
  - Special media for fastidious or atypical agents
- Serologic testing
- Molecular methods
  - In situ hybridization
  - DNA amplification

especially when correlated with available clinical and laboratory data. Because histology laboratories do not generally observe the same level of caution in reagent preparation and storage as microbiology laboratories, it is worth remembering that tissue sections are prone to false-positive results from in vitro bacterial contamination.

In those cases where bacteria are visible on H&E-stained sections, the Gram stain can be helpful in confirming a presumptive etiology. For example, pairs and chains of gram-positive cocci in a necroinflammatory background suggest a streptococcal pneumonia, whereas numerous slender gram-negative bacilli investing and infiltrating blood vessels are characteristic of *Pseudomonas* pneumonia (Fig. 7.47). Other types of gram-negative pneumonias (Fig. 7.48) can also be confirmed with well-prepared Gram stains.[81] In the case of an abscess, a mixture of gram-positive cocci and gram-negative bacilli in tissue (illustrated earlier in Fig. 7.29) is a useful finding that is helpful in supporting a diagnosis of an anaerobic infection.

When organisms are sparse, other stains such as Giemsa or silver impregnation may highlight the organisms in the exudates (Fig. 7.49). The Gram stain is also useful for evaluating infections with granules and allows differentiation of the agents of botryomycosis (the gram-positive cocci or gram-negative bacilli) from the filamentous *Actinomyces* organisms (Fig. 7.50).

Staining with methenamine silver is the best procedure for detecting *Nocardia* organisms. The modified Ziehl–Neelsen stain allows for differentiation of *Nocardia* (positive) from the anaerobic *Actinomyces* (negative).[114]

Commercially available immunohistochemical reagents exist for relatively few bacterial species. Immunohistochemistry testing for the potential bioterrorist agents discussed in this chapter is available through the CDC in Atlanta, Georgia. It is expected that commercial reagents will become increasingly available for the common etiologic agents in the near future.[33]

Culture media that will allow recovery of common bacterial species causing pneumonia from various types of respiratory samples (secretions, washings, brushings, aspirates, and tissues) include sheep blood agar, chocolate agar, and McConkey agar. These media also will support growth of *B. anthracis* and *Y. pestis*. Buffered charcoal yeast extract (BCYE) agar is the primary medium for *Legionella* species. Because *Legionella* organisms survive poorly in respiratory secretions, rapid

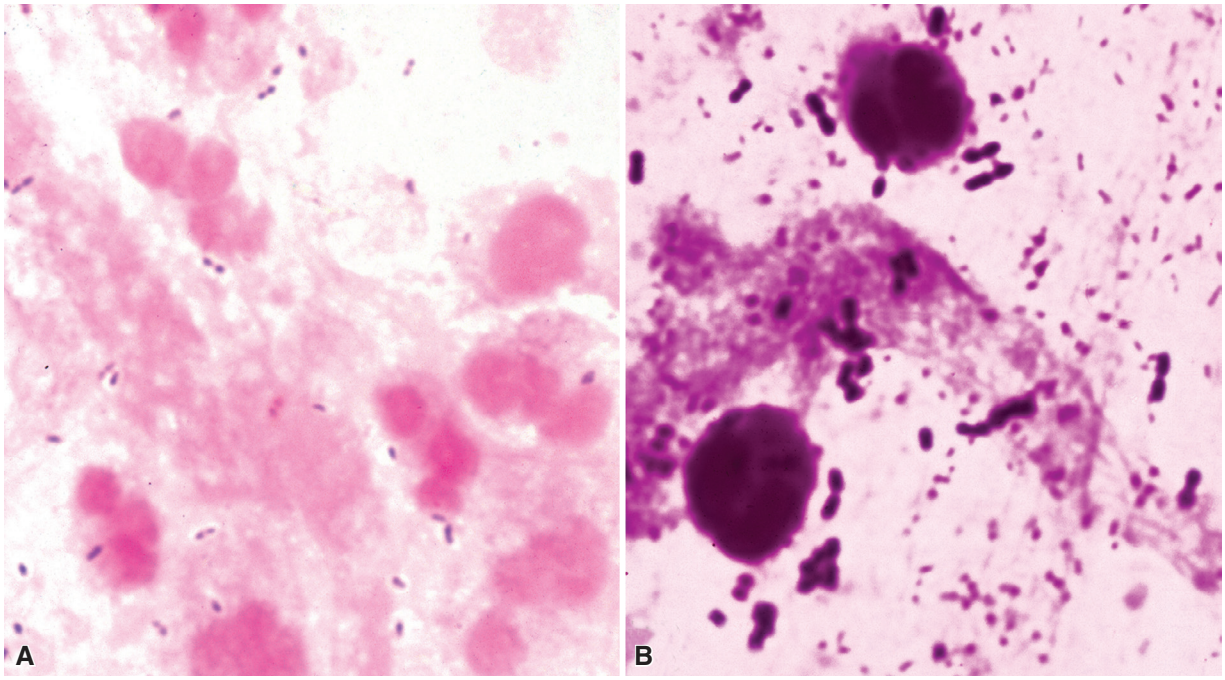


Figure 7.46 Sputum Gram stain. (A) Gram-positive diplococci *(Streptococcus pneumoniae)* with neutrophils, but no squamous cells. (B) Gram-positive diplococci *(S. pneumoniae)* and gram-negative coccobacilli *(Haemophilus influenzae).*

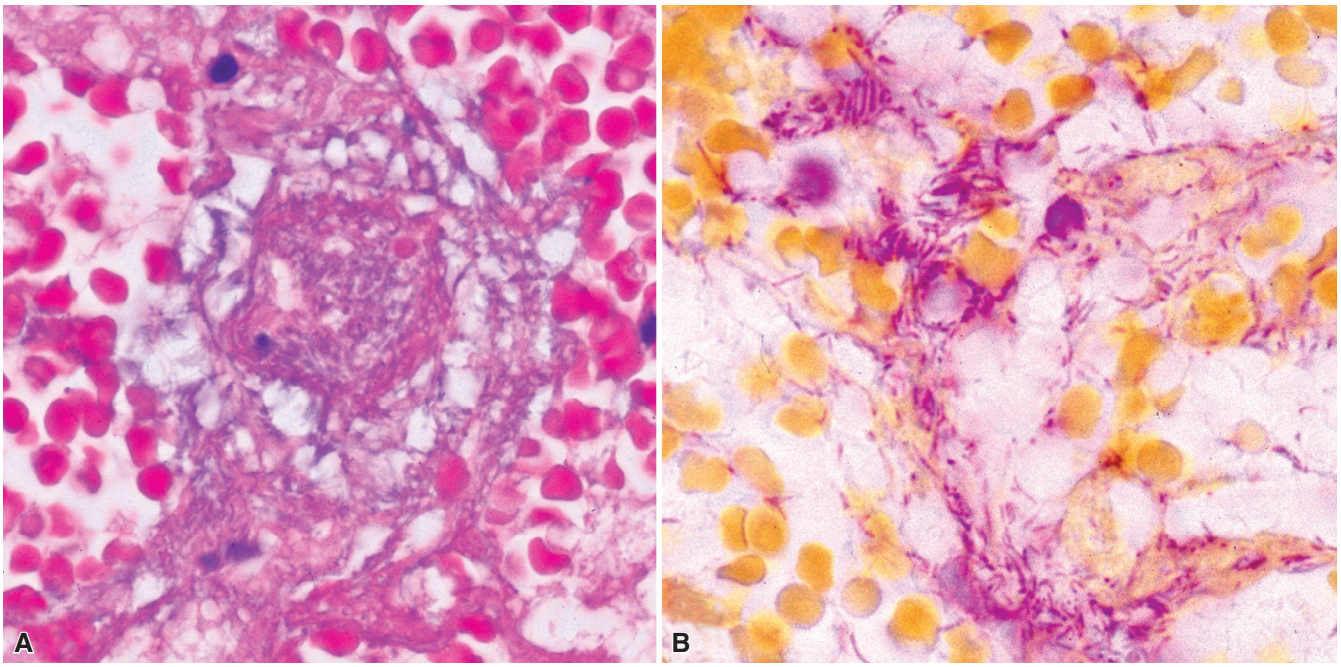


Figure 7.47 (A) *Pseudomonas aeruginosa* bacilli investing interstitial vessels (Brown and Hopps stain). (B) The slender gram-negative bacilli are nicely demonstrated on Gram stain.

transport and immediate plating is essential for recovery. BCYE is also a good all-purpose medium for growing other fastidious species, including *F. tularensis*. However, *F. tularensis* grows best in cysteine-enriched media.[127]

In addition to respiratory samples, blood can be obtained for cultures in patients sick enough to lead to suspicion of bacteremia; pleural fluid culture can be used when effusions are present. Positive cultures of these normally sterile fluids circumvent the interpretive problems associated with bacterial growth in sputum samples.

The Actinomycetes are best isolated from invasive specimens such as needle aspirates and transbronchial and lung biopsy specimens. The laboratory should be alerted to search for these agents because special consideration must be given to culture setup and incubation conditions.[89] The Actinomycetes responsible for actinomycosis require anaerobic media and atmosphere as well as prolonged incubation. *Nocardia*, an aerobic Actinomycete, grows well on most nonselective media but requires extended incubation. Determination of colonial morphology, Gram and acid-fast stains, and a few biochemical tests generally suffice

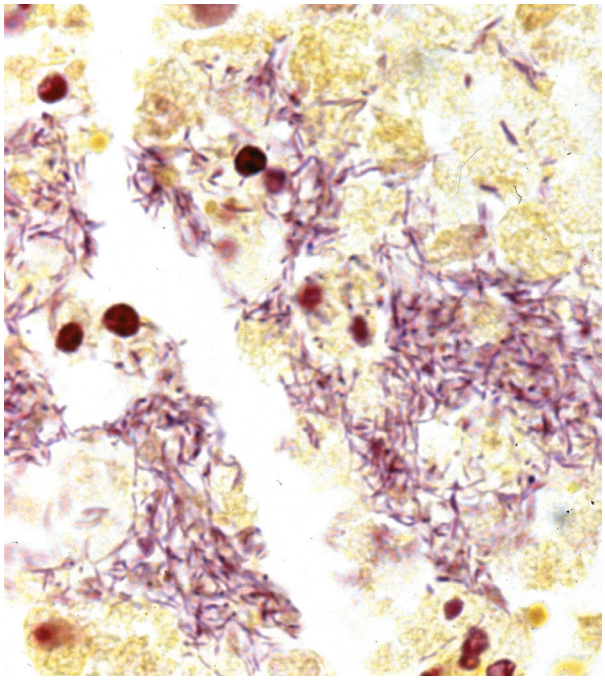


Figure 7.48 *Burkholderia cepacia* bacilli (Brown and Hopps stain).

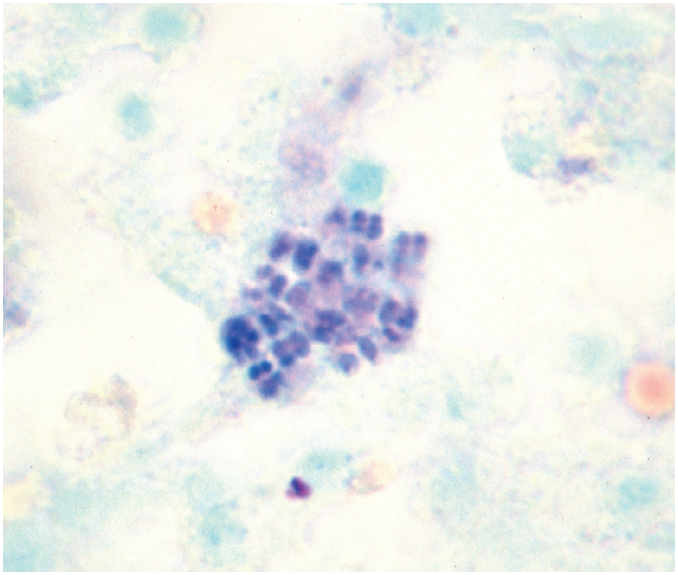


Figure 7.49 Bacterial tetrads in alveolar exudate (Giemsa stain).

to identify these organisms at the genus level. However, genotype rather than phenotype characteristics are required to identify newly emergent species.[128]

In general, the laboratory diagnosis of pneumonia caused by most of the atypical agents is difficult because systems are not routinely available or are costly, cumbersome, or unsafe. For the atypical agents (*Mycoplasma*, *Chlamydia*, and *Coxiella* species), serologic testing has been the method of choice for diagnosis.[67,129] Classic cold agglutinin and complement fixation tests for these agents have largely been replaced by enzyme immunoassay and microimmunofluorescence testing.[87,130,131] Serologic methods are also useful for the diagnosis of tularemia because of the difficulty in culturing the fastidious bacterium.

Legionella pneumonia is a common form of severe pneumonia that is not readily diagnosed for a number of reasons, including the organism's

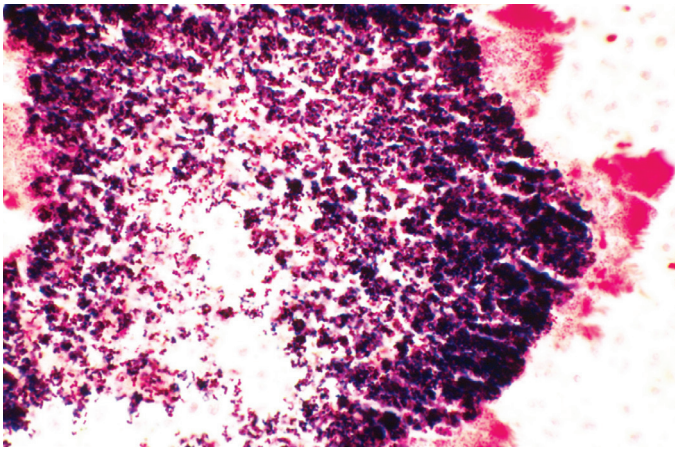


Figure 7.50 Botryomycosis. Cluster of gram-positive cocci *(Staphylococcus aureus)* invested by gram-negative–staining Splendore-Hoeppli material (Brown and Brenn stain). (Courtesy Dr. Francis Chandler, Augusta, Georgia.)

fastidiousness.[132] In the microbiology laboratory, the direct fluorescent antibody test and culture on buffered BCYE agar have been the mainstays of diagnosis. Culture is considered the diagnostic gold standard but is only 60% sensitive. Serologic testing is available for most of the *Legionella pneumophila* serotypes, which account for 90% of the pneumonia cases; however, the need to collect paired sera weeks apart limits its usefulness in the acutely ill patient. Antigen detection in urine has become commercially available for both *L. pneumophila* and *S. pneumoniae*; because the need to collect acute and convalescent sera is obviated, it has become a frequently used diagnostic test.[132,133] Its advantage lies in its potential to effect early treatment decisions through rapid diagnosis. Its disadvantage lies in the fact that it identifies only patients infected with *L. pneumophila* serogroup 1 (LP1), the most prevalent species and serotype, but none of the non-LP1 serotypes or cases due to other *Legionella* species.[134-136]

The use of molecular diagnostic tools (in situ hybridization and nucleic acid amplification by PCR or other methods) to detect these agents has been reported.[85,136,137] PCR nested assays are replacing more and more of the above classical methods with sensitive, specific, and rapid diagnostic technique. Multiplex assay, to detect multiple agents in a single reaction, would seem to be an ideal pursuit for the laboratory diagnosis of the most common community-acquired pneumonias, including those due to the atypical pneumonia agents.[36,138-141]

Differential Diagnosis

The key morphologic and microbiologic features of the bacterial pneumonias are summarized in Table 7.5. The presence of purulent exudates or significant numbers of neutrophils in biopsy or cytologic samples should always trigger a search for bacterial infection. Because lung biopsies are usually performed late in the clinical course after procedures have been performed and bacterial infections have been excluded and/or treated with antibiotics, neutrophilic exudates may not signify bacterial infection unless accompanied by necrosis, as in an abscess. Instead, consideration should be given to one of several noninfectious acute inflammatory diseases, with an immunologic basis, that can mimic bacterial infection. Some of these include granulomatosis with polyangiitis, Goodpasture syndrome, systemic lupus erythematosus, and microscopic polyangiitis, all conditions that can produce acute inflammation predominantly involving alveolar septal blood vessels ("capillaritis"). On occasion, capillaritis can result in airspace accumulation of neutrophils, further raising concern for bronchopneumonia. Centrally necrotic or cavitary neoplasms of various types may mimic

Table 7.5 Bacterial Pneumonias: Summary of Pathologic Findings

Assessment Component	Findings
Pyogenic Bacteria	
Surgical pathology	Acute purulent inflammation with/without necrosis; organization; diffuse alveolar damage may be present
Cytopathology	Acute inflammation with/without visible bacteria on Diff-Quik–stained smear
Microbiology	Gram stain reactivity and morphology (visual detection requires heavy bacterial burden: 10^6 organisms/g of tissue); culture-sterile lung tissue on standard nonselective and selective media (blood, chocolate and MacConkey agars); anaerobic broth and agars for abscesses; urinary antigen for *Streptococcus pneumoniae*
Atypical Pneumonia Agents	
Surgical pathology	*Legionella* pneumonia: fibrinopurulent with bacilli visible in silver-stained (Dieterle; Warthin-Starry) sections DAD often present *Chlamydia* and *Mycoplasma* infection: polymorphous bronchiolar and interstitial infiltrate
Cytopathology	Acute inflammation with bacilli stained with silver or by immunofluorescence (*Legionella* pneumonia)
Microbiology	DFA for *L. pneumophila* serotypes; culture on selective (BCYE) agar for *Legionella*; urinary antigen for *Legionella*; serologic testing and/or PCR assay for *Mycoplasma* and *Chlamydia*
Filamentous Granule Group	
Surgical pathology	Granules or loose filamentous aggregates in purulent exudate with abscess formation and poorly formed granuloma in some cases
Cytopathology	Filamentous tangles or aggregates or granules with neutrophils and/or necroinflammatory background
Microbiology	Gram-positive branching filaments: *Nocardia* (aerobic actinomycete) and *Actinomyces* (anaerobic actinomycete); *Nocardia* partially acid-fast and GMS-positive Gram-positive cocci or gram-negative bacilli (botryomycosis); culture on standard nonselective media and selective (BCYE) media; anaerobic culture broths and media for *Actinomyces*

BCYE, Buffered charcoal yeast extract; *DAD*, diffuse alveolar damage; *DFA*, direct fluorescence assay; *GMS*, Grocott methenamine silver.

abscesses grossly and microscopically, and exceptionally well-differentiated adenocarcinomas containing glands filled with detritus may mimic inflammatory and bacterial diseases. Suppurative granulomas can have a bacterial, mycobacterial, or fungal etiology. Even the miliary necroinflammatory lesion typical of bacterial infection can be produced by viruses, some fungi, and even protozoa (e.g., *Toxoplasmagondii*). Aspiration, common in hospitalized patients, may manifest with an acute bronchopneumonia/bronchiolitis type of pattern, and it should also be in the differential diagnosis as a contributing or etiologic factor when considering most pneumonias; microscopic identification of foreign material is a definitive diagnostic clue, frequently gained only from histopathologic examination.[78]

Mycobacterial Infections

The surgical pathologist tends to encounter mycobacterial infections in lung biopsies when standard clinical diagnostic approaches to pulmonary infiltrates are unsuccessful and the lesions persist or progress. Tuberculosis is but one of several different types of lung infection that can manifest clinically as community-acquired pneumonia, resulting in delay until an invasive procedure such as transbronchial biopsy, transthoracic needle biopsy, or surgical lung biopsy is performed, often as a "last resort" effort.[142,143] In recent years, delays in the diagnosis of mycobacterial infection have markedly decreased, thanks in part to recommendations from the CDC for improving laboratory turnaround time and to the response of the diagnostics industry with better methods and technology. Because direct acid-fast smears of respiratory specimens yield negative findings in at least half of the cases,[144] and because many mycobacterial species are fastidious and slow-growing, the biopsy results may be the first suggestion of a mycobacterial infection. The biopsy findings can also define the organism's relationship to a histopathologic lesion and host response—important information in evaluating the significance of a culture result. Although an isolate of *M. tuberculosis* is always taken seriously, obtaining a single isolate of a nontuberculous mycobacterium from the respiratory tract does not necessarily implicate the organism as the cause of disease.[145]

Box 7.8 Classification of Tuberculosis

- Primary tuberculosis
  - Exogenous first infection
  - Exogenous reinfection
- Progressive primary tuberculosis
- Postprimary tuberculosis
  - Endogenous reactivation
  - Exogenous infection in BCG-vaccinated persons
  - Exogenous superinfection

BCG, Bacille Calmette-Guérin.

Data from Allen E. Tuberculosis and other mycobacterial infections of the lung. In: Churg AM, Thurlbeck WM, eds. *Pathology of the Lung,* 2nd ed. New York: Thieme; 1995:233, Table 13.1.

Etiologic Agents

The mycobacterial species can be categorized in two clinically relevant groups: *Mycobacterium tuberculosis* complex (MTC) and the nontuberculous mycobacteria (NTM). MTC includes the subspecies *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microti*. The last three species produce tuberculosis in some areas of the world, but in the United States the prevalence of such disease is very low.

Mycobacterium tuberculosis

M. tuberculosis is the most virulent mycobacterial species and an unequivocal pathogen that is responsible for numerous deaths worldwide. This organism is the etiologic agent of tuberculosis in its various forms, which are listed in Box 7.8.

Primary tuberculosis occurs in patients without previous exposure or with the loss of acquired immunity. Progressive primary tuberculosis occurs in patients with inadequate acquired immunity—that is, impaired cellular immunity. Postprimary tuberculosis, also referred to as *secondary* or *reinfection-reactivation tuberculosis*, occurs in patients with previous immunity to the organism and accounts for most clinical cases of tuberculosis.[146,147] Many clinical experts consider that most cases of active tuberculosis in adults with normal immunity arise from reactivation of latent infection (postprimary tuberculosis), whereas reinfection with a new strain derived from the environment (primary or postprimary tuberculosis) can occur in the immunocompromised patient. More recently, DNA fingerprinting methods (genotyping) have challenged this dogma by showing that exogenous reinfection accounts for a significant percentage of cases in some areas of the world.[148] Miliary tuberculosis and extrapulmonary disease can occur with any of these forms.[146,149]

Primary tuberculosis is usually a mild illness that is often not recognized. The bacillemia that occurs during its development can seed extrapulmonary organs and set the stage for subsequent reactivation.

Approximately 5% of patients pass through latency to postprimary disease within 2 years of primary infection, and another 5% do so later in their lives.[150]

Nontuberculous Mycobacteria

Recognized NTM species, many of which were identified during the past decade, number more than 125.[151,152] However, relatively few cause pulmonary disease.[145,153-155] These organisms are acquired from the environment, where they are ubiquitous. In contrast with *M. tuberculosis*, the NTM are not spread from person to person. In most instances, patients in whom NTM infection develops have chronic lung disease and other risk factors, such as AIDS, alcoholism, or diabetes. Reports of NTM infections in nonimmunocompromised patients are increasing.[16,156] MAC and then *Mycobacterium kansasii* are the most frequent isolates in all settings. Among a growing number of species causing lung disease are *Mycobacterium abscessus*, *Mycobacteriumfortuitum*, *Mycobacterium szulgai*, *Mycobacterium simiae*, *Mycobacterium xenopi*, *Mycobacterium malmoense*, *Mycobacterium celatum*, *Mycobacterium asiaticum*, and *Mycobacterium shimodii*. These species manifest marked geographic variability with respect to prevalence and severity. Of note, however, since 1985, more MAC isolates than *M. tuberculosis* have been reported in the United States.[145]

Histopathology

The histopathologic patterns produced by mycobacteria are listed in Box 7.9. The radiologic, gross, and microscopic patterns of mycobacterial disease reflect the virulence of the various mycobacterial species as well as the patient's prior exposure and immune status.[157-160]

Box 7.9 Histopathologic Patterns in Mycobacterial Lung Injury

- Large nodules with or without cavities
  - Well-formed granuloma
  - Poorly formed granuloma
  - Suppurative granuloma
  - Histiocytic aggregates
- Miliary nodules
- Calcified nodules
- Granulomatous interstitial pneumonitis
- Bronchitis/bronchiectasis
- Spindle cell pseudotumors

Primary Tuberculosis

Mycobacterium tuberculosis occurs typically in the best-aerated lung regions (anterior segments of the upper lobes, lingua and middle lobe, or basal segments of the lower lobes).[158] The disease passes through progressive phases of exudation, recruitment of macrophages and T lymphocytes, and granuloma formation followed by repair with granulation tissue, fibrosis, and mineralization.[147,161] Macrophage-laden bacilli also travel to the hilar lymph nodes, where the phases are repeated. This combination of events produces the classic Ghon complex, consisting of a peripheral 1- to 2-cm lung nodule (Fig. 7.51) and an enlarged, sometimes calcified hilar lymph node. In both locations, the histopathologic hallmark is a necrotizing granuloma (Fig. 7.52) composed of epithelioid cells with variable numbers of Langhans giant cells, a peripheral investment of lymphocytes, and a central zone of caseation necrosis, a form of necrosis attributed to apoptosis.[146,162] A spectrum of lesions may be seen, from the tuberculoid "hard" granuloma without

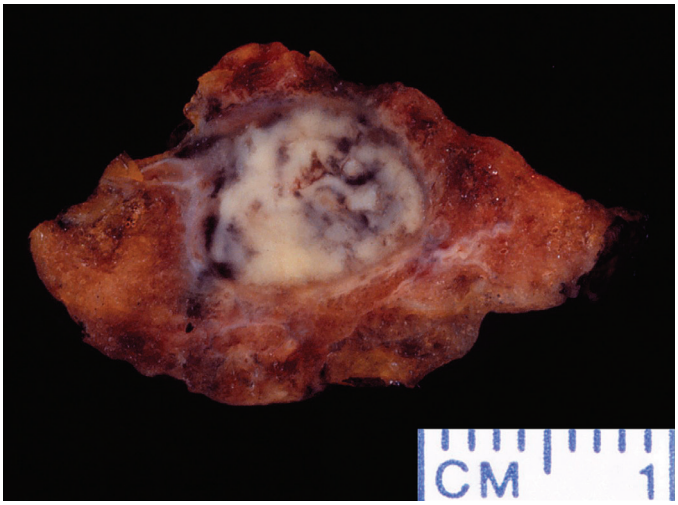


Figure 7.51 Tuberculoma removed from right upper lobe.

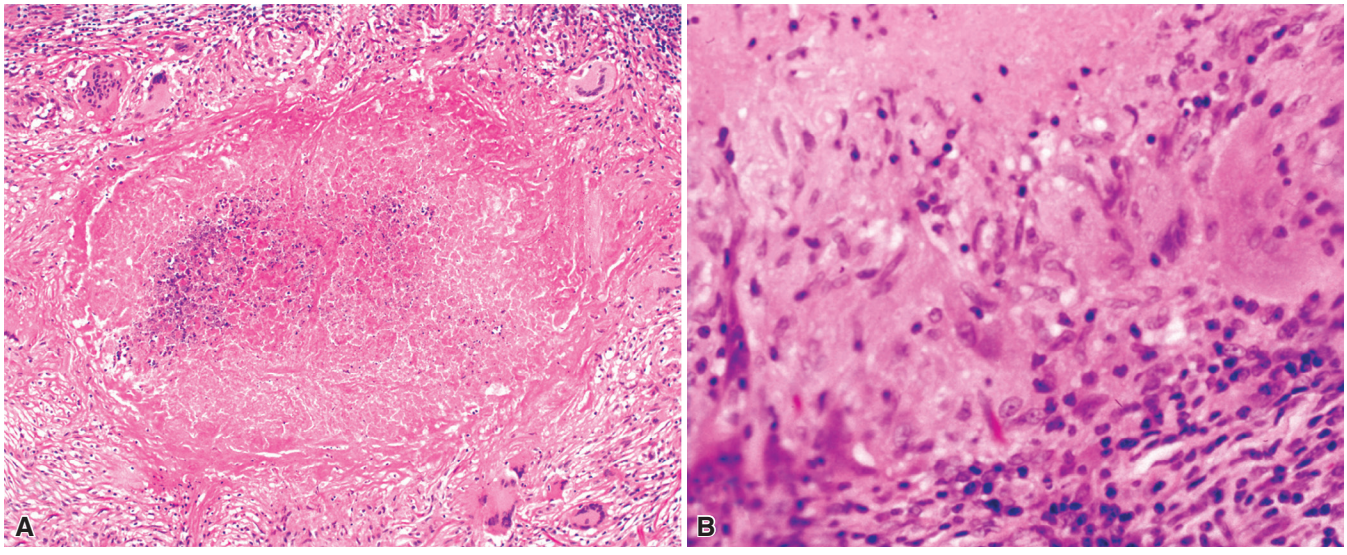


Figure 7.52 (A) Tuberculoid granuloma with central zone of caseation necrosis surrounded by epithelioid cells, giant cells, and outer investment of lymphocytes. (B) Palisade of epithelioid histiocytes in giant cells at edge of necrotic zone.

necrosis and rare organisms to the multibacillary necrotic lesion with scant epithelioid cells.[163] In a minority of patients the lesions enlarge and progress as a result of increased necrosis or liquifaction.

The complications of tuberculosis are listed in Box 7.10 and illustrated in Fig. 7.53. Other complications may include extension into blood vessels with miliary (Fig. 7.54) or systemic dissemination, lymphatic drainage into the pleura with granulomatous pleuritis and effusions, involvement of bronchi by bronchocentric granulomatous lesions (Fig. 7.55), or tuberculous bronchopneumonia. Granulomas may also encroach upon blood vessels, mimicking a "granulomatous" vasculitis. The hemophagocytic syndrome, which has been implicated in a variety of bacterial, viral, and parasitic infections, has also been associated with tuberculosis.[164]

Postprimary Tuberculosis

Postprimary tuberculosis, the most common form in adults, typically involves the apices of the upper lobes, producing granulomatous lesions with greater caseation, often with cavities and variable degrees of fibrosis and retraction of the parenchyma.[149,160] Fibrosis and bronchiectasis occur with the healing of cavities and is the major cause of pulmonary disability in this disease.[165] Recent studies have proposed that postprimary disease begins as a form of lipoid pneumonia, with bacilli-laden foamy alveolar macrophages and bronchiolar obstruction progressing to caseating cavitary disease and microvascular occlusion due to delayed-type hypersensitivity.[166] Extension to other lobes or hilar/mediastinal lymph nodes and miliary spread through the lungs and to extrapulmonary sites can occur. Other presentation patterns include acute and organizing diffuse alveolar damage with advanced or miliary disease, acute tuberculous bronchopneumonia, and the solitary pulmonary nodule

Box 7.10 Complications of Tuberculosis

- Miliary tuberculosis
- Granulomatous pleuritis and effusions
- Tuberculous bronchopneumonia
- Extrapulmonary dissemination to
  - Meninges
  - Kidney
  - Bone
  - Other

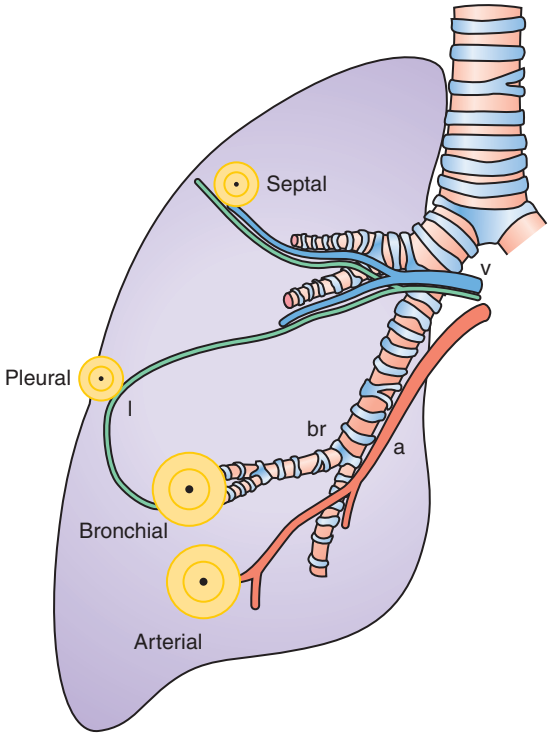


Figure 7.53 Complications of tuberculosis. Invasion of arteries *(a)* with miliary spread; bronchi *(br)* with tuberculous bronchopneumonia; lymphatics *(l)* with granulomatous pleuritis and effusions. Invasion of septal *(s)* veins *(v)* leads to extrapulmonary dissemination.

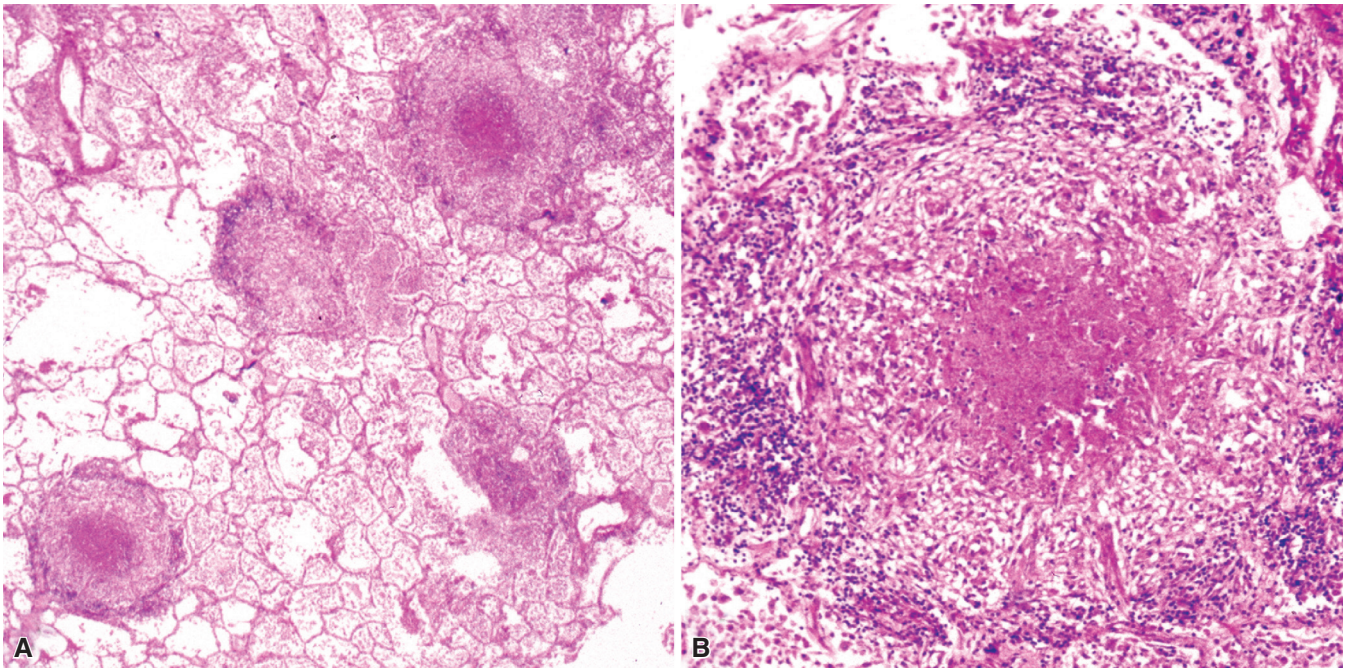


Figure 7.54 Miliary tuberculosis. (A) Miliary pattern. (B) Epithelioid granulomas with necrotic central zones.

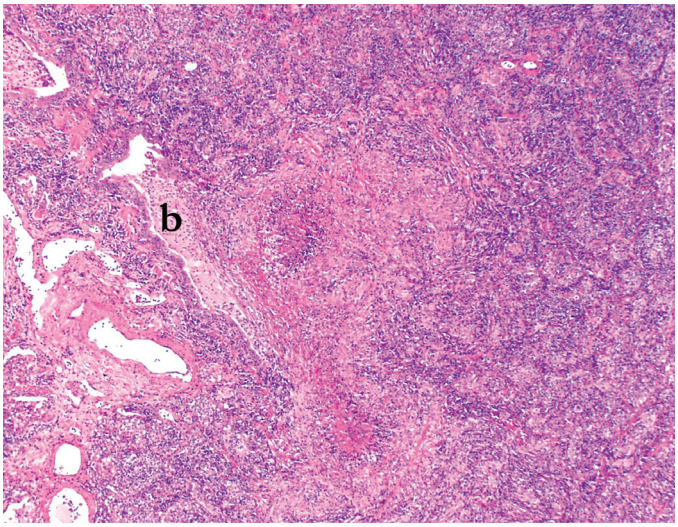


Figure 7.55 Bronchocentric granuloma in mycobacterial infection. Only a small focus of residual bronchial epithelium (b) remains.

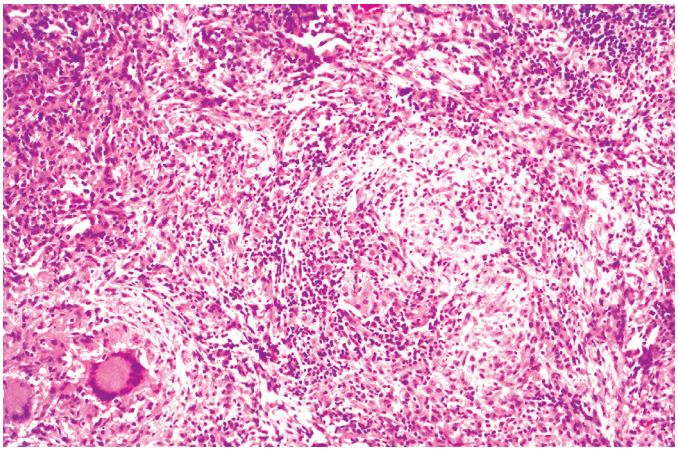


Figure 7.56 Nonnecrotizing granuloma in infection due to *Mycobacterium avium* complex (MAC).

(tuberculoma). A proximal endobronchial form may mimic a neoplasm, noteworthy for its necrosis and large numbers of bacilli.[167] Because characteristic granulomatous morphology may not be visible around the necrotic material, stains for mycobacteria should be considered for all necrotic endobronchial samples.

Tuberculous Pleurisy

Tuberculosis is a rare cause of chronic pleural effusion. Pleural biopsy may be included when clinical suspicion for tuberculosis is high, both to improve recovery of the organisms and to visualize granulomas. The presence of pleural caseating granulomas can be considered nearly diagnostic of tuberculous pleural effusion and a powerful indication for treatment[168]; lack of true caseation in the granulomas expands the differential diagnosis to include sarcoid, fungal infection, and rheumatoid disease.

Nontuberculous Mycobacterial Infections

NTM infections may be similar to those due to *M. tuberculosis*, but certain differences have been noted. For example, the NTM pathogens do not cause the same sequence of primary or postprimary disease, and systemic dissemination does not occur except in the immunocompromised patient. *M. kansasii* is more virulent than MAC, and the infection-associated histopathologic pattern is more like that produced by *M. tuberculosis.*[169]

Infections due to MAC and other common pulmonary NTM pathogens generally manifest as one of five clinicopathologic entities: solitary pulmonary nodule, chronic progressive pulmonary disease, disseminated disease, chronic bronchiolitis with bronchiectasis, and hypersensitivity-like pneumonitis.[147,170] Solitary pulmonary nodules generally exhibit granulomas resembling those caused by *M. tuberculosis*.

Chronic progressive disease also resembles tuberculosis, with upper lobe thin-walled cavities and granulomatous inflammation with or without caseous necrosis (Fig. 7.56). Multiple confluent granulomas in fibrosis can mimic sarcoidosis. Organisms are usually sparse and more difficult to find in the immunocompetent patient. This presentation most often is seen in patients with underlying chronic lung disease, such as COPD, bronchiectasis, cystic fibrosis, pneumoconiosis, reflux disease, or preexisting cavitary lung disease of any cause (including old tuberculous cavities).

Disseminated disease is typically associated with the immunocompromise produced by human immunodeficiency virus (HIV) infection, in which the disease tends to target the gastrointestinal tract (the likely portal of entry) and pulmonary and reticuloendothelial disease signifies dissemination.[171] In this setting, NTM bacilli (predominantly MAC) proliferate characteristically to high levels in poorly formed granulomas or in sheets and clusters of plump, finely vacuolated macrophages ("pseudo-Gaucher" cells) containing abundant phagocytosed intracytoplasmic bacilli (Fig. 7.57).

A distinctive form of NTM disease occurs as the "Lady Windermere syndrome." In the classic clinical scenario, an elderly, nonsmoking, immunocompetent woman of particular habits, demeanor, and body type presents with multiple pulmonary nodules, preferentially involving the middle lobe and lingula. The airway-centric granulomas and bronchiectasis can be subtle or pronounced (Fig. 7.58); these findings have been recognized as one of the patterns of middle lobe syndrome.[172] NTM bacilli can also colonize bronchiectatic lung from any cause, with resultant granulomatous inflammation predominantly affecting the airway walls—presumably as a result of localized decreased mucociliary clearance.

Hypersensitivity-like pulmonary disease has been associated with contaminated water in hot tubs ("hot tub lung") and other environmental sources such as humidifiers and air conditioners.[16] Biopsy reveals a miliary bronchiolocentric and interstitial granulomatous pattern, similar to that produced by hypersensitivity pneumonitis (Fig. 7.59). A similar infection-colonization-hypersensitivity syndrome has been described in workers exposed to metalworking fluid aerosols.[173] The clinical, radiologic, and pathologic findings are similar to disease associated with hot tub use and other water sources except that a distinctive rapid-growing NTM species, *M. immunogenum*, has been recovered almost exclusively. Organisms are difficult to find in these cases but can sometimes be recovered in culture or with molecular techniques. Whether this entity represents an infection, a colonization, a hypersensitivity reaction, or a hybrid condition remains unresolved at this time.

A rare morphologic manifestation of mycobacterial infection is the so-called *spindle cell inflammatory pseudotumor* (Fig. 7.60), which may occur in lung, skin, lymph nodes, and a number of other sites in immunocompromised patients.[174] The etiologic agents usually are NTM (MAC and *M. kansasii*), but *M. tuberculosis* has also been identified in some cases. Another uncommon variant is proximal endobronchial disease, discussed earlier in the spectrum of postprimary tuberculosis.

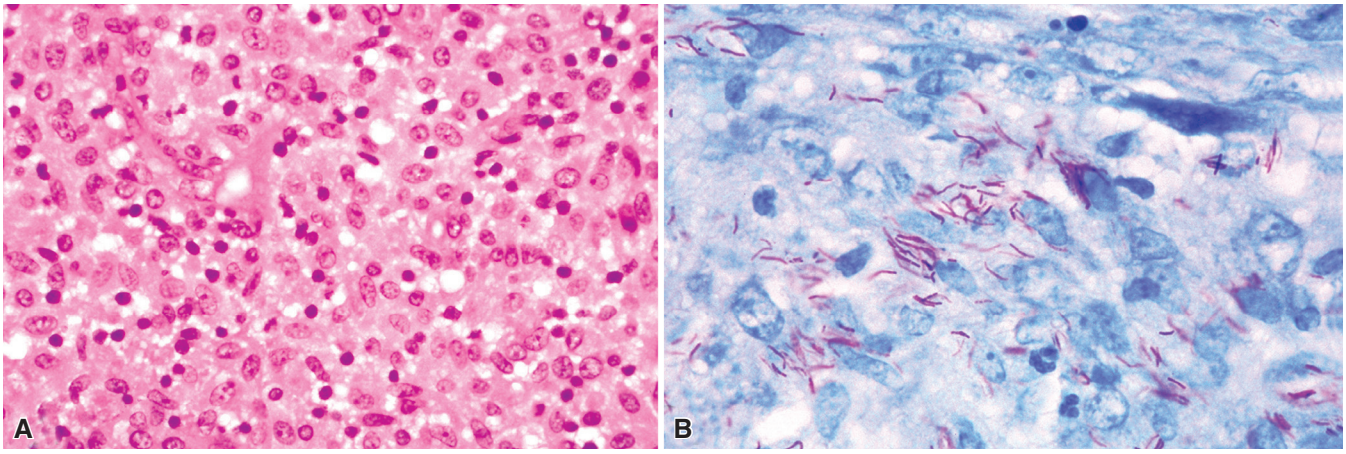


Figure 7.57 (A) Clusters of macrophages in *Mycobacterium avium* complex (MAC) infection in a patient with AIDS. (B) Myriad acid-fast bacilli (MAC) in histiocytic infiltrate (Ziehl–Neelsen stain).

