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Lung Infections

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Lower respiratory tract infections constitute a leading cause of morbidity and death worldwide.^{1,2} Included in this category of infections are bronchitis and bronchiolitis, community-acquired and nosocomial pneumonias, and pneumonias in the immunocompromised patient. A relatively small percentage of these infections come to the attention of the surgical pathologist, because most are diagnosed in the microbiology laboratory. Nevertheless, as summarized in [Box 6-1](#), the anatomic pathologist can play a pivotal role in the diagnosis of lung infections by identifying reaction patterns in tissue, and sometimes in the identification of an organism that microbiologic techniques fail to detect.³ Despite significant advances in laboratory techniques, culture diagnosis is not always possible; the organism may not reproduce in culture, a culture study may not have been requested, or the culture technique may have failed for any of various technical reasons. Even when culture is successful, the time frame for diagnostic purposes may not be clinically useful, or the culture result, in the absence of an expected tissue response, may not permit distinction of pathogens from innocent bystanders, be they colonizers or contaminants. For all of these reasons, the pulmonary infection for which biopsy is performed often is one that 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,^{4,5} if not for providing an immediate report intraoperatively (by frozen section or cytologic imprints or smears), then for dramatically improving diagnosis turnaround time with the use of newer rapid tissue-processing systems⁶ ([Table 6-1](#)).

Box 6-1. 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 Watts J, Chandler F. The surgical pathologist’s role in the diagnosis of infectious disease. *J Histotechnol.* 1995;18:191–193.

Table 6-1. 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

Diagnostic Tools and Strategies

The history of the field of pathology is intertwined with the discovery of pathogenic bacteria and the development of the science of microbiology.⁷ Today, pathologists and microbiologists approach the diagnosis of infection with techniques and methods that share some aspects but have important differences.⁸ The surgical pathologist and the cytopathologist are in a position to apply the tools of both disciplines to achieve a clinically relevant diagnosis by correlating the histopathologic or cytopathologic examination findings with data obtained using microbiology techniques (Table 6-2). Unfortunately, the diagnostic workup and reporting of biopsy findings in surgical or cytopathology departments and those in the microbiology laboratory typically run along nonintersecting paths, often without one group knowing (or acknowledging) the findings of the other. An interdisciplinary approach that is based on mutual understanding and communication would seem to be a logical, if not ideal, scenario for optimal clinical management.⁹ Our concept of an integrated morphologic and microbiologic approach is presented schematically in Figure 6-1, and with greater detail for specific situations in which bacterial (Fig. 6-2), mycobacterial (Fig. 6-3), fungal (Fig. 6-4), or viral (Fig. 6-5) pathogens are suspected.

In current medical practice, identification of the genus or species of an infectious organism can have important prognostic and therapeutic implications. Because histopathologic examination alone rarely provides this information, the findings should always be cor-

Table 6-2. 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

related with results of cultures. Accordingly, foresight is required on the part of the intraoperative pathologist in obtaining and properly handling tissues for culture.¹⁰ The correlation of the morphologic and microbiologic data can be facilitated in the surgical pathology report by appending a comment that seeks to enhance the morphologic diagnosis by suggesting a specific etiologic disorder or agent, considerations for the differential diagnosis, or additional workup with culture, serology, or molecular studies. In certain situations, it is also appropriate to include the preliminary results of microbiology stains and cultures, and to correlate this information with the morphologic findings whenever possible.

Knowledge of the Clinical Setting

Identification of risk factors and determination of the immune status of the patient are of primary importance, because these parameters typically influence the spectrum of histopathologic changes and the type of etiologic agents and pathogen burden.¹¹⁻¹⁶ Also, because the degree of immunosuppression often influences the burden of organisms, different efforts may be required to identify the pathogen. 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.¹⁷ By contrast, persons infected with the human immunodeficiency virus (HIV) in whom the acquired immunodeficiency syndrome (AIDS) or *Mycobacterium avium* infection develops typically manifest poorly formed granulomas, or simply histiocytic infiltrates, despite an overabundance of organisms identified by tissue acid-fast stains. *Pneumocystis* organisms may be easily identified in patients with AIDS, who manifest diffuse alveolar damage accompanied by abundant, foamy alveolar casts but when immunosuppression is less severe (such as that produced by corticosteroids therapy for arthritis), the morphologic features can be less typical, and the organisms sparse. The relationship among the level of immunity, burden of organisms, and patterns of disease is illustrated for cryptococcosis in Figure 6-6.

In the immunocompromised patient, one must also consider a broader differential diagnosis. In addition to infection, other disorders come into consideration such as pulmonary involvement by pre-existing 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

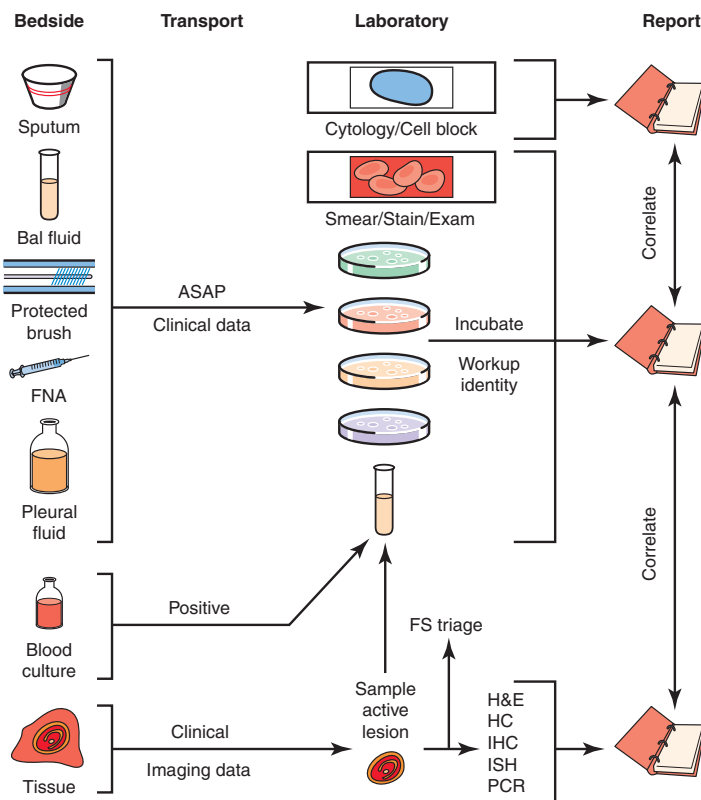


Figure 6-1. Schematic for workup of respiratory specimen for suspected infection. BAL, bronchoalveolar lavage; FNA, fine-needle aspiration; FS, frozen section; HC, histochemistry; H&E, hematoxylin-eosin [stain]; IHC, immunohistochemistry studies; ISH, in situ hybridization; PCR, polymerase chain reaction [assay].

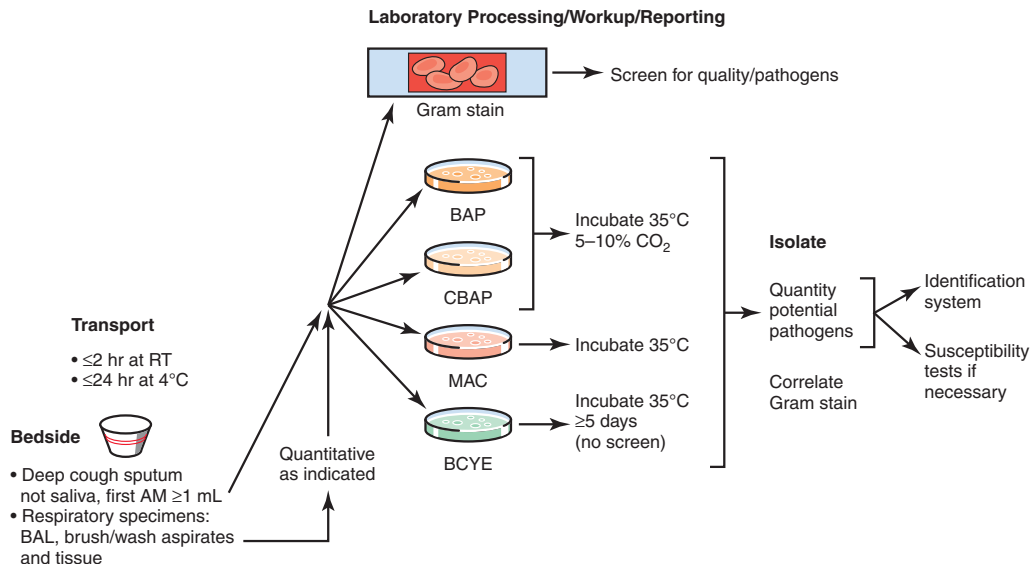


Figure 6-2. Integrated morphologic and microbiologic approach to laboratory diagnosis of bacterial infection. BAL, bronchoalveolar lavage; BAP, blood agar plate; BCYE, buffered charcoal yeast extract; CBAP, chocolate blood agar plate; MAC, MacConkey agar; RT, room temperature.

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. 6-7).

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

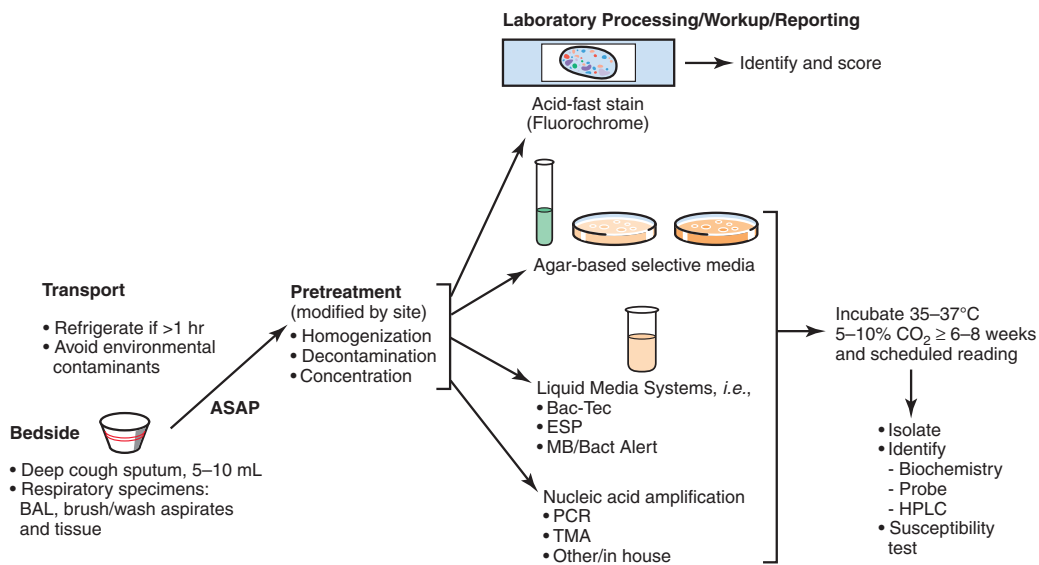


Figure 6-3. Integrated morphologic and microbiologic approach to laboratory diagnosis of mycobacterial infection. Bac-Tec, BD BACTEC Instrumented Mycobacterial Growth Systems; BAL, bronchoalveolar lavage; ESP, ESP Culture System; HPLC, high-performance liquid chromatography; MB/Bact Alert, Biomerieux Bact/alert 3D; PCR, polymerase chain reaction [assay]; TMA, transcription-mediated amplification.

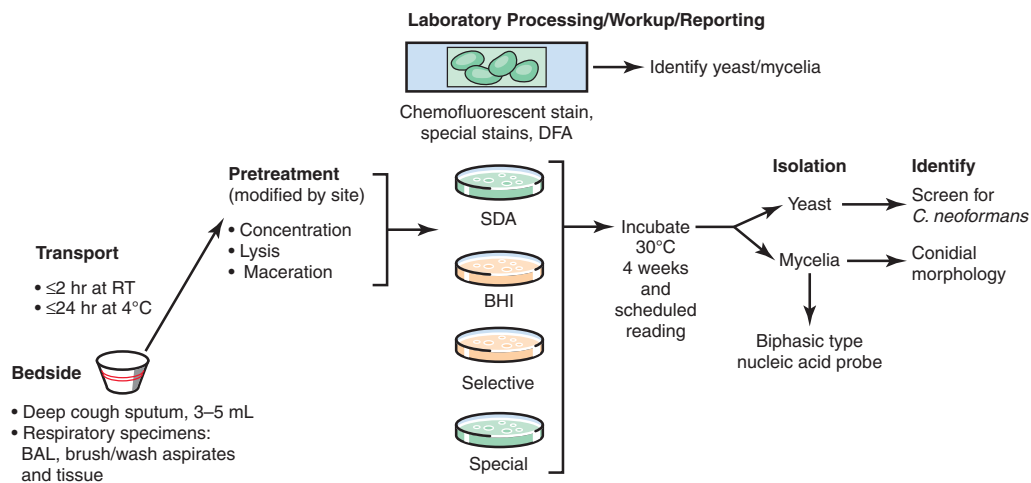


Figure 6-4. Integrated morphologic and microbiologic approach to laboratory diagnosis of fungal infection. BAL, bronchoalveolar lavage; BHI, brain-heart infusion; DFA, direct immunofluorescence assay; RT, room temperature; SDA, Sabouraud dextrose agar.

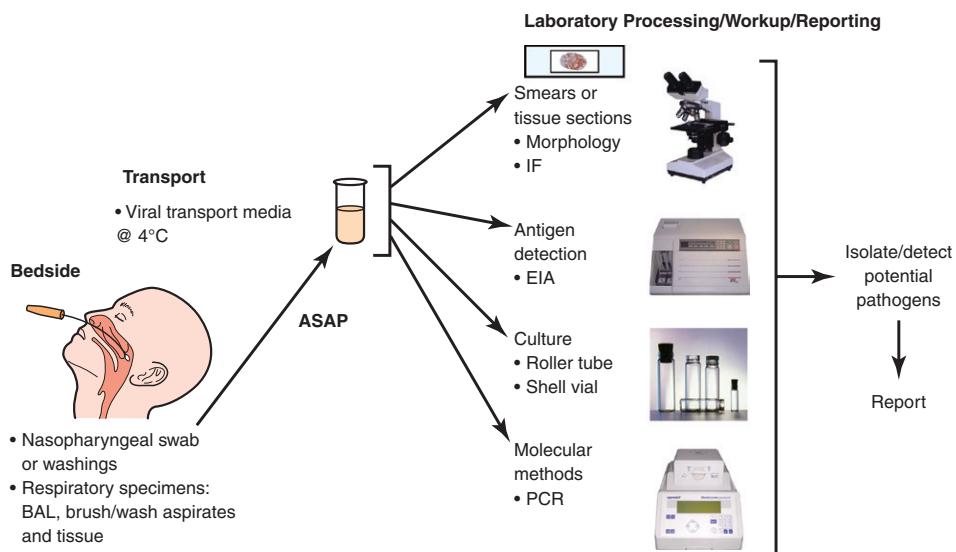


Figure 6-5. Integrated morphologic and microbiologic approach to laboratory diagnosis of viral infection. BAL, bronchoalveolar lavage; EIA, enzyme immunoassay; IF, immunofluorescence; PCR, polymerase chain reaction assay.