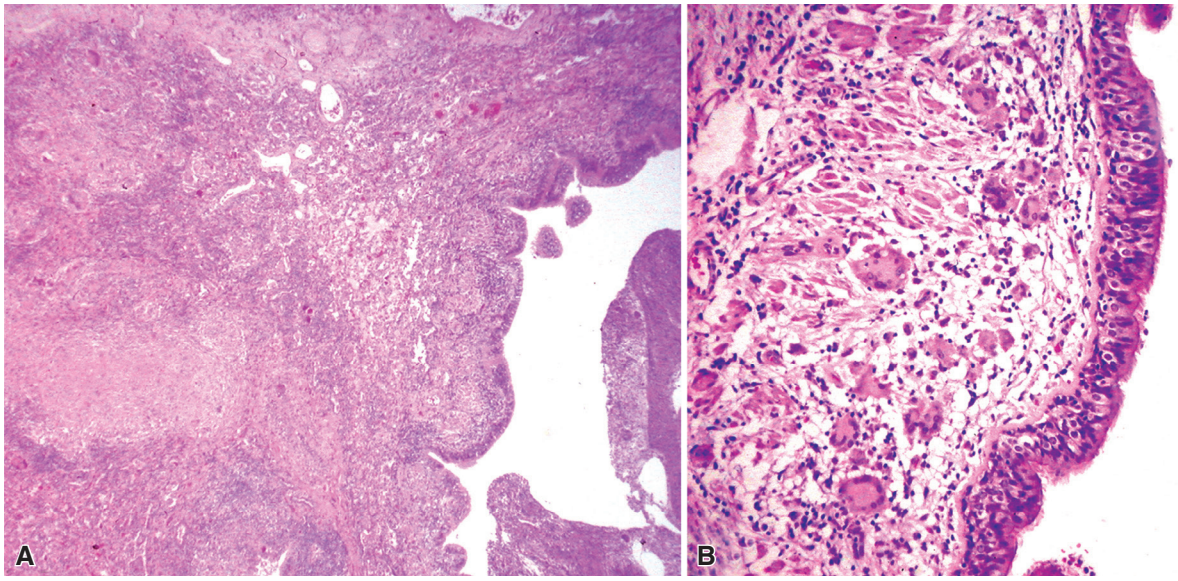


Figure 7.58 Middle lobe syndrome. (A) Bronchiectasis with peribronchial granulomas containing *Mycobacterium avium* complex. (B) Airway mucosa with granuloma.

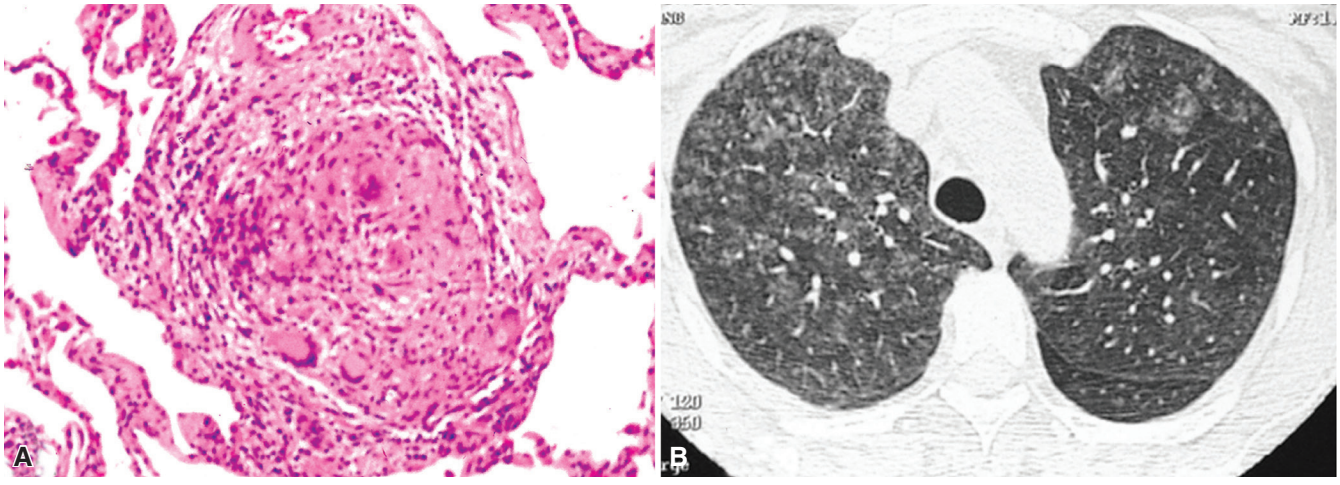


Figure 7.59 Hot tub lung. (A) Nonnecrotizing granuloma. (B) Computed tomographic image with features resembling those of hypersensitivity pneumonitis.

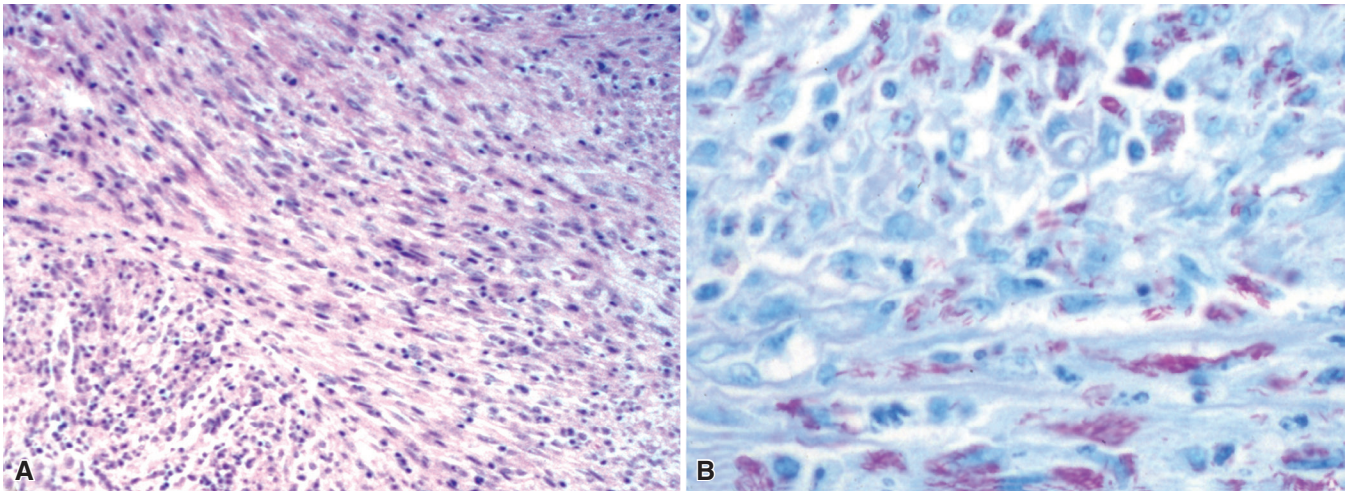


Figure 7.60 Spindle cell pseudotumor. (A) The fascicles of fibroblasts with scattered lymphocytes. (B) Myriad acid-fast bacilli (Ziehl–Neelsen stain).

Most cases are due to *M. avium* complex and manifest as polypoid lesions in immunocompromised HIV-infected patients, but this lesion may also be seen in immunocompetent persons.[175]

Certain species of rapidly growing mycobacteria (RGMs) are capable of producing pulmonary disease, albeit infrequently.[145,176,177] *M. abscessus* is the third most frequently recovered NTM respiratory pathogen in the United States, after *M. avium* complex and *M. kansasii*. *M. abscessus* produces chronic lung infection that has a striking clinical and pathologic similarity to *M. avium* complex infection, including the propensity to involve the lungs of patients with bronchiectasis. The RGMs have also been thought to colonize lipoid pneumonia[178]; however, it is more likely that the pathogenesis of the lung injury pattern caused by the RGMs is similar to that seen in skin and soft tissue cases, in which various combinations of suppurative foci, poorly formed or necrotizing granulomas, scattered multinucleated giant cells, and vacuoles are typical (termed *pseudocysts*).[179] These combined features may mimic lipoid pneumonia and constitute an important clue to the presence of RGM infection.

Cytopathology

Fine-needle aspiration biopsy has been successfully used to diagnose both tuberculous pulmonary lesions and nontuberculous mycobacterial infections.[180,181] The finding of finely granular amorphous necrotic debris associated with aggregates of epithelioid histiocytes (with or without multinucleate giant cells; Fig. 7.61) is suggestive of a mycobacterial or fungal infection.[182] In this setting, necrotic cancers must be excluded by a thorough search for atypical cells. Epithelioid granulomas manifest a similar cellular pattern, but the granular necrotic debris is absent. Another pattern that may be seen, particularly in specimens from the immunocompromised patient, is a pure histiocytic or macrophage reaction with few or no epithelioid or multinucleate giant cells or necrotic debris. Numerous bacilli may be present in the distended cytoplasm of histiocytes and in the extracellular background. In air-dried (Diff-Quik) and alcohol-fixed (H&E- or Papanicolaou-stained) smears, the bacilli may be recognized as negative images (Fig. 7.62)

The use of fine-needle aspirate to target and harvest potential microbiologically positive diagnostic specimens is an important technique, especially in underdeveloped countries where bronchoscopy may not be available. Fine-needle aspiration, especially of affected lymph nodes, combined with an automated rapid PCR diagnostic platform where available (such as Xpert MTB/RIF, Cepheid, Sunnyvale, California) is a public health opportunity for faster diagnosis and containment of disease in areas with a large incidence.[181,183]

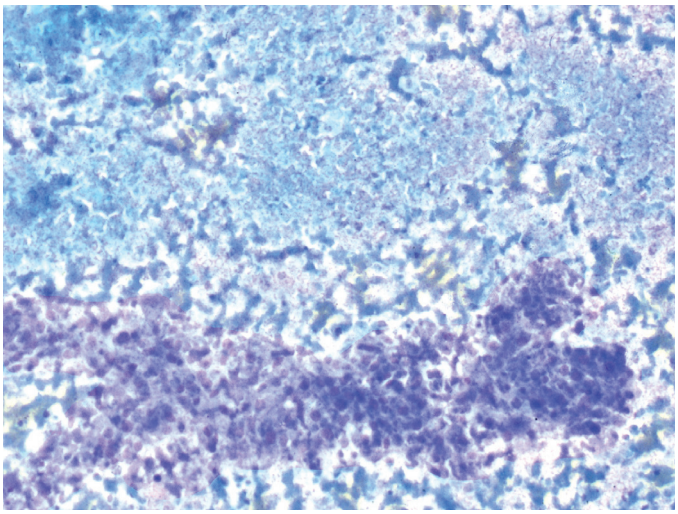


Figure 7.61 Necrotizing granuloma in *Mycobacterium kansasii* infection. Sheets of epithelioid cells in a background of granular necroinflammatory debris are evident in this fine-needle aspirate (Diff-Quik preparation).

Microbiology

The traditional as well as newer molecular approaches to the laboratory diagnosis of mycobacterial lung infection are outlined in Box 7.11. The mycobacterium is a slender, slightly curved bacillus 4 µm in length, often with a beaded appearance; the length, curvature, and beading are sometimes accentuated in *M. kansasii*.[184] In tissue sections or on smears, the Ziehl–Neelsen acid-fast stain or auramine-rhodamine fluorescent stains are most often recommended for best visualization; practice among pathologists in the use of acid-fast stains, quality control, and their perception of the value of such stains varies considerably.[185] Organisms are most often found within the area of granulomatous reaction at the immediate periphery of the necrotic zone of the granulomas or the cellular reactive process in the lining of cavities. Sections from several tissue blocks may be required to find organisms. Bacilli are rarely found in the absence of necrosis except in smears from immunocompromised

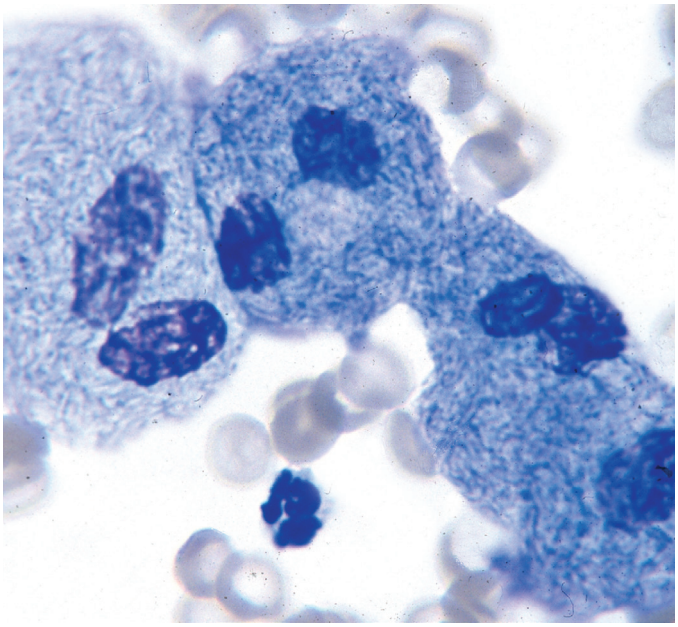


Figure 7.62 Pseudo-Gaucher histiocytes filled with myriad mycobacteria are seen as negative images in this fine-needle aspirate (Diff-Quik preparation).

Box 7.11 Laboratory Diagnosis of Mycobacterial Lung Infection

- Direct detection of organisms
  - Ziehl–Neelsen; Kinyon acid-fast stains
  - Auramine O fluorescent stain
  - Histopathologic/cytopathologic examination
  - Immunohistochemical studies
- Culture
  - Conventional solid and broth media
  - Radiometric liquid media system
  - Nonradiometric (fluorescent; colorimetric) liquid media systems
- Molecular methods
  - In situ hybridization
  - DNA amplification

patients, in which they are visible and abundant within pseudo-Gaucher cells on H&E-stained sections, or as ghosted intracellular outlines with Giemsa-type stains. Dead bacilli lose their acid-fast character but may sometimes be identified with the GMS stain. The NTM, especially the RGM, may be more sensitive to acid alcohol decolorization and may not stain well or at all with the auramine-rhodamine method.[145] There are several recent series of confirmed identification using anti-MPT64 for immunostaining of the *M. tuberculosis* complex in pathology and cytology specimens.[186-188] Differentiation of mycobacterial species in Ziehl–Neelsen–positive, formalin-fixed sections has also been achieved by in situ hybridization techniques with specific nucleic acid probes.[189-191] PCR amplification plus identification is likely to be the most sensitive technique in those cases in which the lesion is suspected to harbor mycobacteria but yields a negative result on acid-fast staining.[192] This technique may also be useful in cases in which the characteristic granulomatous pattern of inflammation is lacking or mycobacteria have been identified in acid-fast–stained sections but culture results remain negative or cultures were not performed.[193,194]

Conventional wisdom states that culture is more sensitive than direct examination; however, the literature clearly documents cases where acid-fast stains on tissue biopsies succeeded when cultures of tissue failed—an outcome that speaks to the virtue of perseverance in the face of compelling histopathologic findings.[195] Furthermore, tissue culture is prone to sampling error unless more than one site is sampled.[196] Specimens may also be smear-positive and culture-negative in patients whose disease has been treated. When only a rare bacillus is found, strict criteria must be maintained and artifactual pseudo acid-fast bacilli excluded. As a general rule, a cutoff value of three organisms for a positive result seems prudent. False-positive smears can also result from contamination with local tap water, which may harbor mycobacteria.

Traditional solid media (Lowenstein–Jensen, Petragani, and Middlebrook agars) have given way to liquid media (radiometric and nonradiometric) as the first-line systems. Liquid media have demonstrated increased recovery of mycobacteria and decreased time to detection. They also facilitate rapid and accurate susceptibility testing.[144,197] Some of these liquid systems are manual with visual inspection, whereas others are fully automated and continuously monitored. Most laboratories back up liquid systems with conventional media because no system, at this time, is capable of identifying all isolates. Commercially available DNA probes that hybridize to the mycobacterial RNA have largely replaced traditional biochemical testing, and these methods have significantly shortened the time to identification of *M. tuberculosis* and selected NTM.[198] For identification of the less frequently isolated species of NTM, for which probes are not available, it usually is necessary to send specimens to reference or state laboratories, where identification is accomplished by either biochemical testing, cell wall analysis using chromatographic techniques, or genotypic sequencing.[151]

The rapid differentiation of *M. tuberculosis* from NTM species is clinically very important because the latter are much less infectious. In this context, molecular techniques have decreased the time to detection and identification of mycobacteria to less than 3 weeks in most instances. Direct nucleic acid amplification testing of clinical specimens using commercially available PCR or transcription-mediated amplification (TMA) methods can reduce detection and identification times to less than 8 hours.[196] Immunochromatographic techniques based on the detection of secreted mycobacterial proteins have the potential to reduce these times even further.[199] Although nucleic acid amplification is faster, its overall accuracy is higher than that of smears but less than that of culture.[198] In fact, no single test at this time has sufficient sensitivity and specificity to stand alone; therefore the use of a combination of available techniques, depending on the clinical and economic setting, may be the best overall strategy.[200,201]

Interpretation of a culture isolate can sometimes be difficult. The presence of *M. tuberculosis* is always significant. *M. kansasii* is an important pathogen, and its isolation is usually also significant, although it may represent colonization. The significance of other NTM isolates is variable depending on whether there is clinical and radiologic evidence of disease. It is in this setting that histopathologic examination plays an important role. *M. avium* complex can be isolated from the respiratory tract of otherwise healthy adults as well as HIV-infected patients with no clinical or radiologic evidence of disease. The American Thoracic Society has proposed diagnostic criteria requiring that certain clinical, radiologic, and laboratory parameters be met in order to prove pathogenicity.[145]

A synopsis of the key morphologic and microbiologic attributes of mycobacterial lung infections is presented in Table 7.6. Mycobacteria produce a wide spectrum of inflammatory patterns, both granulomatous and nongranulomatous. Although the potential differential diagnostic listing is long, in practical terms major considerations are fungal infections, sarcoidosis, granulomatosis with polyangiitis, and bacterial infections that produce suppurative granulomas, such as those due to *Nocardia*, *Actinomyces*, *Brucella*, and *Francisella* species. Generally, the use of special stains and cultures will resolve most diagnostic dilemmas. Granulomatosis with polyangiitis can usually be excluded based on the

Table 7.6 Mycobacterial Pneumonias: Summary of Pathologic Findings

Assessment Component	Findings
Mycobacterial Tuberculosis	
Surgical pathology	Necrotizing (tuberculoid) granulomas
Cytopathology	Epithelioid cells and necroinflammatory debris; acid-fast bacilli detected with Ziehl–Neelsen or auramine O stains of cell block sections, more sensitive than smears
Microbiology	Acid-fast bacilli detected with Ziehl–Neelsen, Kinyon stains, or fluorescent bacilli with auramine O stain; culture on Lowenstein-Jensen and Middlebrook selective and nonselective agar and/or liquid media systems, DNA probes, or NAA for identification
Nontuberculous Mycobacteria (MOTT)	
Surgical pathology	Granulomas generally with less necrosis; often epithelioid only; unusual patterns (e.g., pseudo-Gaucher and spindle cell proliferation in immunocompromised patients)
Cytopathology	Epithelioid cells; pseudo-Gaucher or spindle cells with little or no necrosis; negative images in Diff-Quik, confirmed as acid-fast bacilli with Ziehl–Neelsen organisms sparse, except in immunocompromised patient
Microbiology	As for *Mycobacterium tuberculosis*

MOTT, Mycobacteria other than *M. tuberculosis*; *NAA*, nucleic acid amplification.

lack of the characteristic tinctorial properties of the necrosis in the granulomas and absence of vasculitis or capillaritis. When necrosis is absent or sparse in a mycobacterial infection, sarcoidosis can be difficult to exclude. Radiologic evidence of bilateral hilar adenopathy and other systemic findings of sarcoidosis often resolve the issue.

Fungal Pneumonias

The pathologist examining fungi can provide at least a provisional diagnosis at the group or genus level and make a judgment about fungal invasion or the presence of fungi as a pathogen, saprophyte, or allergen. One effective diagnostic strategy available is the rapid identification of fungi in frozen sections, routine sections, or cytologic samples.[45,202,203] This approach is especially important when opportunistic infection is being considered in the immunocompromised patient. Prudent practice requires caution in morphologic diagnosis alone; integration of microbiologic data and histopathologic findings is required.

Etiologic Agents

Nearly 70,000 fungi are known, and approximately 100 have been recovered from respiratory infections.[204] A small number are implicated as pathogenic on a consistent basis; these are listed in Box 7.12.

Histopathology

Like mycobacterial species, fungal pathogens typically produce one or more nodular lesions in the normal host (Fig. 7.63); these may become cavitary as the lesions evolve (Fig. 7.64). Inflammatory histopathologic patterns that suggest the presence of a fungal infection are summarized in Box 7.13. As is the case for other etiologic agents, there are no absolutely diagnostic patterns. Overlap is common and atypical reactions occur, ranging from overwhelming diffuse alveolar damage, little or no reaction, or sheets of organisms in the immunocompromised patient. Proximal endobronchial disease mimicking a neoplasm has also been described for various fungal species.[205] Detection of the etiologic agent in tissue by microscopic examination, ancillary tests, or culture confers specificity and significance to the listed patterns. Large spherules with endospores characteristic of *Coccidioides immitis* or yeast with large

Box 7.12 Common Fungal Pathogens in the Lung

Dimorphic fungi (mycelia at 25–30°C; yeast at 37°C)
- *Blastomyces dermatitidis*
- *Coccidioides immitis*
- *Histoplasma capsulatum*
- *Paracoccidioides braziliensis*
- *Sporothrix schenckii*
- *Penicillium marneffei*

Yeasts
- *Cryptococcus neoformans*
- *Candida* spp.

Hyaline (nonpigmented) molds
- *Aspergillus* spp.
- *Zygomycetes* organisms

Phaeoid (pigmented; dematiaceous) molds
- *Bipolaris* spp., *Alternaria, Curvularia*
- *Pseudoallescheria boydii/Scedosporium apiospermum*

Miscellaneous pathogens
- *Pneumocystis jirovecii*

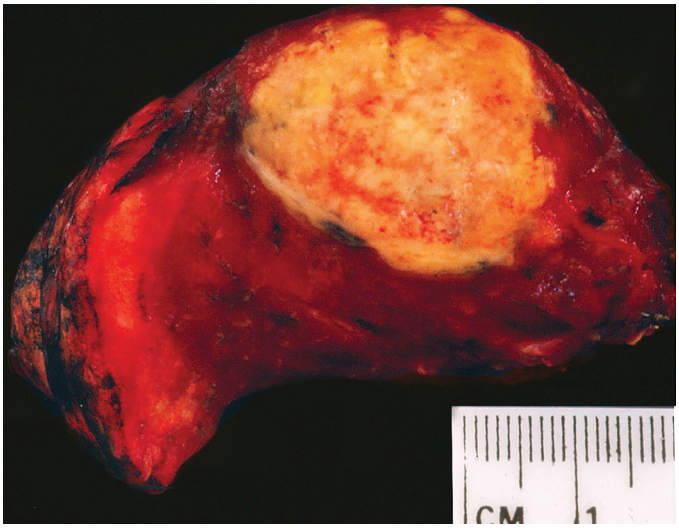


Figure 7.63 *Coccidioides* granuloma.

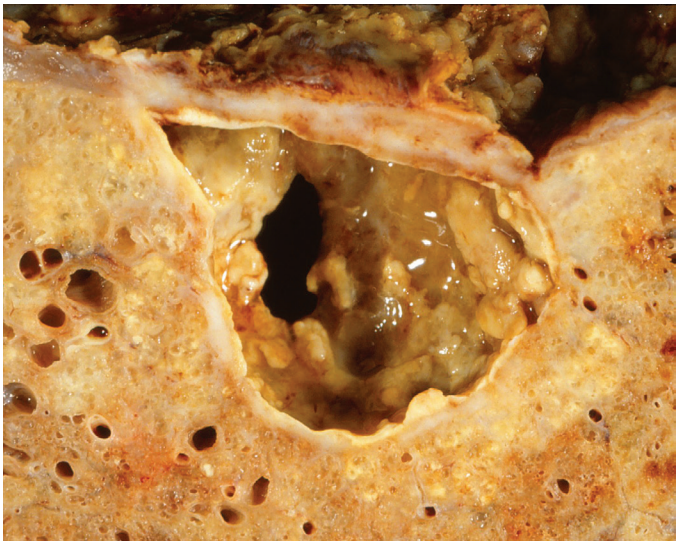


Figure 7.64 Cavitary aspergilloma.

mucoid capsules of *C. neoformans* can be diagnostic. However, atypical forms of these organisms can be misleading and challenging. For example, in aerated cavities or in the setting of bronchopleural fistula, *Coccidioides* species may produce branching septate and moniliform hyphae or immature morula-like spherules mimicking other fungi (e.g., hyaline molds and *Blastomyces dermatitidis*).[40] Similarly, *C. neoformans*, *H. capsulatum*, and *S. schenckii* have been reported to produce hyphae or pseudohyphae in tissue, whereas acapsular *C. neoformans* may mimic other yeasts or *Pneumocystis* organisms.[206]

Mycelial morphology is helpful when it is characteristic of a specific genus or group. For example, broad, sparsely septate, nonparallel, twisted or irregular in diameter, thin-walled mycelia with variable wide-angle branching characterize Zygomycetes. Progressively proliferating, regularly septate, 45-degree angle, dichotomously branching mycelia with parallel walls are typical of *Aspergillus* species (Fig. 7.65). In the case of *Aspergillus*, an important point is that only the presence of a fruiting body (conidiophore with sterigmata and conidia) permits diagnosis at the genus level, and there are many *Aspergillus* look-alikes in tissue, such as *Fusarium*, *Paecilomyces*, *Acremonium*, *Bipolaris*, *Pseudallescheria boydii*, and its asexual anamorph *Scedosporium apiospermum*.[206] Sometimes careful examination of tissue with special stains under high magnification or oil immersion will reveal clues, such as in situ sporulation, allowing a more definitive diagnosis.[41] However, these clues are often subtle, and it is important to defer to culture whenever possible.[207] Typical morphologic injury patterns and related etiologic agents are detailed next.

Box 7.13 Histopathologic Patterns in Fungal Lung Injury

- Large nodules
  - Nonnecrotizing granulomas
  - Necrotizing granulomas
  - Suppurative granulomas
  - Poorly formed granulomas
- Cavitary lesions
- Miliary nodules
- Acute bronchopneumonia
- Airway disease
- Intravascular changes/infarct
- Diffuse alveolar damage, acute and organizing
- Foamy alveolar casts

Blastomycosis

Blastomycosis, the chronic granulomatous and suppurative infection produced by *B. dermatitidis*, is essentially a North American disease, concentrated in the Ohio and the Mississippi River valleys. The prevalence of infection is particularly high in the state of Mississippi. Blastomycosis is the third most common endemic mycosis in North America, following histoplasmosis and coccidioidomycosis. It may occur in patients with normal immunity as well as those immunocompromised by diseases or medical therapy.[208-210] The isolated nodular manifestation can simulate lung cancer radiologically.[211]

The disease almost always begins in the lungs, although skin and bone are other common sites of involvement. In the lung, pathologic manifestations include focal or diffuse infiltrates, rare lobar consolidation, miliary nodules, solitary nodules, and acute or organizing diffuse alveolar damage (Box 7.14).[208,211-213] Necrotizing granulomas are characteristic and often of the suppurative type (Fig. 7.66A), but nonnecrotizing granulomas may be found as well.

The broad-based budding yeast forms of *Blastomyces* are refractile and have double-contoured walls. Multinucleate yeast cells are

Box 7.14 Histopathologic Patterns in Pulmonary Blastomycosis

- Acute pneumonia
  - Lobular
  - Lobar
- Diffuse alveolar damage
- Miliary nodule
- Solitary nodule

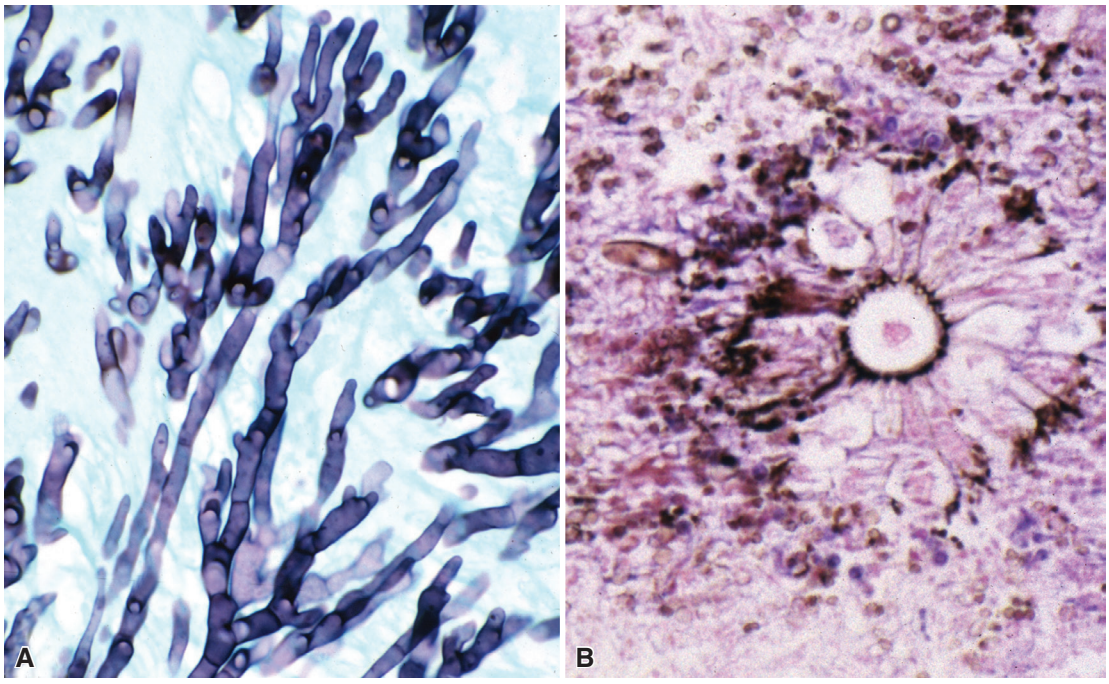


Figure 7.65 Aspergillus species. (A) Septate mycelia with 45-degree angle branching (Grocott methenamine silver stain). (B) Fruiting body (conidiophore with sterigmata and conidia) (Grocott methenamine silver stain).

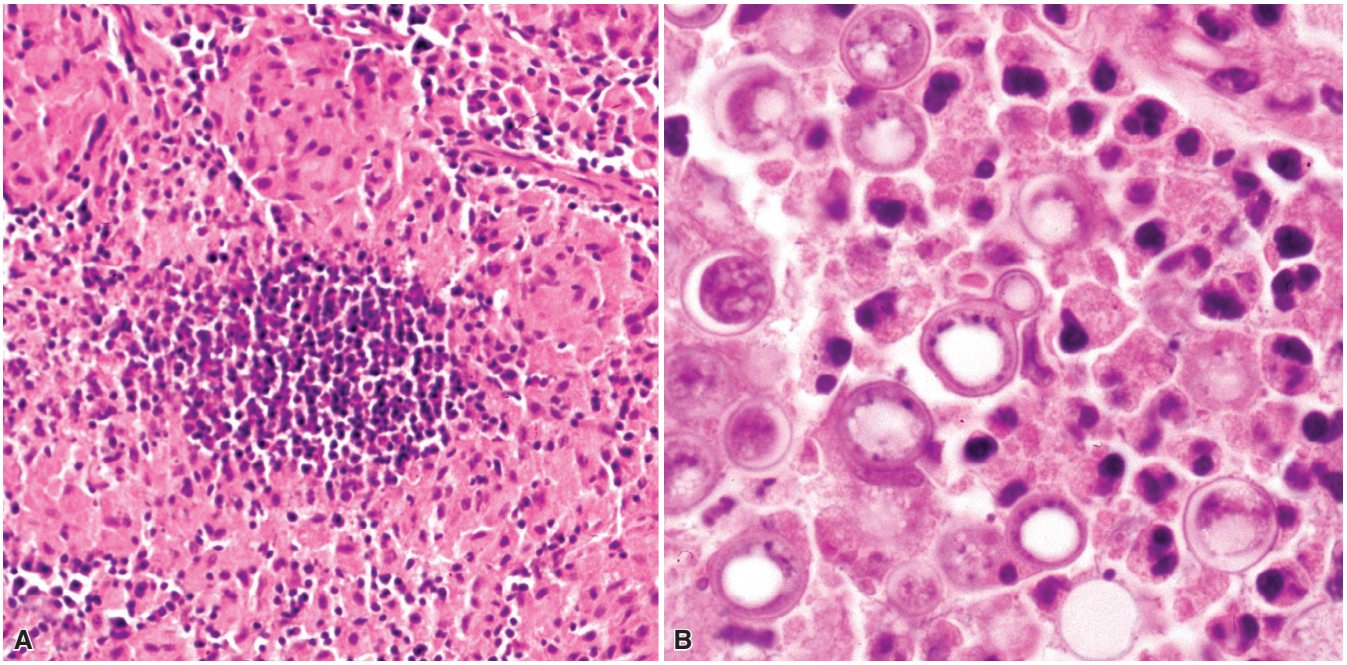


Figure 7.66 Blastomycosis. (A) The suppurative granuloma is characteristic. (B) Double-contour-wall yeast with broad-based budding.

Box 7.15 Histopathologic Patterns in Coccidioidal Respiratory Tract Disease

- Airway disease
  - Pharyngeal granuloma
  - Laryngeal granuloma
  - Tracheobronchial granuloma
- Pulmonary parenchymal disease
  - Acute pneumonia
  - Eosinophilic pneumonia
  - Chronic progressive infection
- Fibrocavitary lesions
- Bronchopleural fistula; empyema
- Solitary pulmonary nodule
- Disseminated disease
  - Miliary
  - Extrapulmonary

typically 8 to 15 µm in diameter, with some forms measuring up to 30 µm (Fig. 7.66B). These large forms can mimic small *Coccidioides* spherules,[214] whereas smaller forms ("microforms") can mimic *C. neoformans*.[213]

Coccidioidomycosis

Endemic in the Lower Sonoran life zone of the southwestern United States, the soil fungus *C. immitis* and the more recently recognized, morphologically identical, and genomically similar species *Coccidioides posadasii*[215] may be encountered outside the endemic area as a result of fomite transmission of arthroconidia (e.g., Asian textile workers handling imported Arizona cotton) or in travelers who have returned from an endemic area. Most primary pulmonary infections are asymptomatic. The exceptionally wide spectrum of pulmonary pathology in patients with clinically evident disease is outlined in Box 7.15. The true prevalence of the disease is significantly underestimated in endemic regions of the Southwest, where it is thought to account for nearly 30% of community-acquired pneumonias in some metropolitan areas.[216-219] Granulomas are characteristic and may occur with or without necrosis. Intact spherules induce fibrocaseous granulomas (Fig. 7.67A), whereas ruptured spherules may incite suppurative and bronchocentric granulomatosis (BCG)-like reactions (Fig. 7.67B).[216]

The large mature spherule (up to 40 to 60 µm in diameter) has a thick refractile wall lined by or filled with endospores; it constitutes the key diagnostic finding (Fig. 7.67C). This finding allows the distinction of coccidioidomycosis from other fungal infections such as blastomycosis and histoplasmosis, which are associated with similar histopathologic reaction patterns. In aerated cavities or in the setting of bronchopleural fistula, mycelia resembling various hyaline molds may be seen with or without a variety of mature and immature spherules (Figs. 7.20 and 7.67D). *Coccidioides* spherule look-alikes include large-variant *B. dermatitidis,* adiaspiromycosis, pollen grains, and pulses (legume seeds).

Histoplasmosis

Histoplasmosis, the most common pulmonary fungal infection worldwide, is endemic in the Ohio and the Mississippi River valleys of North America and is the most common endemic mycosis in AIDS.[220] The clinical forms of *H. capsulatum* infection[55,203,221,222] are presented in Box 7.16. The histopathologic correlates include a spectrum ranging from an exudative to a granulomatous process influenced by such factors as the fungal burden and the immune status of the patient. In patients with normal defenses, the characteristic histopathology is dominated by well-formed necrotizing and nonnecrotizing granulomas occurring as solitary lesions indistinguishable from other granulomatous infections. Other presentations include miliary nodules (Fig. 7.68), cavitary lesions, and laminated fibrous solitary nodules (Fig. 7.69) that may be partially calcified (sometimes referred to as *residual granulomas*). In patients with impaired immunity, striking macrophage response with numerous intracellular yeasts is a characteristic pattern (Fig. 7.70A). The exudative lesion resembles acute lobular pneumonia with fibrinopurulent exudates.[223]

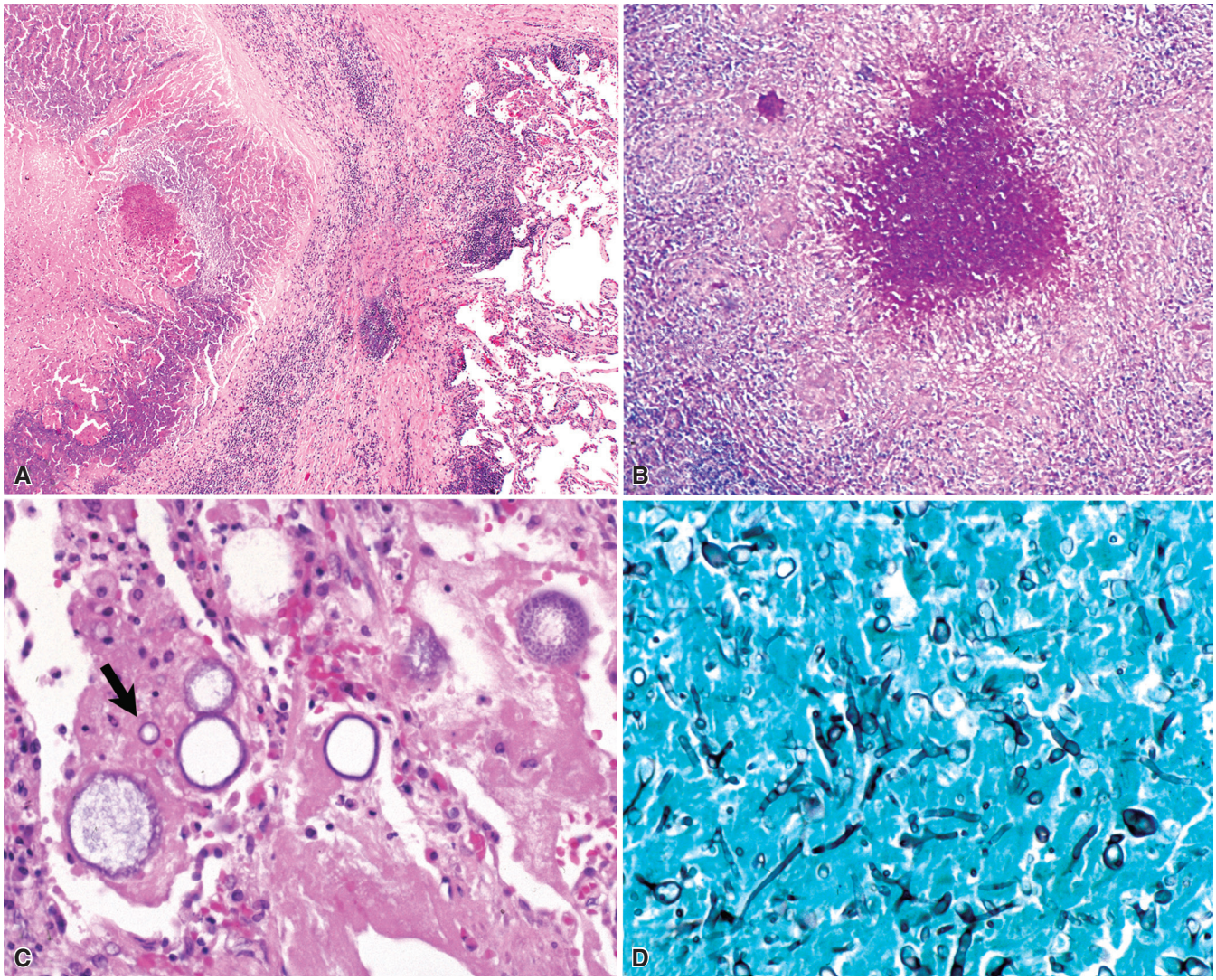


Figure 7.67 Coccidioidomycosis. (A) Fibrocaseous granuloma. (B) Bronchocentric granulomatosis–like granuloma. (C) *Coccidioides immitis*. Both small *(arrow)* and large spherules with and without endospores can be seen. (D) Biphasic pattern with mycelia and spore-like swellings (Grocott methenamine silver stain).

Box 7.16 Clinical Forms of Pulmonary Histoplasmosis

- Benign, self-limited
- Acute
  - Acute respiratory distress syndrome
  - Acute self-limited, upper lobe (in smokers with emphysema)
- Chronic
  - Asymptomatic pulmonary nodule, with or without calcification ("histoplasmoma")
  - Progressive (chronic cavitary) pulmonary
- Progressive disseminated
- Mediastinal
  - Lymphadenopathy
  - Middle lobe syndrome
  - Fibrosis

Reprinted with permission from Travis WD, Colby TV, Koss MN, et al. Lung infections. In: King D, ed. *Atlas of Non-Tumor Pathology, Fascicle 2. Non-neoplastic Disorders of the Lower Respiratory Tract.* Washington, DC: American Registry of Pathology; 2002:539–728, Table 12.6.

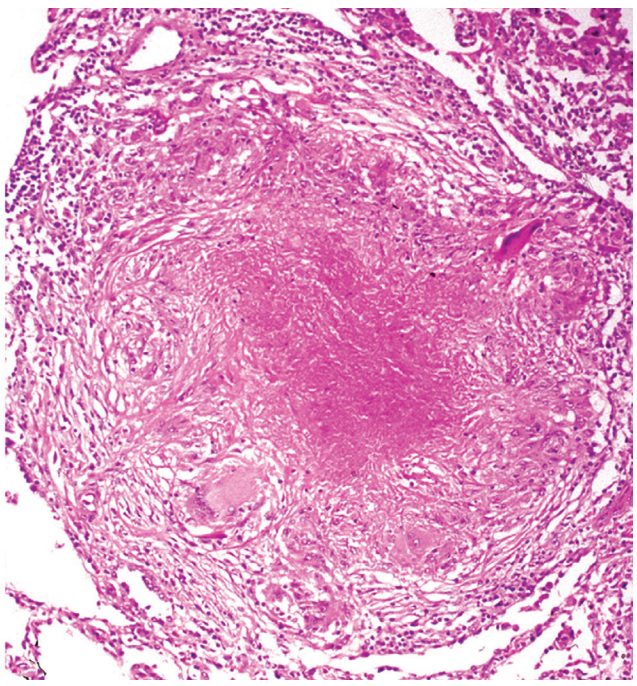


Figure 7.68 Histoplasmosis. Miliary nodule with central zone of necrosis invested by epithelioid histiocytes, multinucleate giant cells, and outer collarette of lymphocytes.

H. capsulatum organisms are yeasts (2 to 5 µm), with narrow-based unequal budding (Fig. 7.71; Fig. 7.70B). They may be seen on H&E-stained sections and, when numerous, appear as small refractile ovoid structures within macrophages. Yeasts typically occur in clusters but may be rare in old granulomas. A search for budding organisms in these situations may prove futile. Sometimes, yeasts may have dark-staining foci resembling *Pneumocystis* organisms. Also, some yeast cells may be surrounded by a clear space and may be mistaken for *Cryptococcus*.[55] Other look-alikes include *Candida* species, *P. marneffei*, capsule-deficient cryptococci, intracellular *B. dermatitidis*, and Hamazaki-Wesenberg bodies.

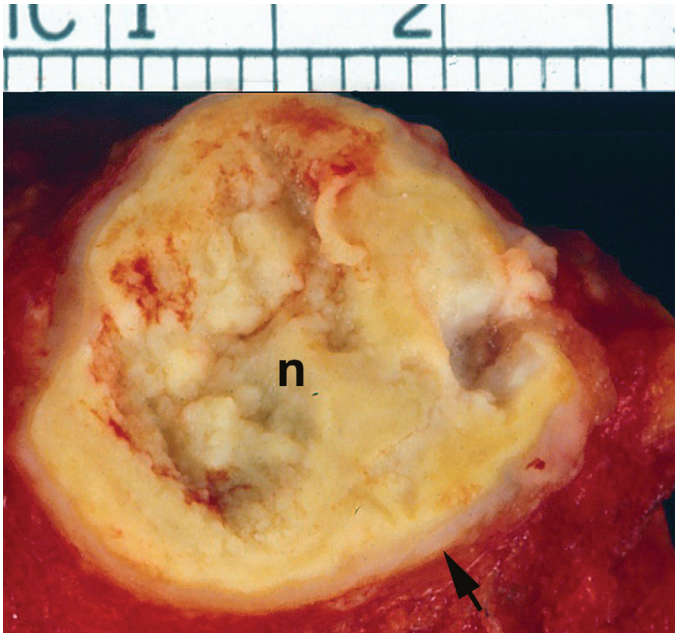


Figure 7.69 Histoplasmoma. Characteristic gross appearance of the persistent granulomatous nodule. Note the fibrous wall *(arrow)* surrounding caseous necrosis *(n)*.

Paracoccidioidomycosis (South American Blastomycosis)

Seven clinical forms occur, but they rarely cause lung infections in North America. In areas of high endemicity, such as Brazil, the several forms can mimic malignancy or sarcoidosis.[224,225] The histopathology resembles that of other mycoses and can be exudative or granulomatous. *Paracoccidioides braziliensis* appears as a large spherical yeast (10 to 60 µm) with multiple buds attached by narrow necks (a "steering wheel" or "ship's wheel" appearance).[226] When budding is sparse, look-alikes include *H. capsulatum* with small intracellular forms, *B. dermatitidis* and capsule-deficient cryptococci for medium-sized forms, and *C. immitis* or *C. posadasii* for large forms.

Sporotrichosis

Infection by *Sporothrix schenckii* is usually confined to the skin, subcutis, and lymphatic pathways, but the organism can disseminate to the lungs. Rarely, *S. schenckii* is a primary pulmonary pathogen.[227] The organism can produce cavitary disease in the form of a single lesion. Infection may be bilateral and apical, progressive and destructive, or it may be identified clinically as a solitary pulmonary nodule. Microscopically, caseous and suppurative granulomas (Fig. 7.72A) occur with variable numbers of round to oval, small (2 to 3 µm), narrow budding yeast (Fig. 7.72B) or cigar-shaped forms.[228] Nonnecrotizing granulomas also occur. Asteroid bodies are an important clue, especially when organisms are sparse, as is often the case. Look-alikes include *H. capsulatum*, acapsular cryptococci, *Candida* organisms, and Hamazaki-Wesenberg bodies.

Penicilliosis

Southeast Asia is the endemic setting of the unique dimorphic fungus *Penicillium marneffei*. The disease it produces is not seen in North America except in travelers, especially immunocompromised persons. It is a common opportunistic infections in AIDS patients in Southeast Asia and a significant clue to the presence of AIDS in that area.[229] The respiratory tract is the portal of entry, with pulmonary infiltrates and disseminated disease, especially involving the skin. Microscopically, alveolar macrophages stuffed with spherical to oval yeast-like cells (2.5 to 5 µm) are seen, each with a single transverse septum; short hyphal

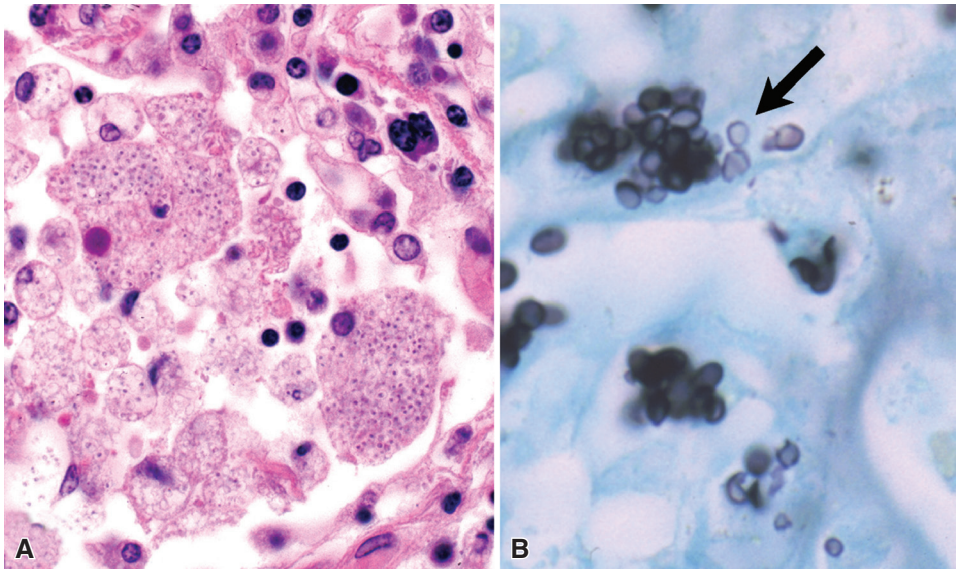


Figure 7.70 Histoplasmosis in an immunocompromised patient. (A) Numerous *Histoplasma capsulatum* yeast cells in macrophages. (B) Clusters of *H. capsulatum* yeast cells in macrophages. Note the narrow-based budding *(arrow)* (Grocott methenamine silver stain).

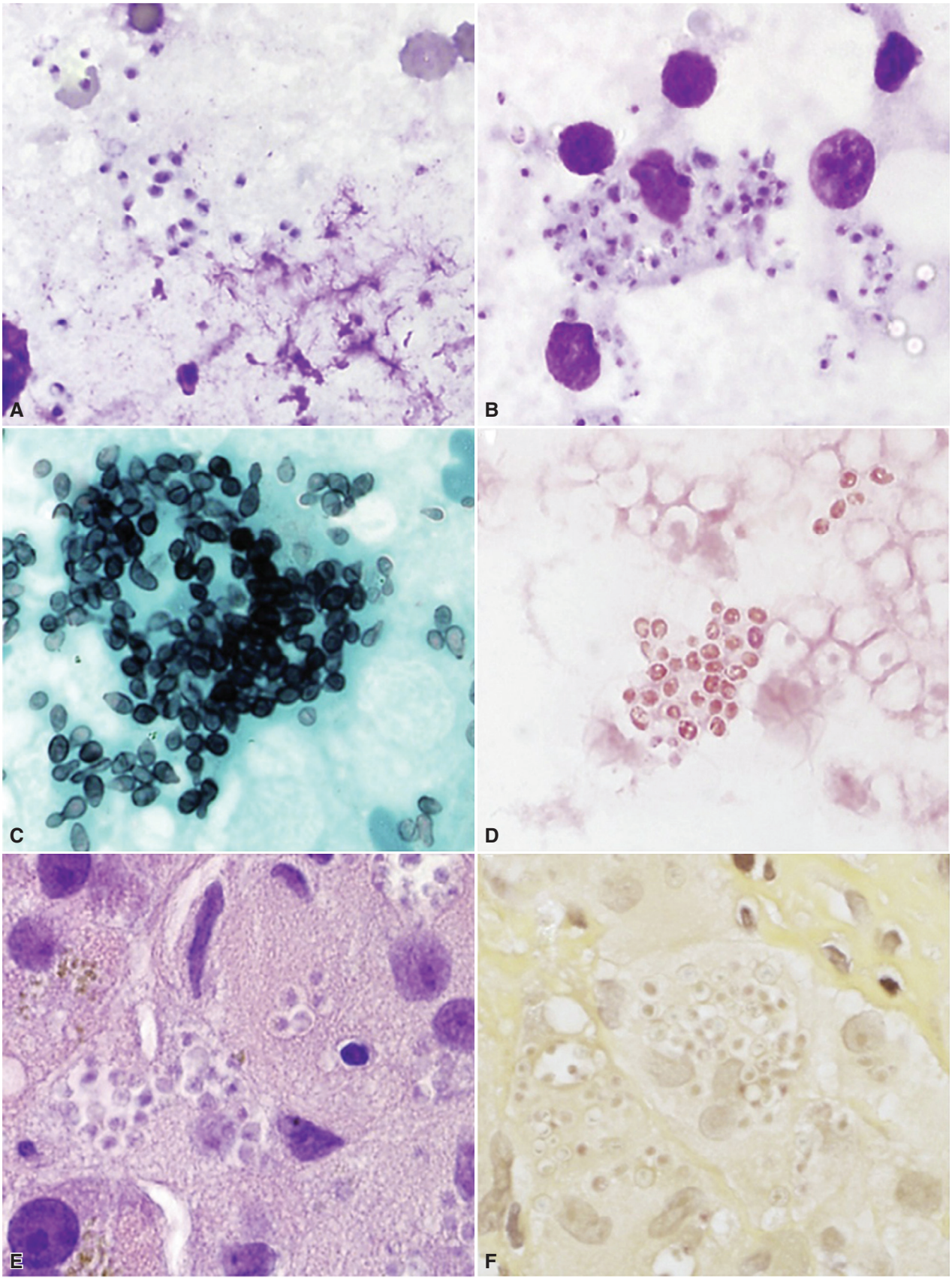


Figure 7.71 Tinctorial and morphologic attributes of *H. capsulatum* in stained clinical specimens. (A) Diff-Quik: BAL fluid smear showing extracellular yeast forms. (B) Giemsa stain: tissue touch preparation demonstrating numerous yeast within a histiocyte and in the surrounding space. (C) GMS stain: abundant black-colored yeast cells in a tissue touch preparation. Both extracellular and intrahistiocytic forms are seen. (D) Gram stain: red-colored yeast cells in a blood culture smear. (E) Hematoxylin and eosin stain: liver biopsy containing numerous intracellular and extracellular yeast forms. Note the presence of colorless halos surrounding the yeast. (F) Mucicarmine stain: yeast forms are barely visible without the aid of increased contrast.

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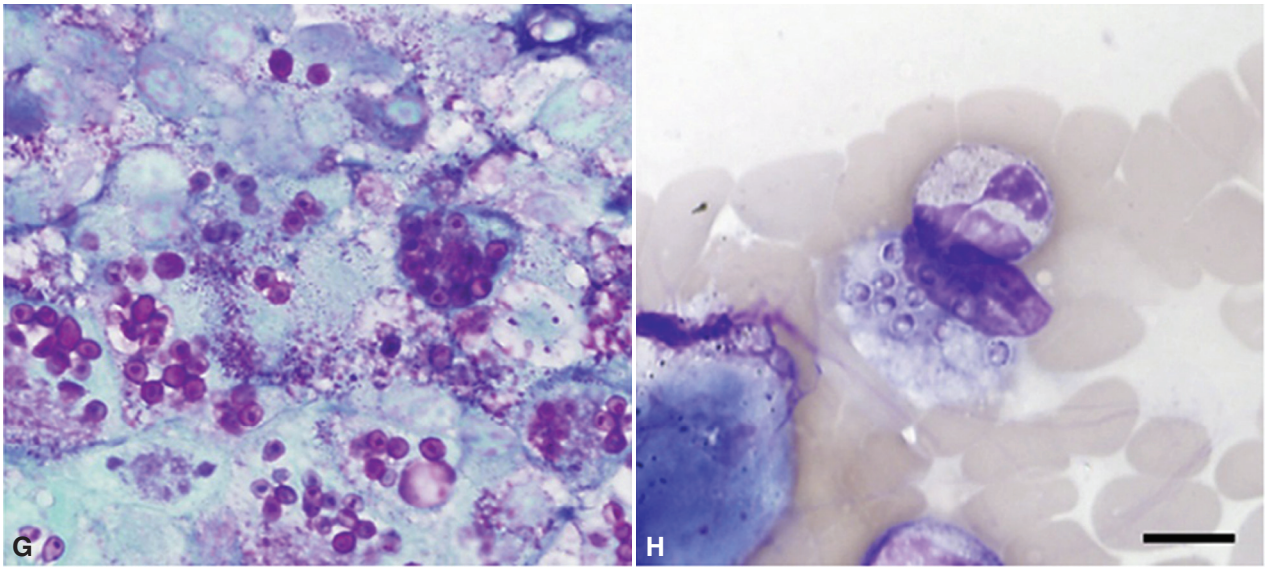

Figure 7.71, cont'd (G) Periodic acid–Schiff stain: magenta-colored yeast cells are evident scattered throughout the epidermis of a skin biopsy specimen. (H) Wright-Giemsa: yeast forms evident within a monocyte in a peripheral blood smear. Scale bar, 10 mm; original magnification, ×1000. (From Wheat LJ, Azar MM, Bahr NC, et al. Histoplasmosis. *Inf Dis Clin NA*. 2016;30:216.)

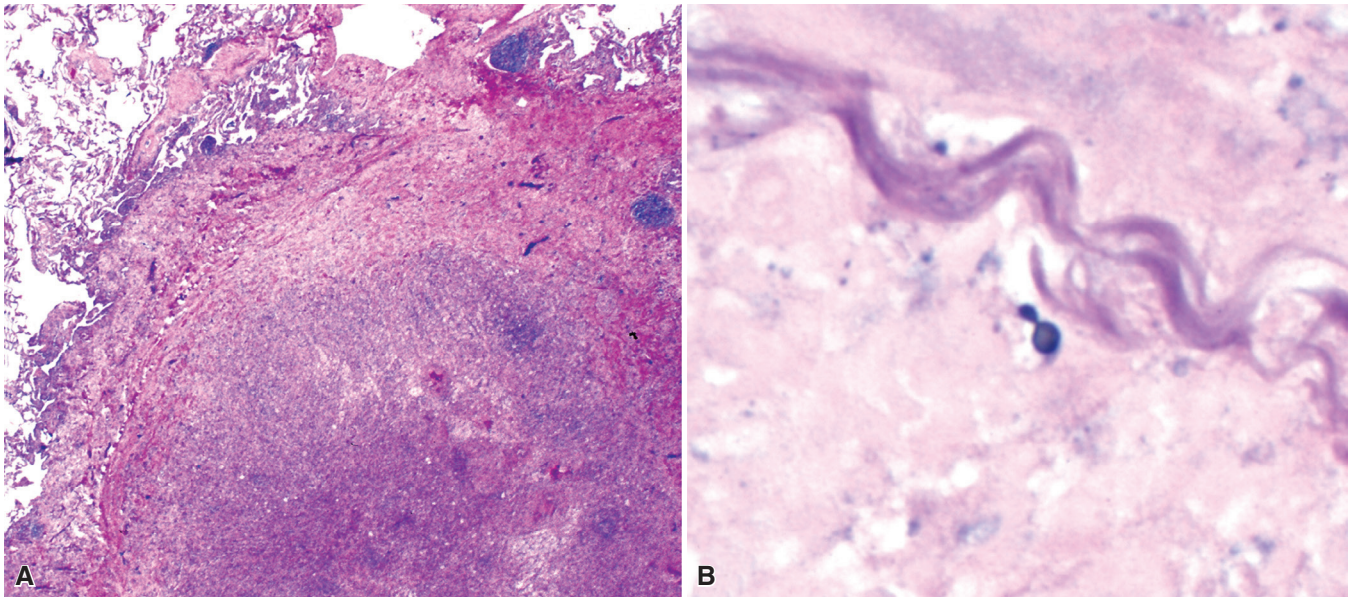

Figure 7.72 Sporotrichosis. (A) Cavitary granuloma manifesting as a solitary pulmonary nodule. (B) A rare, oval, narrow budding yeast (Grocott methenamine silver stain).

forms and elongated, curved "sausage" forms may be formed in necrotic and cavitary lesions.[230,231] The septum distinguishes it from *H. capsulatum*, its look-alike.[229,232]

Cryptococcosis

C. neoformans is a ubiquitous, facultative intracellular yeast. Pulmonary cryptococcosis occurs worldwide but has a particularly high incidence in the United States.[233] The pathogenicity and histopathologic features of lung infection depends largely on the patient's immune status, as illustrated earlier in Fig. 7.7 and summarized in Box 7.17. In the normal host, a substantial proportion of cryptococcal infections are asymptomatic, others are symptomatic, with infiltrates or nodules. Immunocompromised patients are almost invariably symptomatic and often develop disseminated disease with a predilection for the brain and meninges. Pulmonary injury patterns include single or multiple large nodules, segmental or diffuse infiltrates, cavitary lesions, and miliary nodules. Normal hosts most often develop nodules comprising fibrocaseous granulomas (Fig. 7.73A), or granulomatous pneumonia (Fig. 7.73B). Immunocompromised patients are more likely to have histiocytic (Fig. 7.73C) or mucoid infiltrates without inflammation (Fig. 7.73D).

The cryptococcal organisms are round yeast forms ranging in diameter from 2 to 15 μm, with an average size of 4 to 7 μm. Cryptococcal yeasts are visible on H&E-stained sections as pale gray to light blue structures, frequently with attached smaller buds. They often occur in clusters and can sometimes be found within giant cells.[203] The mucicarmine stain highlights the capsule (Fig. 7.74A); but with capsule-deficient forms

(Fig. 7.74B) the pleomorphic appearance can be confused with that of other yeast forms (e.g., *H. capsulatum*, *B. dermatitidis*, *S. schenckii*) and sometimes *Pneumocystis*.

The lungs of patients with the most severe immunodeficiency may show myriad yeasts in alveolar septal capillaries (Fig. 7.74A), with little if any intraalveolar reaction[234]; this form of the disease may also be associated with mucoid pneumonia.[235] The mucoid pneumonia (Fig. 7.75A) of cryptococcal infection can be confirmed with mucin stains such as Alcian blue (Fig. 7.75B). Another microscopic pattern recently described in HIV-infected patients is the inflammatory spindle cell pseudotumor, a lesion much more commonly associated with mycobacterial infection.[236]

Box 7.17 Histopathologic Patterns in Cryptococcal Lung Disease

In order of associated decrease in immune function:
- Fibrocaseous granuloma
- Granulomatous pneumonia
- Histiocytic pneumonia
- Mucoid pneumonia
- Intracapillary cryptococcosis

Reprinted with permission from Mark EJ. Case records of the Massachusetts General Hospital. *N Engl J Med.* 2002;347:518–524.

Candidiasis

Candida organisms are yeasts that can produce pseudohyphae and are the most common invasive fungal pathogens in humans. Secondary *Candida* pneumonia is relatively common, but primary *Candida* pneumonia is rare in other than immunocompromised patients in the intensive care unit.[55] In general, *Candida albicans* is the most frequently isolated of the more than 100 known species, which include a few rare and emerging human pathogens. *Candida glabrata* and *Candida tropicalis*, together with *C. albicans*, account for 95% of bloodstream infections, the principal route for the acquisition of *Candida* pneumonia.[237] A non–blood-borne route to pneumonia results from aspiration of organisms from a heavily colonized or infected oropharynx. When the infection is blood-borne, miliary nodules with a necroinflammatory center and a hemorrhagic rim reflect an intravascular distribution of fungi. In the case of aspiration, the organisms may be found in the airways associated with an alveolar filling pattern of bronchopneumonia (Fig. 7.76A)[238] or, much less commonly, a bronchocentric granulomatosis pattern.

In tissue sections, oval budding yeast-like cells (blastoconidia) 2 to 6 µm in diameter may appear with pseudohyphae that constrict at points of budding, creating the impression of bulging rather than parallel walls (Fig. 7.76B). The pseudohyphae branch at acute angles and can overlap in width with the true hyphae of *Aspergillus*, from which they must be distinguished. Among the medically important species, *Candida glabrata*

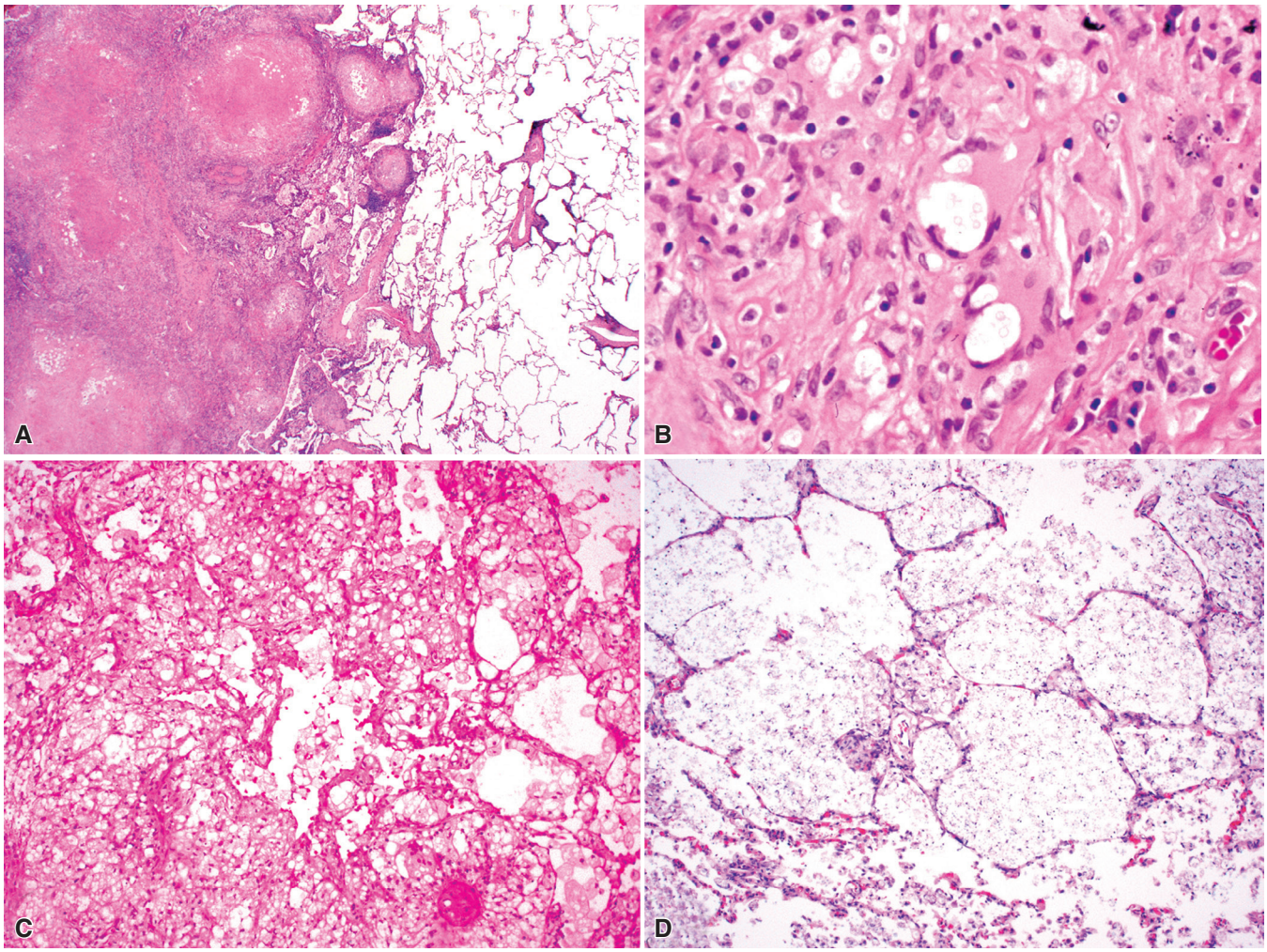

Figure 7.73 Cryptococcosis. (A) Solitary pulmonary nodule with small satellite granulomas. (B) Granulomatous pneumonia with clusters of pale staining yeast in clear spaces surrounded by histiocytes and multinucleated giant cells. (C) Histiocytic pneumonia. (D) Mucoid pneumonia with no inflammatory cell reaction.

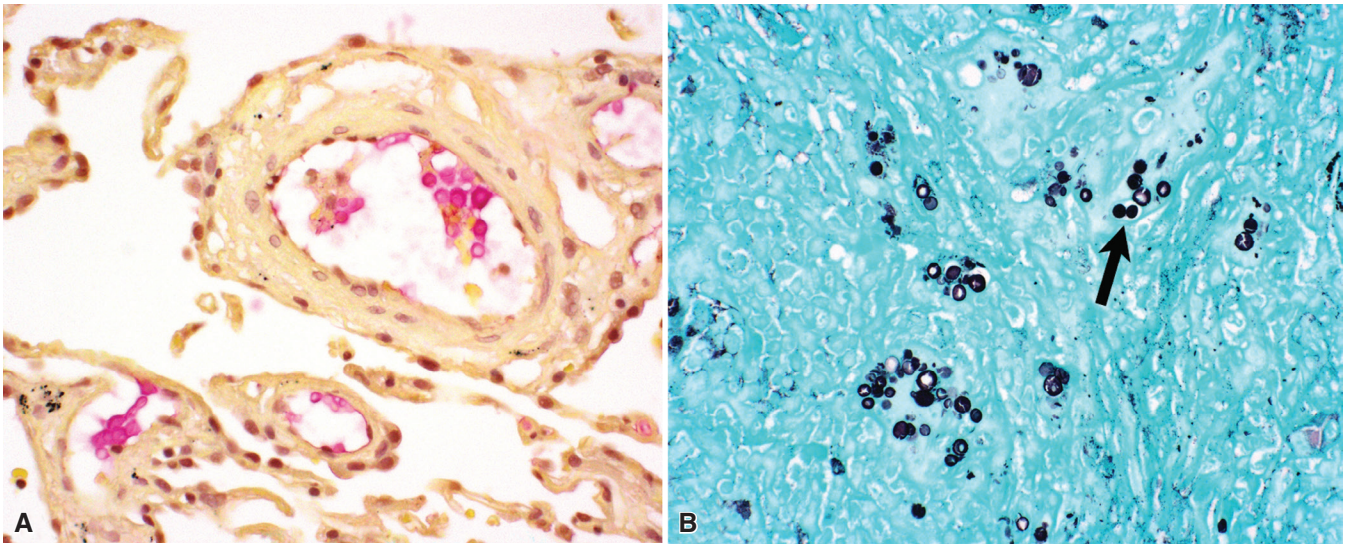

Figure 7.74 (A) Intravascular cryptococcus. Yeast cells with stained capsules (mucicarmine stain). (B) Capsule-deficient cryptococcus (Grocott methenamine silver stain).

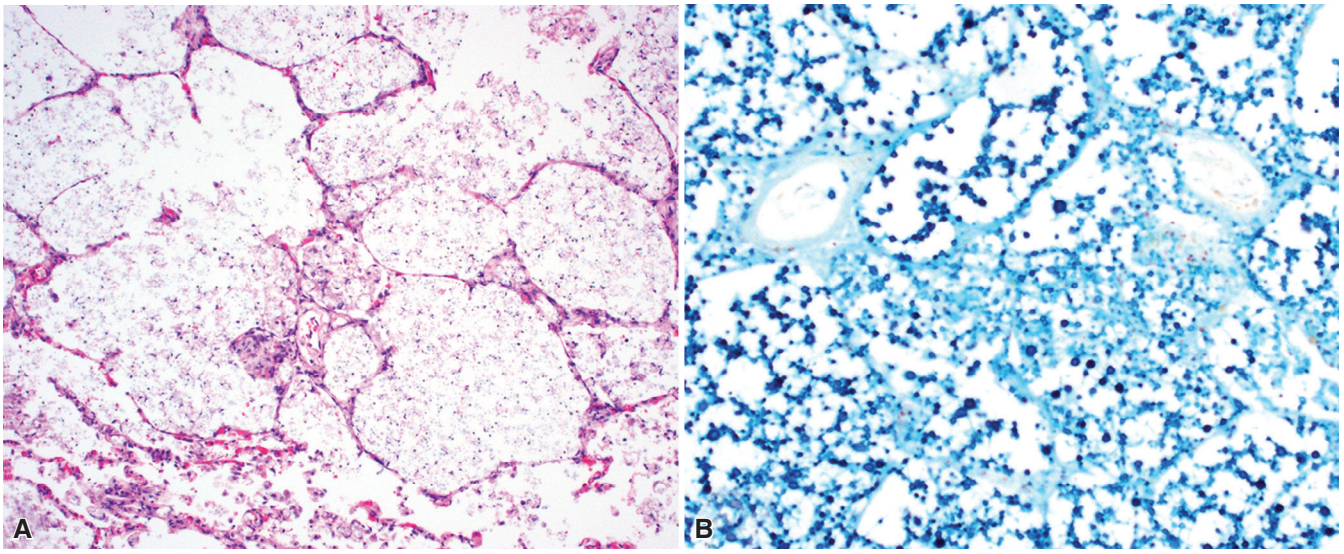

Figure 7.75 Cryptococcal mucoid pneumonia. (A) Myriad blue-gray yeast cells in mucoid matrix. (B) Alcian blue mucin stain accentuates the mucoid matrix.

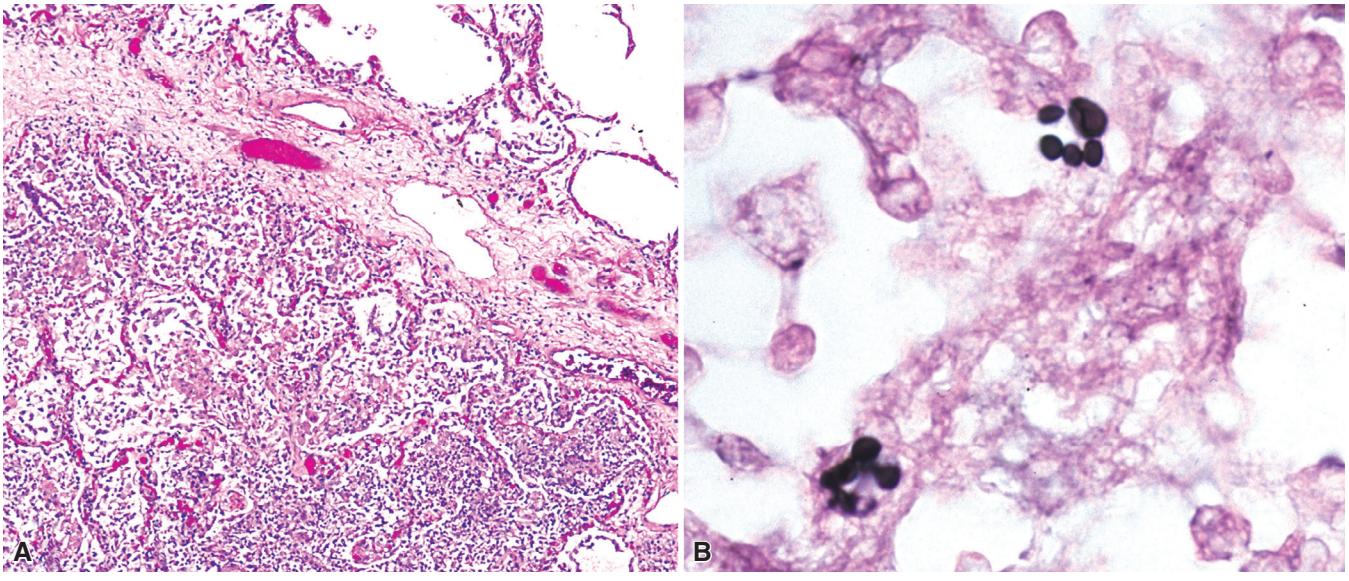

Figure 7.76 (A) *Candida* bronchopneumonia. (B) *Candida* yeast cells—blastoconidia (Grocott methenamine silver stain).

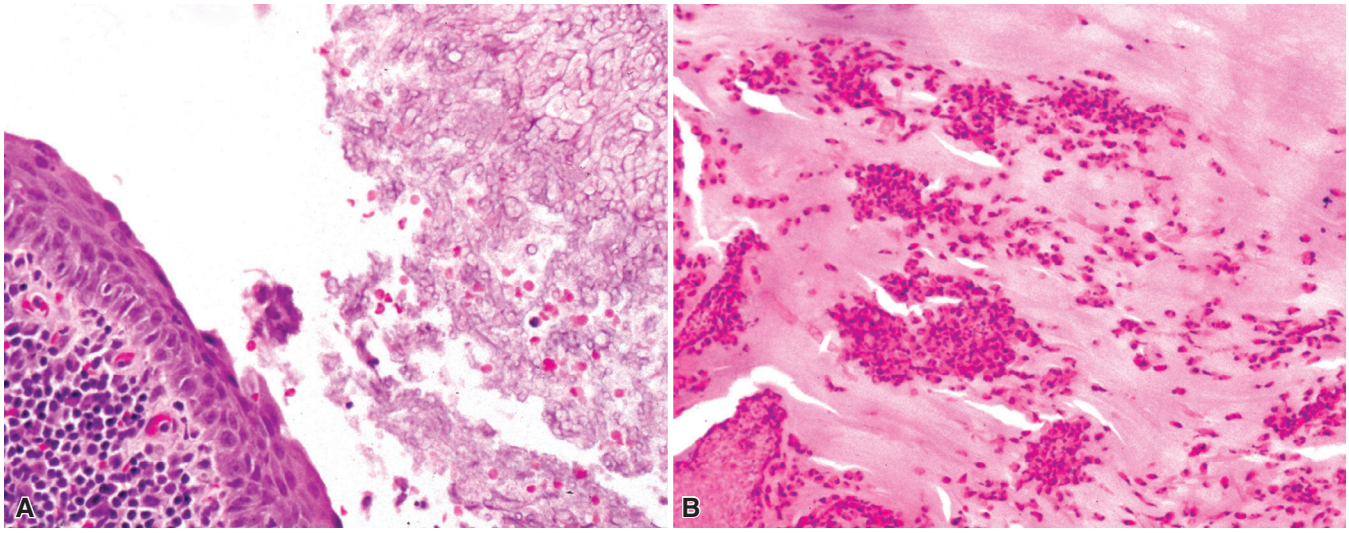

Figure 7.77 (A) Aspergillosis fungus ball. (B) Allergic bronchopulmonary aspergillosis. Intraluminal allergic mucin with laminated clusters of eosinophils can be seen in inspissated basophilic mucin with scattered Charcot–Leyden crystals.

(formerly *Torulopsis glabrata*) and *Candida parapsilosis* produce only yeast cells in tissue, in contrast with most other *Candida* species, which produce both yeast and pseudohyphae.[203]

Other look-alikes include *H. capsulatum*, *Trichosporon beigelii*, and *Malassezia furfur*, depending on whether pseudohyphae or yeast forms alone are present. They can be distinguished from *Histoplasma* by their extracellular location and Gram stain positivity. *T. beigelii* tends to be somewhat larger and more pleomorphic. *Malassezia* is clinically associated with parenteral nutrition, Intralipid, and indwelling catheters. Pulmonary lesions include pneumonia, mycotic thromboemboli, infarcts, and vasculitis. *M. furfur* may be found in small arteries, where the organisms appear as small 2- to 5-µm yeast-like cells. They form distinctive unipolar broad-based buds but no pseudohyphae.[55]

Aspergillosis

Aspergillus species and other hyaline and dematiaceous molds have emerged as significant causes of morbidity and death in the immunocompromised host. Worldwide, species of *Aspergillus* are the most common invasive molds. They are the second most common fungal pathogens after *Candida* species but, in contrast with *Candida*, are more commonly isolated from the lung. Several species are recognized, but *Aspergillus fumigatus* is the one most often seen in the clinical laboratory and most often isolated from the lungs of immunocompromised patients.[239] Respiratory aspergillosis can be classified into a colonizing or saprophytic form (intrabronchial and preexisting cavity fungus ball, Fig. 7.77A); hypersensitivity forms (allergic bronchopulmonary aspergillosis, including mucoid impaction of bronchi and hypersensitivity pneumonitis; Fig. 7.77B); and invasive disease (minimally invasive–chronic necrotizing or angioinvasive–disseminated, Box 7.18).[55,240-243] Invasive disease (Fig. 7.78) tends to occur in immunocompromised patients, including those with prolonged neutropenia, transplant recipients (especially hematopoietic stem cell and lung transplants), advanced AIDS, and the inherited immune deficiency disorder referred to as *chronic granulomatous disease of childhood*. The clinicopathologic features of invasive disease reflect these host-associated risk factors.[244] In patients with neutropenia, a characteristic angioinvasive pattern occurs, with intravascular spread resulting in hemorrhagic infarcts (Fig. 7.79). In the nonneutropenic patient, the necroinflammatory pattern tends to lack this angioinvasive feature.[245] Some cases defy categorization (e.g., bronchocentric and miliary patterns; Fig. 7.80) or may be hybrids of infection and hypersensitivity.[246]

Box 7.18 Histopathologic Patterns in Pulmonary Aspergillosis

- Colonization
  - Fungus ball
- Hypersensitivity reaction
  - Allergic bronchopulmonary aspergillosis
  - Eosinophilic pneumonia
  - Mucoid impaction
  - Bronchocentric granulomatosis
  - Hypersensitivity pneumonitis
- Invasive
  - Acute invasive aspergillosis
  - Necrotizing pseudomembranous tracheobronchitis
  - Chronic necrotizing pneumonia
  - Bronchopleural fistula
  - Empyema

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Microscopically, septate hyphae, dichotomously branched at a 45-degree angle, have uniform, consistent width (3 to 6 µm) without constrictions at points of septation. When numerous, as in some angioinvasive lesions and fungus balls, these features can be readily appreciated in H&E-stained sections. Fruiting heads of *Aspergillus* (shown earlier in Fig. 7.65) are sometimes formed in cavities. Oxalate crystals, visible in plane-polarized light (Fig. 7.81), are an important clue to *Aspergillus* infection when hyphae cannot be identified.

Look-alikes include various hyaline molds such as Zygomycetes and *Candida* species as well as *Pseudallescheria boydii*.[247] Another look-alike is *Fusarium* species. Fusariosis is an emerging mycosis in the immunocompromised host, and *Fusarium* is the second most common opportunistic pathogen after *Aspergillus* species in immunosuppressed patients with hematologic malignancies.[248] The clinical and pathologic features in the lung and at sites of dissemination mimic those of aspergillosis, and the mycelia are essentially indistinguishable. Isolation in culture, immunohistochemistry, or molecular techniques, such as in situ

hybridization or PCR amplification, is required for definitive diagnosis. Other previously uncommon but newly emerging hyaline molds that may be difficult to distinguish from *Aspergillus* in tissue are *Paecilomyces*, *Acremonium*, *Scedosporium*, and *Basidiobolus*.[237,249,250]

Zygomycosis

The taxonomic organization of the fungal phylum Zygomycota includes the class Zygomycetes, which is subdivided into two orders: Mucorales and Entomophthorales. These orders contain the agents of human zygomycosis.[251] The order Mucorales includes the genera *Absidia*, *Apophysomyces*, *Rhizopus*, *Rhizomucor,* and *Mucor*, from which the often taxonomically incorrect term *mucormycosis* is derived. In fact, most infections are due to *Rhizopus* and *Absidia* species.[252] The zygomycete species share clinical and pathologic features with invasive *Aspergillus* species, being angiotropic and capable of inducing hemorrhagic infarcts with sparse inflammation.

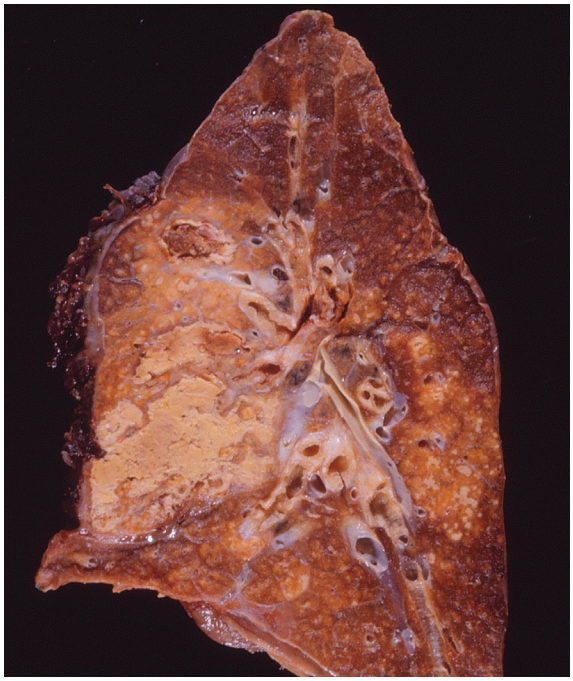

Figure 7.78 Resected lung specimen from an immunocompromised patient with necrotizing *Aspergillus* pneumonia.

Clinical syndromes produced by these fungi include rhinocerebral, pulmonary, cutaneous, and gastrointestinal infections with a predilection for neonates.[253] Hematopoietic malignancies and diabetes mellitus with acidosis underlie most cases of pulmonary infection in children and adults.[254,255] Box 7.19 lists a broad spectrum of pulmonary diseases that includes solitary or multiple and bilateral nodular lesions, segmental or lobar consolidation, cavitary lesions, fistulas, and infarcts (Figs. 7.82 and 7.83); direct extension into mediastinal, thoracic soft tissue, chest wall, and diaphragm; chronic tracheal and endobronchial infection; and fungus balls similar to those seen with aspergilloma.[256] An endobronchial syndrome with a propensity for blood vessel erosion has also been described, sometimes resulting in fatal hemoptysis.[257]

Hyphae are broad (6 to 25 μm), thin-walled, and pauciseptate (Fig. 7.84A). They display variation in width, with twisted, nonparallel contours and random wide-angle branching nearing 90 degrees.[203] They also have a tendency to fragment more commonly than *Aspergillus* organisms, which tend to retain their elongated sweeping profiles. Additional features include variability in tinctorial staining in H&E sections, ranging from basophilia to eosinophilia. In frozen sections, hyphae may show weak staining, and they often have a bubbly or vacuolated appearance.[256] In addition to being angiotropic, they are neurotropic.[258] In lesions exposed to air, the hyphae may form ovoid or spherical thick-walled chlamydoconidia, within or at the terminal ends (Fig. 7.84B).[259] Look-alikes at the lower-width range include *Aspergillus* and other *Aspergillus*-like hyaline molds. The pseudohyphae of *Candida* species can sometimes be similar.