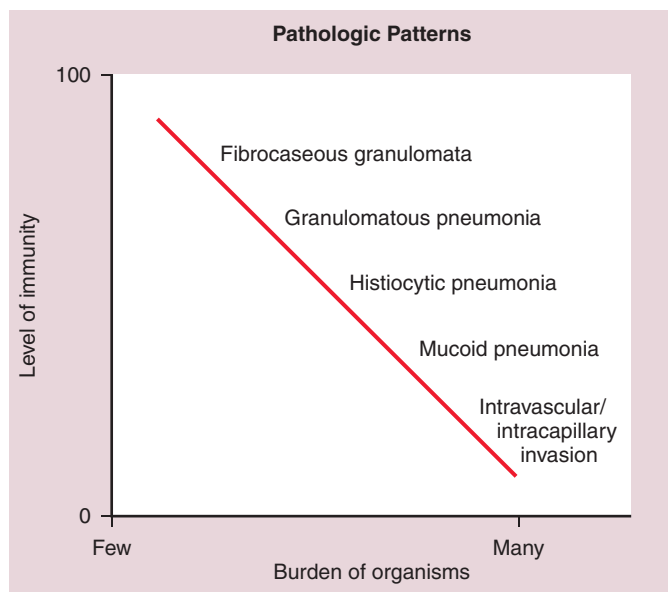


Figure 6-6. Cryptococcosis: Correlation of pathologic patterns with immunity level and organism burden. With cryptococcal pneumonia in patients with normal or near-normal immunity, granuloma formation with few organisms is characteristic. In immunocompromised patients, typical findings include histiocytic infiltrates or mucoïd 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.)

disease of autosomal recessive inheritance involves mutation of the *CFTR* gene that affects the ability of epithelial cells to effectively transport chloride and, secondarily, water across cell membranes. As a result, many organs, including the lungs, develop excessively viscous mucous secretions, which cannot be cleared effectively from the airways. In the lung, retention of such secretions leads to progressive and widespread bronchiectasis with airway obstruction that in turn paves the way for

recurrent infection (Fig. 6-8). Bacterial organisms commonly isolated include *Pseudomonas aeruginosa* (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 also is a risk factor for non-tuberculous 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) typically are dominated by increased attenuation (opacity). Such opacities may occur as one or more localized densities (nodule, mass, or localized infiltrate) or as more extensive infiltrates referred to as either *ground-glass opacities* (attenuation that allows underlying lung structures to be visible) or *consolidation* (attenuation that overshadows underlying structure).³⁰ Review of the chest imaging studies with the radiologist can be very helpful in arriving at a clinically relevant diagnosis. Correlating these data with the clinical history and pace of the disease under scrutiny (acute, subacute, chronic) allows a more accurate interpretation of the observed histopathologic pattern of disease in the tissue (Fig. 6-9). 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 6-3).

Useful Tissue Stains in Lung Infection

Many diagnostic 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

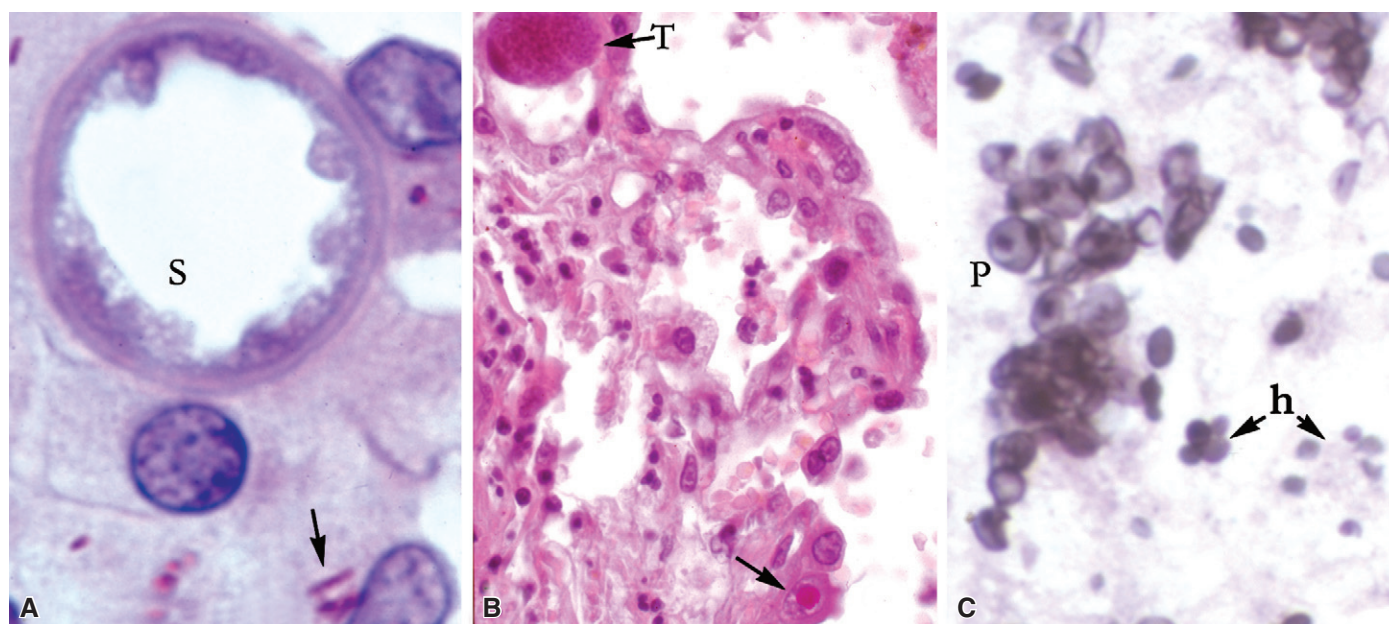


Figure 6-7. Co-infection with dual pulmonary pathogens. **A**, Spherule of *Coccidioides* (S) and *Mycobacterium avium* complex acid-fast bacilli (Ziehl-Neelsen/H&E stains). **B**, *Toxoplasma* pseudocysts (T) and cytomegalovirus-infected alveolar lining cell (arrow). **C**, Clusters of Pneumocystis cysts (P) in the midst of *H. capsulatum* yeast cells (h) (Grocott methenamine silver stain).

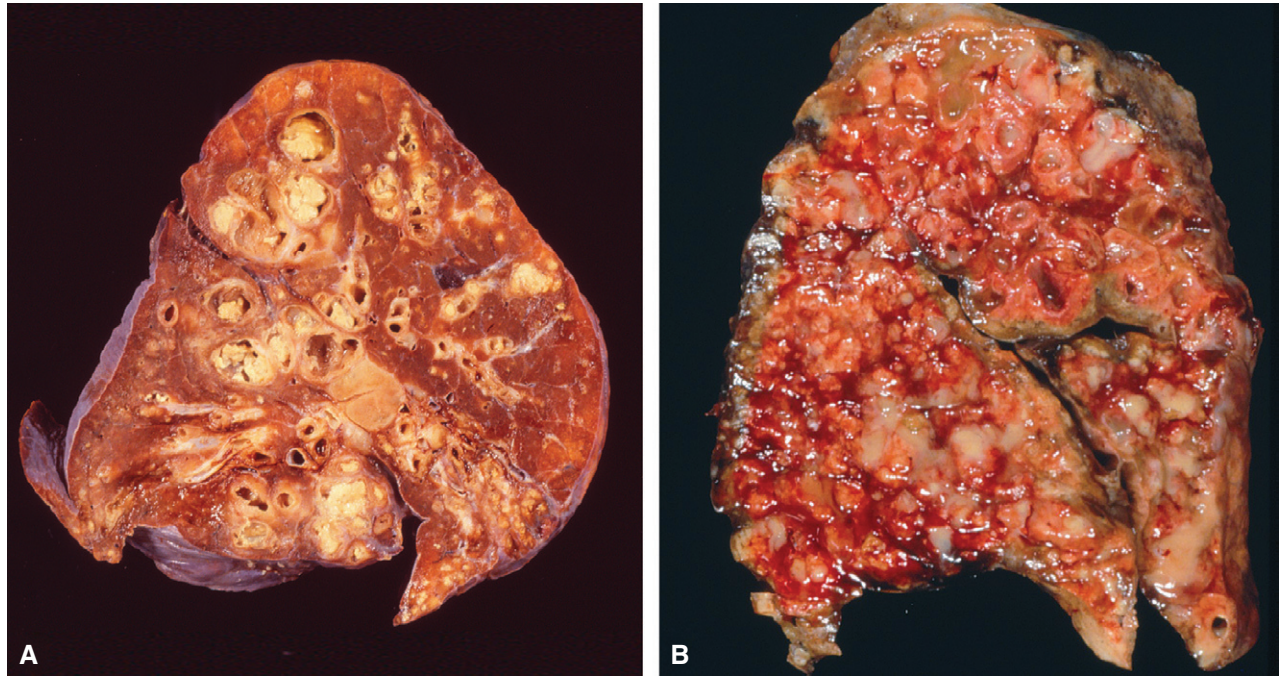


Figure 6-8. Changes of cystic fibrosis in the lung. **A,** Explant from a 13-year-old patient. **B,** Advanced disease at autopsy.