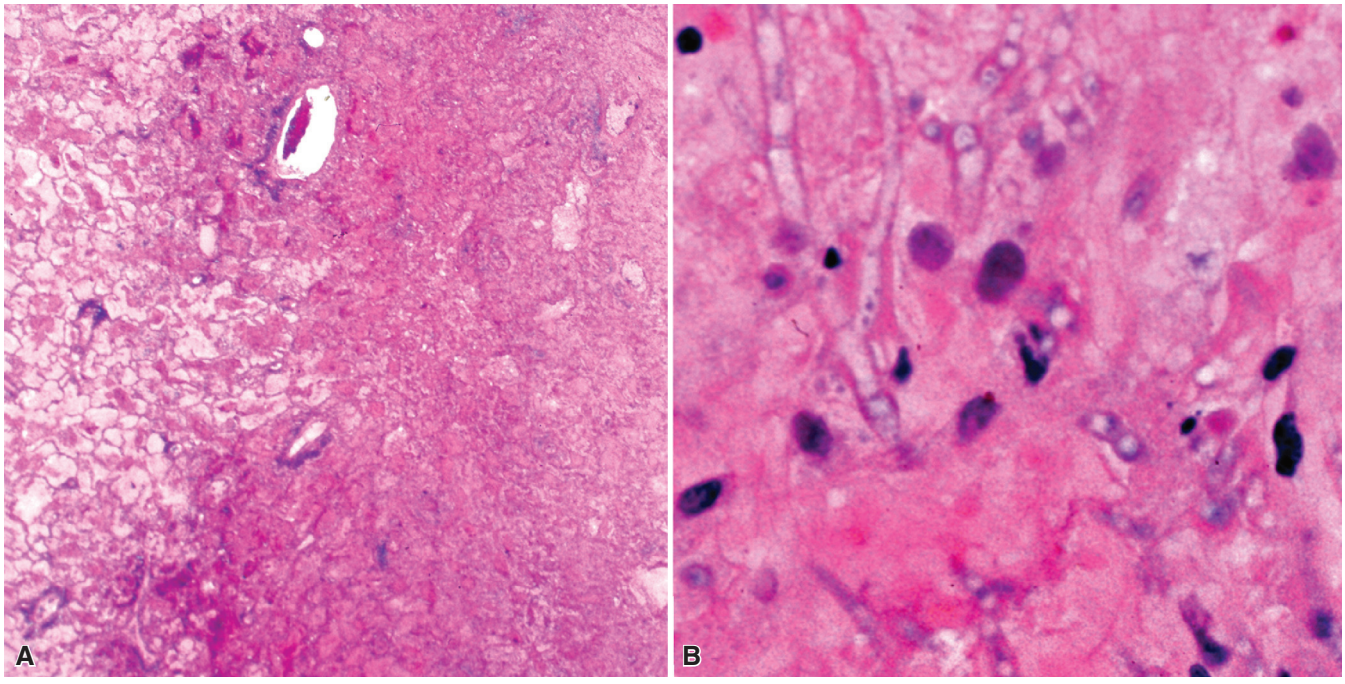

Figure 7.79 Invasive aspergillosis. (A) Hemorrhagic infarct. (B) 45-degree angle branching septate hyphae.

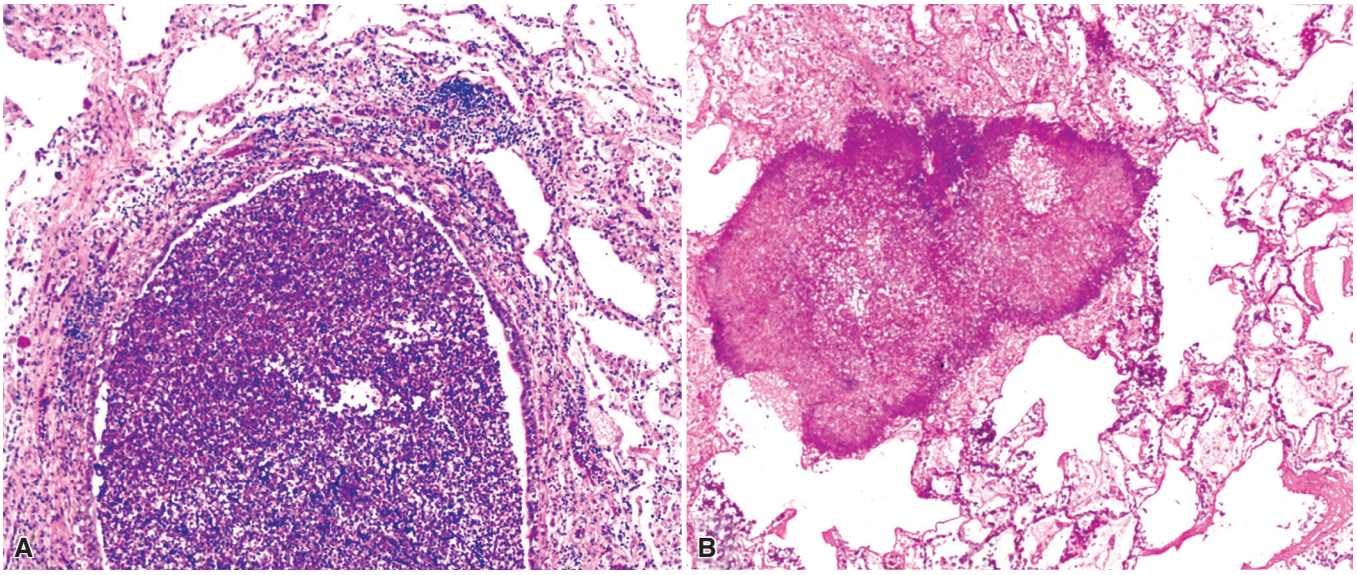

Figure 7.80 Bronchocentric aspergillosis. (A) Bronchiole expanded and filled with purulent exudate. (B) Miliary aspergillosis. Colony of organisms with hyaline membranes evident at periphery of the image *(lower right)*.

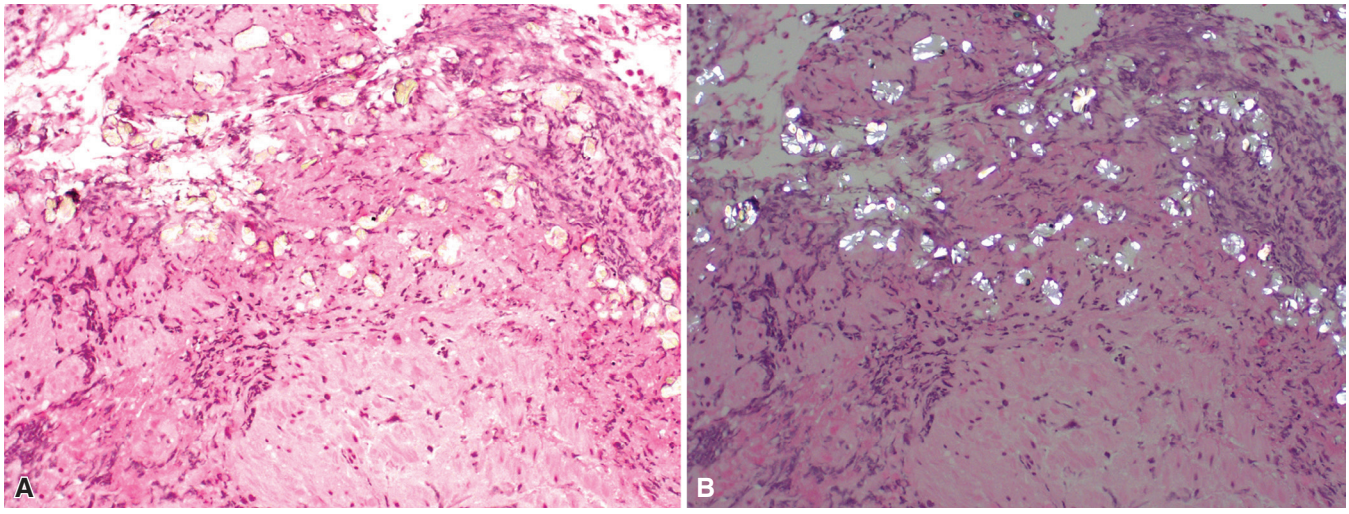

Figure 7.81 (A) Pale yellow oxalate crystal sheaths in necroinflammatory debris. (B) Birefringent oxalates seen under polarized light.

Box 7.19 Histopathologic Patterns in Pulmonary Zygomycosis

Acute lobular or lobar pneumonia
Nodules
Cavities
Endobronchial mass
Fistulas
Infarcts
Thoracic soft tissue/mediastinum
Fungus ball

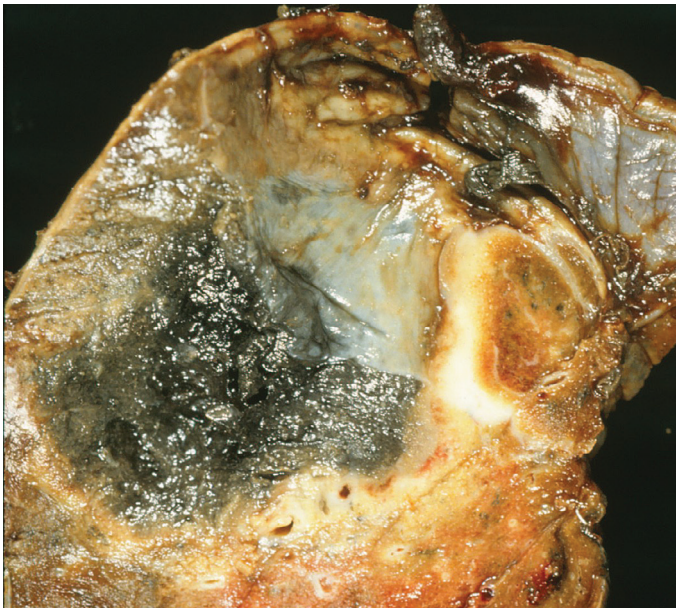

Figure 7.82 Resected lung specimen from patient with necrotizing pneumonia caused by zygomycosis.

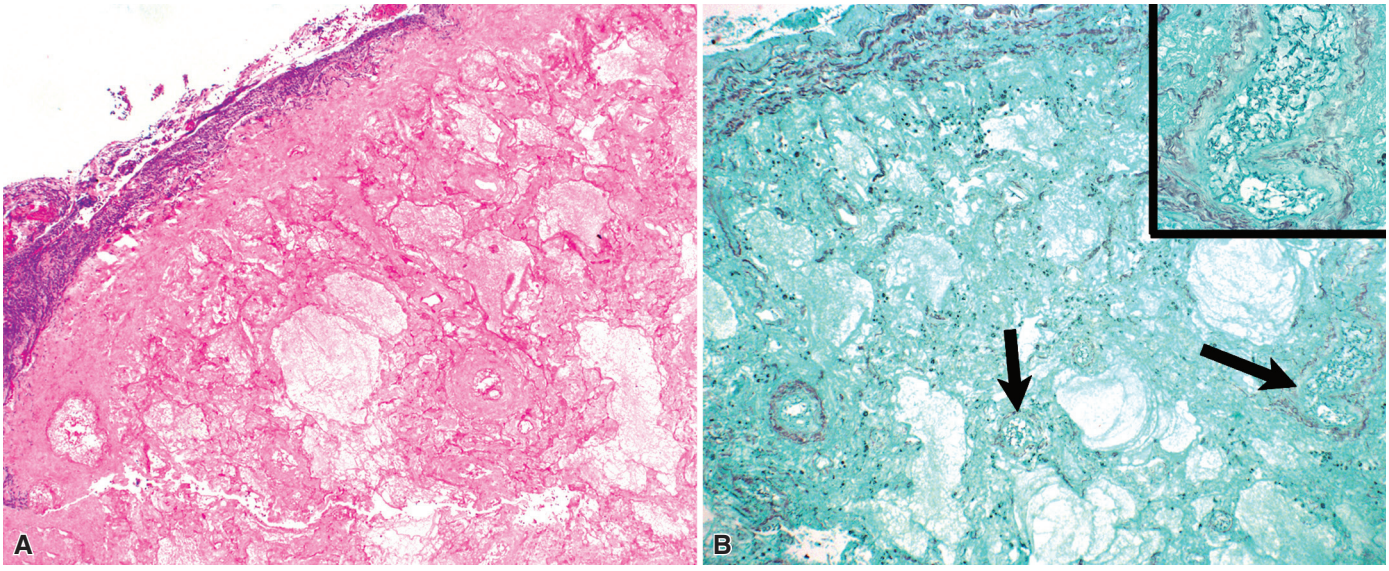

Figure 7.83 Zygomycosis. (A) Nodular infarct. (B) Intravascular organisms *(arrows)*. Vessel at right arrow is shown at high magnification *(inset)* (Grocott methenamine silver stain).

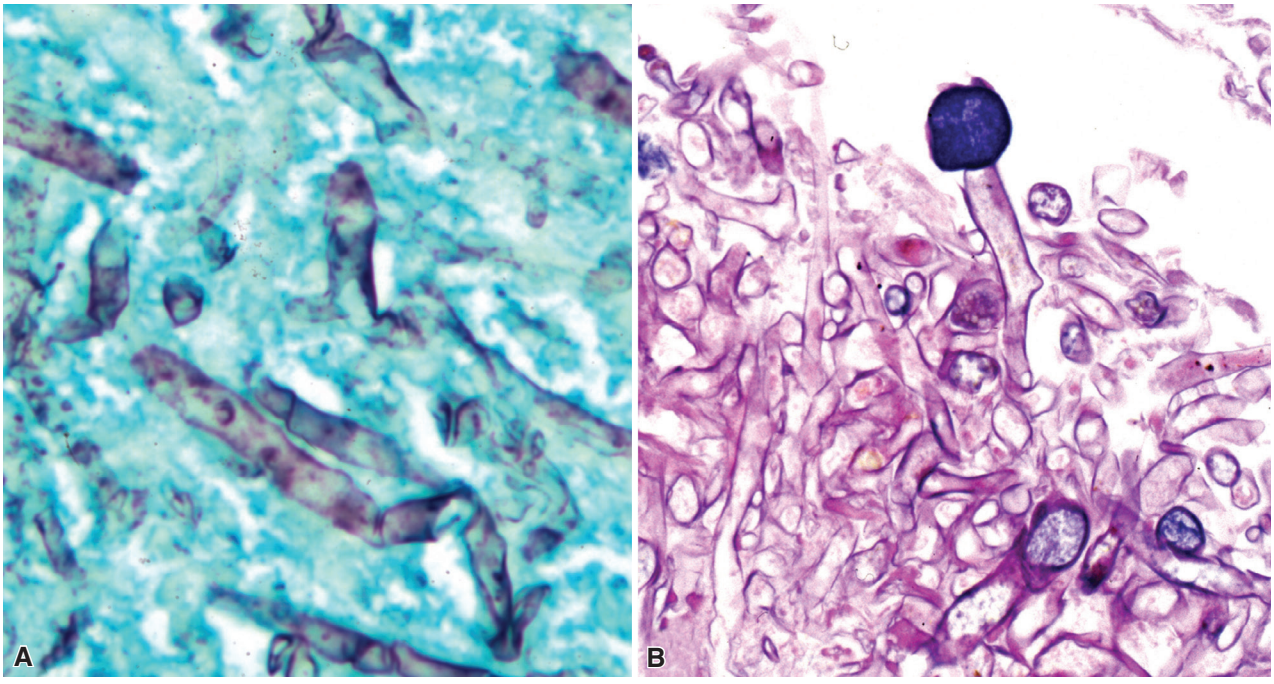

Figure 7.84 Zygomycosis. (A) Twisted pauciseptate, broad mycelia characteristic of Zygomycetes (Grocott methenamine silver stain). (B) Endobronchial zygomycosis with chlamydospores.

Phaeohyphomycosis

A few genera of dematiaceous molds produce infections resembling those of *Aspergillus*, including allergic bronchopulmonary disease (Fig. 7.85A) and bronchocentric granulomatosis patterns.[260,261] The more than 80 genera and species of these saprophytes, which occur naturally in wood, soil, and decaying matter, include *Bipolaris*, *Exserohilum*, *Xylohypha*, *Alternaria*, and *Curvularia*, among others.[203] The unique appearance of these fungi is due to their cell wall melanin content. In the allergic mucin or other deposits of necroinflammatory debris, the phaeoid (dark brown- to black-pigmented) hyphae (2 to 6 µm in diameter) are generally sparse but can resemble *Aspergillus* and other hyaline molds, especially when lightly pigmented or nonpigmented. Typically only small mycelial fragments are seen, which may be mistaken for artifacts, sometimes with terminal swellings resembling chlamydoconidia (Fig. 7.85B). The dematiaceous agents of subcutaneous forms of chromoblastomycosis appear as pigmented muriform cells in granulomas, and they do not form mycelia. Chromoblastomycosis is rarely encountered in the lung. Another *Aspergillus* look-alike is *P. boydii*, an organism that is sometimes grouped with the dematiaceous fungi. *P. boydii* usually exhibits a more ragged, disorganized, and densely clustered pattern of mycelia. Clinically, localized disease may be cured by excision alone; systemic disease is often refractory to treatment.[262]

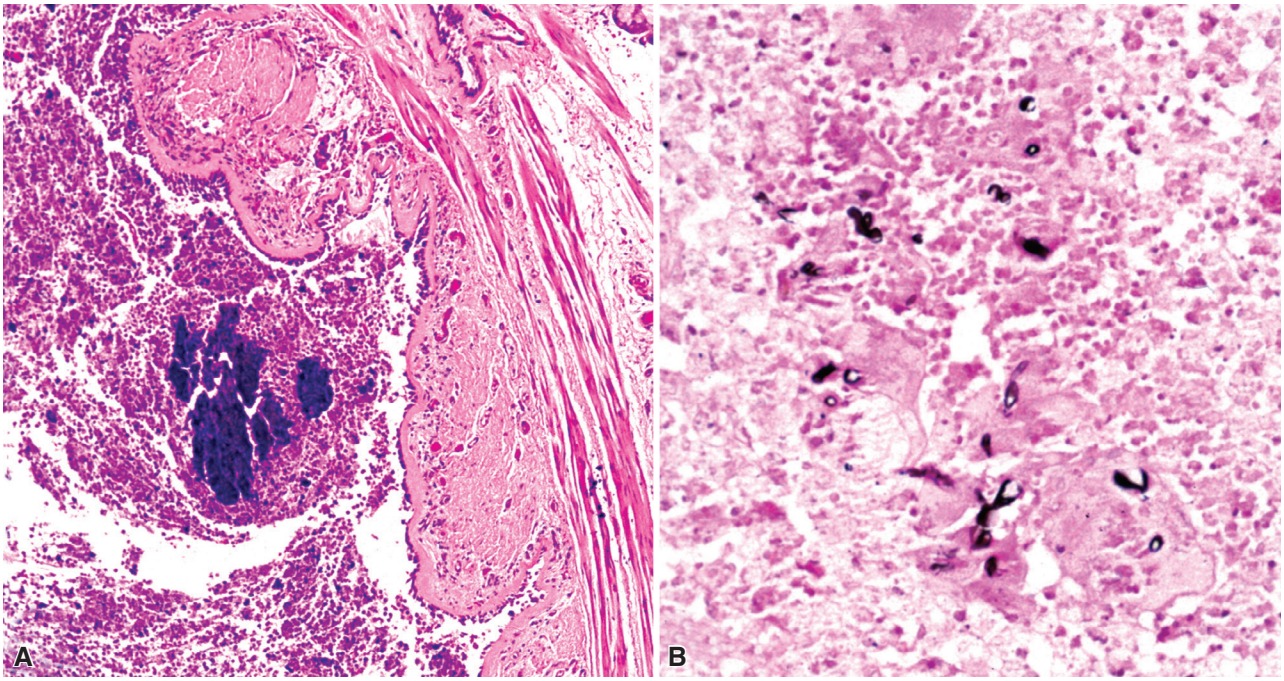

Figure 7.85 Allergic bronchopulmonary fungal disease. (A) Ectatic bronchus with thick eosinophilic basement membrane and intraluminal necroinflammatory debris. (B) Mycelial fragments of *Bipolaris* organisms (Grocott methenamine silver stain).

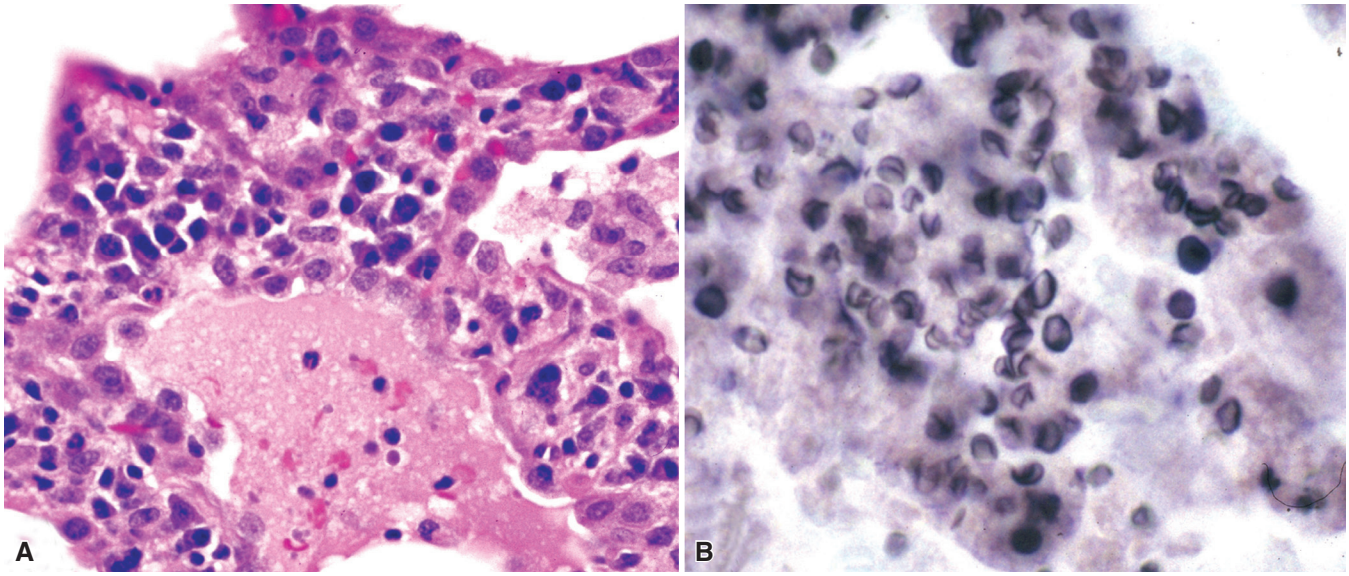

Figure 7.86 *Pneumocystis* pneumonia. (A) Lymphoplasmacytic interstitial infiltrate and intraalveolar foamy alveolar cast. (B) Numerous yeast-like cells of *Pneumocystis jirovecii* of various shapes (Grocott methenamine silver stain).

Pneumocystosis

The face of *Pneumocystis* pneumonia continues to change. Once considered to be a protozoan, this organism is now classified as a fungus, and the species infecting humans has been renamed *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*).[263] Once a disease of malnourished or leukemic children, today *Pneumocystis* infection is identified most commonly in patients with defective immunity, especially AIDS, or those on immunosuppressive therapies for hematopoietic malignancies, organ transplants, and collagen vascular diseases. With the success of contemporary therapy for AIDS, the pathologist is now more likely to encounter the disease in the latter group of patients in whom it is apt to be more subtle.[264] The classic pattern during the HIV epidemic was the foamy alveolar cast (Fig. 7.86) with moderate to numerous organisms, type II pneumocyte hyperplasia, and a scant to moderate interstitial lymphoplasmacytic infiltrate.[265,266]

In recent years a number of atypical and unusual patterns have been described that are worth recognizing.[55,267,268] These are listed in Box 7.20. *P. jirovecii* infection can mimic any lung injury pattern, ranging from acute diffuse alveolar damage with hyaline membranes (Fig. 7.87) and minimal or no foamy exudates to an organizing phase with sparse organisms. There is also a spectrum of granulomatous infection, both nonnecrotizing and necrotizing, that may overlap morphologically with

Box 7.20 Histopathologic Patterns in Pulmonary *Pneumocystis* Infection

Foamy alveolar cast
Diffuse alveolar damage
"Id" reaction (minimal-change reaction)
Granulomas
Miliary disease
Vascular invasion/vasculitis/infarct
Lymphoid interstitial pneumonia
Cavities and cysts
Subpleural blebs and bullae
Microcalcification

mycobacterial or other fungal infections, particularly histoplasmosis (Fig. 7.88). Cavitary disease, solitary pulmonary nodules that may be relatively fibrotic, cysts, and dystrophic calcification are also described.[268-270]

Microscopically, the three life stages of the organism are still referred to by protozoan terminology as sporozoites, trophozoites, and cysts. The cyst is the most common form seen by pathologists. On silver stains the cyst is seen as an oval (4 to 7 μm) yeast-like cell that may be collapsed, helmet-shaped, or variably crescentic. The intracystic dot or paired–comma structures are important keys to distinguishing *P. jirovecii* cysts from look-alikes such as *Histoplasma*, the capsule-deficient form

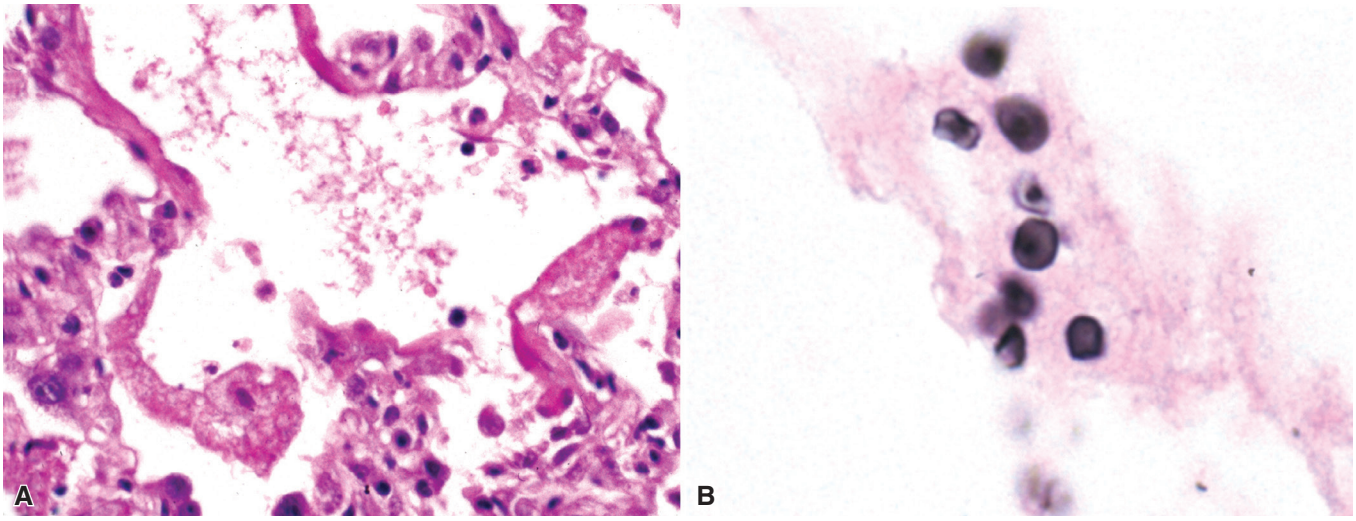

Figure 7.87 *Pneumocystis* pneumonia. (A) Diffuse alveolar damage pattern with hyaline membranes. (B) Cysts in hyaline membrane (Grocott methenamine silver stain).

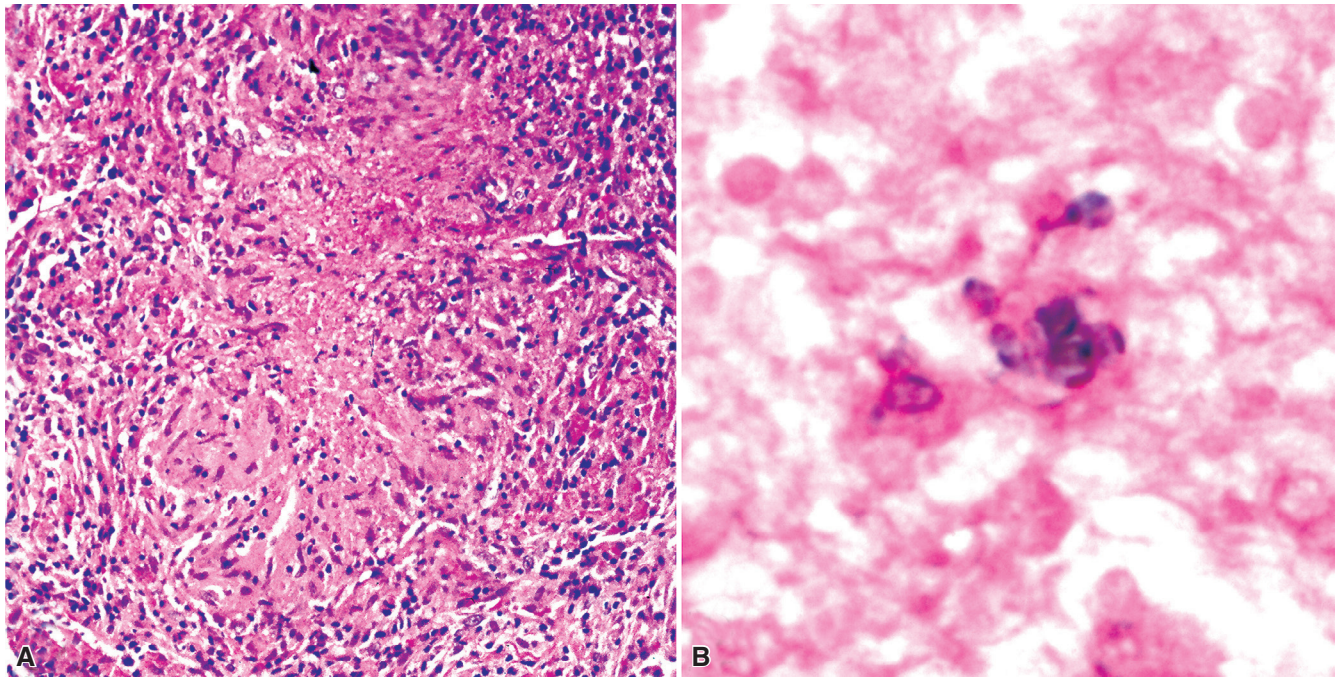

Figure 7.88 *Pneumocystis* pneumonia. (A) Miliary granuloma with central necrosis. (B) Sparse organisms in granuloma (Grocott methenamine silver stain).

Table 7.7 Morphologic Features of Selective Yeast Forms

	Small			Intermediate		Large
Feature	***Candida***	***Pneumocystis***	***Histoplasma***	***Cryptococcus***	***Blastomyces***	***Coccidioides***
Size (μm)	3–4	5–8	2–5	5–15	8–20	20–200
Shape	Oval	Pleomorphic	Oval	Pleomorphic	Round	Round
Budding	None	None	Narrow-based	Narrow-based	Broad-based	None
Wall thickness	Thin	Thin	Thin	Thin	Thick	Thick
Hyphae/pseudohyphae	Common; characteristic	Absent	Rare	Rare	Rare	Occasional
Other features	Single and chains	Intracystic body Trophozoite forms	Intracellular Refractile	Mucicarmine + capsule Acapsular forms	Double-contour wall	Endospores, immature spherules

Modified from Chandler FW, Watts JC. *Pathologic Diagnosis of Fungal Infections.* Chicago: ASCP Press; 1987:87.

of *Cryptococcus*, *Candida* species, and even overstained red blood cells. Sporozoites and trophozoites are seen to best advantage in touch imprints and cytologic preparations of respiratory samples.

Cytopathology

Many of the fungal pathogens involving the respiratory tract can be detected by cytologic techniques in sputum samples, bronchial washings and brushings, BAL fluid samples, and needle aspirates.[46] The aspirates and other samples can also be submitted for culture and ancillary studies.[271] The four most common yeast forms—*C. neoformans*, *C. immitis* or *C. posadasii*, *H. capsulatum*, and *B. dermatitidis*—must be distinguished from each other, and *P. jirovecii* can also enter the differential diagnosis.[45] Morphologic features of these organisms are often better visualized in cytologic preparations than in tissue sections, usually permitting a rapid and definitive diagnosis on smears prepared using routine stains (Papanicolaou, Diff-Quik, and H&E). More specific fungal stains (GMS, Gridley, and Fontana–Masson) can often be held in reserve.

Amorphous granular debris and epithelioid cells characterize many necrotizing granulomas. Typically a background of neutrophils is seen when suppurative granulomas are aspirated. *Histoplasma* infections may manifest an epithelioid or phagocytic cell population. Cryptococcal infections can be similar or may be associated with little or no accompanying inflammation in the immunocompromised patient.

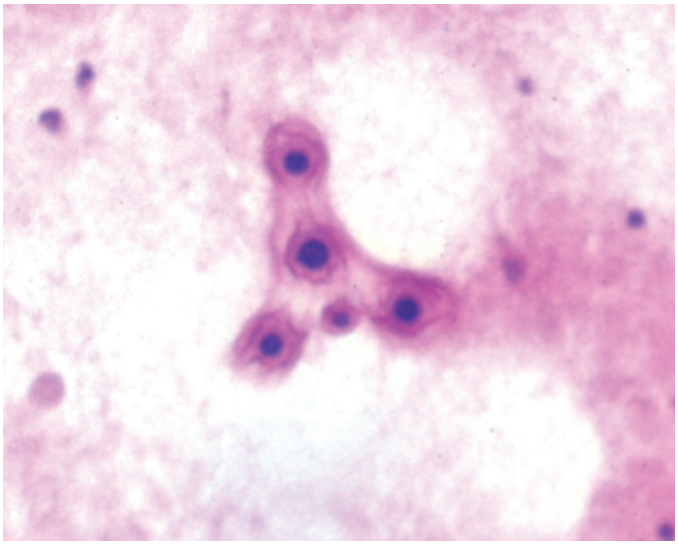

Figure 7.89 *Cryptococcus neoformans.* In this fine-needle aspirate, clusters of yeast cells resembling "spare tires" are invested by capsule in a sparse inflammatory background (alcohol-fixed).

Cytology of Common Yeast Forms

Morphologic features of some of the more common yeast forms that the pathologist may encounter in cytologic material are presented in Table 7.7.

C. neoformans organisms are seen as are single budding yeast forms with a narrow, pinched-off base, approximately 4 to 7 μm in diameter but ranging in size from 2 to 15 μm. In needle aspirates, the mucoid capsule investing the yeast imparts a "spare tire" appearance (Fig. 7.89).

B. dermatitidis organisms are refractile, double-contoured yeast forms and range in diameter from 8 to 15 μm with broad-based budding (Fig. 7.90). An internal amorphous mass can be appreciated in some stained preparations. Smaller or larger yeast cells can be mistaken for *C. neoformans* or *C. immitis*, respectively.

C. immitis/C. posadasii spherules exhibit a variety of sizes and shapes, ranging from large spherules packed with endospores (Fig. 7.91A) to empty, collapsed spheres and small immature spherules.[272] The latter may overlap with *Blastomyces* and other yeasts. Mycelial forms of *Coccidioides* species, with arthrospores, may be found in aspirates of cavitary nodules exposed to air (Fig. 7.91B).

H. capsulatum yeast cells are small (2 to 5 μm) and stain poorly in routine smears, but the presence of this pathogen can be suspected on the basis of the dot-like refractile appearance of these cells in the cytoplasm of macrophages. In Diff-Quik–stained smears, the characteristic purple, polarized yeast forms (Fig. 7.92) are discernible, and they are outlined entirely in GMS-stained smears.

P. jirovecii is most commonly identified in exfoliative samples and aspirates by the presence of the foamy alveolar cast, which varies from eosinophilic to basophilic and is highly characteristic (Fig. 7.93A). These organisms rarely occur singly. The GMS stain outlines the characteristic cysts (Fig. 7.93B).

Cytology of Common Mycelial Forms

The cytopathologist's most frequent challenge is the interpretation of mycelial forms in exfoliated material, especially the distinction between *Aspergillus* look-alikes—Zygomycetes and *Candida* hyphae. The morphologic features of some of the more common agents are compared in Table 7.8.

Candida species are readily seen and easily diagnosed when both yeasts and pseudohyphae are present. However, interpretation of their significance is difficult in all except transthoracic needle aspirates, where the presence of any mycelial structure, particularly in the setting of mass-like and cavitary infiltrates, provides strong morphologic evidence of infection.

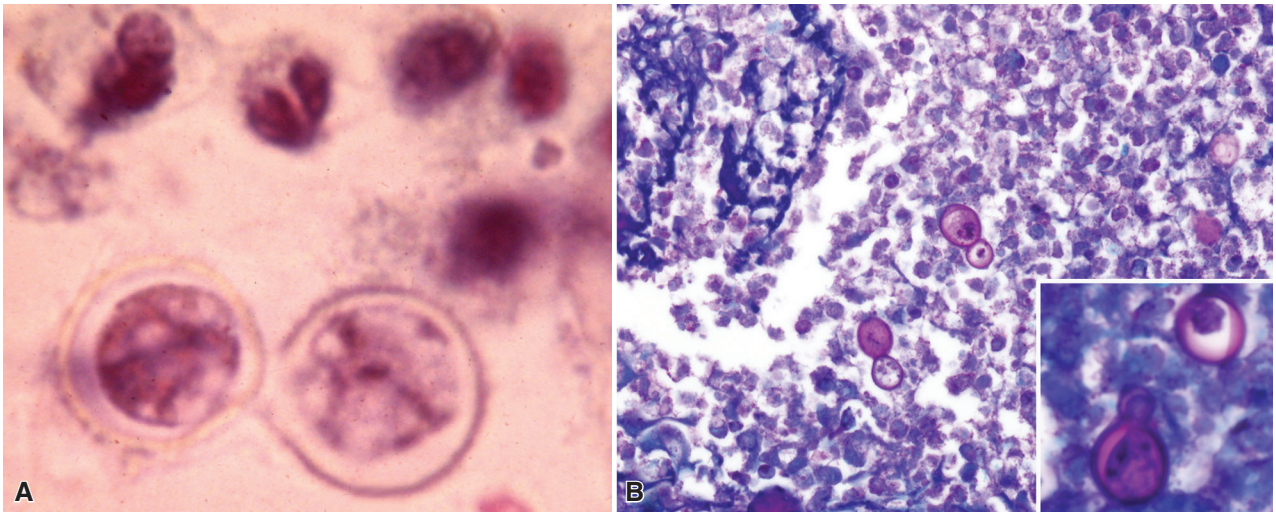

Figure 7.90 *Blastomyces dermatitidis.* (A) Necroinflammatory infiltrate with refractile yeast forms. (B) Periodic acid–Schiff staining highlights the double-contoured yeast with broad-based budding (see *inset* for greater detail).

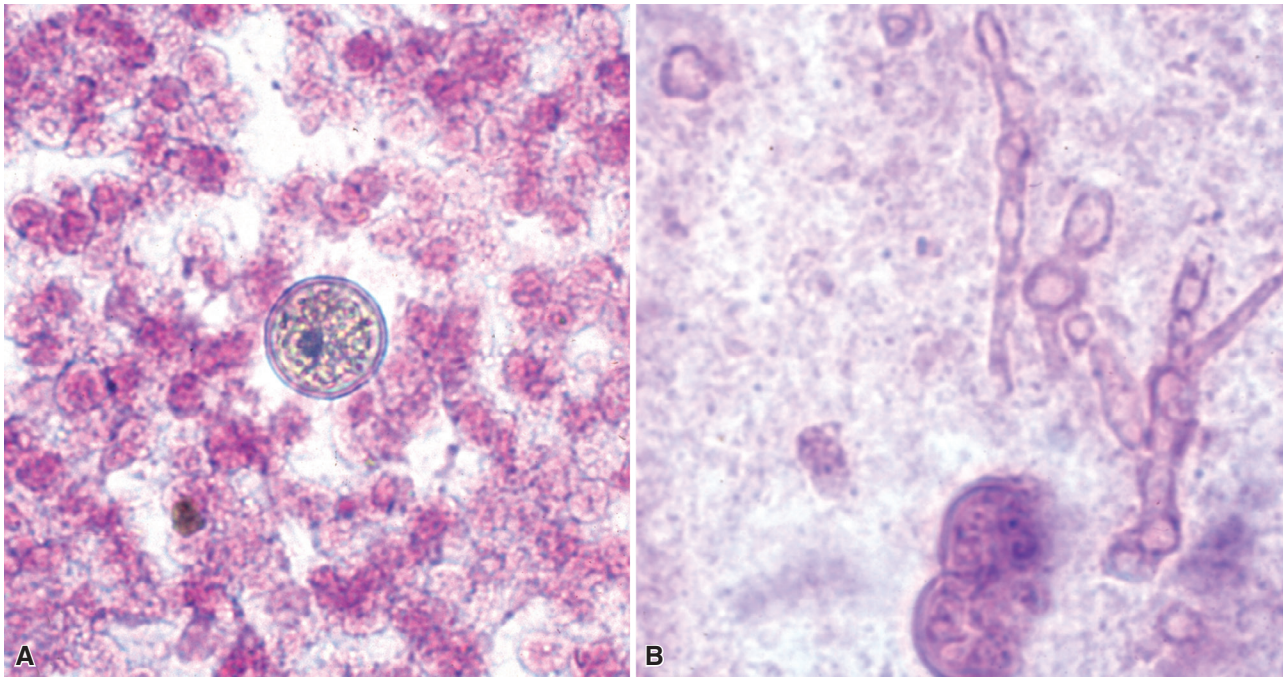

Figure 7.91 *Coccidioides species.* (A) Negative-staining spherule in suppurative inflammatory background in a fine-needle aspirate (alcohol-fixed). (B) Ruptured spherules and mycelia with arthrospores in granular necrotic background in another fine-needle aspirate (alcohol-fixed).

Aspergillus species are characterized by septate mycelia that branch at angles approaching 45 degrees (Fig. 7.94). *Aspergillus* hyphae lack constrictions at points of septation. However, *Aspergillus* organisms cannot be differentiated from one of their mimics by morphology alone unless accompanied by a fruiting body. A rapid in situ hybridization technique specific for *Aspergillus* species can be performed on pulmonary cytocentrifuge preparations, as well as on tissue.[273]

Zygomycete mycelia are distinguished from *Aspergillus* and *Candida* forms by their often broader width and their pleomorphic, twisted ribbon–like, pauciseptate features. Of note, however, in aspirates of aspergilloma, the mycelia may also have a twisted appearance.

A potential pitfall in the evaluation of cytopathologic specimens in fungal infections (both exfoliative samples and needle aspirates) is the confounding presence of atypical reactive squamous cells and type II pneumocytes, which can mimic the cytologic atypia of malignant neoplasms.[48] Furthermore, the pathologist interpreting lung biopsy findings, especially with transbronchial specimens, should always attempt to correlate such findings with samples that may have been collected for cytologic or microbiologic study. This is especially advisable because etiologic agents that escape detection in tissue, such as *Pneumocystis*, *Aspergillus*, and CMV, may be found in washings or lavage fluid.[274]

Microbiology

Complementary laboratory methods are often required for the diagnosis of fungal infection; these are listed in Box 7.21.[204] Under the microscope, many fungi are readily apparent in H&E-stained sections, where they appear colorless (negative staining) or phaeoid (naturally pigmented). The GMS stain is the best histologic stain for demonstrating fungi when they are sparse or not visible on H&E sections. However, some fungi, notably the Zygomycetes, may stain poorly with GMS. The GMS preparation can be counterstained with H&E, allowing coevaluation of the host inflammatory response. The Fontana–Masson stain has been used to detect melanin in *C. neoformans* and phaeoid fungi, but many *Aspergillus* species and some Zygomycetes will also stain with this reagent.[259,275] The PAS stain can be useful in select circumstances, and histochemical stains for mucin (Alcian blue or mucicarmine) are useful for *C. neoformans* infections. The PAS and mucin preparations can also be counterstained with GMS or Fontana–Masson to simultaneously highlight cell walls and capsules of cryptococci. It is important to recognize that not everything that stains with the silver methods is a fungus, and care must be taken to distinguish organisms from pseudomicrobes, such as overstained red cells, white blood cell nuclei, reticulin and elastic fibers, calcium deposits, and even Hamazaki-Wesenberg bodies.[42]

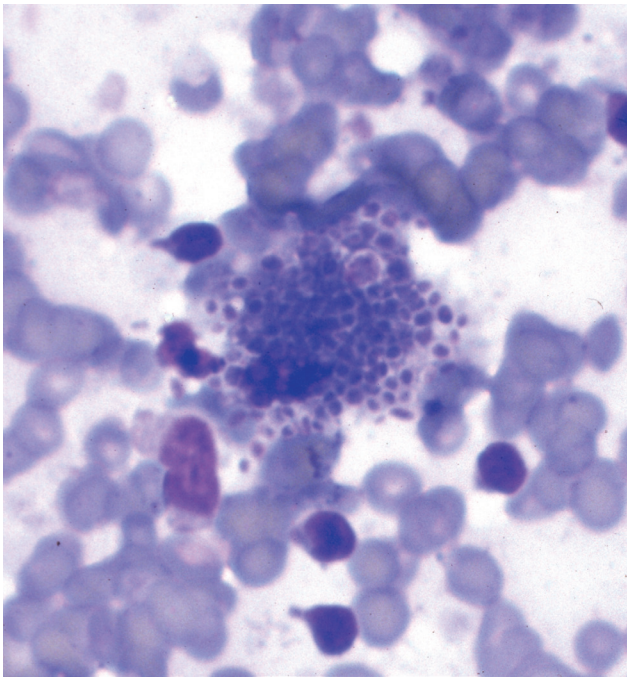

Figure 7.92 *Histoplasma capsulatum.* Clusters of purple polarized yeast cells are readily seen in this fine-needle aspirate (Diff-Quik preparation).

In the microbiology laboratory, the age-old technique of direct light microscopic visualization of fluids, exudates, and tissue homogenates treated with potassium hydroxide (the KOH wet prep) is being replaced by chemofluorescent cotton-brightening agents (such as calcofluor white and fungiqual). Fluorescence microscopy with these reagents can detect a wide variety of fungi in wet mounts as well as frozen sections and paraffin-embedded tissue.[276,277]

Laboratory techniques for the identification of fungi (gross colonial and microscopic morphologic analysis after isolation on fungal media, followed by biochemical testing) are the principal means to a species-specific etiologic diagnosis. For deep tissues, including the lung and other sterile sites, the Emmons modification of Sabouraud glucose agar with chloramphenicol is recommended by many mycologists.[278] Additional use of enriched media such as brain-heart infusion agar can improve recovery of *C. neoformans*, *B. dermatitidis*, and *H. capsulatum*. Selective media containing cycloheximide are not recommended for normally sterile sites because they are potentially inhibitory for yeasts, such as *Cryptococcus* and *Candida* species, and molds, such as *Aspergillus* and the Zygomycetes.

The interpretation of a positive fungal culture must be made in the clinical context. In the absence of proof of tissue invasion or compelling ancillary data, the interpretation of laboratory results requires considerable judgment. Many fungi are ubiquitous in the environment, and most fungal isolates from nonsterile respiratory samples do not represent disease unless there are also significant risk factors such as

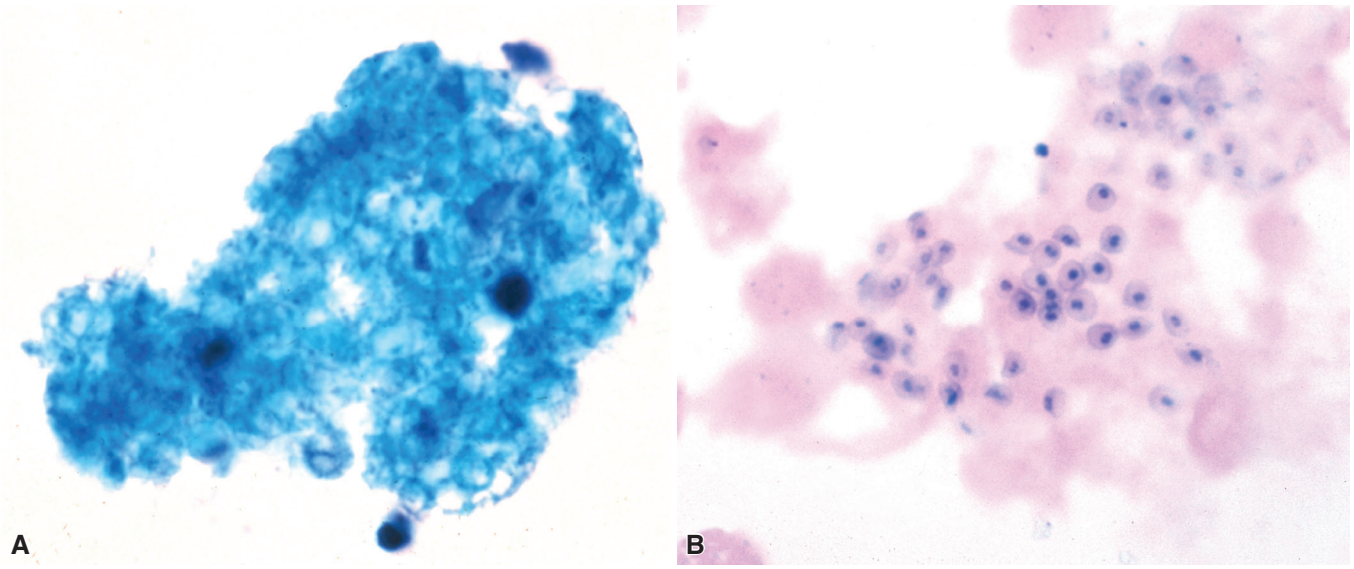

Figure 7.93 *Pneumocystis jirovecii.* (A) Foamy alveolar cast in bronchial washing (ThinPrep Papanicolaou stain). (B) Cysts with intracystic dot in bronchial washing (ThinPrep, Grocott methenamine silver stain).

Table 7.8 Morphologic Features of Selected Fungal Mycelia

Feature	*Aspergillus*	*Bipolaris*	Zygomycetes	*Pseudallescheria Boydii*	*Fusarium*
Width (μm)	3–6	2–6	5–20	2–5	3–8
Contour	Parallel	Parallel	Irregular	Parallel	Parallel
Branching	Dichotomous	Haphazard	Wide angle	Haphazard	90-degree angle
Branch orientation	Parallel	Random	Random	Random	Random
Septation	Frequent	Frequent	Infrequent	Frequent	Frequent
Phaeoid (Brown)	No	Yes	No	Usually not	No
Angioinvasive	Yes	No	Yes	Yes	Yes
Other features	Fruiting body; oxalate crystals sometimes	Chlamydoconidia sometimes One of many dematiaceous genera	Rarely chlamydoconidia	*Aspergillus* "lookalikes"	*Aspergillus* "lookalikes"

Modified from Chandler FW, Watts JC. *Pathologic Diagnosis of Fungal Infections.* Chicago: ASCP Press; 1987:204.

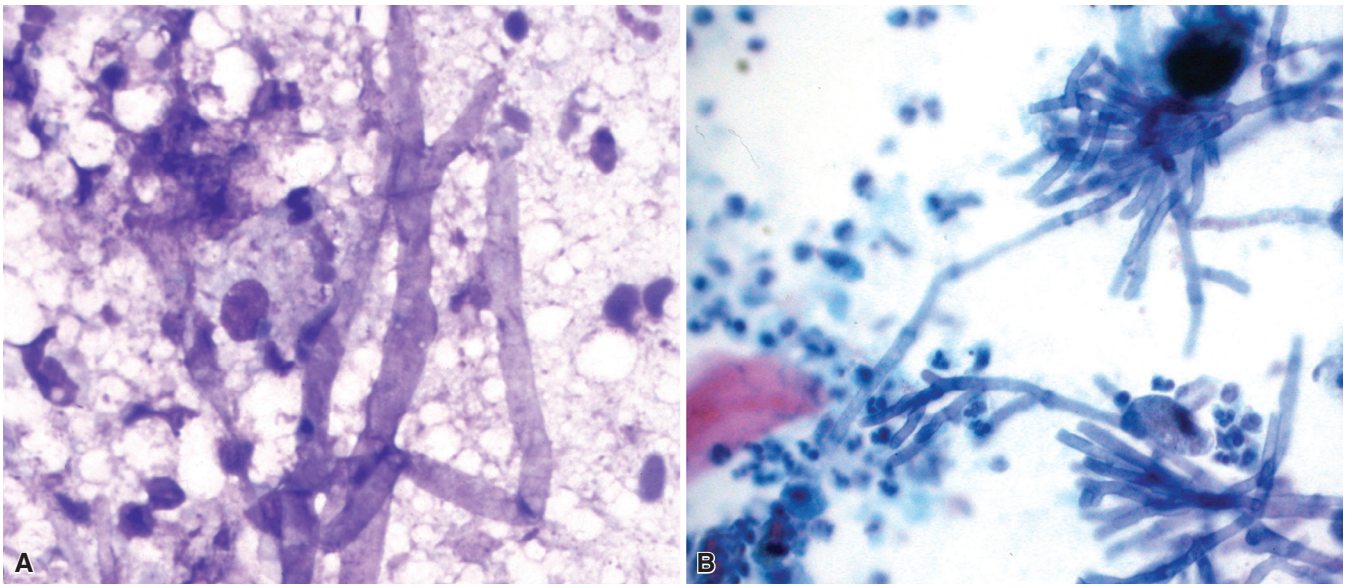

Figure 7.94 *Aspergillus species.* (A) Twisted, sparsely septate mycelia are difficult to differentiate from mimics, including Zygomycetes, in this fine-needle aspirate (Diff-Quik preparation). (B) Characteristic mycelia in a bronchial washing (Papanicolaou stain).

Box 7.21 Laboratory Diagnosis of Fungal Pneumonia

- Direct detection of organisms
  - Chemofluorescence stains
  - Direct fluorescent antibody stain
  - Histopathologic/cytopathologic examination
  - Immunohistochemical studies
- Antigen detection (in suspected histoplasmosis and cryptococcosis)
- Culture
  - Emmons modified Sabouraud agar
  - Brain-heart infusion agar
  - Special and selective media
- Serologic testing
- Molecular methods
  - In situ hybridization
  - DNA amplification

HIV infection, organ transplantation, or immunocompromising drug therapy.[279]

For most of the dimorphic fungi, in vitro hyphae-to-yeast conversion studies have given way to commercially available nucleic acid probes for rapid specific identification. Procurement of tissue for culture before formalin fixation is important whenever fungal infections are suspected. The tissue sample should be kept moist using sterile, nonbacteriostatic saline or Ringer's solution. Specimens are minced but not ground before plating.

The value of bringing multiple, often complementary laboratory methods to bear on inconclusive morphologic findings cannot be overemphasized. In this context, while culture has been considered the most reliable method for definitive diagnosis and histopathology often the fastest, the greatest yield results from combining histopathology with traditional culture and one or more of the newer molecular methods.[280,281] Culture may fail to yield an isolate even in the face of positive microscopic findings. In fact, the yield from tissue specimens, needle aspirates, BAL fluid samples, and bronchial washings is quite low for molds and other fungi for reasons that are not entirely clear.[49,282] Immunofluorescence testing using specific monoclonal antibodies can

achieve a rapid and specific diagnosis in selected infections, especially when tissue has not been submitted for culture. Antibodies directed against the antigens of *Aspergillus* species and selected other fungi have been described, but most are not yet commercially available. For the problematic case, the mycology section of the CDC can provide assistance. Immunohistochemical identification of fungi can be accomplished fairly easily for those species for which reagents are commercially available.[33,283,284]

Molecular techniques, including in situ hybridization and amplification technologies such as PCR, are other powerful tools that can provide rapid, accurate diagnosis for yeasts and molds that may be present in small numbers or manifest overlapping histologic features.[277,285-287] A few laboratories (including the CDC) are performing such assays. Use of quantitative real-time PCR assays on blood, body fluids, and other samples holds promise for relatively rapid definitive diagnosis when routine methods of isolation and identification fail in critical situations.[288,289]

Serologic tests can support a morphologic diagnosis when positive titers are present, but effective serodiagnosis of systemic fungal infections is not available for most fungi.[290] Unfortunately an antibody response does not necessarily correlate with invasive disease, and an antibody response may be lacking for various reasons. False-positive results due to cross reactions and false-negative results due to a variety of reasons plague many of these assays. Some of the most accurate serologic tests (with high sensitivity and specificity) for fungal infections are those for histoplasmosis and coccidioidomycosis, yet tests for both have limitations that must be recognized in interpreting results.[291,292]

The detection of macromolecular antigens shed into various body fluids requires a relatively large microbial burden, which tends to limit sensitivity for most fungal infections except histoplasmosis and cryptococcosis.[280] For these two fungi, useful antigen detection techniques are available using serum, urine, cerebrospinal fluid, and BAL fluid. They are especially sensitive in patients with defective immunity.[271,292] In patients with pneumonia and normal immunity, however, these tests may be positive in lavage fluid but negative in urine unless the disease has disseminated. Other assays designed to detect antigens or metabolites of invasive fungi include those for 1,3β-D-glucan, a cell wall component of several fungi such as *Aspergillus*, *Candida*, *Fusarium*, and others, and for galactomannan, a polysaccharide antigen in the cell wall of *Aspergillus*; these assays have shown fair sensitivity and specificity.[203,293,294]

Differential Diagnosis

A synopsis of the key morphologic and mycologic features of the fungal pneumonias is presented in Table 7.9.[295] When H&E and GMS stains fail to detect fungal elements, the use of ancillary procedures may provide the specific diagnosis. Sometimes, if tissue or other patient specimens have been submitted for culture, the answer may lie in the mycology section of the microbiology laboratory, as many species begin to grow in a matter of days. When fungi are not readily identified by any of these techniques or strategies, other granulomatous infections should be considered, especially mycobacterial, uncommon bacterial (e.g., tularemia, brucellosis), and parasitic infections. Noninfectious necrotizing and nonnecrotizing granulomatous disorders also enter the differential diagnosis. These include granulomatosis with polyangiitis, idiopathic bronchocentric granulomatosis, aspiration, sarcoidosis, rheumatoid nodules, pyoderma gangrenosum–like lung lesions in patients with inflammatory bowel disease, and Churg-Strauss syndrome.[224]

Table 7.9 Fungal Pneumonias: Summary of Pathologic Findings

Assessment Component	Findings
Blastomycosis	
Surgical pathology	Suppurative granuloma most characteristic; also, tuberculoid (necrotizing) types; round, thick-walled (double-contour) yeast with broad-based budding
Cytopathology	Neutrophils and epithelioid cells with characteristic refractile yeast cell with double-contoured wall and broad-based budding
Microbiology	Characteristic yeast seen on wet mount, KOH- and calcofluor-stained smear; culture-sterile lung tissue on nonselective fungal media (e.g., Emmons modified Sabouraud) and enriched media (e.g., brain-heart infusion); add selective media for bronchial/transbronchial samples; colonies produce oval conidia on terminal ends of conidiophore at right angle to mycelium; confirm with DNA probe; serologic studies not useful
Coccidioidomycosis	
Surgical pathology	Fibrocaseous granuloma; large intact and/or ruptured spherules, full or partially or completely empty of endospores; mycelial forms in aerated cavities and fistula
Cytopathology	Necroinflammatory debris with epithelioid histiocytes; intact, viable, colorless spherules with variable number of endospores and/or ruptured degenerating forms with stained wall; range in size from large mature to small immature types
Microbiology	Characteristic mature spherules in wet mount, KOH- and calcofluor-stained smear; culture of sterile lung tissue on nonselective fungal media yields mycelia with characteristic arthroconidia; confirm with DNA probe; serologic diagnosis with tests for IgG and IgM antibodies by immunodiffusion, EIA; complement fixation for titers
Histoplasmosis	
Surgical pathology	Macrophage reaction and/or granulomas, based on immunity, including miliary and solitary pulmonary, variably hyalinized nodule; small, thin-walled, oval yeasts with narrow-based buds, often refractile
Cytopathology	Macrophage and epithelioid cells with characteristic yeast cell, often intracellular, stained purple with Diff-Quik, black with GMS
Microbiology	Rarely detected by direct examination of most clinical specimens; culture-sterile lung tissue on nonselective and enriched fungal media produces tuberculate macroconidia; confirm with DNA probe; antigen detection by EIA available for BAL fluid, CSF, serum, and urine
Paracoccidioidomycosis	
Surgical pathology	Exudative or granulomatous lesion with large, globose yeast cell with multiple buds
Cytopathology	Suppurative or granulomatous reaction with characteristic yeast cell
Microbiology	Direct detection in wet mount, KOH- and calcofluor-stained smear; culture-sterile lung tissue on standard nonselective fungal media; serologic testing by immunodiffusion, EIA; complement fixation for titer

Continued

Table 7.9 Fungal Pneumonias: Summary of Pathologic Findings—cont'd

Assessment Component	Findings
Sporotrichosis	
Surgical pathology	Necrotizing granuloma, often cavitary with small, usually round, sometimes cigar-shaped yeast with sparse, narrow buds
Cytopathology	Suppurative or necrotizing granuloma pattern; yeast cells generally sparse or absent
Microbiology	Rarely detected by direct examination of most clinical specimens; culture of sterile lung tissue on nonselective fungal media yields thin, hyphae-bearing conidia in a rosette pattern; converts to a yeast phase at 37°C on blood agar; no serologic tests
Penicilliosis	
Surgical pathology	Alveolar macrophages stuffed with yeast cells resemble *Histoplasma* species, but with septum reflecting binary fission, not budding reproduction
Cytopathology	Macrophage with intracellular characteristic yeast forms
Microbiology	Culture of sterile lung tissue on nonselective fungal media yields a mold with a red pigment evident as culture ages; erect conidiophores, sometimes branched with metulae bearing one or several phialides with long, loose chains of oval conidia; new urinary antigen test
Cryptococcosis	
Surgical pathology	Granulomas, histiocytic infiltrate or mucoid pneumonia, based on immunity with pale, round, budding pleomorphic yeast cells, often in clusters; mucoid capsules usually present; acapsular types sometimes seen
Cytopathology	Yeast cell with mucoid capsular halo resembles "spare tire"; combination of mucicarmine and GMS or Fontana–Masson outlines capsule and cell wall; background of epithelioid cells or necroinflammatory debris may be sparse or absent
Microbiology	Oval to lemon-shaped calcofluor-positive yeast cell with capsule in India ink–stained touch imprint; culture on nonselective fungal media yields mucoid yeast-type colonies; no pseudohyphae; germ tube–negative; dark brown pigment on birdseed (niger) agar; confirm with biochemical tests; antigen detection test (latex agglutination or EIA) on serum, BAL fluid, CSF, and needle aspirates
Candidiasis	
Surgical pathology	Miliary necroinflammatory lesions or bronchopneumonia with small, oval, budding yeasts with or without pseudohyphae; *C. glabrata* yeast only
Cytopathology	Yeasts and/or pseudohyphae in a necroinflammatory background
Microbiology	Budding yeasts and pseudohyphae in wet mounts, KOH- and calcofluor-stained smears; cultures on selective and nonselective fungal media yield creamy tan to white yeast-type colonies; identification by germ tube production, carbohydrate assimilation, and cornmeal agar morphology
Aspergillosis	
Surgical pathology	Various forms include saprophytic (fungus ball), allergic (ABPA and mucoid impaction), hypersensitivity pneumonitis, and invasive disease ranging in severity from minimal chronic necrotizing to extensive pneumonia; angiotrophic with necrotizing infarcts; also hybrid forms of disease; septate, dichotomous, 45-degree angle mycelia; oxalate crystals; presence of fruiting body is genus-specific
Cytopathology	Tangled clusters of septate mycelia in a necroinflammatory background; may appear sparsely septate and twisted, mimicking *Zygomycetes*
Microbiology	Positive staining of mycelia with calcofluor and GMS; culture of sterile lung tissue on nonselective fungal media produces mold-type colonies in a range of colors; species differentiation by conidial and conidiophore morphology
Zygomycosis	
Surgical pathology	Nodular lesions, lobar consolidations, cavitary lesions, fungus balls, and airway infections commonly necrotizing and ischemic secondary to angioinvasion; broad pauciseptate mycelia with 90-degree angle, branching, often with twisted-ribbon morphology
Cytopathology	Pauciseptate mycelia, often with twisted-ribbon morphology in a necroinflammatory background
Microbiology	Positive staining of mycelia with calcofluor and GMS; rapidly growing cottony colonies are grown on most nonselective fungal media, but "controlled baiting" with bread sometimes necessary; identification based on presence and locations of rhizoids, shape of sporangia, presence of columellae, and shape of sporangiospores
Phaeohyphomycosis	
Surgical pathology	Allergic bronchopulmonary fungal disease similar to aspergillosis
Cytopathology	Similar to ABPA pattern—"allergic mucin" with eosinophils, Charcot–Leiden crystals in inspissated mucus; fungal mycelial fragments sparse or absent
Microbiology	Dematiaceous (phaeoid) dark brown to black colonies on nonselective fungal media; identified by shape and cross walls of multicell, pigmented conidia
Pneumocystosis	
Surgical pathology	Pneumonia with foamy alveolar cast is classic; other patterns include diffuse alveolar damage, granulomatous lesions, and minimal changes; variable numbers of cysts noted in GMS-stained sections
Cytopathology	Foamy alveolar cast with characteristic cysts outlined by GMS
Microbiology	Causative organism: formerly *Pneumocystis carinii*, classified as a fungus and renamed *Pneumocystis jirovecii*; cannot be cultured; Detection is with fluorescent monoclonal antibody assay or GMS-stained smears

ABPA, Allergic bronchopulmonary aspergillosis; *BAL,* bronchoalveolar lavage; *CSF,* cerebrospinal fluid; *EIA,* enzyme immunoassay; *GMS,* Grocott methenamine silver; *IgG, IgM,* immunoglobulins G and M; *KOH,* potassium hydroxide.

Table 7.10 Viral Pathogens of the Lung

RNA Viruses	DNA Viruses
Influenza virus	Adenovirus
Parainfluenza virus	Herpes simplex virus
Respiratory syncytial virus	Varicella-zoster virus
Measles virus	Cytomegalovirus
Hantavirus	Epstein–Barr virus

Box 7.22 Histopathologic Patterns in Viral Lung Injury

Diffuse alveolar damage
Bronchitis and bronchiolitis
Diffuse interstitial pneumonia
Perivascular lymphoid infiltrates
Miliary small nodules
Airspace organization—*Bronchiolitis obliterans*–organizing pneumonia (BOOP) pattern
Calcified nodules

Viral Pneumonia

Viruses cause more infections than all other types of microorganisms combined and involve the respiratory tract more commonly than other organ systems.[296] Fortunately, the lung diseases produced by viruses are usually mild and self-limited. Nevertheless, viruses cause major public health illnesses and account for many of the new and emerging diseases in current headlines. At times viruses are also capable of producing serious and life-threatening infections that come to the attention of pathologists in both immunocompromised patients and young, healthy persons.[297] The viruses that commonly infect the lung are listed in Table 7.10.[298]

Etiologic Agents

The conventional respiratory viruses—influenza virus, parainfluenza virus, RSV, and adenovirus—cause outbreaks of respiratory illness in the general population each year. In infants, the elderly, and in patients with chronic diseases, these pathogens can cause serious pneumonias. Pneumonia in immunocompromised persons is usually attributed to the herpesviruses (HSV and CMV). Less appreciated is that the conventional respiratory viruses are also frequent causes of respiratory illness in these patients and that such infections result in high rates of morbidity and mortality.[299]

Newly recognized respiratory viruses [300,301] include H5N1, a highly pathogenic strain of influenza. First detected in 1997 in Hong Kong, it has since spread to Europe, the Middle East, and Africa. Another unique, triple-reassortment swine-origin influenza virus A, H1N1 (S-OIV), emerged in 2009 as the cause of outbreaks sustained by person-to-person transmission in multiple countries. It was characterized by respiratory illness of variable severity ranging from self-limited disease resembling seasonal flu to severe illness requiring hospitalization and occasionally eventuating in death from respiratory failure.[302] An acute cardiopulmonary syndrome in the southwestern United States was etiologically linked to a new hantavirus referred to as *Sin Nombre* ("without a name"). The severe acute respiratory syndrome (SARS) epidemic, which began in southern China and was carried by travelers to 33 other countries and 5 continents, was caused by SARS-CoV, a newly recognized coronavirus. Four other coronaviruses linked to respiratory illnesses (HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) have since been reported.[303] Human metapneumovirus, a paramyxovirus closely related to RSV clinically and pathologically, has become recognized as one of the leading causes of respiratory illness in children and can also cause illness in adults and immunocompromised patients.[304] Human bocavirus (HBoV) has been isolated in several countries from children with wheezing.[305] Other viruses such as the picornavirus group (rhinovirus and enterovirus) can cause pneumonia, as can polyomavirus (BK virus).[306] Parvovirus B19, an Erythrovirus, has long been known to cause disease, primarily in maternal–fetal and pediatric patients. Recently an autoimmune-type pneumonitis associated with serologic evidence of parvovirus B19 has also been described.[308] The evolution of diagnostic laboratory methods and large-scale molecular screening suggests that more viruses will be linked to respiratory tract disease in the future.

Histopathology

The respiratory tract viruses have a tendency to target specific regions of the tracheobronchial tree and lungs, producing characteristic clinical syndromes. However, sufficient overlap clinically, radiologically, and pathologically often limits a strict interpretation of findings for a definitive diagnosis. The information in Box 7.22 can sometimes be useful in narrowing the search for a specific etiologic agent. The microscopic findings in most pulmonary viral infections include the direct effect of the virus as well as the host's inflammatory response. The clinical outcome depends upon the virulence of the organism and the nature of the host response, be it diffuse alveolar damage, diffuse or patchy bronchiolitis and interstitial pneumonitis, giant cell reactions, or even minimal change.[309]

The histopathologic diagnosis of viral infection is impossible without identification of the characteristic CPE. The term *cytopathic effect* has traditionally been used by virologists to describe cellular changes in unstained cell culture monolayers seen by light microscopy,[310,311] but it can be applied to all virus-associated nuclear and cytoplasmic alterations seen on H&E-stained slides or highlighted by immunohistochemical staining, molecular in situ–based methodology, or ultrastructural localization.[312,313] Diffuse alveolar damage, often with bronchiolitis, is the most typical pattern of viral lung injury. As noted earlier, however, diffuse alveolar damage also occurs in bacterial, mycobacterial, and fungal pneumonias, so a careful search for specific viral CPE becomes important in this setting. For the surgical pathologist, CPE manifests mainly as the viral inclusion present in the nucleus or cytoplasm of an infected cell. Viral inclusions confer diagnostic specificity to the pathologic pattern of injury in which they are found, and for the common respiratory tract viruses, the features are presented in Table 7.11. Finally, it is worth mentioning that most clinically significant viral pneumonias that have CPE also show necrosis somewhere in the biopsy.

Influenza Virus

Influenza viruses are the most pathogenic of the respiratory viruses and predispose patients most commonly to secondary bacterial pneumonia. These viruses also account for the greatest public health burden. Annually they cause epidemic outbreaks of respiratory disease that are often associated with considerable morbidity; periodically, they produce pandemics with high mortality rates. These viruses target the ciliated epithelium of the tracheobronchial tree, producing necrotizing bronchitis and bronchiolitis and a spectrum of changes that vary depending on the stage of the disease (early vs. late), outcome (fatal vs. nonfatal), and the presence or absence of secondary bacterial pneumonia. Uncomplicated influenza pneumonia is rarely biopsied today. Based on historical data from bronchoscopic biopsies performed in the 1950s and early 1960s, the histopathologic findings in nonfatal uncomplicated influenza

Table 7.11 Cytopathic Effects in Pulmonary Infections With Selected Viruses

Virus	Presence of Inclusions		Inclusion Characteristics
	Intranuclear	Intracytoplasmic	
Herpes simplex virus; varicella-zoster virus	+	–	Early ground-glass appearance; later eosinophilic (Cowdry A type) multinucleate cells
Adenovirus	+	–	Early eosinophilic (Cowdry A); later basophilic, smudged nucleus
Cytomegalovirus	+	+	Cytomegaly with large "owl eye" amphophilic (Cowdry A) nuclear and multiple smaller basophilic (GMS-positive), cytoplasmic type
Respiratory syncytial virus	–	+	Eosinophilic smooth, small, often indistinct; multinucleate syncytia in some cases
Measles virus	+	+	Eosinophilic nuclear (Cowdry A) in multinucleate cells; cytoplasmic type—eosinophilic, pleomorphic
Parainfluenza virus	–	+	Rarely observed, pleomorphic, eosinophilic; multinucleate syncytia rarely
Influenza virus	–	–	No inclusions or other distinctive cytopathic effects

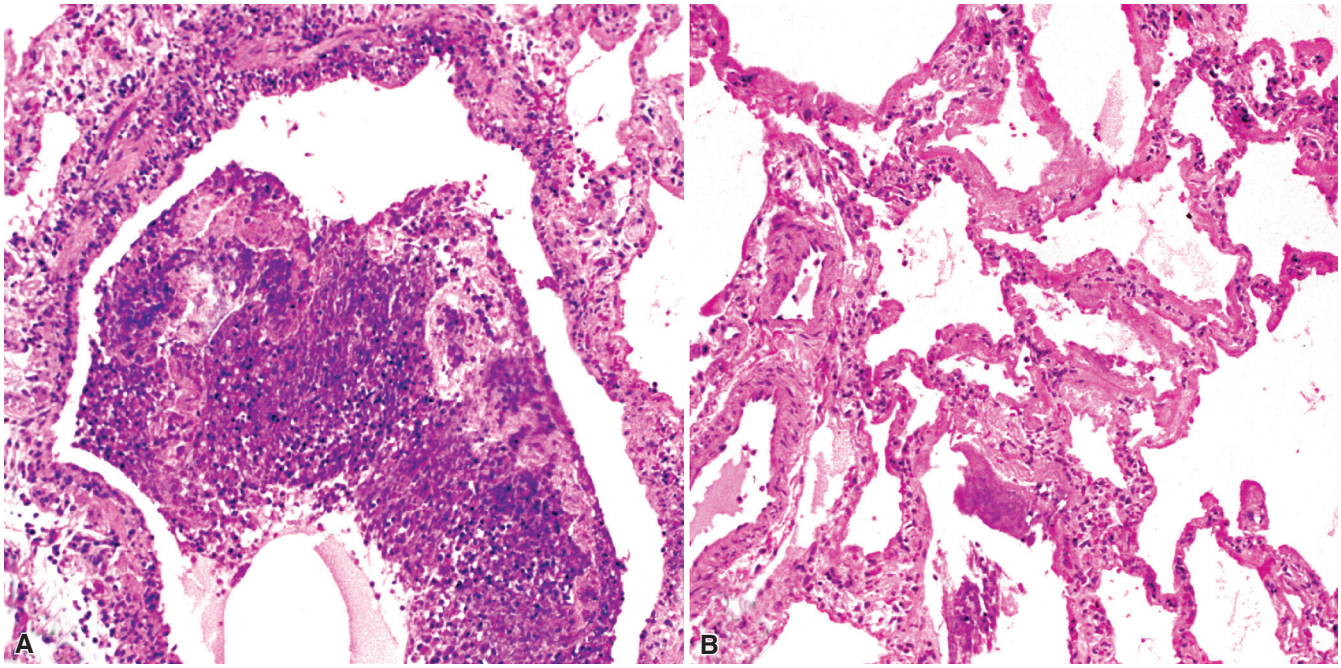

Figure 7.95 Influenza virus. (A) Bronchiolitis with intraluminal necroinflammatory debris. (B) Acute diffuse alveolar damage pattern with hyaline membranes.

are those of active tracheobronchitis.[314] Necrosis and desquamation of the epithelial cells to the basement membrane is associated with a relatively scant lymphocytic infiltrate; however, in more severe cases, the virus and its attendant inflammatory response spread more distally into the respiratory bronchioles and alveoli, with hemorrhage, edema, fibrinous exudate with hyaline membranes, and patchy interstitial cellular infiltrates (Fig. 7.95). This constellation of findings comprises the *lesion of characterization*.[315] In contemporary pathologic terms this would correspond to diffuse alveolar damage and, clinically, a primary viral pneumonia. Depending on the clinical course and time of lung biopsy (or autopsy) within the first 2 weeks of illness, the process may be in the acute and/or organizing phase.[316,317] Later, the airway epithelial damage may pave the way for secondary bacterial pneumonia, which accounts for much of the morbidity and mortality of influenza and may obscure the features of primary viral pneumonia.[318]

From 2003 through 2008, 391 human cases of highly pathogenic avian influenza involving the H5N1 strain were recorded with 247 deaths.[319] The histopathologic changes observed in the few autopsied cases fall within the spectrum of findings described during the pandemics of 1918, 1957, and 1968 and in fatal cases of interpandemic (seasonal) influenza.[317] A characteristic feature of the H5N1 and 1918 cases is the high mortality rate, especially among previously healthy older children and young adults. Excessively high levels of cytokines and chemokines are thought to play an important role in the pathogenesis of the acute lung injury pattern seen in these fatal cases of influenza.[320] Because these viruses produce no characteristic cellular inclusions, etiologic diagnosis is not possible by morphology alone and requires antigen detection by immunofluorescence, immunohistochemistry, in situ hybridization, or culture.[321] The influenza virus genome steadily shifts over the passage of time; infections occur in those without previous immunity to each new strain. Rapid characterization of each viral variant is a continuous challenge, with numerous virologic methods concentrating on rapid point-of-care methods needed for effective prophylaxis.[322]

Parainfluenza Virus

Parainfluenza virus comprises four serotypes (I to IV) that typically target the upper respiratory tract, classically in the form of croup.[323] Some cases involve distal airways, as in infections due to RSV and

influenza virus, but are milder, with less morbidity and requiring fewer hospitalizations. A few documented cases have been described with a diffuse alveolar damage pattern or an interstitial pneumonitis with giant cells, the latter resembling those of measles and RSV infection. The giant cells of parainfluenza tend to be larger and have more intracytoplasmic inclusions.[55] Parainfluenza virus is a potential opportunist in immunocompromised patients, especially children with congenital immunodeficiency disorders,[324] in whom fatal pneumonitis with disseminated disease may occur.[325]

Respiratory Syncytial Virus

RSV causes more significant respiratory infections in early childhood than those attributable to either influenza viruses or parainfluenza viruses.[326,327] The annual outbreaks of bronchiolitis and pneumonia in infants are especially severe during the first year of life and in those of low birth weight or with cardiopulmonary disease.[323] Considered primarily a childhood virus, RSV has more recently been recognized as the etiologic agent of pneumonia in community-dwelling and high-risk adults with chronic lung disease requiring hospitalization.[324,328,329] Also, RSV is often an unsuspected opportunistic pathogen in immunocompromised patients.[299,330] RSV targets the epithelium of the distal airway, producing bronchiolitis with disorganization of the epithelium and epithelial cell sloughing (Fig. 7.96A).[307] In fatal cases, airway obstruction due to sloughed cell detritus, mucus, and fibrin is compounded by airway lymphoid hyperplasia.[331] Diffuse alveolar damage may be seen in immunocompromised patients. Giant cells (syncytia), similar to the cytopathic changes seen in cell culture, may be present in alveolar ducts and airspaces around areas of bronchiolitis (Fig. 7.96B). Eosinophilic inclusions in cytoplasm may be seen in tissues and cytology specimens from immunosuppressed patients, but these are difficult to confirm as diagnostic of RSV without immunohistochemistry.

Human Metapneumovirus

Human metapneumovirus, a newly recognized paramyxovirus, is a leading cause of respiratory tract disease in infants, with annual epidemics occurring during the winter and early spring months.[304,332] The virus also causes disease in immunocompromised patients[333] and likely explains some lower respiratory tract infections in the elderly. The clinical spectrum of croup, bronchiolitis, and pneumonia is similar to that for infections due to other paramyxoviruses, such as RSV and parainfluenza virus. The pathologic features are not well characterized because few well-documented cases have included biopsy in the evaluation. However, histopathologic assessment of lung tissue in severe cases has revealed acute and organizing diffuse alveolar damage as well as smudge cell formation.[334,335] The definitive identification of the virus can be established in tissue culture, but monoclonal antibody reagents and molecular techniques (real-time PCR assay) are the current diagnostic methods of choice.

Measles Virus

The measles virus causes a highly communicable childhood viral exanthem worldwide that, unlike varicella (chickenpox), leads to complications that are common and serious.[336] Measles pneumonia accounts for the vast majority of measles-related deaths, and most of these are a consequence of secondary pneumonia (bacterial or viral) or attributable to an aberrant immune response. Despite vaccination, measles is still a global pathogen and has resurfaced due to variation in vaccination rates, even in the United States.[337,338] Primary viral pneumonia occurs but is uncommon, even in immunocompromised hosts. Microscopically, bronchial and bronchiolar epithelial degeneration and reactive hyperplasia with squamous metaplasia is typically accompanied by peribronchial inflammation. Diffuse alveolar damage may occur, and quantitative immunohistochemical studies have revealed severe immune dysfunction with loss of key effector cells and their cytokines.[339] Characteristic giant cells show distinctive intranuclear eosinophilic inclusions surrounded by halos (Fig. 7.97). This is the classic measles injury pattern[307] and is referred to as *Hecht giant cell pneumonia*. Minute intracytoplasmic eosinophilic inclusions precede the development of the intranuclear inclusions and are often difficult to identify. Pneumonia with giant cells should always suggest measles,

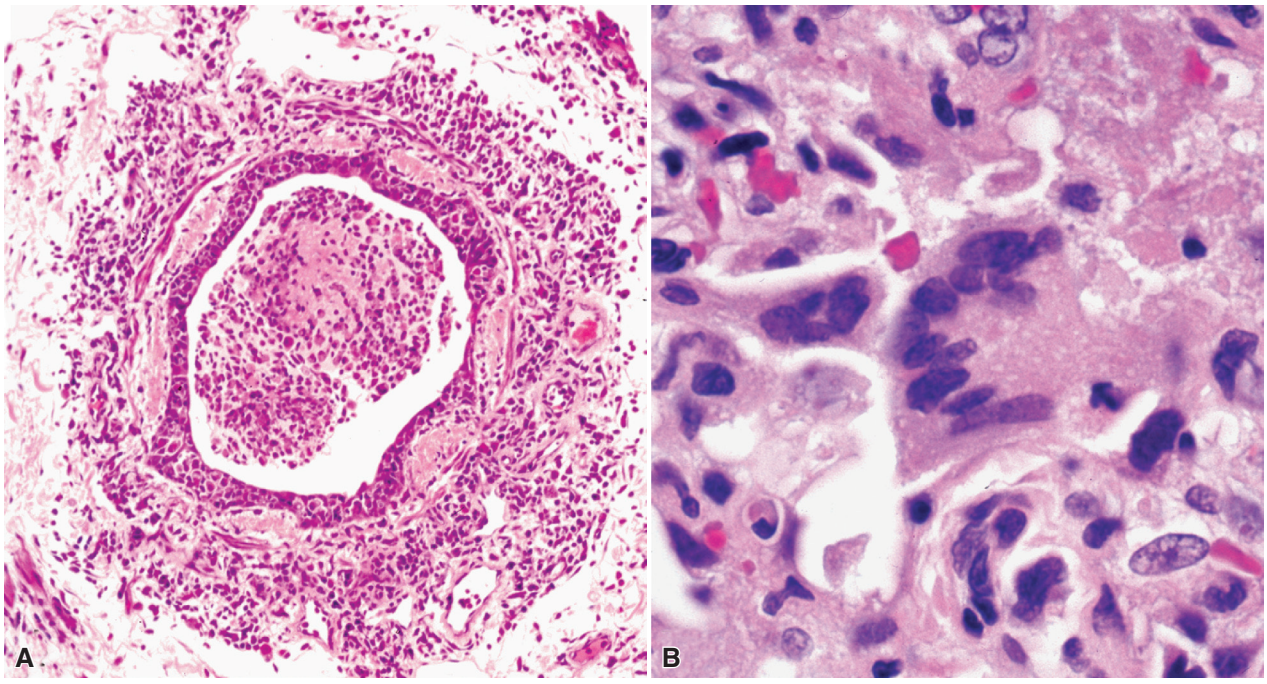

Figure 7.96 Respiratory syncytial virus. (A) Bronchiolitis with intraluminal sloughing. (B) Bronchiolitis with giant cell syncytia.

but similar changes can be seen in RSV and parainfluenza pneumonias, and not all cases of measles pneumonia have these giant cells.[307] Hard metal pneumoconiosis (giant cell interstitial pneumonia) is in the differential diagnosis, but the overall appearance of hard metal disease is one of a chronic disease with some fibrosis and few if any acute changes. In the absence of giant cells, the cellular interstitial pneumonia must be differentiated from those caused by other viruses and atypical pneumonia agents as well as from nonspecific interstitial pneumonia.

Hantavirus

The recently identified hantavirus produces a rapidly evolving cardiopulmonary syndrome with a high mortality rate. This disorder first came to public attention as an emerging infection following an outbreak in the southwestern United States in 1993; it was causally linked to a previously unrecognized hantavirus. All members of this genus are zoonotic and are found in rodents around the world. The specific type responsible for the cardiopulmonary syndrome, *Sin Nombre*, is present in rodent feces and is acquired from the environment through inhalation. It produces florid pulmonary edema with pleural effusions, variable fibrin deposits, and focal wispy hyaline membranes (Fig. 7.98A).[340] Immunoblast-like cells are present in vascular spaces and in the peripheral blood (Fig. 7.98B). Morphologic diagnosis is presumptive because hantaviral antigen in endothelial cells, detected by immunohistochemistry, is required for definitive diagnosis.[341] In the appropriate clinical setting, clues to the diagnosis can sometimes be found in a constellation of morphologic findings on a peripheral blood smear, and confirmation can be achieved serologically by the detection of hantavirus-specific immunoglobulin M (IgM) antibodies or by the detection of hantavirus RNA by PCR assay in peripheral blood leukocytes.[342-344]

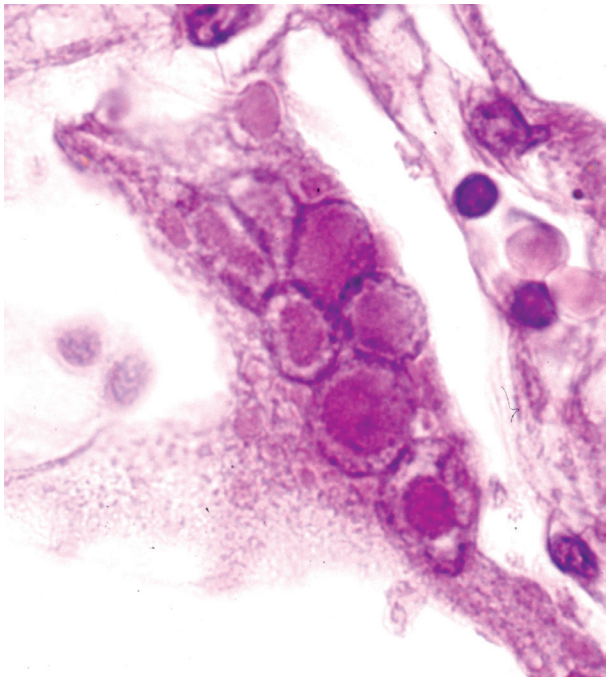

Figure 7.97 Measles virus pneumonia with characteristic eosinophilic intranuclear inclusions in giant cell.