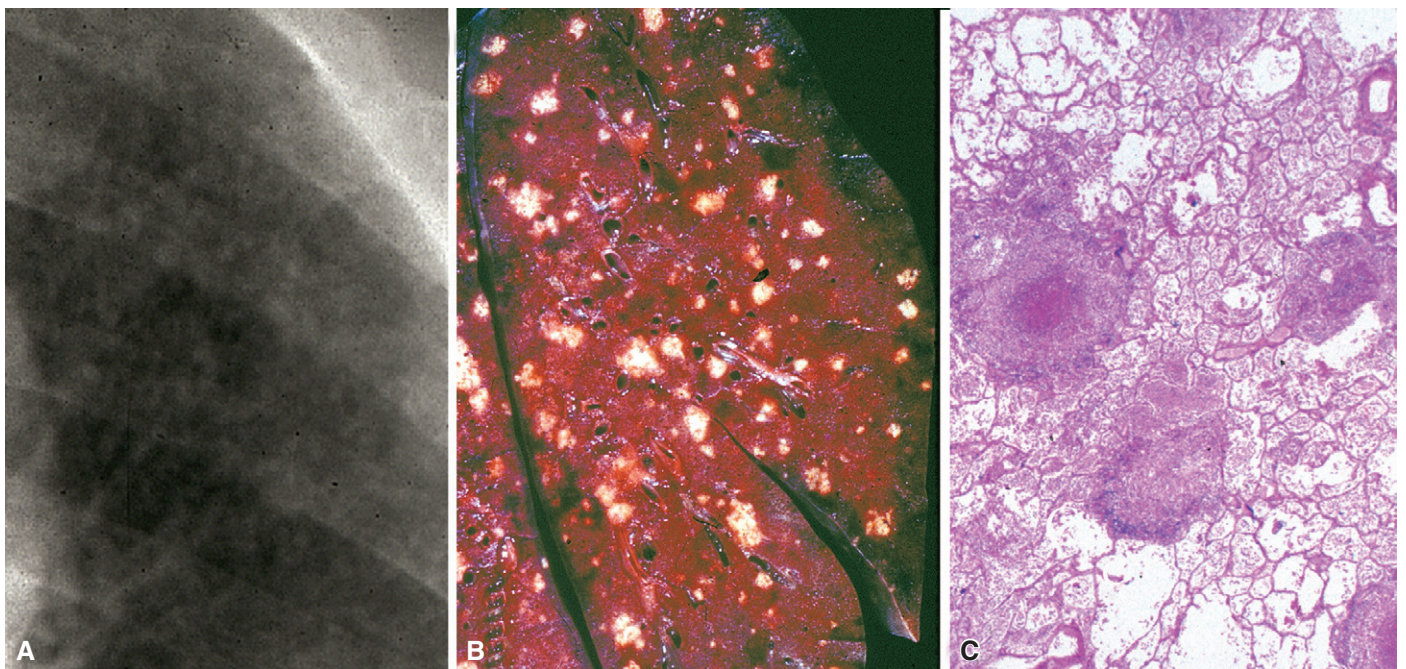


Figure 6-9. Miliary pattern of tuberculosis. **A,** Chest film, close-up view of miliary infiltrate. **B,** Gross cut surface of pulmonary parenchyma with miliary nodules. **C,** Histopathologic features of miliary necrotizing granulomas.

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 in [Box 6-2](#). These stains should always be applied as part of an algorithmic strategy for acute lung injury, but especially in the immunocompromised patient.³ 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)

([Fig. 6-10](#)), 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. 6-11](#)) and become more visible.³ Other stains (e.g., Giemsa) will sometimes detect bacteria that do not stain well with more conventional stains ([Fig. 6-12](#)). The Grocott methenamine silver (GMS) stain ([Fig. 6-13](#)) is the best stain for most fungi in tissue and also stains actinomycetes,

Table 6-3. Histopathologic Patterns and Most Agents of Pulmonary Infection

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

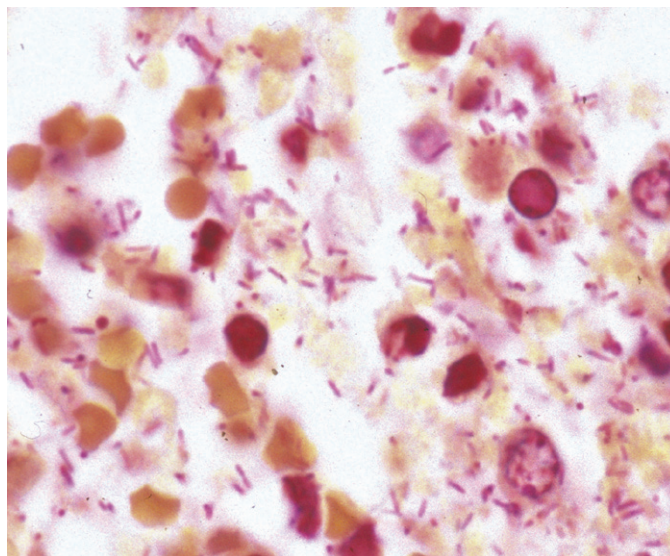
Nocardia, *Pneumocystis* (cysts), free-living soil amoebae, algal cells, the spores of certain microsporidia, and the cytoplasmic inclusions of cytomegalovirus (CMV).⁸

Most mycobacteria stain well with the Ziehl-Neelsen procedure (Fig. 6-14), but the auramine-rhodamine fluorescent procedure is superior in terms of sensitivity (Fig. 6-15). *Nocardia* organisms, *Legionella micdadei*, and *Rhodococcus equi* are weakly or partially acid-fast, and use of modified acid-fast stains such as in the Fite-Faraco technique is more satisfactory for identification of these organisms. Some mycobacterial species, such as *M. avium* complex (MAC), also are periodic acid/Schiff reagent (PAS)-positive, GMS-positive, and weakly gram-positive.

Finally, for completeness, it can be said that for 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 often can detect and even distinguish between bacterial cocci and bacilli when the burden of organisms is high (Fig. 6-16).