Coronaviruses

Coronaviruses are ubiquitous RNA viruses known to cause disease in many animals. At least five different coronaviruses are known to infect humans, and these cluster into two antigenic groups.[303] They, along with the rhinoviruses, are responsible for a majority of common colds. Coinfections with other respiratory viruses occur in infants and children presenting with more severe respiratory disease. In certain epidemiologic situations, they can cause pneumonia in children, frail elderly individuals, and immunocompromised adults.[345,346]

In November 2002, the appearance of an atypical pneumonia in China, subsequently labeled SARS, became an alarming global health problem in the period of a few months.[347] The disease was linked (Koch's postulates were fulfilled) by means of tissue culture isolation, electron microscopy, and molecular analysis to an emergent novel coronavirus, proposed as the Urbani strain of SARS-associated coronavirus.[348]

Clinically the disease ranges from a nonhypoxemic febrile respiratory disease (with minimal symptoms in some patients) to one of severe pulmonary dysfunction, manifesting as ARDS and eventuating in death for approximately 5% of the patients affected.[349] In the reported cases, the chest x-ray appearance on presentation was either normal or the chest film showed unilateral, predominantly peripheral areas of

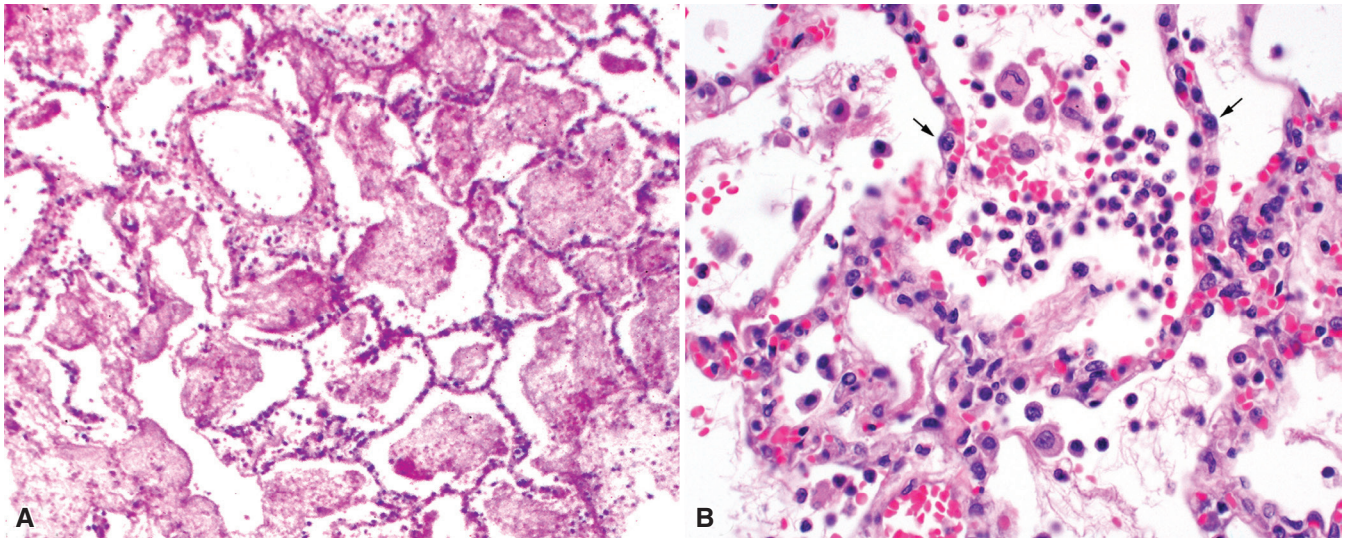

Figure 7.98 Hantavirus. (A) Pulmonary edema with fibrin deposits. (B) Immunoblast-like cells in alveolar capillaries at arrows.

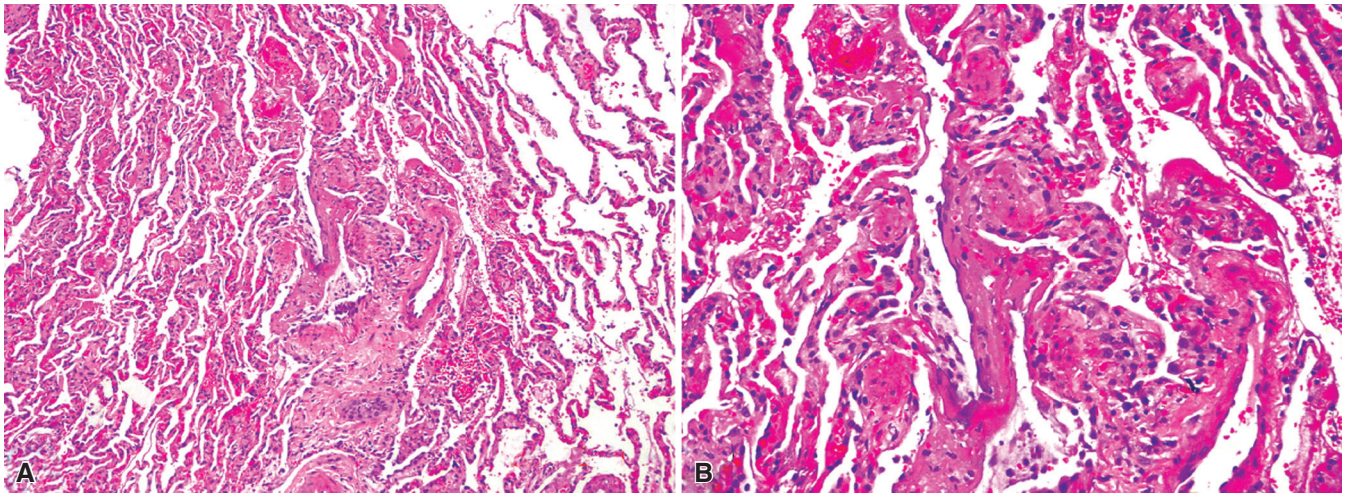

Figure 7.99 Coronavirus pneumonia: Severe acute respiratory syndrome. (A) and (B) Acute fibrinous lung injury is evident. (Courtesy Dr. Oi-Yee Cheung, Queen Elizabeth Hospital, Hong Kong, China.)

consolidation that progressed to bilateral, patchy consolidation, the degree and extent of which correlated with the development of respiratory failure. In patients who presented with a normal x-ray appearance, CT scans often revealed bilateral ground-glass consolidation resembling that in bronchiolitis obliterans with organizing pneumonia (cryptogenic organizing pneumonia). Lymphopenia and elevated LDH were helpful clues, but the clinical, radiologic, and laboratory features, although characteristic, were not distinguishable from those in patients with pneumonia caused by other viruses and bacteria and various atypical agents.

Histopathologic findings in lung biopsy and autopsy tissues included acute lung injury (diffuse alveolar damage) in various stages of organization.[350,351] Lung biopsy specimens in milder cases showed relatively scant intraalveolar fibrin deposits with some congestion and edema (Fig. 7.99). However, the spectrum of findings included acute fibrinous pneumonia, hyaline membrane formation, interstitial lymphocytic infiltrates, desquamation of alveolar pneumocytes, and areas undergoing organization of the acute-phase injury.[352] In some patients, multinucleate syncytial cells reminiscent of the CPE seen in influenza virus, RSV, and measles virus infections were noted. Viral inclusions were not identified, and initial immunohistochemical studies failed to reveal viral antigen. Subsequent investigations detected virus in epithelial cells (predominantly type II pneumocytes) and alveolar macrophages using immunohistochemical staining, in situ hybridization, RT-PCR methods, and electron microscopy. A unique coronavirus (Fig. 7.100) was finally implicated as the etiologic agent.[352,353] Comparative histopathologic studies in fatal cases of SARS and H5N1 avian influenza reveal similarities and differences.[354] Both infections feature acute and organizing diffuse alveolar damage, but SARS appears to be more frequently associated with subacute injury with intraalveolar organization, whereas H5N1 virus causes a more fulminant diffuse alveolar damage pattern with patchy interstitial inflammation and paucicellular fibrosis.

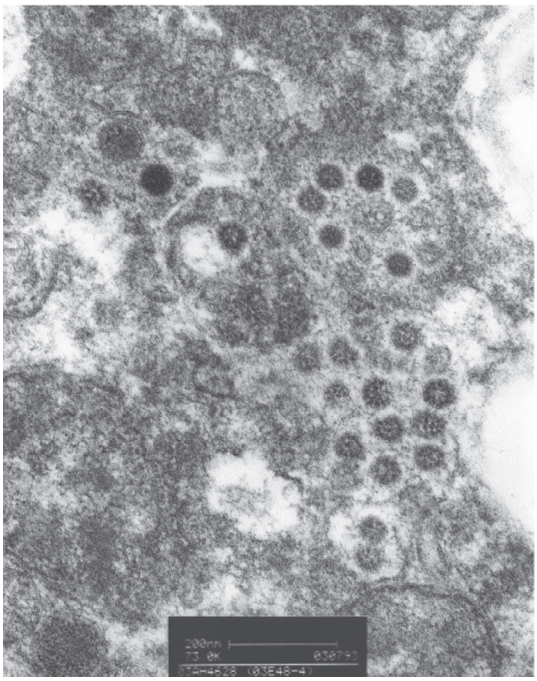

Figure 7.100 Coronavirus-infected cell can be seen in this electron photomicrograph. (Courtesy Dr. Oi-Yee Cheung, Queen Elizabeth Hospital, Hong Kong, China.)

Adenovirus

Adenovirus comprises several genera, with multiple serotypes that cause infections of the upper and lower respiratory tract, conjunctiva, and gut. Respiratory tract infections are most common and account for approximately 5% to 10% of pediatric pneumonias. These can be especially severe in neonates, children, and immunocompromised persons.[307,355] In the lung, adenovirus infection produces two patterns of lung injury: diffuse alveolar damage with or without necrotizing bronchiolitis and pneumonitis with "dirty" or karyorrhectic necrosis [356] (Fig. 7.101). These patterns may coexist in some cases, and the pneumonia may be accompanied by hemorrhage secondary to adenovirus-induced endothelial cell damage.[357] Two types of adenoviral CPE may be seen. Initially an eosinophilic (Cowdry A) intranuclear inclusion occurs surrounded by a halo with marginated chromatin, similar to HSV (Fig. 7.102A). This later enlarges and becomes amphophilic and then more basophilic, obliterating the nuclear membrane and producing the characteristic smudge cell (Fig. 7.102B).[307]

Herpes Simplex Viruses

HSVs types I and II have had traditional assigned roles as etiologic agents of mucocutaneous disease of the head and neck (type I) and

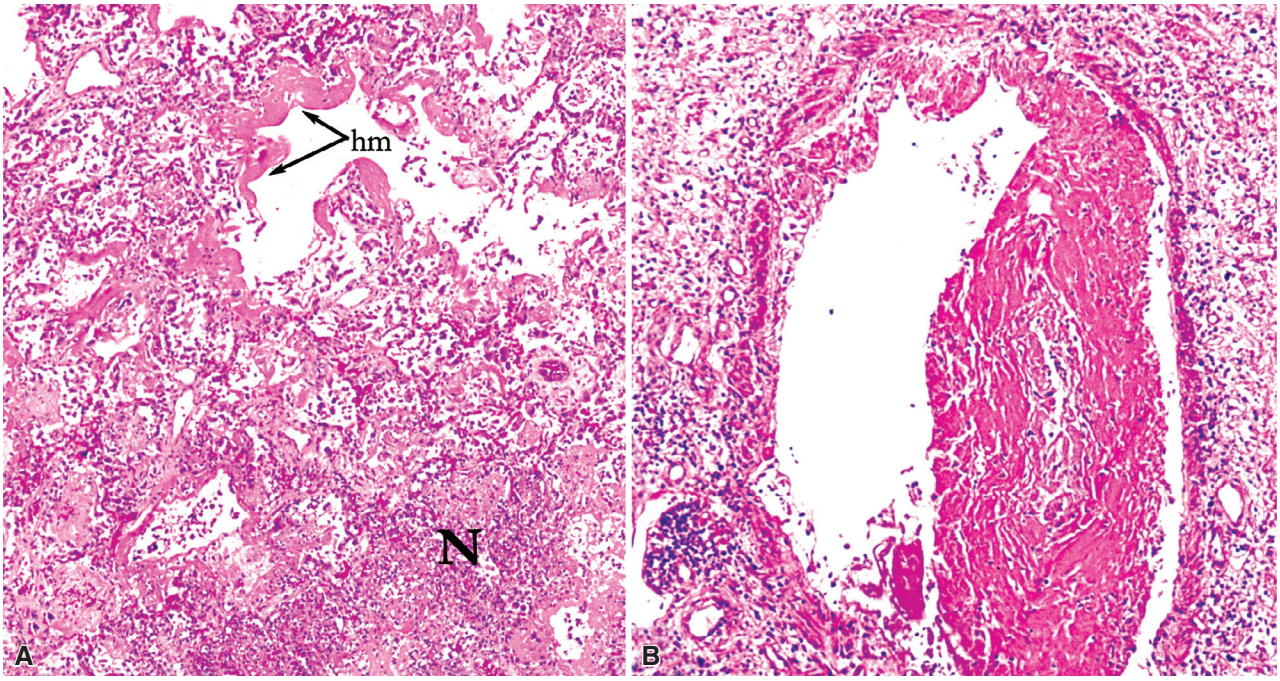

Figure 7.101 Adenoviral pneumonia. (A) Necrosis *(N)* and diffuse alveolar damage *(hm)*. (B) Necrotizing bronchiolitis. *hm,* hyaline membrane.

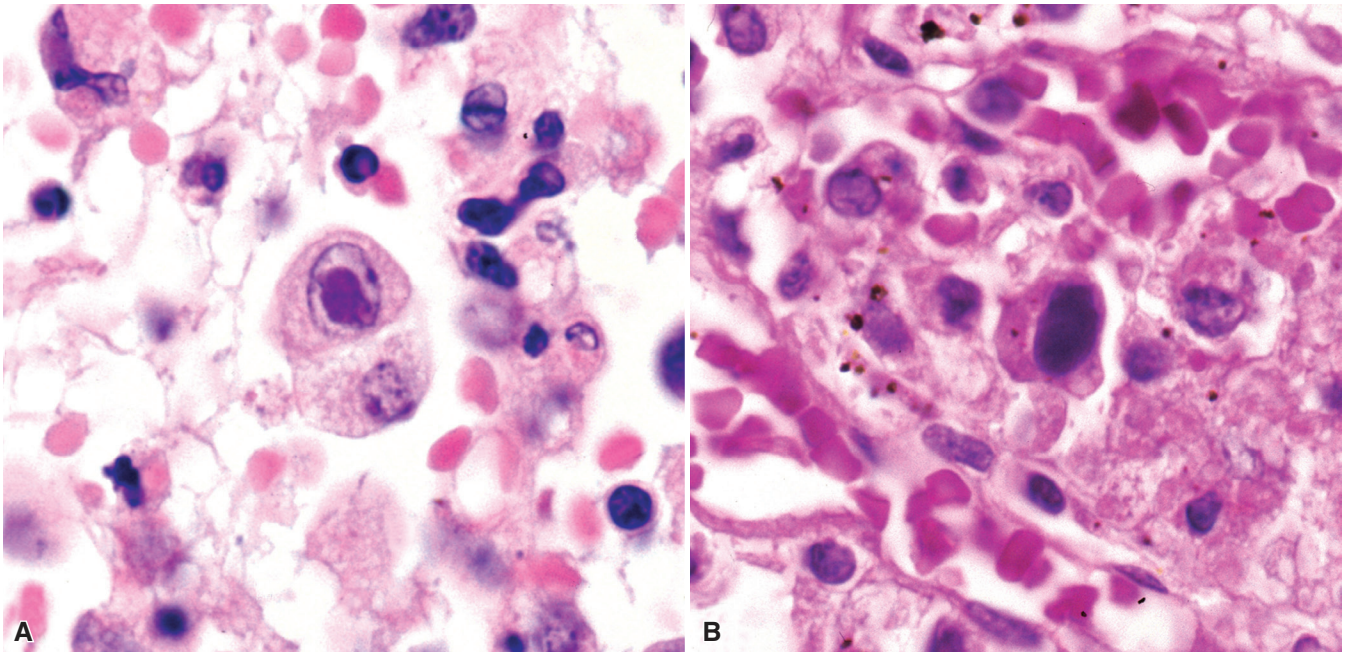

Figure 7.102 Adenovirus. (A) Cowdry A intranuclear inclusions. (B) Smudged cell.

genitalia (type II). Considerable crossover has been documented, however, with both types isolated from patients with disease at either site. Tracheobronchitis and pneumonia due to these viruses are rare in healthy adults with intact immune systems. They occur primarily in patients with underlying pulmonary disease and in association with inhalational and intubational trauma. They also occur in neonates and in patients who are immunosuppressed or compromised by various chronic diseases. Characteristic lesions include tracheobronchitis (Fig. 7.103A) with ulcers and hemorrhagic diffuse alveolar damage. Necrosis in a miliary small (or rarely large) nodular pattern is a helpful clue and the best location to identify CPE (Fig. 7.103B).[73] Like adenovirus, HSV also has two types of CPE: Initially a ground-glass amphophilic intranuclear inclusion, *Cowdry B*, appears with marginated chromatin; later a single eosinophilic *Cowdry A* inclusion (Fig. 7.104) surrounded by a halo, similar to that seen with adenovirus, develops. The Cowdry A inclusion is considered noninfectious, as it is devoid of nucleic acid protein and is thought to represent the nuclear "scar" of HSV infection.[307] In the absence of smudge cells, HSV and adenoviral infections can look identical. Fortunately, immunohistochemistry or in situ hybridization can often resolve this differential diagnosis.

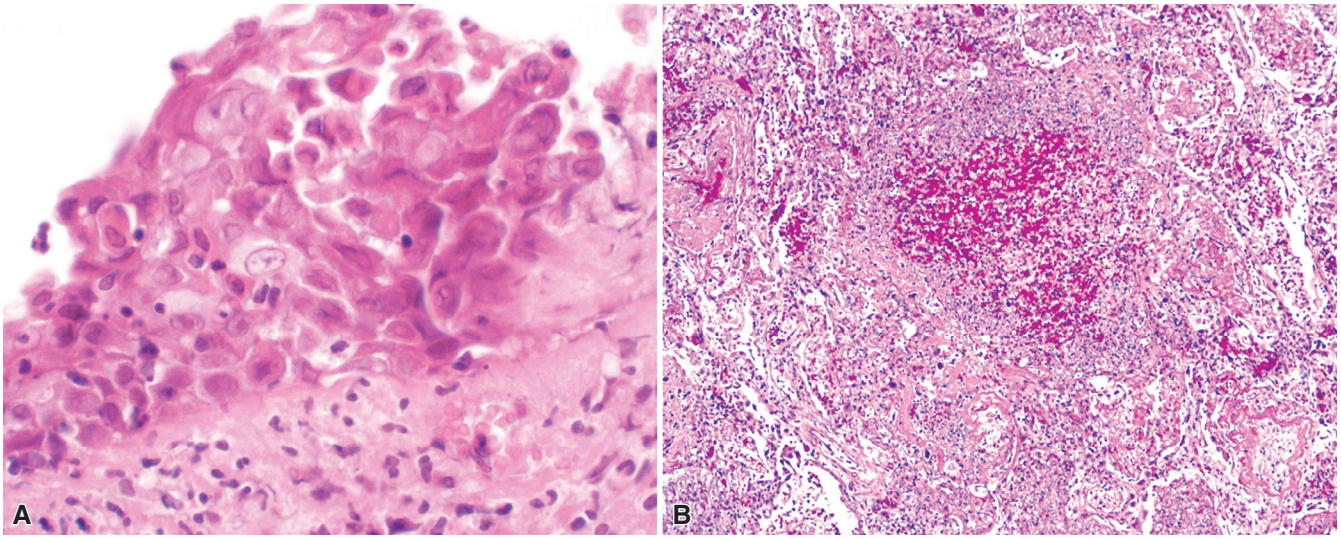

Figure 7.103 Herpes simplex virus pneumonia. (A) Tracheobronchitis. Note cells with ground-glass inclusion. (B) Miliary nodular pattern of hemorrhagic necrosis.

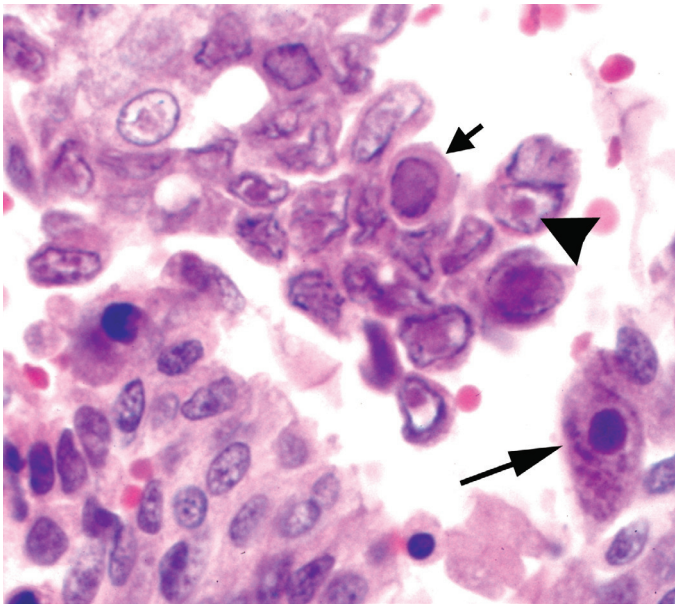

Figure 7.104 Herpes simplex virus pneumonia. Note two types of nuclear cytopathic effects: Cowdry A ground-glass type *(short arrow)* and Cowdry B eosinophilic inclusion *(arrowhead)*. Compare with cytomegalovirus intranuclear and cytoplasmic inclusion at long arrow.

Varicella-Zoster Virus

Varicella-zoster virus (VZV) infection produces considerable morbidity in the newborn, the adult, and the immunocompromised host, both in its primary form (varicella) and in its reactivated form (zoster). Varicella pneumonia is rarely observed in otherwise healthy children but is a major complication of adult varicella, occurring in approximately 10% to 15% of adults with VZV. In affected adults without underlying diseases and normal immunity, the course is generally mild and self-limited. Nevertheless, fatality rates of up to 10% have been reported.[307] By contrast, high mortality rates (25% to 45%) have been noted among some cohorts of immunosuppressed patients. Microscopically, small miliary nodules of necrosis are seen, associated with interstitial pneumonitis, edema, fibrin deposits, or patchy hyaline membranes (Fig. 7.105A). HSV-like intranuclear inclusions are present but may be sparse and difficult to identify. A miliary pattern of calcified nodules (Fig. 7.105B) may be present in the healed phase.[358]

Cytomegalovirus

CMV infections are acquired throughout life. This virus can cause considerable morbidity and even death in the neonate, but infection is generally asymptomatic in older healthy children and adults. As in the case of other herpesviruses, primary infection is followed by latency, which persists until immune deficiency or immunosuppressive therapy causes it to reactivate and disseminate. CMV has therefore become one of the most common opportunists in patients with AIDS and those who receive organ transplants. In these settings, CMV can produce a variety of patterns, including one with minimal changes where only scattered alveolar lining cells with typical viropathic changes are seen. The CPE of CMV produces cytomegalic cells with large, round to oval, smooth "owl eye" eosinophilic to basophilic intranuclear inclusions surrounded by a clear halo (Fig. 7.106A).

Later, multiple eosinophilic cytoplasmic inclusions develop that may be positive on staining with PAS and GMS (Fig. 7.106A, inset). The more numerous the cytomegalic cells, the greater the clinical significance. In some cases, atypical inclusions may be seen in cells that are not significantly enlarged, and the nuclei may contain dark-staining homogeneous inclusions that may lack a clear halo. Despite their atypical appearance, these inclusions will usually be highlighted with immunohistochemical stains.[359] Another typical pattern that suggests viral infection is the presence of small miliary nodules with a central hemorrhage surrounded by necrotic alveolar walls (Fig. 7.106B).[73] Interstitial pneumonitis is the least common pattern of CMV infection. Ulcers may be seen in the trachea and bronchi, but they occur less often than in herpetic infections. In CMV pneumonias, it is advisable to look for other pathogens, typically *P. jirovecii* (Fig. 7.107); but bacteria, fungi, protozoa, and other viruses are all possible coinfecting organisms.[360]

Epstein–Barr Virus

EBV infections are usually acquired in childhood and are generally asymptomatic. The pathologist most often encounters this virus in the lung in the context of pulmonary lymphomas or in other EBV-associated lymphoproliferative disorders that can occur in transplant recipients and other immunocompromised patients. However, the most common

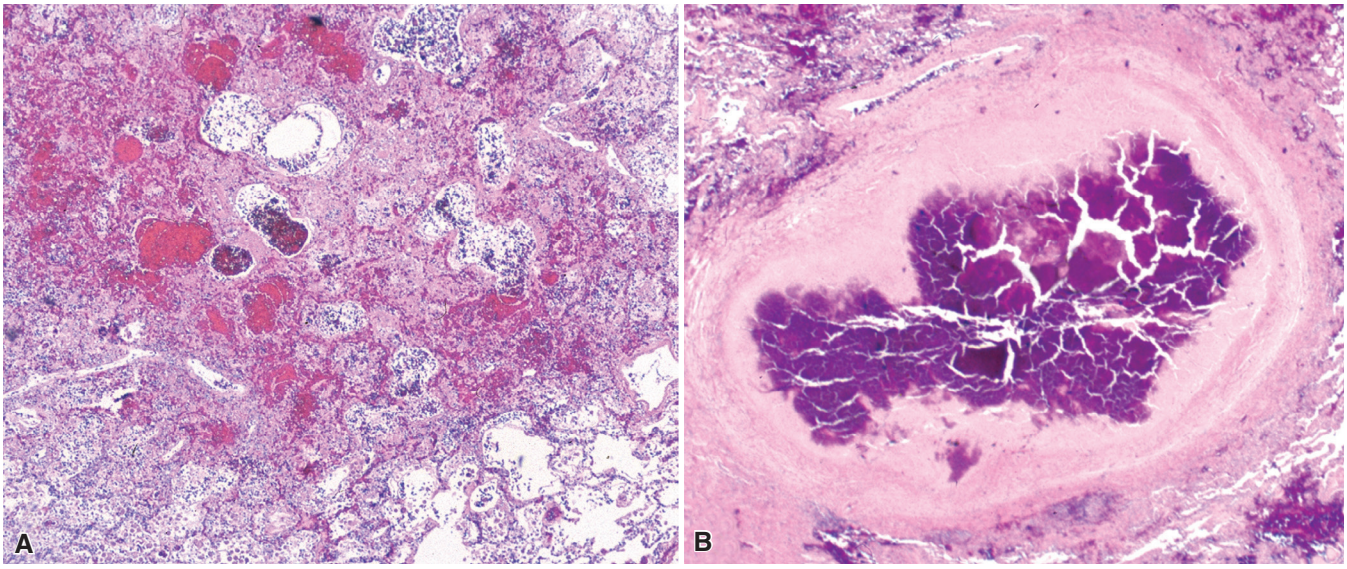

Figure 7.105 Varicella pneumonia. (A) A hemorrhagic miliary nodule. (B) Late phase with calcified nodules.

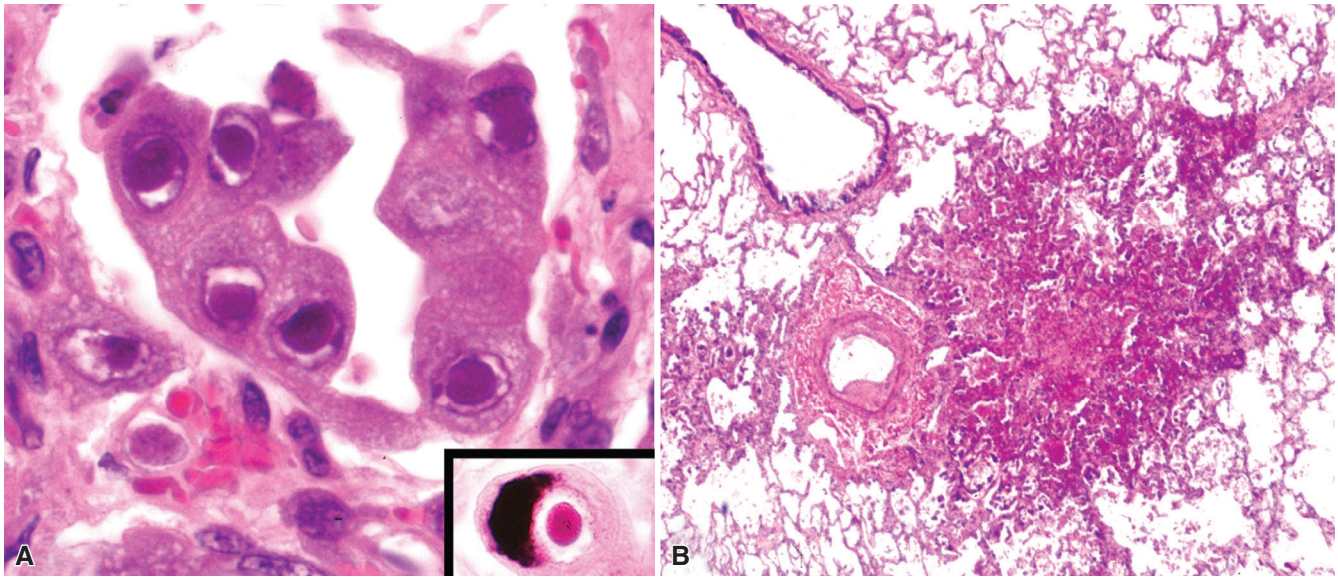

Figure 7.106 Cytomegalovirus (CMV) pneumonia. (A) Multiple characteristic intranuclear and intracytoplasmic inclusions in alveolar lining cells. Note Grocott methenamine silver–positive staining of inclusions *(inset)*. (B) Miliary nodule pattern of CMV pneumonia. (A, Courtesy Dr. Francis Chandler, Augusta, Georgia.)

symptomatic primary EBV infection is infectious mononucleosis. Most of these patients recover uneventfully, but a few develop one or more complications. Pneumonitis is one of them, albeit rare and not well characterized. The few reports describing pathology indicate a nonspecific lymphocytic interstitial pneumonitis, which may be bronchiolocentric (Fig. 7.108).[361,362] CPE is absent, and although serologic studies can be supportive of a clinicopathologic diagnosis, etiologic proof of EBV infection requires the demonstration of the virus in lymphoid cells by in situ hybridization for EBV-encoded RNA-1 (EBER-1).

Cytopathology

The cytologic features of viral infections in the respiratory tract are most likely to be found in exfoliative specimens, such as bronchial washings and BAL fluid samples, rather than needle aspirates, although viral diagnosis has been achieved with this technique.[363,364] This is because viral infections are less likely to produce radiologic mass–like infiltrates, which are the most common targets of needle biopsy procedures. Herpes simplex virus, CMV (Fig. 7.109), and adenovirus are the most commonly identified viral pathogens in respiratory cytologic specimens, but varicella virus, parainfluenza virus, RSV, human metapneumovirus, and measles virus have also been detected.

Characteristic CPE produced by these viruses is often better appreciated in cytologic smears than in tissue sections, which may, in fact, yield a negative result. Therefore review of any cytology sample taken at the time of biopsy can be valuable. Other, less specific changes may be found. These include ciliocytophoria (free cilia complexes with terminal bars) and cytologic atypia mimicking cancer.[46]

Microbiology

Diagnostic virology is the newest of the microbiology and infectious disease specialties to have benefited from the technologic revolution in laboratory medicine. Rapid and accurate diagnosis can often be achieved

today using practical, convenient laboratory methods that employ reliable, commercially available mammalian cells, media, and reagent systems.[297,365,366] This has allowed many rural and small urban hospital laboratories to provide timely viral diagnostic services not possible a short time ago. It is predicted that self-contained, rapid-cycle real-time PCR methods will one day account for the majority of viral assays in laboratories of all sizes. As a result, the pathologist who suspects a viral infection will increasingly have a variety of tools to obtain an etiologic diagnosis when morphologic manifestations are suggestive of viral infection.

The basic approaches to viral diagnosis in the laboratory are listed in Box 7.23. In questionable cases, confirmation by immunohistochemical studies (Fig. 7.110A), in situ hybridization (Fig. 7.110B), or electron microscopy may be helpful.[32,367] Many of the traditional methods of viral detection, detailed later, are being augmented by respiratory panel assays based on the detection of nucleic acid and compiled around common respiratory viral and bacterial pathogens.[368,369]

The diagnosis of viral respiratory infections can also be based on antigen detection and culture (Fig. 7.111). Direct antigen detection in clinical specimens collected by nasopharyngeal swabs, nasal washings, and aspirates or BAL fluid (but not sputum samples or, with rare exception, throat swabs) is performed using monoclonal antibodies by either immunofluorescence microscopy or enzyme immunoassay. By using a single reagent containing the monoclonal antibodies against several viruses and dual fluorochromes, the common respiratory viruses can be rapidly screened by direct immunofluorescence testing. Positive specimens can then be tested with individual reagents to determine the specific etiologic agent, while negative specimens can be submitted for culture.[370] Enzyme immunoassay includes methods that offer speed and convenience at the point of care. However, they are less sensitive than standard virologic methods, which must still be used to test negative specimens. Direct detection can also be accomplished in cellular samples, including tissue, by in situ hybridization or amplification techniques such as PCR. For RNA viruses, PCR amplification uses a reverse transcriptase (RT) step. PCR methodology has recently evolved into multiplex formats, and novel systems have been introduced that combine multiplex PCR chemistry with electron microarray (DNA chip) technology or fluid microsphere-based systems, permitting the simultaneous detection of a wide array of respiratory viruses and other pathogens.[371-376]

These systems have the potential to more rapidly and accurately diagnose acute infections and may also allow the study of complex coinfections and the active monitoring of outbreaks of influenza and other viral illnesses.[377] Panels composed of common respiratory bacterial and viral pathogens are available; these are based on nucleic acid detection by nested PCR and come by several brand names. Such panels typically encompass many of the respiratory viruses detailed previously with specimens obtained through sampling with a nasopharyngeal swab.[378,379]

Traditional viral cultures in tubes with various types of cell monolayers are currently performed with greater sensitivity and turnaround time using the shell vial technique. This technique uses centrifugation of clinical specimen suspensions onto coverslipped cell monolayers followed by brief incubation (1 to 2 days) and antigen detection.[365] It is important, therefore, to preserve a portion of tissue from a bronchial or transbronchial

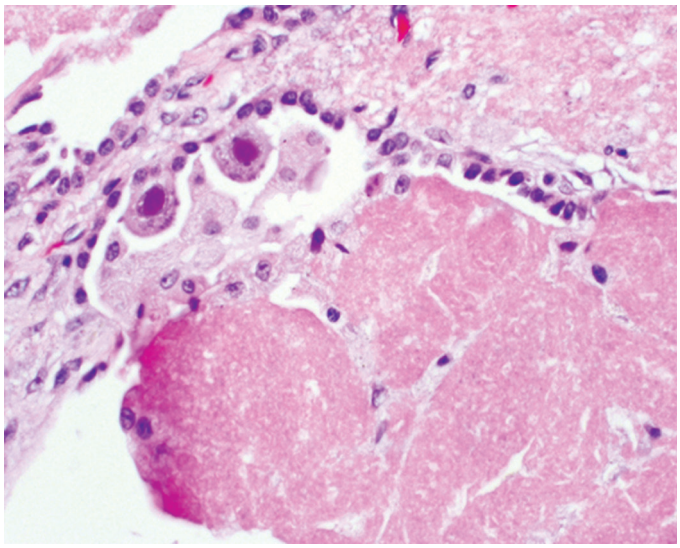

Figure 7.107 Cytomegalovirus-infected alveolar lining cells associated with the foamy alveolar casts of *Pneumocystis jirovecii*.

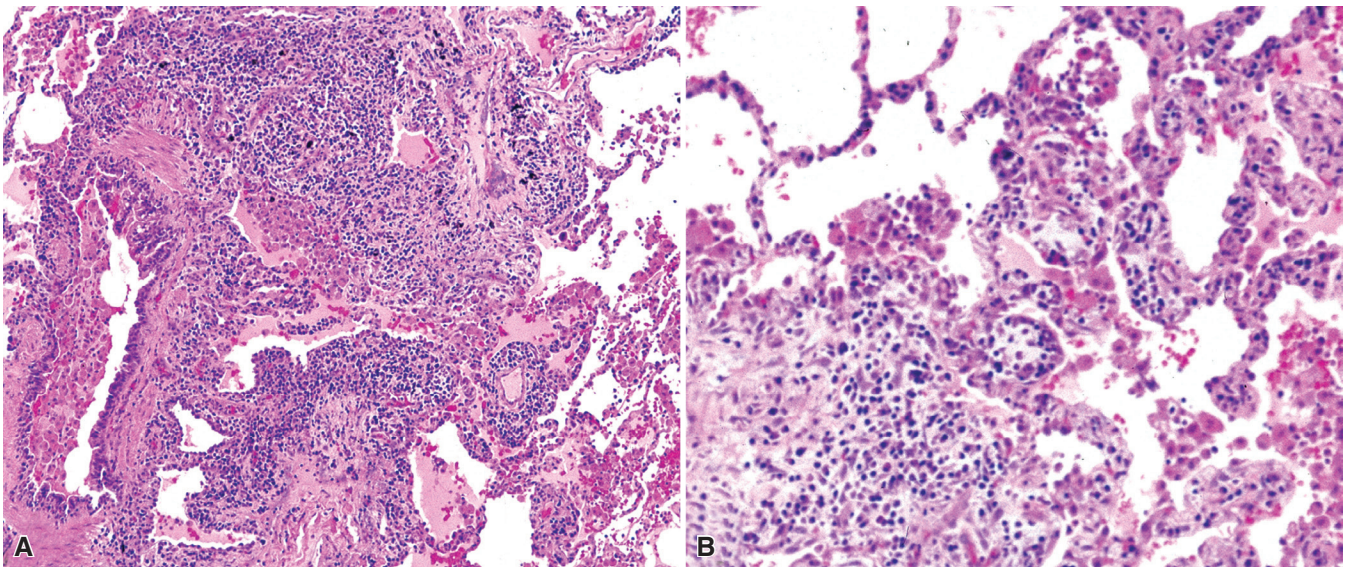

Figure 7.108 Epstein–Barr virus pneumonitis. (A) Nonspecific cellular interstitial pneumonitis. (B) Patchy interstitial infiltrate.

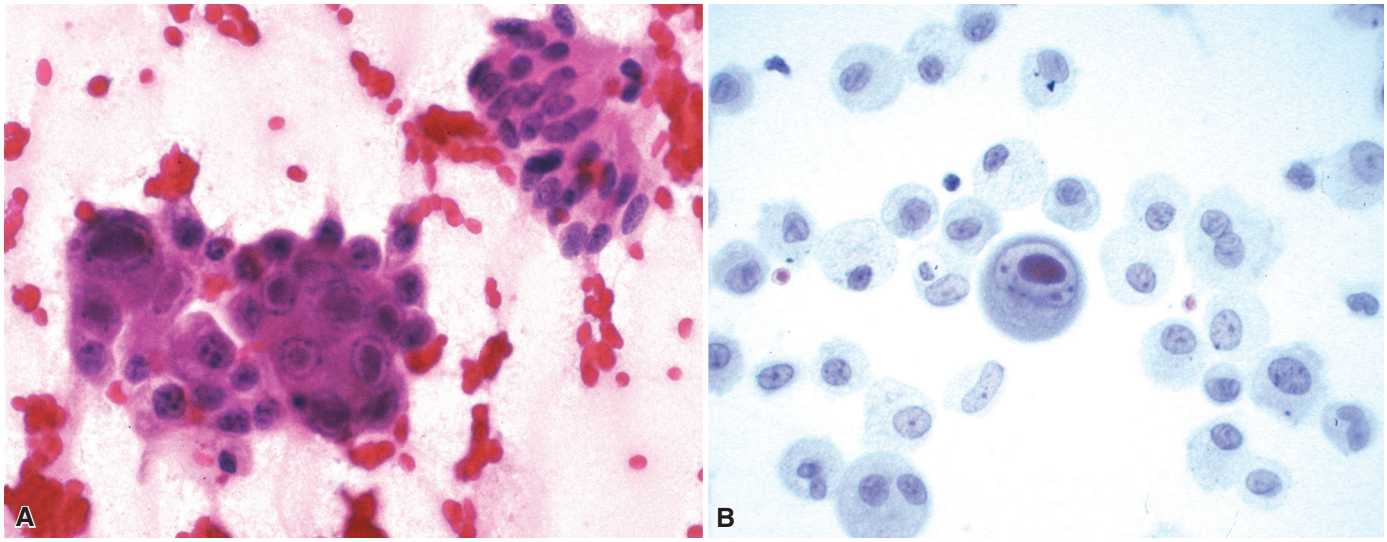

Figure 7.109 Cytomegalovirus pneumonitis with characteristic cytopathic effect. (A) Fine-needle aspirate. (B) Bronchoalveolar lavage specimen.

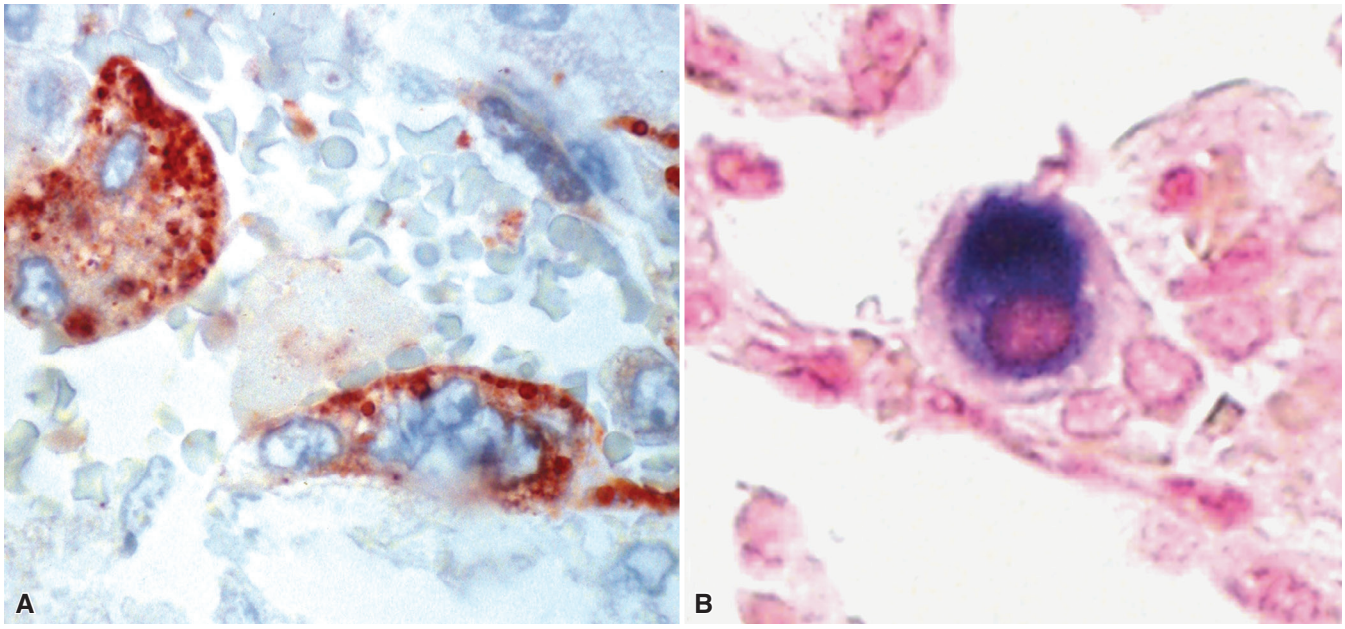

Figure 7.110 (A) Respiratory syncytial virus cytoplasmic inclusions detected by immunohistochemical staining. (B) Cytomegalovirus-infected cell with cytoplasmic inclusions detected by in situ hybridization. (Courtesy R.V. Lloyd, MD, Rochester, Minnesota.)