Box 6-2. 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-Ferraco (peanut or mineral oil)
Other tissue stains
 Giemsa; Diff-Quik
 Mucicarmine
 Modified trichrome (Weber)
 Fontana-Masson
 Chemofluorescent (optical brighteners)
 Immunofluorescent antibodies
 Immunohistochemical

**Figure 6-10.** Gram-negative bacilli (*Escherichia coli*) in alveolar exudate (Brown and Hopps stain).

Immunologic and Molecular Techniques

The application of ancillary studies, such as immunohistochemistry, in situ hybridization³¹ (Fig. 6-17), or nucleic acid amplification technology, can provide a specific etiologic diagnosis in certain cases. These techniques have the best chance for diagnosing infections caused by fastidious species that are difficult or impossible to culture from fresh samples, and also for situations in which only formalin-fixed, paraffin-embedded tissues are available. Immunohistochemical reagents for microbiological detection are becoming increasingly available and provide added power to determining specific diagnoses on formalin-fixed paraffin-embedded tissue³² (Fig. 6-18). Although these techniques provide the diagnostic equivalence of culture confirmation, they are

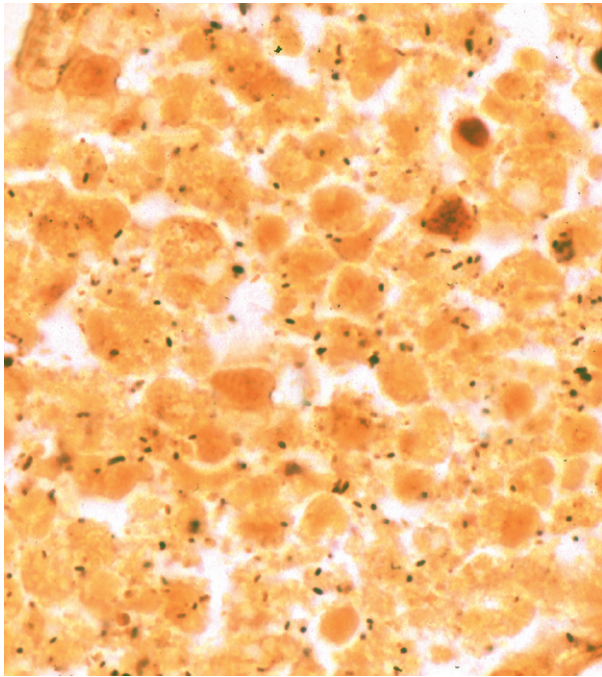


Figure 6-11. Black (silver-coated) bacilli (*Legionella pneumophila*) in alveolar exudate (Dieterle stain).

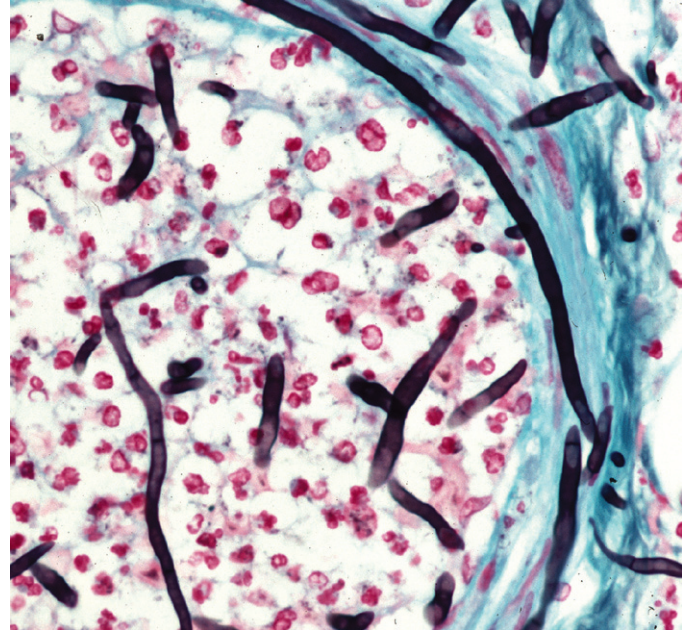


Figure 6-13. Angioinvasive *Aspergillus* species (Grocott methenamine silver stain). (Courtesy of Dr. Francis Chandler, Augusta, GA.)

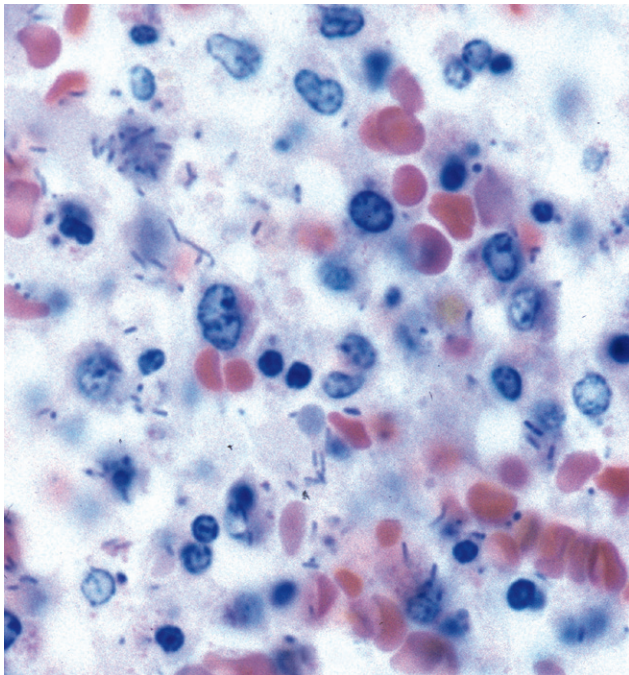


Figure 6-12. Bacillary organisms in alveolar exudates (Giemsa stain).

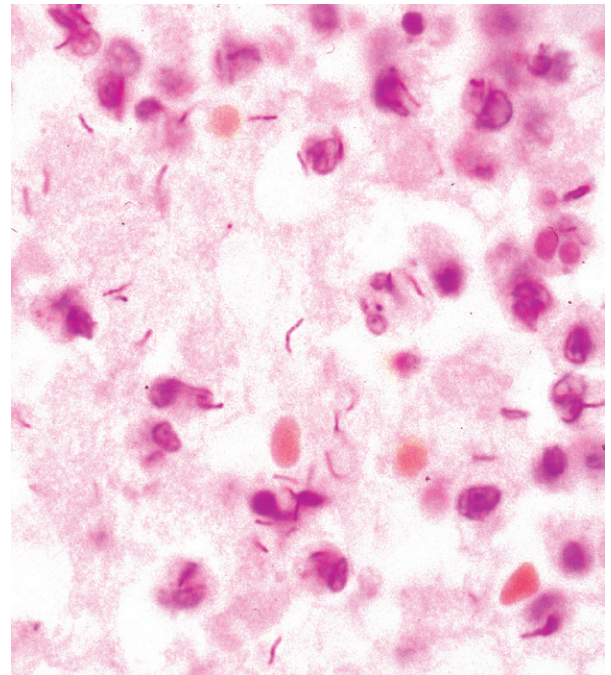


Figure 6-14. Acid-fast bacilli: *Mycobacterium tuberculosis* (Ziehl-Neelsen stain).

not without limitations and diagnostic pitfalls. The 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.³³ 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.^{34–37}

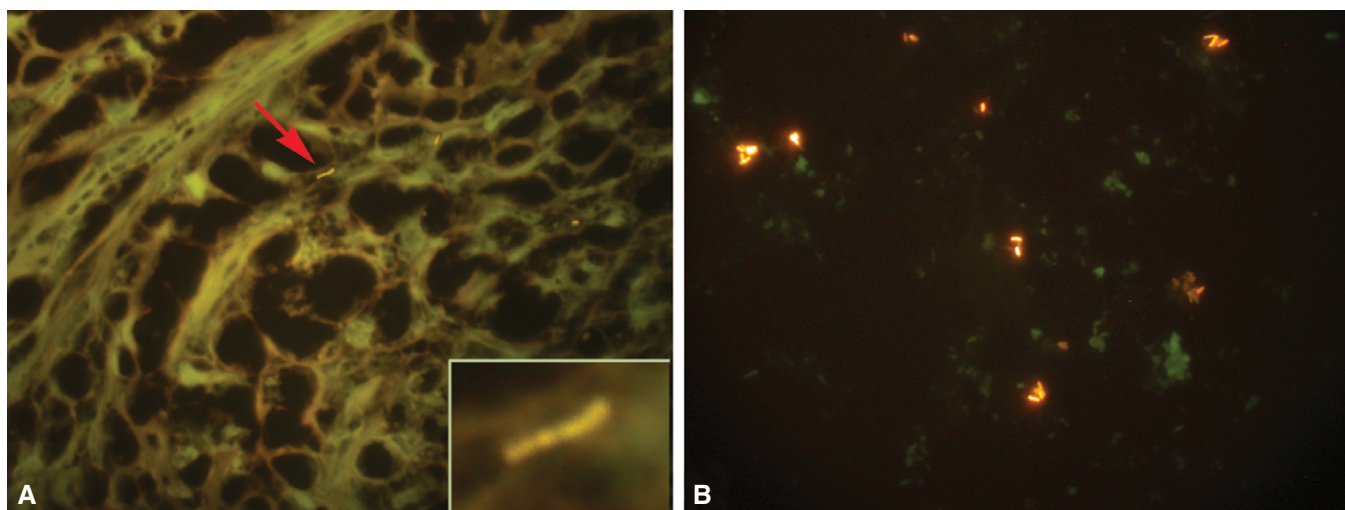


Figure 6-15. Fluorescent bacillary organisms: *Mycobacterium tuberculosis*. **A**, Tissue section with two bacilli. Note beaded character in closeup view (*inset*). (Auramine-rhodamine stain.) **B**, Low-power view.

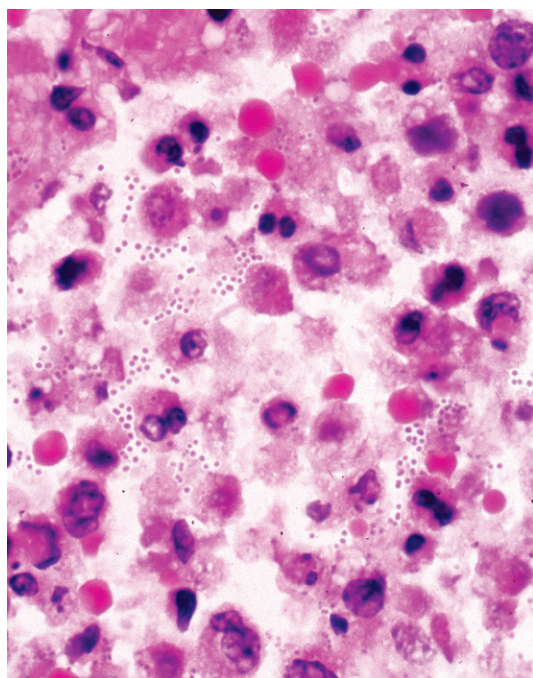


Figure 6-16. Streptococci in necrotizing pneumonia.

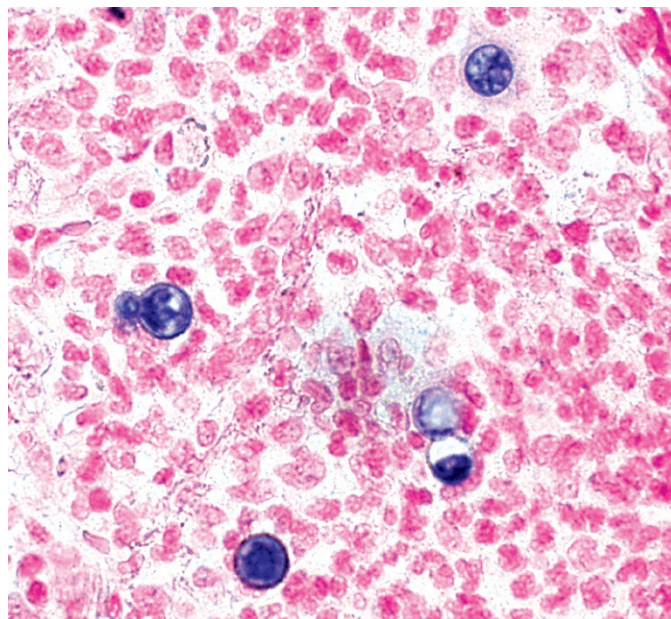


Figure 6-17. *Blastomyces dermatitidis*. In situ hybridization. (Courtesy of Ricardo Lloyd, MD, Rochester, MN.)

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.⁸ Some common tools are listed in [Box 6-3](#). Culture alone cannot distinguish contamination from colonization, or in the case of viruses, asymptomatic shedding from true infection. Molecular tests also suffer from some of these problems; require specialized, often costly equipment; and are susceptible to false positive and false negative results.³⁶ If a surgical biopsy is available, correlation of the histopathologic features can help assign an etiologic role to an agent recovered in culture. 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 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 small budding yeasts of *Histoplasma capsulatum* are seen, or yeasts with large mucoid capsules of *Cryptococcus neoformans* are identified. However, atypical forms of these organisms can be confusing.³⁸ Similarly, hyphal morphology is helpful when it is characteristic of a specific genus or group, but the many look-alikes ([Fig. 6-19](#)) require separation by searching for subtle differences under high magnification (or oil immersion), or reliance on special techniques and culture.³⁹

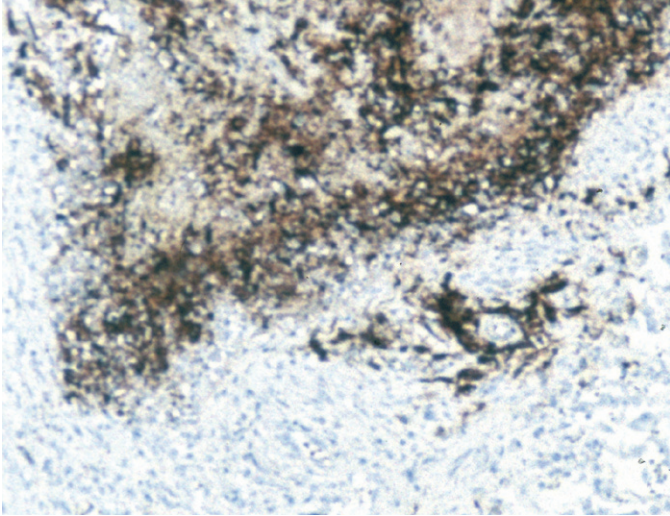


Figure 6-18. Herpes simplex virus necrotizing pneumonitis (immunohistochemical stain).

Box 6-3. 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

Certain viruses may have characteristic inclusions in tissue, but there are notable pitfalls. For example, eosinophilic intranuclear inclusions of adenovirus may resemble the early inclusions in herpes simplex virus or CMV, especially when the typical smudged cellular forms of adenovirus are absent. Also, simulators of viral cytopathic effect (CPE) can occur in a number of conditions and need to be recognized, such as macronucleoli, optically clear nuclei, and intranuclear cytoplasmic invaginations (Fig. 6-20).

Pseudo-microbe artifacts also have been recognized on routine and special stains for identification of bacteria and fungi. Such potential artifacts include fragmented reticulin fibers, pigments, calcium deposits, Hamazaki-Wesenberg (yellow-brown) yeast-like bodies (Fig. 6-21),

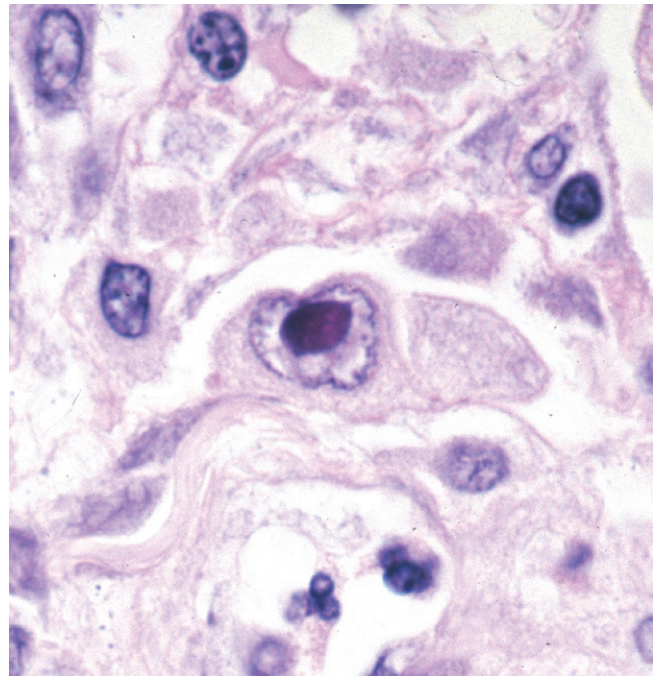


Figure 6-20. Macronucleolus mimicking a viral inclusion in an alveolar lining cell.