Box 7.23 Laboratory Diagnosis of Viral Pneumonia

- Direct detection of organisms
  - Histopathologic/cytopathologic examination for cytopathic effect (CPE)
  - Immunohistochemical studies
  - Electron microscopy
- Antigen detection
  - Direct fluorescent antibody test
  - Enzyme immunoassay
- Culture
  - Conventional roller tube technique
  - Shell vial technique
- Serologic studies
- Molecular methods
  - In situ hybridization
  - DNA amplification

biopsy or thoracotomy specimen in viral transport medium, especially with an immunocompromised patient, who may not have had BAL fluid submitted for culture. Shell vials, although faster than the traditional tube culture method, are still a slow method based on viral growth and are being replaced by direct nucleic acid detection.

Viral serologic testing has commonly been used for diagnosis but may be the least sensitive approach. A positive serodiagnosis is typically based on a fourfold rise in titer between acute and convalescent sera and therefore cannot be achieved by this means in the acutely ill patient; antigen detection or culture of respiratory tract specimens is much preferred. However, a serologic strategy, utilizing a panel of antigens in an immunofluorescence or enzyme immunoassay format on a single specimen, is useful in suspected EBV infections.[380]

A case also can be made for the benefit of CMV serologic testing for assessing the antibody status of organ donors and recipients for

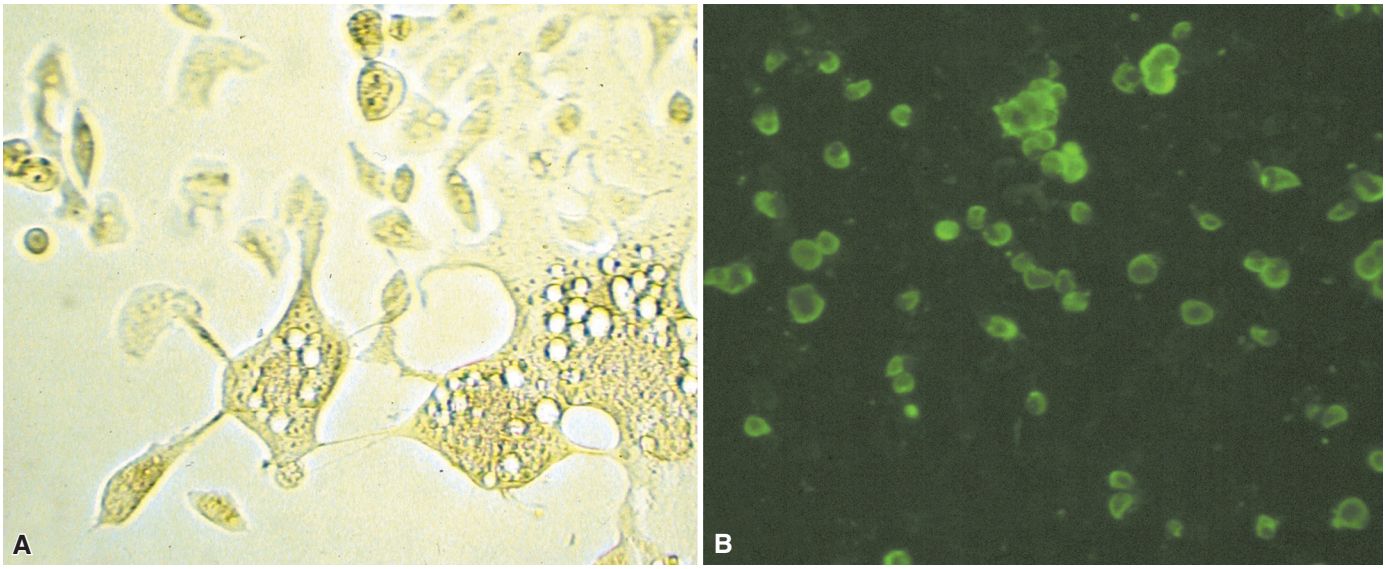

Figure 7.111 Respiratory syncytial virus (RSV) infection. (A) RSV cytopathic effect in tissue culture. (B) RSV antigen in nasopharyngeal swab specimen detected by direct immunofluorescence microscopy.

predicting the risk of posttransplantation CMV disease. When tissue is not available or findings are inconclusive, tests for the detection of actual disease in these transplant recipients include the p65 antigenemia assay on peripheral blood leukocytes and amplification or quantitation of CMV DNA in various peripheral blood compartments (plasma, whole blood, and leukocytes).[381] These assays may eventually replace culture of BAL fluid for surveillance of CMV infection in such patients.[382] The detection of virus in respiratory secretions (including BAL fluid), urine, or blood establishes the presence of virus but does not necessarily implicate it as the etiologic agent of a pneumonia. Quantitation of viral load by real-time PCR amplification, however, can be useful in this regard by linking high viral load with infection.[383]

Differential Diagnosis

A synopsis of the key morphologic and microbiologic features of the viral pneumonias is presented in Table 7.12. In the absence of CPE, diffuse alveolar damage and other patterns of lung injury are not diagnostic of viral infection. Diffuse alveolar damage is a nonspecific response to many types of infection, including bacterial, mycobacterial, fungal, and protozoal, all of which must be considered in the differential diagnosis. In addition, other noninfectious causes include reactions to drugs, radiation, toxic inhalants, and shock of any type. Occasionally, CPE may not be diagnostic; for example, the early inclusions of adenovirus, HSV, and CMV may be quite similar. In most cases, immunohistochemistry or molecular techniques can resolve the diagnostic dilemma. Mimics of CPE that must be ruled out include macronuclei in both reactive processes and occult neoplastic infiltrates and intranuclear cytoplasmic invaginations, which can occur in a variety of cells. Cytoplasmic viral inclusions can also be simulated by aggregated altered protein and particulate matter.

Parasitic Infections

Approximately 300 species of helminth worms and 70 species of protozoa have been acquired by humans during our short history on Earth.[384] Most of these are rare, but approximately 90 are relatively common and some have been found in the lung.[385-389] With travel to endemic areas and emergence (or reemergence) of parasitic pathogens in immunocompromised patients, pathologists will see these organisms[36] as exotic pulmonary conditions.

Etiologic Agents

Several parasite species migrate through the lungs as part of their normal life cycle, but few preferentially infect the human lung.[390] Most are aberrant pulmonary localizations in the human host, where they become lost in transit or are part of a secondary disseminated infection from another organ system, often in the setting of compromised immunity. The listing of etiologic agents in Box 7.24 is selective, based on the more common pathogens known to be associated with pulmonary involvement.

Histopathology

When parasites in the form of adult worms, larvae, or eggs invade or become deposited in lung tissue, they usually provoke an intense inflammatory reaction with neutrophils, eosinophils, and various mononuclear cells. One or more of the patterns listed in Box 7.25 may be identified. When the predominant site of involvement is the bronchial mucosa, a bronchitis and bronchiolitis pattern is observed; when they become impacted in pulmonary arteries, a nodular angiocentric pattern is observed, although it may be overshadowed by thrombosis and infarction. Some parasites invade the alveolar parenchyma, resulting in a pattern of miliary small nodules or pneumonitis. Naturally none of these patterns are consistently present and combinations of patterns may be seen. In some cases, an acute Loeffler-like eosinophilic pneumonia may reflect an allergic reaction to the transient passage of larvae through the pulmonary vasculature.

The various patterns, although nondiagnostic, can be suggestive of a parasitic infection, particularly when they incorporate a heavy eosinophilic infiltrate or granulomatous component. Eosinophilic lung disease, with or without blood eosinophilia, has a diverse etiology but is particularly characteristic of parasitic infection, especially in the tropics.[385] In the United States, other infections, such as coccidioidomycosis, must be considered, in addition to the many noninfectious causes of pulmonary eosinophilia. The challenge for the pathologist is the identification of a parasite, distinguishing it from artifact or foreign body, and classifying it as precisely as possible based on its size and unique morphologic features. Once the presence of suggestive morphologic features has been confirmed, the patient's travel or avocation history can help to further narrow the scope of the differential diagnosis.

Table 7.12 Viral Pneumonias: Summary of Pathologic Findings

Assessment Component	Findings
Influenza Virus	
Surgical pathology	Diffuse alveolar damage, bronchitis, and bronchiolitis; secondary acute purulent pneumonia; antigen detection by immunofluorescence, immunohistochemical, or in situ hybridization studies
Cytopathology	Nonspecific changes may include presence of reactive-type pneumocytes; ciliocytophoria
Microbiology	Antigen detection by DFA or EIA; culture on primary monkey kidney cells: noncytopathic; detection by hemadsorption
Respiratory Syncytial Virus	
Surgical pathology	Bronchiolitis with lumen detritus; may be associated with syncytial giant cells; diffuse alveolar damage in immunocompromised patients; confirm with immunohistochemistry
Cytopathology	Giant cell syncytia characteristic but often not seen; eosinophilic inclusions may be seen in bronchial epithelial cells of immunocompromised patients; rarely in those of normal hosts; rarely diagnosed by cytology alone
Microbiology	Antigen detection by DFA and EIA usually more sensitive than culture; cultures on continuous epithelial cell lines (Hep-2) and primary monkey kidney yield characteristic syncytial CPEs
Measles Virus	
Surgical pathology	Bronchitis, bronchiolitis, diffuse alveolar damage with giant cells containing Cowdry A inclusions and small cytoplasmic inclusions
Cytopathology	Eosinophilic intranuclear and cytoplasmic inclusions; rarely diagnosed by cytology
Microbiology	Antigen detection by DFA and EIA; culture on primary monkey kidney produces spindle cell or multinucleate CPE; serologic testing (for measles-specific IgM) available
Hantavirus	
Surgical pathology	Pulmonary edema pattern with variable fibrin deposits; immunoblast-like cells in vascular spaces; confirm by immunohistochemistry
Cytopathology	Noncytopathic
Microbiology	Serology: Hantavirus-specific IgM or detection of specific RNA by PCR assay in peripheral blood leukocytes
Adenovirus	
Surgical pathology	Diffuse alveolar damage with or without necrotizing bronchiolitis and/or pneumonitis with necrosis and karyorrhexis
Cytopathology	Early Cowdry A intranuclear inclusions, later smudge cell; reactive and reparative-type atypia in background
Microbiology	Antigen detection by EIA and DFA; culture on continuous epithelial cell lines produces characteristic grape-like clustered cytopathic effect
Herpesvirus	
Surgical pathology	Tracheobronchitis; diffuse alveolar damage; miliary necroinflammatory lesions
Cytopathology	Ground-glass (Cowdry B) intranuclear inclusions; later Cowdry A inclusions in multinucleated cells, often with a "seeds in a pomegranate" appearance on Pap-, H&E-, and Diff-Quik–stained smears Background reactive and reparative atypia
Microbiology	Antigen detection by immunofluorescence; culture on diploid fibroblasts produces characteristic cytopathic effect, sometimes within 24 hours;serologic testing less useful
Varicella-Zoster Virus	
Surgical pathology	Miliary necroinflammatory lesions; calcified nodules in healed phase
Cytopathology	Intranuclear Cowdry A inclusions sparse and less welldefined than with herpes simplex
Microbiology	Antigen detection by immunofluorescence; culture on human embryonic lung or Vero cells produces CPE more slowly than for herpesviruses (3–7 days); serologic testing available
Cytomegalovirus	
Surgical pathology	Minimal changes with scattered cytomegalic cells; miliary necroinflammatory lesions; interstitial pneumonitis
Cytopathology	Large "owl eye" Cowdry A inclusions with halo; cytoplasmic inclusions stained with GMS
Microbiology	Culture on human diploid fibroblasts produces characteristic CPE slowly in traditional tube cultures but more rapidly with use of shell vial technique p65 antigenemia assay; PCR assay; selective application of serology useful
Epstein–Barr Virus	
Surgical pathology	Polymorphous lymphoid interstitial pneumonitis; confirm by in situ hybridization
Cytopathology	Noncytopathic
Microbiology	No routine culture; diagnosis by serologic testing using panel of antibodies (EA; IgG and IgM VCA; EBNA)

CPE, Cytopathic effect; *DFA*, direct immunofluorescence antibody (test); *EA*, early antigen; *EBNA*, Epstein–Barr virus–determined nuclear antigen; *EIA*, enzyme immunoassay; *GMS*, Grocott methenamine silver; *H&E*, hematoxylin and eosin; *IgG, IgM*, immunoglobulins G and M; *Pap*, Papanicolaou; *PCR*, polymerase chain reaction; *VCA*, viral capsid antigen.

Of interest, a common "parasite" encountered in clinical practice is not a parasite at all but aspirated vegetable material simulating the complex structure of an organism.[391]

Toxoplasmosis

T. gondii is an obligate intracellular protozoan and a common opportunist in patients with AIDS, the disease underlying most cases of toxoplasmosis seen in recent years. The brain and retina are most commonly involved in these patients, but pulmonary lesions may also be present in cases of disseminated disease. These often take the form of miliary small nodules with fibrinous exudates, which may progress to a confluent fibrinopurulent pneumonia.[392] Free forms (crescent-shaped tachyzoites) and cysts may be identified (Fig. 7.112). Pseudocysts packed with tachyzoites can be distinguished from true cysts with bradyzoites by staining of the latter with PAS and GMS.[393] Serology is the main method of diagnosis in the acute phase, and serology with concomitant radiologic findings in appropriate settings in immunocompromised hosts usually obviates the need for direct demonstration of the organisms. Either PCR on the specimen or immunohistochemistry can be used to demonstrate the organisms.[394]

Box 7.24 Some Common Parasitic Lung Pathogens

Protozoa

Toxoplasma gondii
Entamoeba histolytica
Cryptosporidia
Microsporidia

Metazoa (Helminths)

Nematodes
- *Dirofilaria immitis*
- *Strongyloides stercoralis*

Cestodes
- *Echinococcus* spp.

Trematodes
- *Paragonimus* spp.
- *Schistosoma* spp.

Box 7.25 Histopathologic Patterns in Parasitic Lung Injury

Eosinophilic pneumonia
Large nodule(s)
Miliary small nodules
Bronchitis and bronchiolitis
Abscess, cavities, and cysts
Intravascular reaction

Amebiasis

Amebic dysentery becomes invasive in a small percentage of patients. When the trophozoites leave the gut, they most commonly travel to the liver. From the liver, either by direct extension or rarely by hematogenous spread, the lungs may become involved. In this scenario, abscesses composed of liquefactive debris—with few neutrophils, distinguishable from bacterial abscess where neutrophils are dominant—may be seen, most often in the right lower lobe adjacent to the liver.[395,396] Trophozoites can be best seen at the margin of viable tissue (Fig. 7.113). They resemble histiocytes but are usually larger, with a lower nucleocytoplasmic ratio. A tiny central karyosome within a round nucleus having vesicular chromatin is characteristic.[397,398] Bronchial fistula formation and empyema can occur as complications; amebas may be found in sputum and pleural fluid, respectively, in these situations. For free-living amebic species (those of the genera *Acanthamoeba*, *Balamuthia*, *Naegleria*), the central nervous system is the principal focus of infection. However disseminated disease including lung infection (Fig. 7.114) may occur in certain epidemiologic situations, especially those involving compromised immune status, or in lung transplants.[399-401]

Cryptosporidiosis

Ten species of the intracellular coccidian protozoa are currently recognized, but one of them, *Cryptosporidium parvum*, causes most human infections.[402] Clinically, infection due to this organism may have three major manifestations: asymptomatic shedding, acute watery diarrhea that lasts for approximately 2 weeks, and persistent diarrhea that lasts several weeks. Patients with AIDS have a wider spectrum of disease severity and duration that includes a fulminant cholera-like illness.[402] These patients are most likely to manifest extraintestinal disease. In the lung, the organism targets the epithelium of the airways, just as it does the surface epithelium of the gut and biliary tract.[403] In H&E sections,

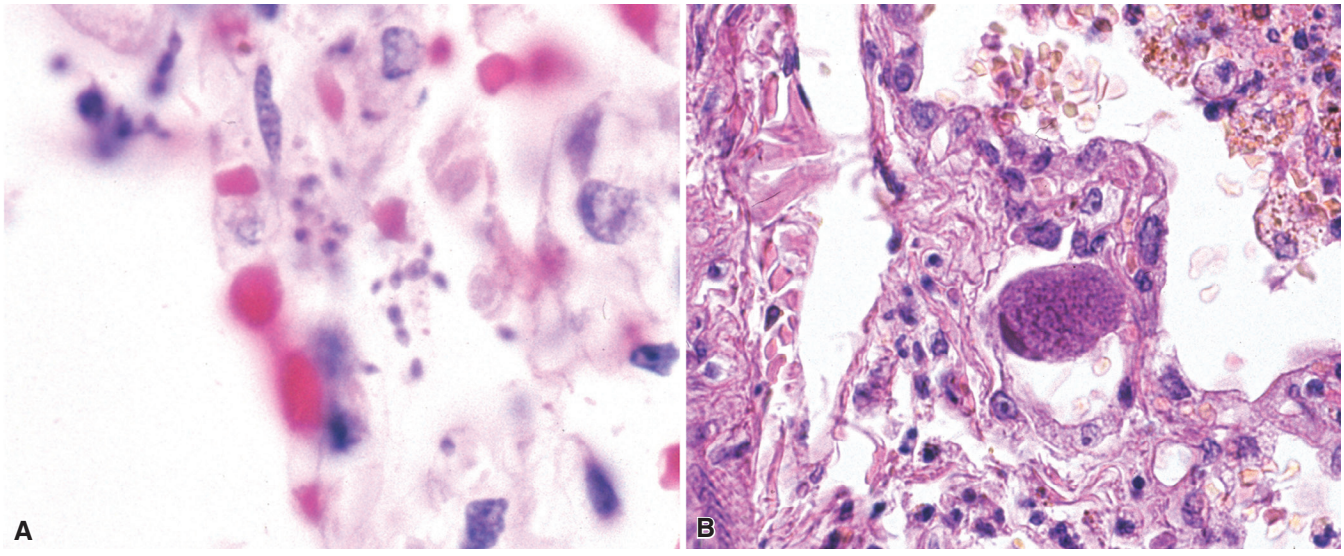

Figure 7.112 Toxoplasmosis. (A) Tachyzoites. (B) Pseudocysts packed with tachyzoites.

cryptosporidia appear as small (4 to 6 µm in diameter) round to oval protrusions from the cell surface. Electron microscopy reveals that they are intracellular but extracytoplasmic. In addition to H&E, they stain with Giemsa, PAS, GMS, and acid-fast stains. A mild to moderate chronic inflammatory cell infiltrate is usually present in the submucosa. Pulmonary cryptosporidiosis is largely a case report event, most reports being from earlier phases of the AIDS epidemic[404]—a surprise in reviewing acid-fast stains for more common organisms.[405] Newer reports suggest that respiratory cryptosporidiosis may occur in immunocompetent children with cryptosporidial diarrhea and cough.[406,407]

Microsporidiosis

The microsporidia are obligate intracellular spore-forming protozoa. More than 140 genera and 1200 species are recognized, but only 7 genera and a few species have been confirmed as human pathogens.[408] They are opportunists that have recently emerged in severely immunocompromised patients, AIDS patients, and transplant recipients, with case reports of pulmonary infections in the immunocompromised population.[409-411] Clinically they primarily cause chronic diarrhea and cholangitis. In the lung, they cause bronchitis or bronchiolitis (or both), usually in patients who also have intestinal infections or disease at other sites, especially the biliary tract.[412] The predominant pathologic changes are in the airways, which show a mixed inflammatory cell infiltrate of mononuclear and polymorphonuclear leukocytes.[413] The organisms are found within vacuoles in the apical portion of epithelial cells lining the airways. They appear as very small (1 to 1.5 µm in diameter) basophilic dots whose recognition depends on organism load. However, even when heavy, the findings can be subtle. Also, as with cryptosporidiosis, their presence is often overlooked or obscured by coexistent pneumonias. Special stains—such as modified trichrome, Warthin-Starry–type silver, and Gram stains—are more sensitive and specific, especially when used in combination.[414]

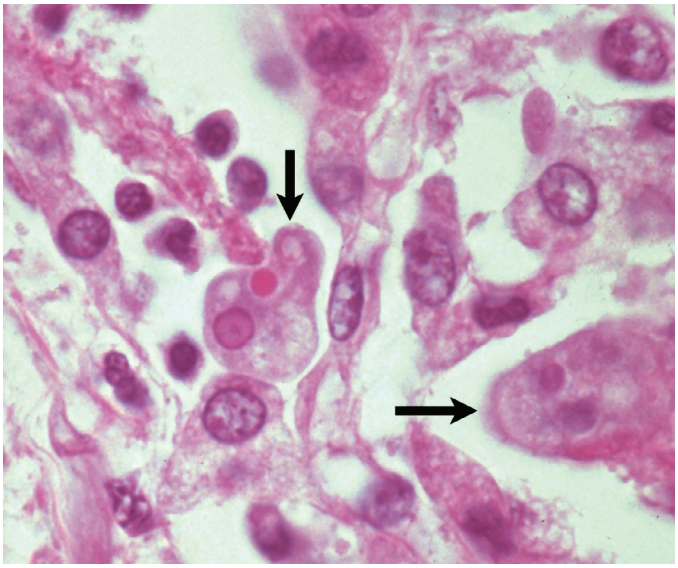

Figure 7.113 Amoebic trophozoite in lung tissue *(arrows)*. Note delicate marginal nuclear chromatin with small central karyosome and small red blood cell in cytoplasm. (Courtesy Ronald Neafi, Armed Forces Institute of Pathology, Washington, DC.)

Leishmaniasis

Leishmaniasis (*Leishmania donovani* infection) is transmitted to humans by several species of the *Phlebotomus* sand fly.[415] Pulmonary leishmaniasis has been reported in HIV-infected patients and transplant recipients.[385,416,417] The organisms (*L. donovani* amastigotes) can be found in the alveoli and alveolar septa and may be recovered in BAL fluid from these patients.[418] They also can be found in bronchoscopic biopsies (Fig. 7.115). Serologic testing for leishmaniasis has been suggested as part of the pretransplantation work-up in endemic areas.[419] A rapid PCR-amplified diagnostic method has been described.[420]

Dirofilariasis

The zoonosis caused by *Dirofilaria immitis*, a parasite of dogs and other mammals, is transmitted by mosquitos and black flies to humans.[421-423] Larvae injected by these insect vectors migrate from the subcutis into veins and travel to the heart, where they die before maturing into adult worms. They are then washed into the lungs by the pulmonary arterial blood flow, where they form the nidus of a thrombus. Formation of an infarct follows, typically manifesting as an asymptomatic solitary pulmonary nodule ("coin lesion") in the lung

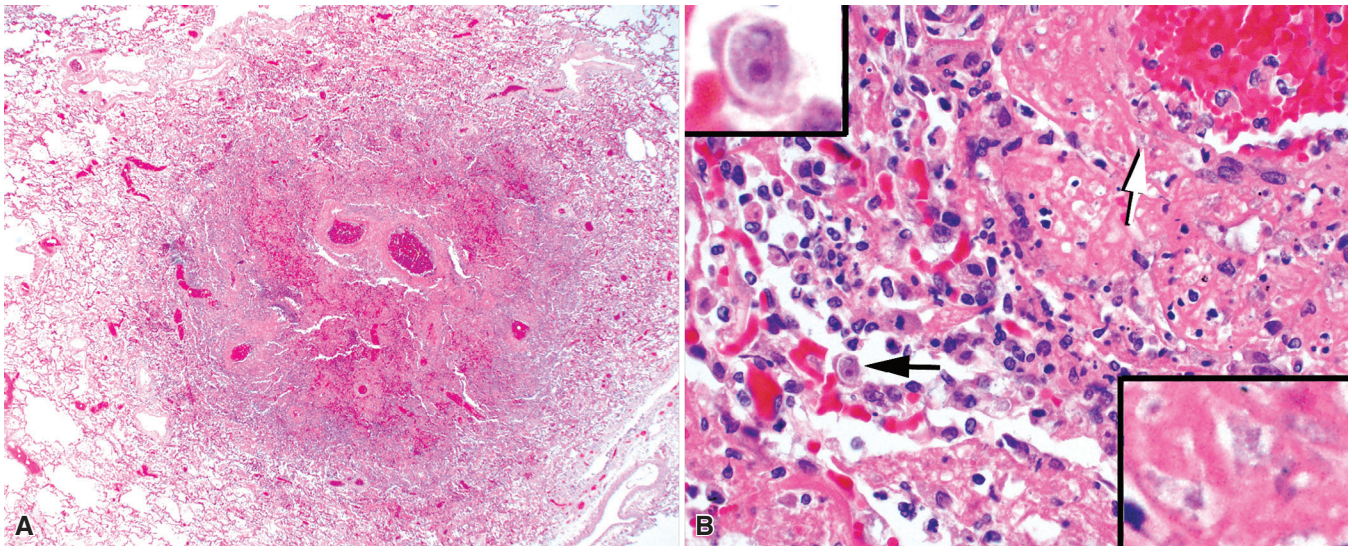

Figure 7.114 Free-living ameba in lung tissue from an immunocompromised patient. (A) Necroinflammatory nodule. (B) Encysted form, *black arrow* and *left upper inset*; trophozoite, *white arrow* and *right lower inset*.

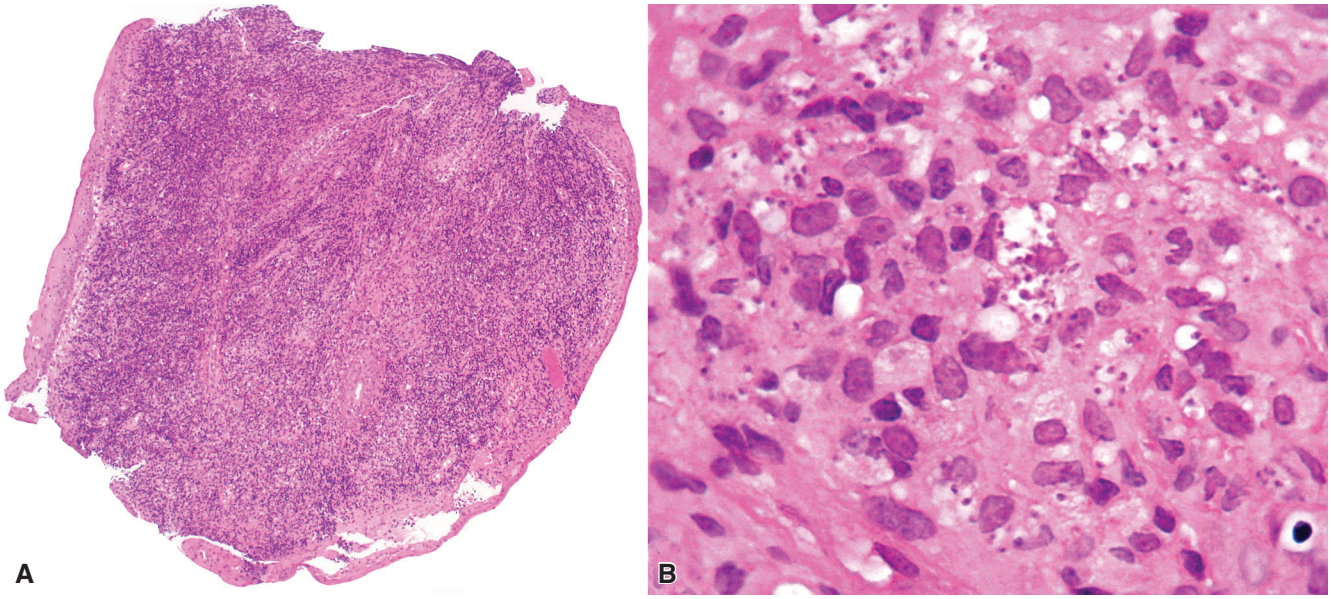

Figure 7.115 *Leishmania donovani* in bronchoscopic biopsy specimens obtained from a North African immigrant to Sicily. (A) Lower-power view of cellular infiltrate. (B) High-power view of dot-like organisms. (Courtesy Dr. Francesca Guddo, Palermo, Italy.)

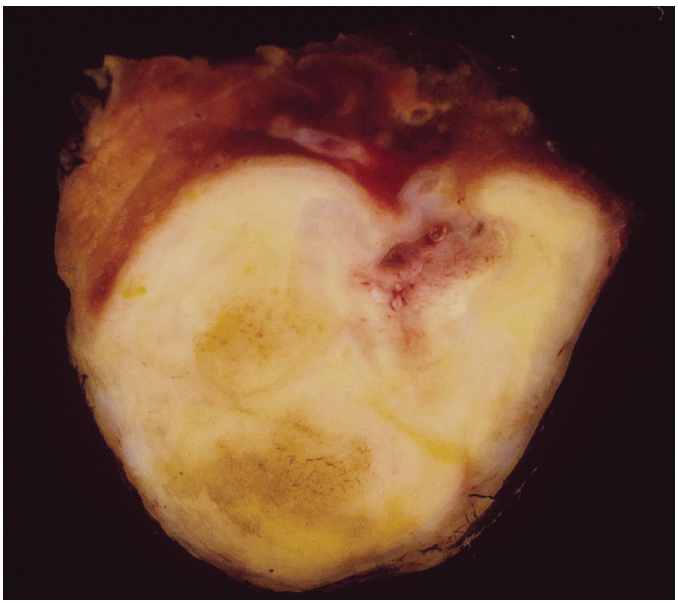

Figure 7.116 Dirofilarial nodule, gross specimen.

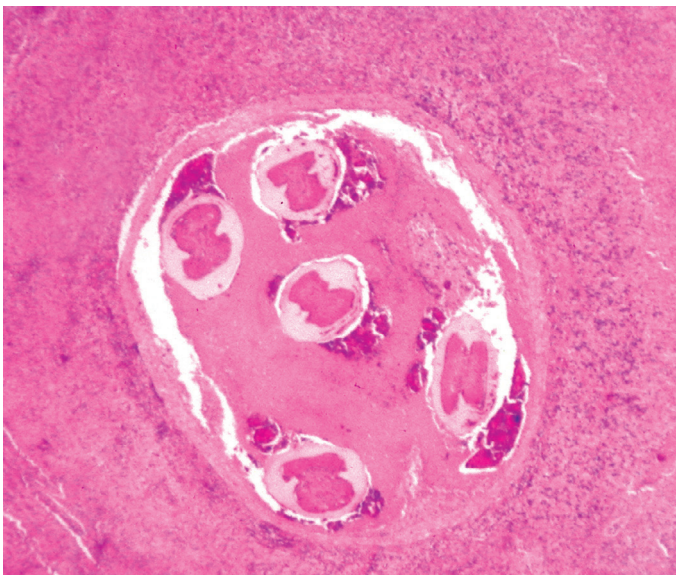

Figure 7.117 Dirofilarial nodule, with worm remnants in organizing thrombosed vessel.

periphery (Fig. 7.116) that may be visualized on a positron emission tomography (PET) scan.[424-426] Microscopically the nodule resembles a typical infarct with a core of coagulation necrosis but also containing degenerated worm fragments in the remnant of an arteriole (Figs. 7.117 and 7.118). A peripheral investment of chronic granulation tissue forms an interface with the alveolated parenchyma. "Step" sections and trichrome stains may be needed when H&E sections do not show the parasite.[427]

Strongyloidiasis

Strongyloides is a parasite most often found in patients or travelers in the tropics, but endemic foci are present in the southeastern United States. Rhabditiform larvae of the nematode *Strongyloides stercoralis*, after hatching from ingested eggs,[428] invade the small intestinal mucosa. At this site occult infection may remain asymptomatic for years. Dissemination typically follows debilitation brought on by immunocompromising diseases and therapies. When this occurs, filariform larvae leave the gut and travel through the pulmonary vasculature. When they penetrate alveoli (Fig. 7.119), they provoke hemorrhage and inflammation.[429-431] Loeffler syndrome, eosinophilic pneumonia, and abscesses may develop. When migration is interrupted, filariform larvae may metamorphose in situ to adult worms, which can produce eggs and rhabditiform larvae. Larvae identified in the sputum indicate hyperinfection.[432] Disseminated strongyloidiasis is but one example of an infection that may become manifest, particularly in immunocompromised patients, years after emigration from or travel to an endemic

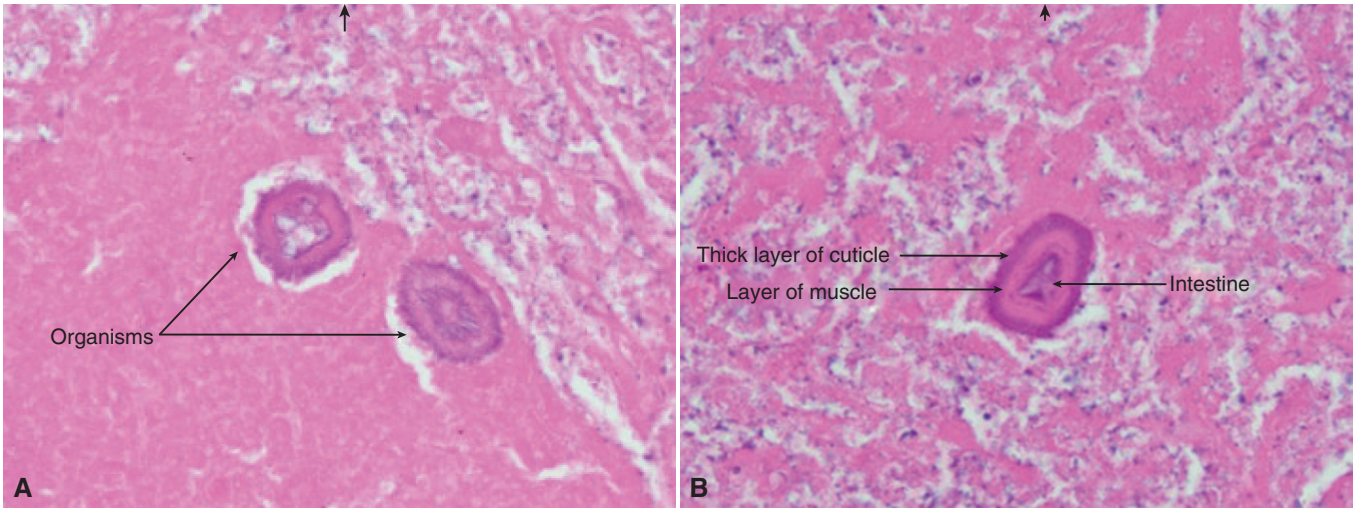

Figure 7.118 Dirofilariasis. (A) Intact worm cross section ×260. (B) Showing body cavity layers ×360. Surrounding necrosis in both figures. (From Abhisek, B, Reilly P, Perez A, et al. Human pulmonary dirofilariasis presenting as a solitary pulmonary nodule: a case report and brief review of literature. *Resp Med Case Rep.* 2013;10:40–42.)

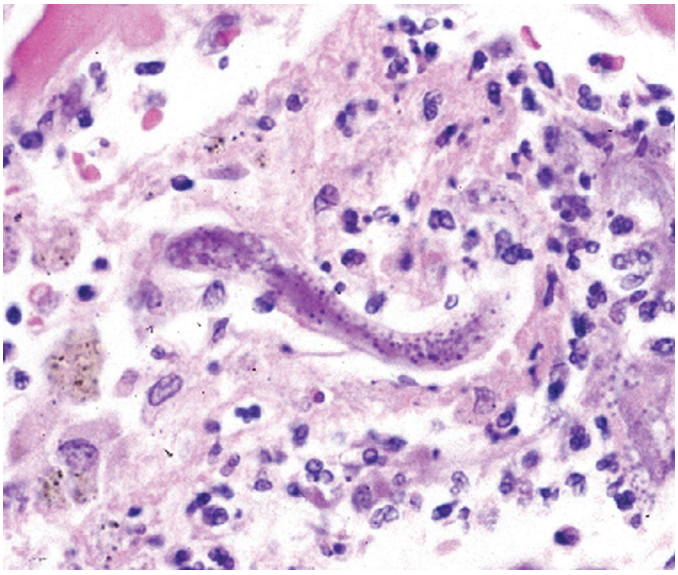

Figure 7.119 Filariform larva of *Strongyloides stercoralis* penetrating into alveolar space with associated inflammation.

area harboring pathogens that are considered unusual or exotic by pathologists in the United States.

Echinococcosis

Echinococcosis is a zoonosis that occurs wherever sheep, dogs or other canids, and humans live in close contact. Ingested eggs of the tapeworm *Echinococcus* hatch in the gut, releasing oncospheres, which then invade the mucosa, enter the circulation, and travel to various sites, where they develop into hydatid cysts.[433,434] In the lung, unilocular slow-growing cysts are produced by *Echinococcus granulosus*.[435] *Echinococcus multilocularis* proliferates by budding, producing an alveolar pattern of microvesicles.[398] The cyst of *E. granulosus* has a trilayered membrane (Fig. 7.120A) with an outer fibrous, middle-laminated hyaline, and inner germinal layer that gives rise to brood capsules containing infective protoscolices with hooklets and suckers (Fig. 7.120B). The layers usually become separated in tissue, with the outer fibrous layer containing chronic inflammatory cells that form an interface with the alveolated parenchyma. Cysts that rupture into bronchi may be expectorated as debris with protoscolices or portions of the cyst wall. Abscesses and granulomas may also form in the lung, pleura, and chest wall.[436]

Paragonimiasis

The parasite *Paragonimus* targets the lung and is acquired by the ingestion of freshwater crabs or crayfish infected with the metacercarial larvae of *Paragonimus* species.[437] Most cases worldwide are due to *P. westermani*, but several other species exist in Asia, Africa, and South and Latin America. In the United States, infections due to *P. kellicotti* have been reported.[390] The disease manifestations are related to the migratory route and the inflammatory response these hermaphroditic flukes stimulate as they enter lung parenchyma and travel to sites near larger bronchioles or bronchi. Typically an area of eosinophil-rich inflammatory reaction surrounds them, and this reactive process may evolve to form a fibrous pseudocyst or capsule containing worms, exudate, and debris (Fig. 7.121A). Cysts rupturing into bronchioles may result in eggs, blood, and inflammatory cells being coughed up in the sputum. Alternatively, eggs may become embedded in parenchyma, producing nodular granulomatous lesions (Fig. 7.121B) that progress to scars.[438] The eggs are yellowish, ovoid, and operculated, measuring 75 to 110 µm by 45 to 60 µm. The opercula unfortunately are not easily seen in tissue; however, the eggs are birefringent under polarized light, which helps to distinguish them from nonbirefringent schistosome eggs (Fig. 7.122).[390]

Schistosomiasis

The public health burden of schistosomiasis is enormous. This parasitic infection affects 200 million people in 74 countries while continuing to expand its geographical range.[439,440] The life cycle and disease manifestations of the three major *Schistosoma* species—*Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*—involve eggs, snail intermediate hosts, and free-swimming cercaria, which penetrate the skin of susceptible animals and people and develop into adult worms. The male and female worms eventually come to reside in various human venous plexuses, depending on the species, where egg deposition occurs. Pulmonary schistosomiasis comprises both acute and chronic forms. The acute disease, referred to as *Katayama syndrome*, manifests with fever, chills, weight loss, gastrointestinal symptoms, myalgia, and urticaria in patients with no previous exposure to the parasite. Acute larval

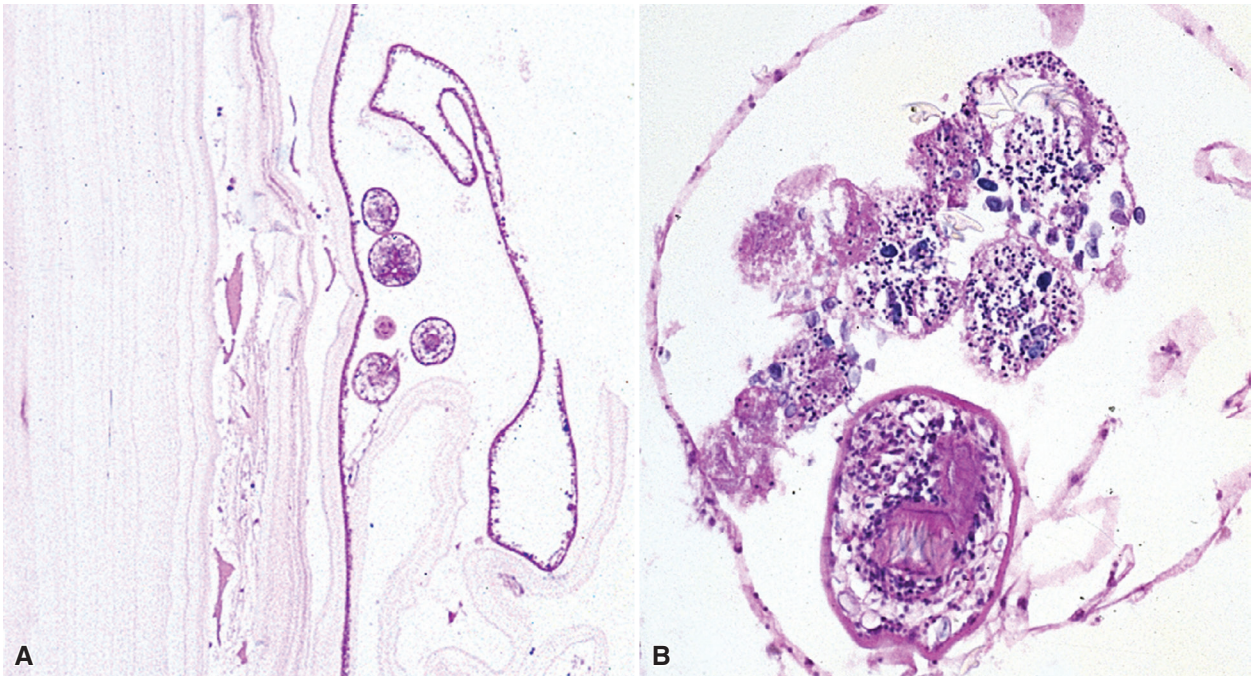

Figure 7.120 *Echinococcus granulosus.* (A) Cyst with trilayered membrane. (B) Brood capsules.