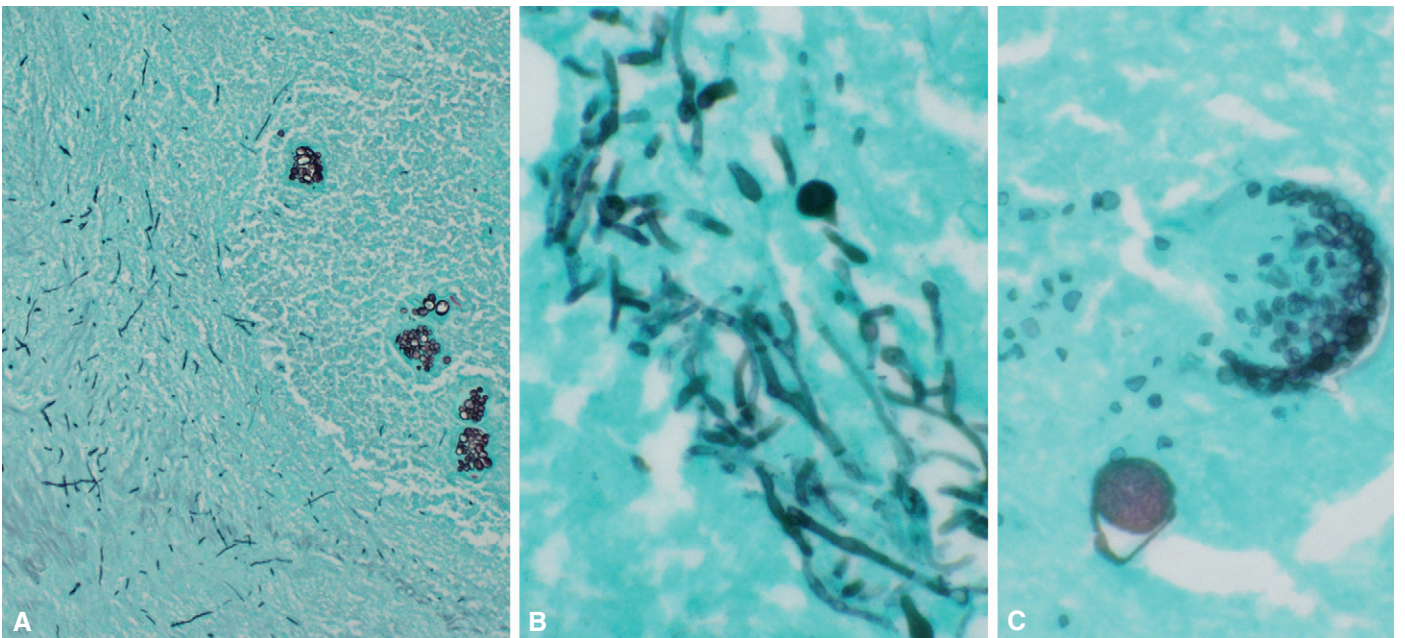


Figure 6-19. *Coccidioides immitis* demonstrating biphasic features versus those of other organisms. Culture grew *C. immitis* and *Fusarium* species. **A**, Spherules and mycelia; **B**, mycelia; **C**, ruptured spherules with endospores (Grocott methenamine silver stain).

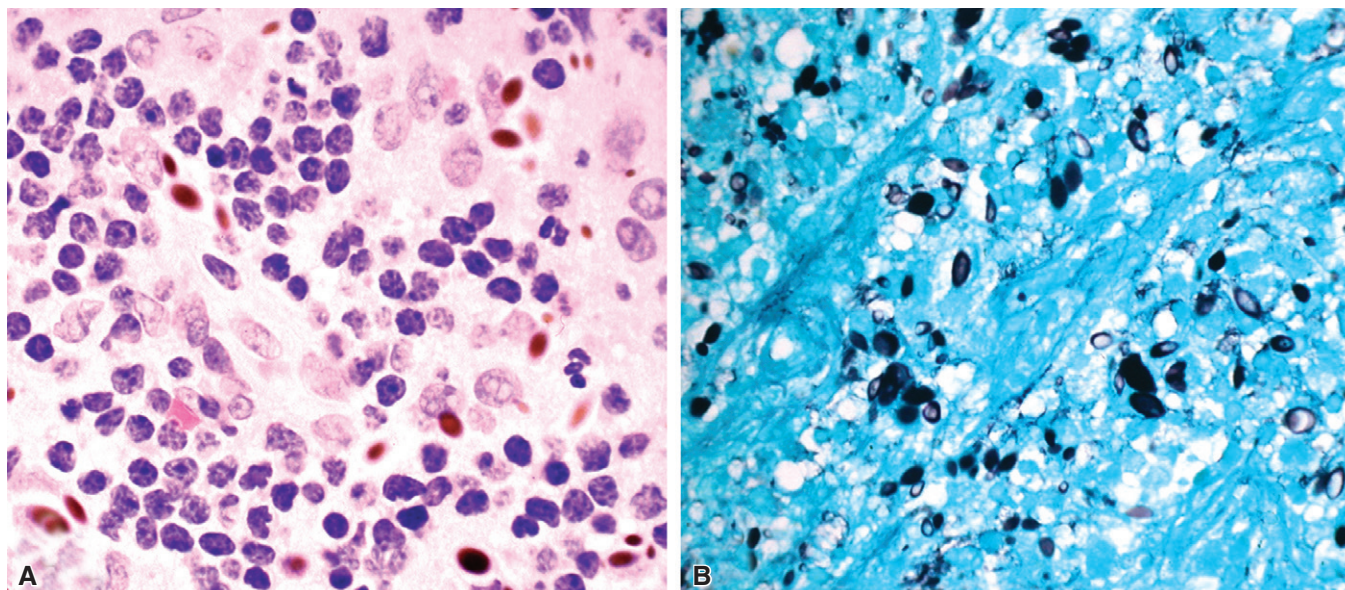


Figure 6-21. Yellow-brown Hamazaki-Wesenberg bodies (A, H&E; B, Grocott methenamine silver stain).

pollen grains, and even lymphoglandular bodies.⁴⁰ 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.

In some instances, presence of a specific infectious disease suggested by the clinical findings cannot be confirmed by the pathologist or the microbiologist, despite thorough microscopic evaluation and culture of the tissue. The histopathologic features indicative of infection may be lacking, and all stains and molecular techniques may yield negative results. Such information is nevertheless useful, however, because the clinical findings may actually reflect a noninfectious disease—for example, a pulmonary infiltrate in an immunocompromised patient may have a noninfectious etiology, such as a drug reaction, lymphangitic neoplasm, or graft-versus-host disease.

The 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.^{41–44} Fine-needle aspiration is an especially powerful tool, compared with exfoliative cytology study of respiratory secretions—sputum samples, bronchial washings, brushings, and bronchoalveolar lavage (BAL) fluid samples. The usefulness of exfoliative cytology examination often is limited owing to problems associated with distinguishing colonizing or oral 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.