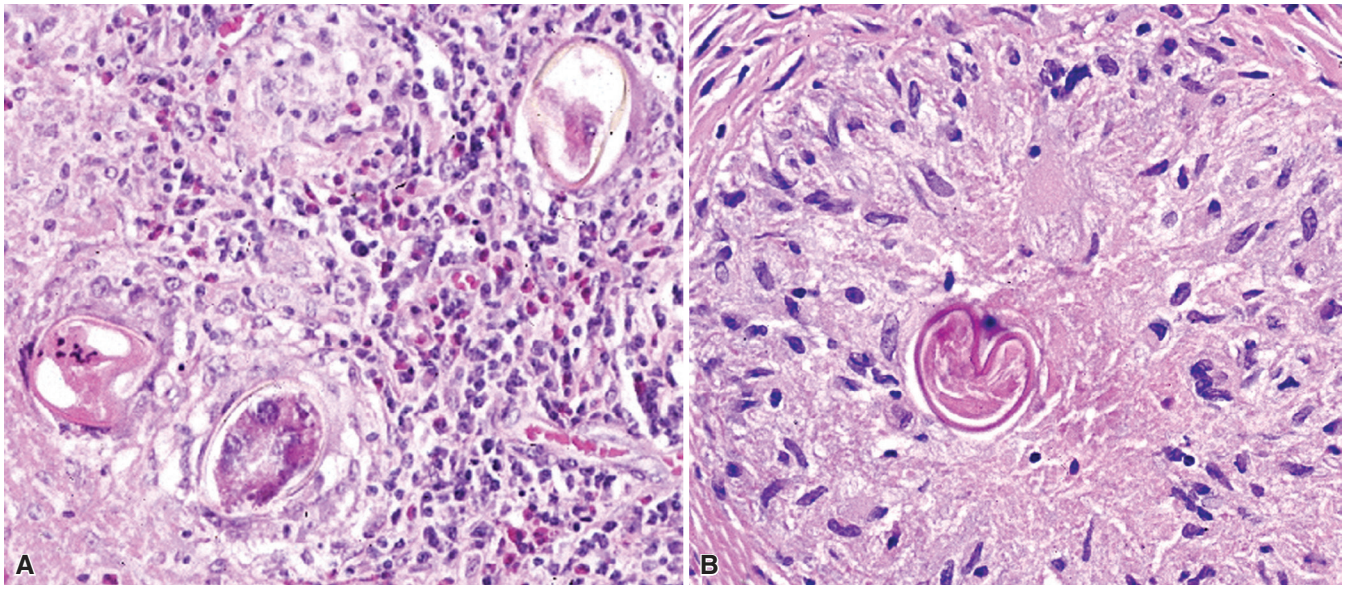

Figure 7.121 (A) *Paragonimus westermani* with yellowish refractile eggs in eosinophil-rich exudates. (B) Distorted egg of *Paragonimus kellicotti* in granuloma.

pneumonitis and a Loeffler-like eosinophilic pneumonia may be seen in this setting.[439,441] Chronic pulmonary disease is almost always secondary to severe hepatic involvement with portal hypertension. In this setting, the eggs *of S. mansoni*, and rarely *S. japonicum* or *S. haematobium*, may be shunted through portosystemic collateral veins to the lungs. The eggs lodge in arterioles, provoking a characteristic granulomatous endarteritis with pulmonary symptoms and radiologic infiltrates.[442,443] When the endarteritis is accompanied by angiomatoid changes, the lesion is considered pathognomonic for pulmonary schistosomiasis.[390]

Eggs typically are surrounded by epithelioid cells and collagen (Fig. 7.123). Most schistosome eggs do not exhibit birefringence and are larger than *Paragonimus* eggs, with which they share a superficial resemblance. Adult schistosomes may rarely be found in pulmonary blood vessels. Worldwide, given the burden of disease in Africa and Asia, chronic disease is associated for unclear reasons with pulmonary hypertension.[444]

Visceral Larva Migrans

The common parasites that cause visceral larva migrans are the dog tapeworm, *Toxocara canis*, and the less common cat tapeworm, *Toxocara cati*. When embryonated eggs are ingested by an intermediate host, typically a child with a history of pica, they hatch into infective larvae in the intestine. Subsequently, the larvae penetrate the intestinal wall, gain access to the circulation, and are carried to many organs, including

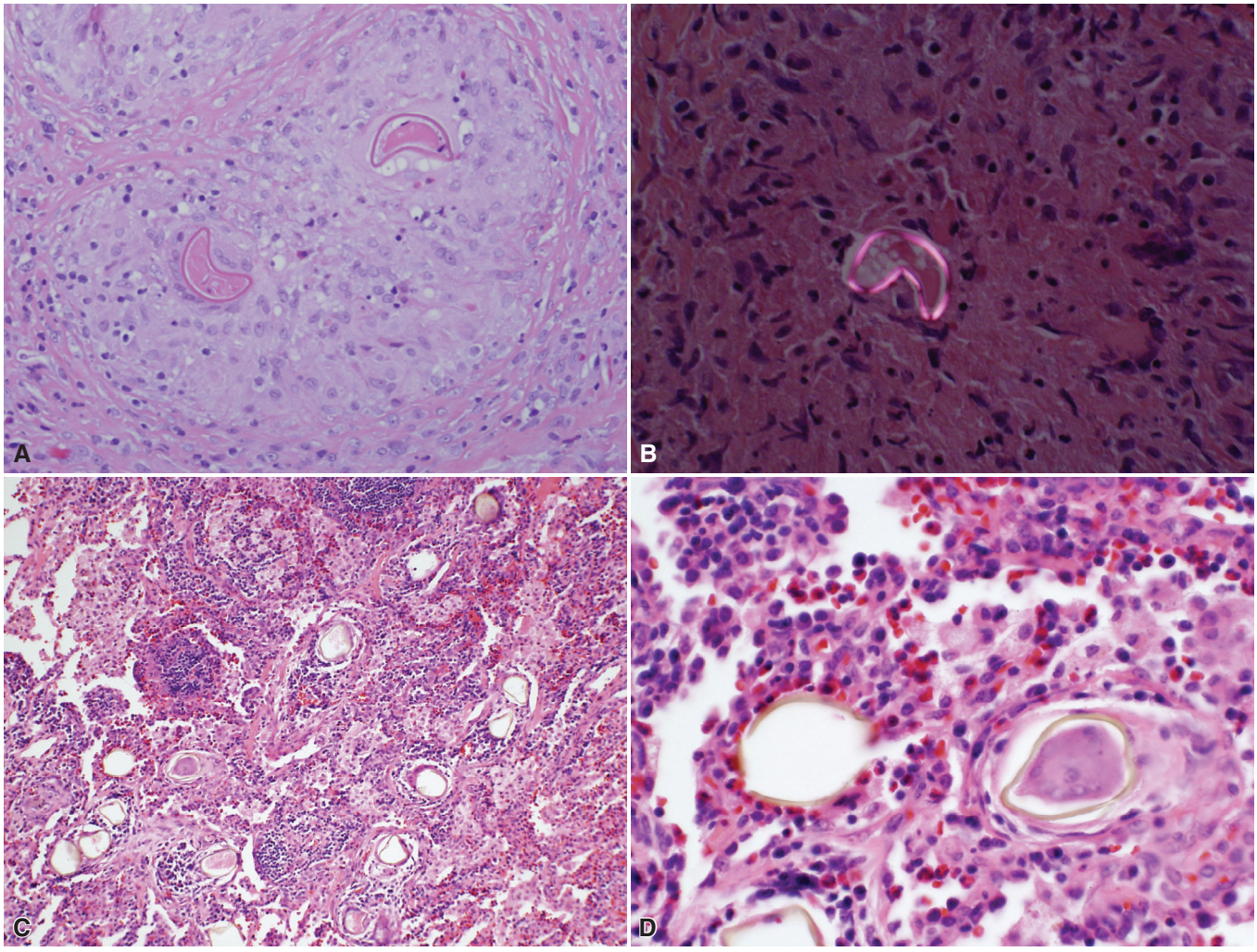

Figure 7.122 Paragonimiasis. (A) Granulomatous reaction to egg. (B) Single egg in polarized light. (C) Chronic eosinophilic pneumonia with many eggs. (D) Giant cell reaction; pigment in eggs. (Courtesy A.E. McCullough, MD.)

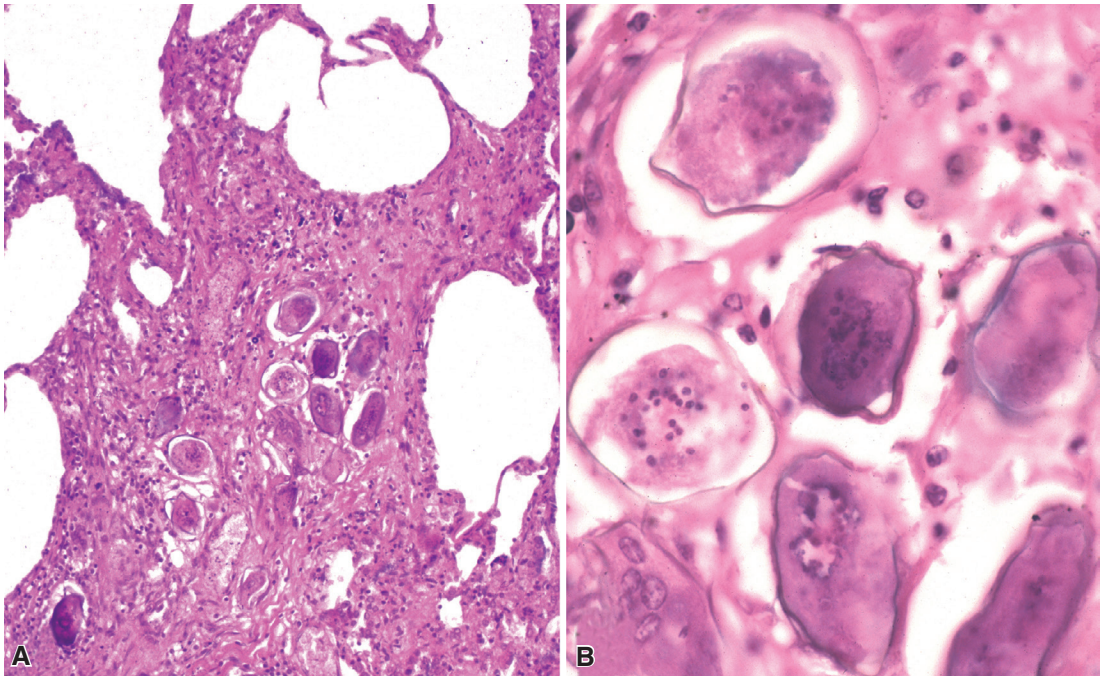

Figure 7.123 (A) Schistosome eggs in lung parenchyma. (B) Eggs of *Schistosoma japonicum.* (A and B, Courtesy Ronald Neafi, Armed Forces Institute of Pathology, Washington, DC.)

the lungs. This is the end point, for their growth is arrested by a granulomatous reaction and they never mature into adult worms. The granulomatous reaction usually has a conspicuous eosinophilic component, and larvae may be seen.[445]

Cytopathology

The cytologic literature contains many reports of the successful identification of parasites in pulmonary specimens recovered by exfoliative (sputum, bronchial washing or brushing, BAL fluid, pleural fluid) and needle aspiration techniques. Some of these are listed in Box 7.26.[418,424-426,436,446-457] Commonly cited in textbooks and reviews is the finding of *Strongyloides stercoralis* larvae in expectorated sputum or bronchial washings of patients with hyperinfections (Fig. 7.124). Also common are reports of *Echinococcus* protoscolices and hooklets in needle aspirates from patients with pleuropulmonary disease.[45,46] Use of large-bore and cutting needle biopsies has traditionally been contraindicated in the setting of suspected *Echinococcus* infections; reports of success with fine-needle aspiration without untoward reactions suggest that this technique is a relatively safe procedure in which the benefits outweigh the risks.[448]

Cytologic analysis is a sensitive and often preferred method to diagnose cryptosporidiosis, microsporidiosis, and other respiratory tract infections in the immunocompromised patient because it has the advantage of being less invasive. Specimens such as bronchial washings and BAL fluids can be prepared by high-speed centrifugation followed by standard smear preparation, cytocentrifugation, or ThinPrep technology. A battery of special stains—including Gram, modified trichrome, Giemsa, GMS, acid-fast, chemofluorescent, and immunofluorescent, depending on reagent availability—can then be applied to detect cryptosporidial oocysts, microsporidial spores, or other etiologic agents.

The morphologic features of many of the aforementioned organisms are usually better defined in cytologic preparations than in tissue biopsy specimens provided that obscuring background debris is limited and that the cytopreparation technique and staining have been well performed. Pseudoparasites such as vegetable matter, textile fibers, pollens, red cell "ghosts," and other extraneous material must be recognized and excluded. Thus, as for all of the various categories of microorganisms cited in this chapter, cytopathologic examination adds synergy to surgical pathologic and microbiologic methods.

Box 7.26 Parasites Reported in Respiratory Cytology Specimens

Toxoplasma
Amebae
Trichomonas
Cryptosporidia
Microsporidia
Leishmania
Paragonimus
Echinococcus
Strongyloides
Schistosoma
Dirofilaria
Microfilariae

Microbiology

The laboratory diagnosis of parasitic disease depends on the collection of appropriate specimens, which, in turn, requires appropriate clinical evaluation. For example, just as stool examination is the most efficient means of diagnosing most intestinal protozoa and helminths, respiratory specimens (e.g., sputum samples, bronchial washings, BAL fluid samples, touch imprints of lung biopsy tissue) can provide a specific etiologic diagnosis when pulmonary infections are suspected.[458] As in the case of cytologic samples, these specimens often reveal the characteristic microanatomic features of parasite larvae and eggs that usually cannot be readily seen when they are embedded in tissue. Moreover, the identification of organisms in respiratory specimens is diagnostic of pulmonary infection, whereas the presence of the organism in the feces of a patient suspected to have pulmonary disease provides only presumptive evidence.

Serodiagnosis with immunologic and molecular methods can be useful when parasites are located deep within tissue, such as the lung, and not easily accessible to biopsy or cytologic sampling.[396] The effectiveness of serodiagnosis of parasitic diseases has been hampered by tests with low sensitivity and specificity, mainly as a result of the complex composition of parasitic antigens and the occurrence of frequent cross reactions.[458] In recent years, however, significant refinements in antigenic preparations and improvements in technology have resulted in assays with greater predictive value. The newer tests are based on enzyme immunoassay and immunoblot methodology. Many test kits are

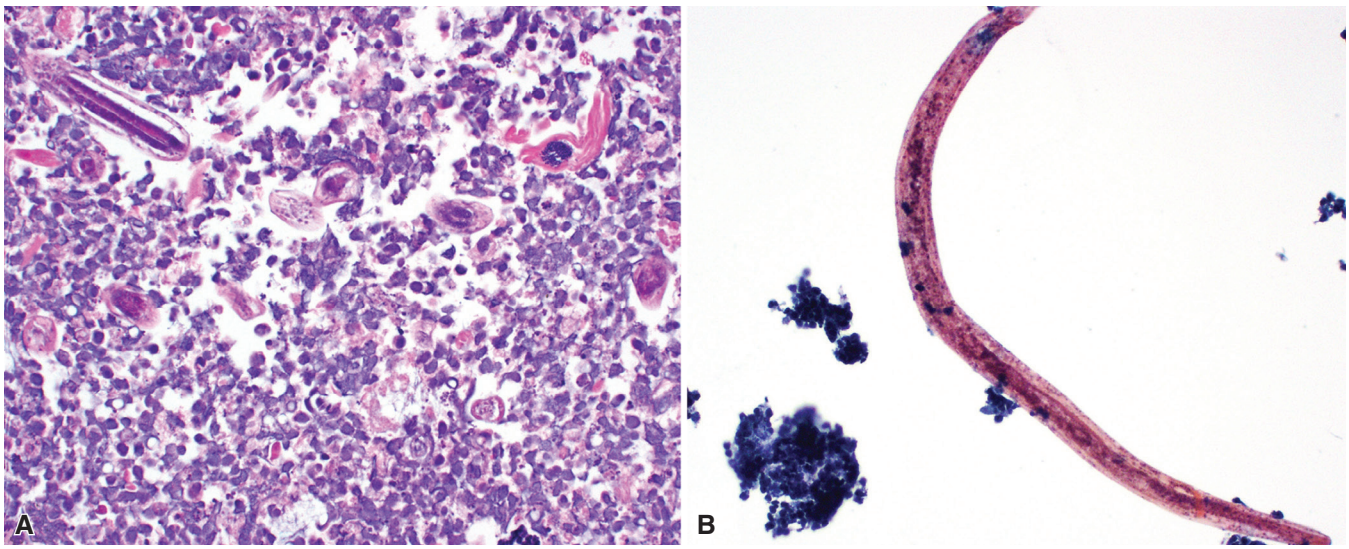

Figure 7.124 *Strongyloides stercoralis* larvae in bronchial washing. (A) Larval fragments in cell block. (B) ThinPrep smear.

commercially available, and diagnostic services are available from the CDC and other reference laboratories.[459]

With protozoal infections, serologic testing is especially useful for the diagnosis of toxoplasmosis. Several commercial kits are available for detection of IgG and IgM antibodies; however, false-negative results are possible in immunocompromised patients, and positive results must be interpreted with caution, especially when the index of clinical suspicion is low.[460] Real-time PCR analysis has been used for the diagnosis of toxoplasmosis in the immunocompromised patient.[461,462] Antibody determinations also have value in cases of pulmonary and other tissue-invasive forms of amebiasis as compared with antigen detection methods, which are more useful for noninvasive amebic intestinal diseases. However, the best diagnostic approach to invasive disease may be the use of serologic testing, antigen detection, and PCR methods in various combinations.[463] For the identification of cryptosporidia, the new immunofluorescence tests and enzyme immunoassays that have been developed for intestinal infections may have application in respiratory infections. Similar tests are not available for the microsporidia, and diagnosis of infection with these organisms continues to rely on direct staining techniques. For the helminths, serodiagnosis is possible for *Echinococcus*, *Paragonimus*, *Strongyloides*, and *Schistosoma* species using enzyme immunoassay methods, which have fair sensitivity and specificity.[385,459] The available tests for *Dirofilaria* suffer from poor sensitivity and specificity and are not clinically useful at this time.

Differential Diagnosis

The key morphologic and microbiologic features of selected parasitic lung infections are summarized in Table 7.13. In the absence of eggs, larvae, worms, or trophozoites, the various inflammatory patterns must be distinguished from those of other infections and various noninfectious processes due to toxins, drugs, and such entities as asthma, allergic bronchopulmonary aspergillosis, and pulmonary vasculitis syndromes including Churg-Strauss and hypereosinophilic syndromes.[464] Acute and chronic forms of eosinophilic pneumonia, as previously emphasized, have a varied etiology that includes parasitic infections.[465] False-positive morphologic diagnosis of a parasitic infection may be based on the presence of objects resembling parasites,[391,466] such as lentils in aspiration pneumonia, pollen grains, or Liesegang rings. These ring-like structures

Table 7.13 Parasitic Pneumonias: Summary of Pathologic Findings

Assessment Component	Findings
Toxoplasmosis	
Surgical pathology	Miliary small necroinflammatory nodules with fibrin; fibrinous pneumonia
Cytopathology	Crescent-shaped tachyzoites, pseudocysts, and true cysts
Microbiology	Serologic diagnosis by IFA or EIA; identification of tachyzoites or pseudocyst in tissue
Amebiasis	
Surgical pathology	Lung abscess
Cytopathology	Trophozoite in necroinflammatory debris resembles histiocytes; confirm with immunohistochemistry
Microbiology	Identification of trophozoite characteristics; serologic methods positive in most cases of extraintestinal disease; DNA probes
Cryptosporidiosis	
Surgical pathology	Bronchitis and/or bronchiolitis with cryptosporidia seen on H&E sections as small, round protrusions along the epithelial surface of the mucosa
Cytopathology	Red oocysts in smears prepared from bronchial washes and BAL fluid stained with modified acid-fast stains
Microbiology	Findings on direct examination of specimens similar to those on cytologic examination; immunofluorescence and enzyme immunoassays developed for intestinal infection
Microsporidiosis	
Surgical pathology	Bronchitis and/or bronchiolitis; small basophilic dots in vacuoles may be visible in H&E-stained sections when burden of organism is heavy; highlighted with Gram and modified trichrome stains; toluidine blue stain on plastic sections; electron microscopy
Cytopathology	Characteristic pink capsule-shaped spores with dark band in modified trichrome-stained preparations of BAL fluid Giemsa, Gram, and chemofluorescence stains also useful
Microbiology	Findings on direct examination of fluids similar to those on cytologic examination; culture in research setting by special arrangement; molecular methods
Dirofilariasis	
Surgical pathology	Solitary pulmonary nodule with infarct pattern and worm fragments
Cytopathology	Intact or fragmented worm in necroinflammatory debris
Microbiology	Identification of characteristic roundworm in tissues; serologic studies not useful
Strongyloides Infection	
Surgical pathology	Eosinophilic pneumonia, abscess, Loeffler syndrome with filariform larvae
Cytopathology	Filariform larvae in sputum indicate hyperinfection
Microbiology	Primary diagnostic stage in stool is rhabitiform larvae; filariform larvae may be seen in sputum and lung tissue; eggs resemble hookworm eggs but are rarely seen
Echinococcus Infection	
Surgical pathology	Trilayered cyst with brood capsules containing protoscolices; fibrous wall forms interface with lung parenchyma; sometimes abscess and granulomas
Cytopathology	Protoscolices with sucker and hooklets or detached hooklets in granular background debris
Microbiology	Identification of hooklets and protoscolices in needle aspirates, pleural fluid, and sputum; serologic testing available

Table 7.13 Parasitic Pneumonias: Summary of Pathologic Findings—cont'd

Assessment Component	Findings
Paragonimiasis	
Surgical pathology	Eosinophilic pneumonia; fibrous pseudocysts containing worms and necroinflammatory debris; egg granulomas
Cytopathology	Yellow ovoid birefringent eggs with flattened operculum
Microbiology	Identification of characteristic egg in sputum or tissue; serologic testing available
Schistosomiasis	
Surgical pathology	Granulomatous endarteritis; eggs in epithelioid granulomas
Cytopathology	Characteristic nonbirefringent, nonoperculated eggs; presence and position of spine determines species
Microbiology	Embryonated eggs may be present in feces or urine; not sputum;serologic testing available

BAL, Bronchoalveolar fluid; *EIA*, enzyme immunoassay; *H&E*, hematoxylin and eosin; *IFA*, immunofluorescence assay.

can simulate various types of nematodes.[467] Careful attention to the microanatomy of an apparent foreign body and comparison with parasites illustrated in atlases can often resolve such diagnostic dilemmas. Some cases, however, may require referral to pathologists with specialized training and experience in parasitic diseases.

Self-assessment questions and cases related to this chapter can be found online at ExpertConsult.com.

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Multiple Choice Questions

1. Which of the following lung injury patterns is NOT found in severe bacterial pneumonias?
   A. Exudative alveolar filling
   B. Abscess
   C. Granuloma
   D. Diffuse alveolar damage
   E. Necrotizing lesions

ANSWER: C

2. Which of the following statements about the atypical pneumonia agents is FALSE?
   A. They do not typically cause lobar consolidation.
   B. Some of them can produce exudative alveolar filling.
   C. They include Mycoplasma, Chlamydia, and Coxiella species.
   D. They produce exudate with filaments and granules.
   E. They can be evaluated with antigen detection and serologic tests.

ANSWER: D

3. The etiologic agent that most commonly causes hemorrhagic mediastinitis is
   A. *Yersinia pestis*
   B. *Bacillus anthracis*
   C. *Francisella tularensis*
   D. *Histoplasma capsulatum*
   E. *Sin Nombre* hantavirus

ANSWER: B

4. The Ghon complex is:
   A. A peripheral lung nodule/granuloma and calcified hilar lymph node
   B. A feature of postprimary/reactivation tuberculosis
   C. A feature of primary tuberculosis
   D. a and b only
   E. a and c only

ANSWER: E

5. Regarding the nontuberculous mycobacteria, all of the following are correct EXCEPT:
   A. They produce histopathologic lesions similar to *Mycobacterium tuberculosis*.
   B. They are not acquired person to person.
   C. They can produce histiocytic infiltrates and spindle cell lesions.
   D. They can cause disseminated disease in the immunocompromised.
   E. *Mycobacterium bovis* and *Mycobacterium africanum* are two of more than 100 species of nontuberculous mycobacteria.

ANSWER: E

6. *Mycobacterium abscessus* is:
   A. Part of the *Mycobacterium tuberculosis* complex
   B. The leading rapid-growing mycobacterium recovered from the lung
   C. The leading slow-growing mycobacterium recovered from the lung
   D. The etiologic agent most associated with middle lobe syndrome
   E. c and d only

ANSWER: B

7. All of the following statements regarding coccidioidomycosis are correct EXCEPT:
   A. It is caused by inhalation of arthrospores in alkaline soil of the Sonoran life zone.
   B. It is caused by a biphasic fungus that forms yeasts in tissue and hyphae only in laboratory media.
   C. Serology offers a sensitive method for laboratory diagnosis.
   D. It can present as community-acquired pneumonia.
   E. It can be associated with blood eosinophilia and eosinophilic pneumonia.

ANSWER: B

8. Clinical forms of histoplasmosis include:
   A. Asymptomatic infection
   B. Solitary pulmonary nodule
   C. Cavitary granuloma
   D. Fibrosing mediastinitis
   E. All of the above

ANSWER: E

9. Aspergillus fungal microscopic look-alikes in tissue include:
   A. Most Zygomycetes species
   B. Most Fusarium species
   C. Bipolaris spicifera
   D. All of the above
   E. a and b only

ANSWER: D

10. Yellowish, oval, birefringent eggs in an eosinophil-rich exudate are characteristic of which of the following parasites?
    A. Paragonimus
    B. Schistosomes
    C. Strongyloides
    D. Ascaris
    E. Echinococcus

ANSWER: A

11. Which of the following statements concerning nontuberculous mycobacterial infection is/are TRUE?
    A. It follows the same sequence of primary and postprimary disease as *Mycobacterium tuberculosis.*
    B. It manifests three distinct clinicopathologic entities.
    C. It is treated aggressively, often with the addition of cytotoxic agents.
    D. Disseminated disease mainly affects human immunodeficiency virus (HIV)–infected individuals.
    E. All of the above.

ANSWER: D

12. True or false: Blastomycosis is endemic to the Pacific Northwest region of the United States.
    A. True
    B. False

ANSWER: B

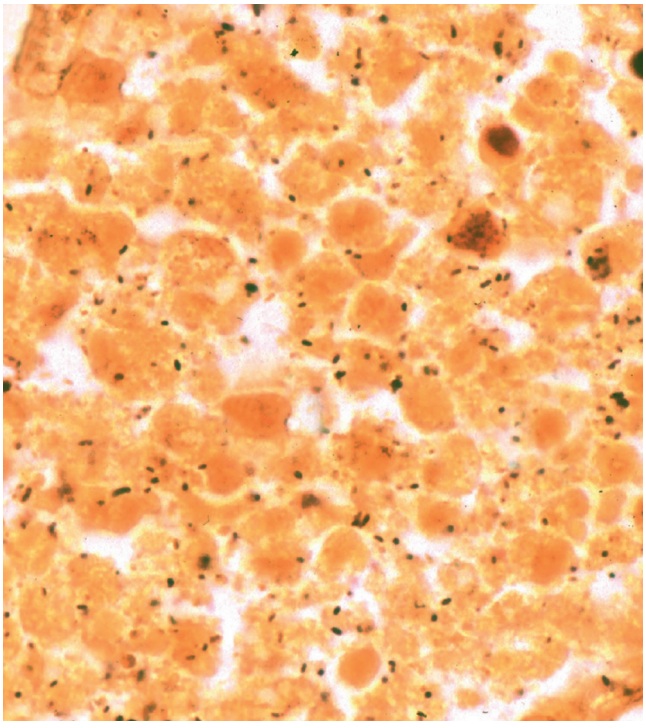
13. True or false: Histoplasmosis is the most common pulmonary fungal infection worldwide.
    A. True
    B. False

ANSWER: A

14. True or false: In children under the age of 1 year, respiratory syncytial virus occurs more frequently than influenza or parainfluenza viral infection.
    A. True
    B. False

ANSWER: A

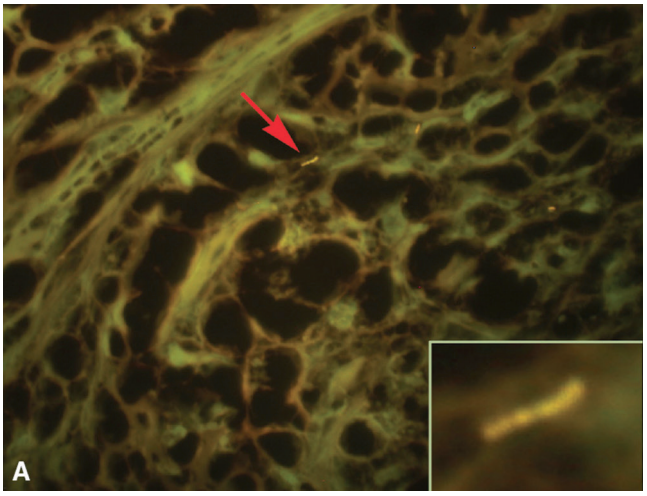
15. What is this?



    A. Giemsa stain of Candida pneumonia
    B. Trichrome stain of Aspergillus infection
    C. Von Kossa stain of malakoplakia
    D. Periodic acid–Schiff stain of Pneumocystis
    E. None of the above

ANSWER: E

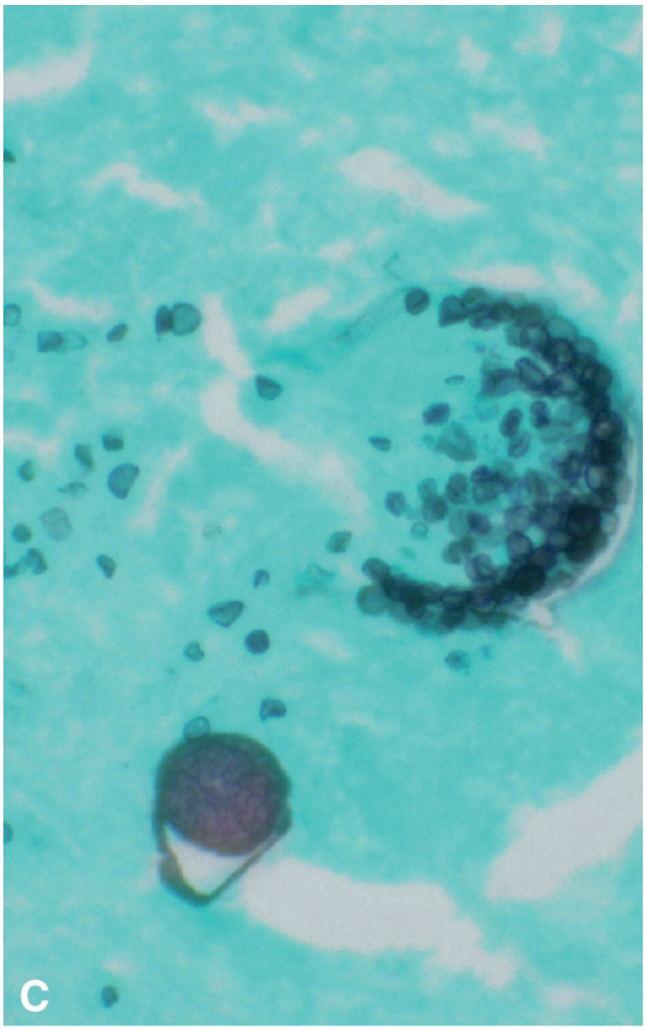
16. What is this?



    A. Osteomyelitis with bacterial stain
    B. Mycobacterial granuloma with rhodamine-auramine
    C. Malakoplakia with silver impregnation technique
    D. Fibrinoid eosinophilia with Strongyloides worms under fluorescence
    E. None of the above

ANSWER: B

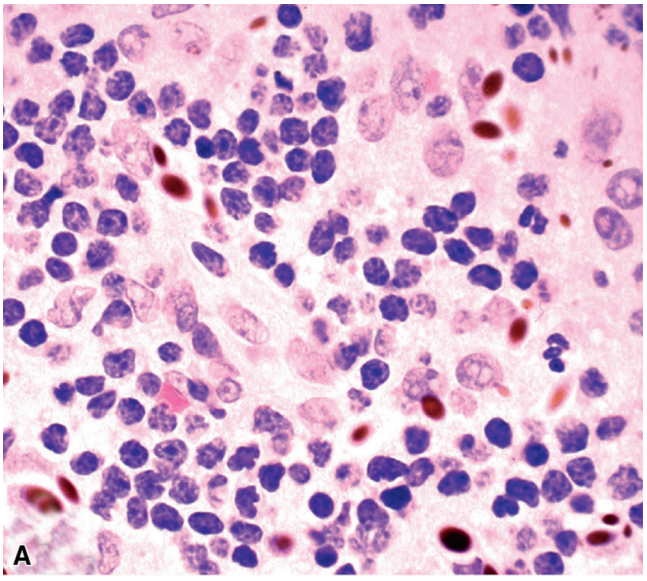
17. What is this?



A. Pneumocystis
B. Hantavirus
C. Blastomyces
D. Coccidioides
E. None of the above

ANSWER: D

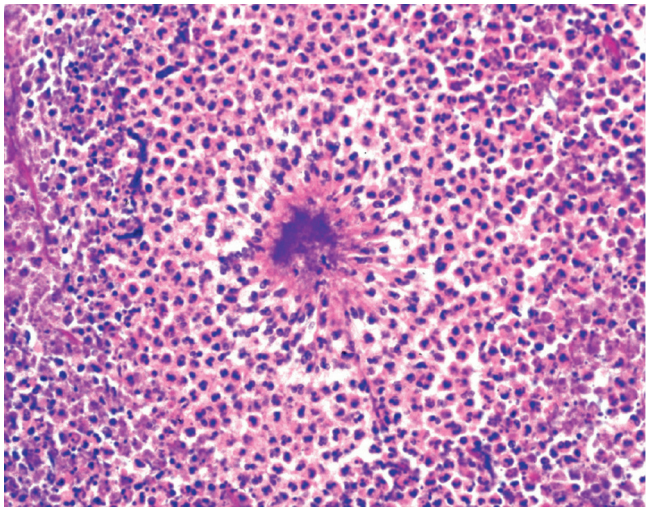
18. What are these brown structures?



A. So-called brown bodies of blastomycosis
B. Hamazaki-Wesenberg bodies
C. Fungal yeast forms of Candida
D. Sideroplanum spores
E. None of the above

ANSWER: B

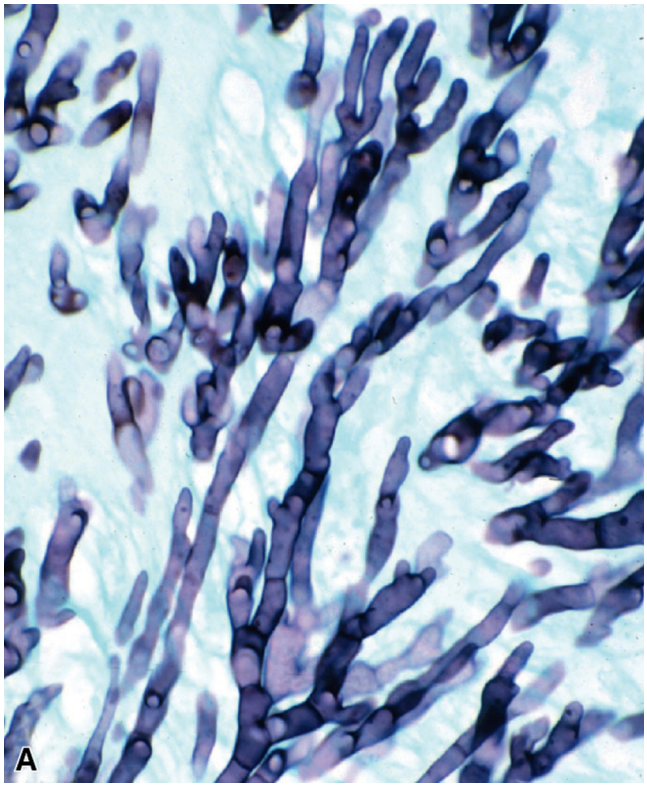
19. What is this?



A. Aspirated vegetable material
B. Migratory parasite
C. Sulfur granule of Actinomyces
D. Eosinophilic pneumonia body
E. None of the above

ANSWER: C

20. What are these?



A. Aspirated vegetable material
B. Migratory parasites
C. Mucor hyphae
D. Aspergillus hyphae
E. None of the above

ANSWER: D

21. Which statement regarding stains for bacterial pneumonias is false?
A. The agent of anthrax pneumonia is a large gram-positive rod that may be seen in alveolar septal vessels.
B. It is difficult to demonstrate the organism of tularemia with a stain.
C. Nocardia species are partially acid-fast.
D. Legionella species can be seen with silver-based stains.
E. Rhodococcus is an animal pathogen that rarely cause pneumonia in humans; it is best demonstrated in a GMS stain.

ANSWER: E

22. With respect to Aspergillus species, which is a true statement?
A. Aspergillus species are nonpigmented.
B. Aspergillus hyphae branch at a right angles.
C. Aspergillus species are positive in a Gram stain.
D. Galactomannan antigen can be used as an adjunct to the diagnosis of Aspergillus in a BAL specimen.
E. Aspergillus hyphae always taper to a thin point.

ANSWER: D

23. You identified yeast cells in a lung biopsy. The yeast has a thick cell wall and in one section there is a yeast cell with wide-based budding. Which statement is most likely to be true about this patient?
A. The patient is from Mississippi.
B. He has a diagnostic serology test.
C. The organism will be recoverable from a culture in about a week.
D. He has a cavitary lung lesion on his chest x-ray.
E. The biopsy shows compact granulomas following alveolar septae.

ANSWER: A

24. Your patient has had a slightly enlarging nodule in the base of the right lower lobe for a year. He says he was sick after an adventure vacation to Arizona last year, where he went spelunking. Serologies for coccidioidomycosis are negative. A wedge biopsy was performed to the remove the nodule and, on frozen section, you saw a large spherule with internal endospores in a thick-walled necrotic granuloma. Which of the following pieces of information can you offer the surgeon?
A. The diagnosis of the nodule is coccidioidomycosis.
B. The nodule is probably due to coccidioidomycosis, but you are qualifying your diagnosis since the serology was negative.
C. The nodule is due to sporotrichosis.
D. The etiology of the nodule is unknown, since the granuloma could have commensal organisms in it and you feel you should wait for culture results.
E. This is a nodule of prior histoplasmosis.

ANSWER: A

25. You have identified a granuloma that you think is probably from histoplasmosis in the biopsy you are examining. It might help to confirm the diagnosis if you:
A. Examine the medical record for results of beta-D-glucan in the serum
B. Examine the medical record for results of serum or urine antigen testing
C. Cut deeper sections and do GMS on multiple levels
D. All of the above
E. b and c

ANSWER: E

26. A bone marrow transplant patient has hemoptysis. You find some ribbon-like hyphae in a hemorrhagic portion of her lung biopsy. Which of these features is most likely to help you differentiate whether this fungus is Aspergillus or Mucor?
A. The morphology of the hyphal branching
B. The negative Gram stain
C. The serum galactomannan result
D. The way the organism is invading vessels
E. Waiting for the culture

ANSWER: C

27. Consider Paracoccidioides infection. Which is untrue?
A. It is common in Brazil.
B. It is most commonly symptomatic after inhalation infection.
C. It has narrow-based budding.
D. It is more common in men.
E. It will have multiple buds in yeast cells.

ANSWER: B

28. The cytopathologic effect of cytomegalovirus is characterized by:
    A. Cellular enlargement
    B. Red-purple inclusions in the nucleus
    C. Red-purple granules in the cytoplasm
    D. Lack of correlation with viral load in blood
    E. All of the above

ANSWER: E

29. Pulmonary dirofilariasis is most typically characterized by:
    A. Eosinophilia in the majority of patients
    B. Degenerating parasites in a histiocyte-rimmed necrotic single pulmonary nodule
    C. Diagnosis by excisional lung biopsy
    D. b and c
    E. All of the above

ANSWER: D

30. Which organism is incorrectly described?
    A. Actinomyces, a filamentous bacterium, is positive in Gram and GMS stains.
    B. Botryomycosis involves a gram-positive collection of rods and cocci.
    C. Legionella is a gram-negative organism that will not grow on standard media owing to a requirement for cysteine.
    D. Cryptococcus is a yeast with a mucicarmine-positive capsule.
    E. Candida is a gram-positive fungus only rarely associated with giant cells or granulomas.

ANSWER: B

Case 1

History

A 40-year-old white male with history of allograft liver transplantation presents after several weeks of dyspnea. Ground-glass infiltrate in chest x-ray. Open lung biopsy performed.

Pathologic Findings

Diffuse alveolar damage (eSlide 7.1A) and characteristic inclusions (eSlide 7.1B).

Diagnosis

Cytomegalovirus pneumonitis.

Case 2

History

A 42-year-old white female on steroids and immune-modifying drugs for rheumatoid arthritis presents with dyspnea and bilateral diffuse lung infiltrates. A right lower lobe wedge biopsy is performed.

Pathologic Findings

Fluffy alveolar infiltrate, somewhat fibrinous appearing (eSlide 7.2A) with some suggestion of internal structure at high power (eSlide 7.2B). GMS stain demonstrates organisms diagnostic of Pneumocystis in exudates (eSlide 7.2C).

Diagnosis

Pneumocystis pneumonia.

Case 3

History

An asymptomatic 81-year-old Japanese white female who had a normal chest x-ray a year earlier is found, on repeat chest x-ray, to have a solitary pulmonary nodule 1.5 cm in diameter in the right middle lobe. The nodule is removed by wedge excision.

Pathologic Findings

Discrete nodule with slightly organized rim and diffuse central necrosis (eSlide 7.3A). Areas in the nodule show necrotic residual organisms in cross section (eSlide 7.3B) surrounded by bland necrosis. A higher-power view suggests a layered body wall with a central lumen (eSlide 7.3C).

Diagnosis

Most consistent with dirofilarial granuloma.

Case 4

History

Three months after heart transplantation a 60-year-old male is admitted with atrial fibrillation, left-sided pleuritic pain, and a 2-week history of nonproductive cough. Chest CT shows two mass-like lesions, one abutting the mediastinum and one on the anterior chest wall, ranging from 2 to 5 cm in maximum diameter. There is no significant adenopathy. BAL without biopsy is performed; nondiagnostic culture results show an Aspergillus antigen index on BAL fluid of >3.75 (normal <0.5). CT guided needle biopsy of anterior mass is then performed.

Pathologic Findings

Acute and organizing pneumonia (eSlide 7.4A) with hemosiderin and neutrophilic exudate (eSlide 7.4B). GMS fungal stain reveals small bits of fragmented hyphae dispersed through the biopsy (eSlide 7.4C). These vary in width and are too fragmented to be definitively identified morphologically (eSlide 7.4D), although they are consistent with Aspergillus species. The greatly elevated Aspergillus antigen from the recent BAL fluid is highly suggestive of Aspergillus infection. Initial calcofluor fungal smear in the microbiology lab made from a needle core biopsy was negative. The culture of the tissue obtained at the lung biopsy subsequently grew Aspergillus fumigatus.

Diagnosis

Acute and organizing pneumonia from Aspergillus fumigatus.

Case 5

History

A 32-year-old HIV-positive black male with AIDS, recently treated *Pneumocystis jirovecii* pneumonia, returns for a follow-up chest x-ray. He has a persistent left upper lobe infiltrate, low-grade fever, and general malaise. Open lung biopsy of the lesion is performed.

Pathologic Findings

Solid infiltrate, slightly nodular with pleural adhesion (eSlide 7.5A), both spindle cell and round cell infiltrate with background lymphocytic infiltrate (eSlide 7.5B). Acid-fast stain shows numerous acid-fast organisms throughout the most cellular portions of the mass (eSlide 7.5C). Culture of tissue obtained at open lung biopsy grew *Mycobacterium avium*.

Diagnosis

M. avium pseudotumor.