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 present, as outlined in [Table 6-4](#). Although nonspecific, such features can suggest certain possibilities to the cytopathologist and assist the microbiology laboratory in triaging the specimen.⁴⁵ To this end, the presence of a cytopathologist, microscope, and staining setup during the aspiration process can be useful. The cytopathologist

Table 6-4. Fine-Needle Aspiration (FNA) 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	Mycobacteria
Suppurative	Bacteria
Epithelial	Parasite
Mixed	Fungi
Foamy alveolar cast pattern	<i>Pneumocystis jirovecii</i>
Histiocytic	Mycobacteria Bacteria Fungi
Chronic inflammation (lymphocyte and plasma cell)	Virus Other agent not otherwise specified
Null (“id”) reaction	Virus Any other

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.⁴⁶ Also, immediate evaluation of smears by rapid stain procedures allows the cytopathologist to either make or suggest a specific diagnosis, as with preparation and evaluation of a frozen section during intraoperative consultation. Smears 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.⁴⁷ 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; while 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.⁴⁷

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 in the best interest of all parties involved 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 types of suspected infections, helping to foster the development of laboratory “foresight” in surgical colleagues and medical consultants. As methods of diagnosis, treatment, and antimicrobial prophylaxis change, the pathologist must remain vigilant to a changing spectrum of etiologic agents and tissue injury patterns. The pathologist capable of integrating clinical and imaging data with morphologic and microbiologic findings can construct a comprehensive report useful for patient management. The microbiology laboratory can be instrumental in delivery of more effective and efficient patient management if the microbiologist can capture this information, in view of its optimal position for choosing the appropriate combination of diagnostic methods (morphologic, culture, immunologic, molecular) for a particular type of sample. An example of such an operational protocol is presented in [Box 6-4](#).

Box 6-4. Workup 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 transport of 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 workup

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.⁴⁸⁻⁵⁰ Patients with mild disease often are not tested but simply 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 type⁵¹ ([Box 6-5](#)). 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*.⁵⁰ 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.⁵² 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.^{53,54} Most nosocomial pneumonias result from aspiration of these bacterial species that colonize the oropharynx of hospitalized patients, and they are often polymicrobial. Any of the bacterial organisms listed (including mixtures with fungi and viruses) can cause pneumonia in immunocompromised patients.^{15,55} Ventilator-associated pneumonia is a special subset of nosocomial pneumonia and an important cause of morbidity

Box 6-5. 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

and mortality in the intensive care unit.⁵⁶⁻⁵⁸ The bacterial etiology in this setting is quite diverse and dependent on such factors as patient characteristics, underlying lung disease, and geographic location.⁵⁹ 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.⁶⁰ In rare nosocomial pneumonias, a number of unusual organisms, such as *Salmonella*, *Rhodococcus*, and *Leptospira* species, may be the etiologic agent.^{61,62}

The **atypical pneumonia agents** are those that 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 *Mycoplasma 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 *F. tularensis* (causing tularemia) as the three more common zoonotic pathogens.^{63,64}

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).⁶⁵ Botryomycosis is caused by nonfilamentous bacteria, especially *Staphylococcus aureus*, or gram-negative bacilli, such as *P. aeruginosa* and *E. coli*, which form organized aggregates referred to as grains or granules.⁶⁶

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 6-6](#) are characteristic, none are diagnostic. Overlap and mixed patterns occur.

Acute Exudative Pneumonia

Acute exudative pneumonia most often is 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 acute lobular pneumonia ([Fig. 6-22](#)), which usually correlates with patchy segmental infiltrates on the chest film (consolidation pattern on HRCT).^{29,67-69}

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. 6-23](#)). 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

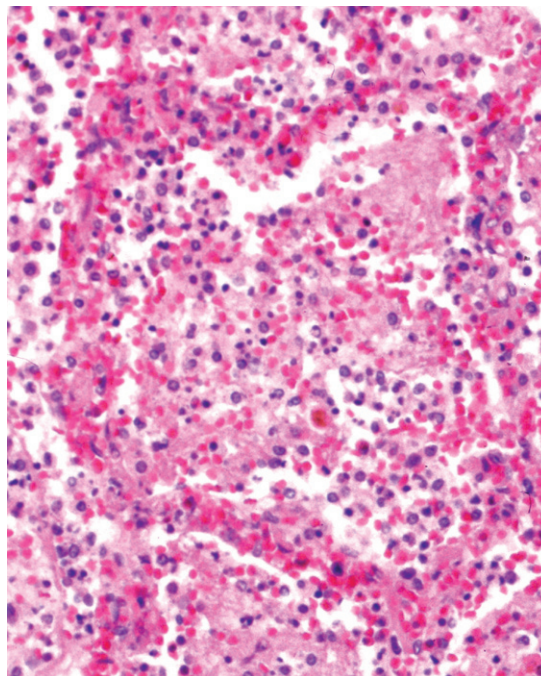


Figure 6-22. Alveoli filled with fibrinopurulent exudate with variable hemorrhage.

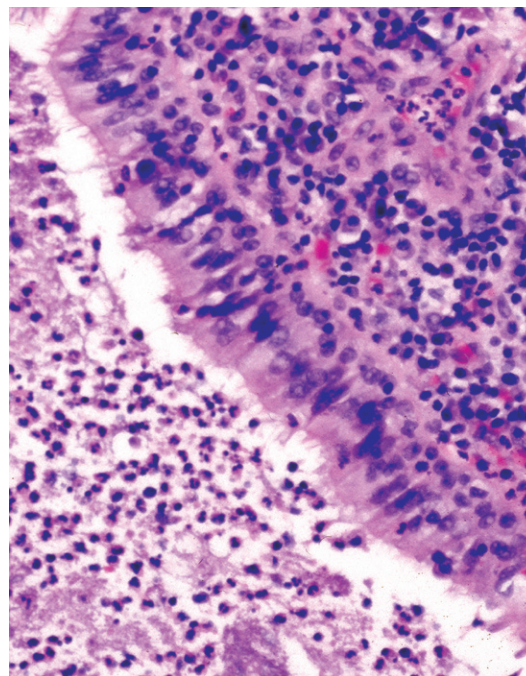


Figure 6-23. Bronchiolitis with intraluminal exudate.

Box 6-6. 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

resulting from viral airway infections, typically resulting from rhinovirus, respiratory syncytial virus (RSV), and human metapneumovirus.⁷⁰

Nodular/Necrotizing Lesions

Nodular inflammatory infiltrates with or without necrotizing features ([Fig. 6-24](#)) are characteristic of infection by certain species, such as *Rhodococcus equi* ([Fig. 6-25](#)).⁷¹ Necrotizing pneumonias also may be produced by pyogenic bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and the gram-negative bacilli—*Klebsiella*, *Acinetobacter*, *Pseudomonas*, and *Burkholderia* species.

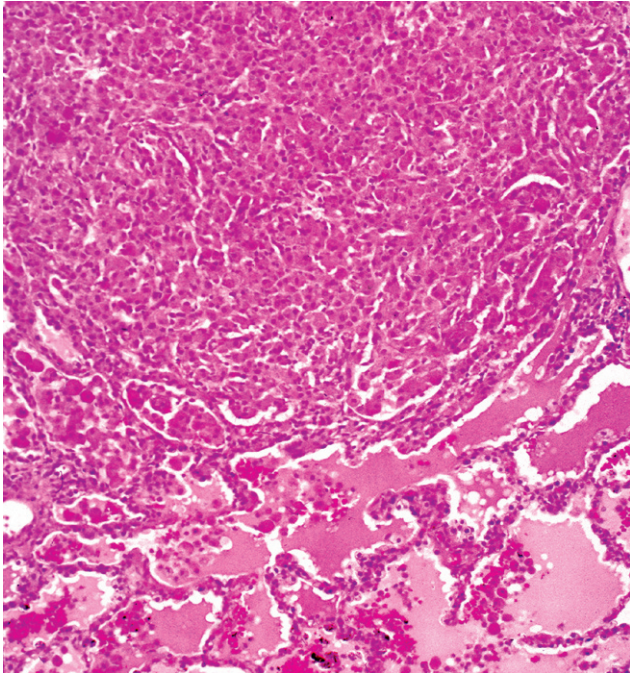


Figure 6-24. Nodular histiocytic infiltrate in rhodococcal pneumonia.

Miliary Lesions

A subset of the nodular histopathologic pattern, miliary infection (Fig. 6-26), strongly implies pneumonia secondary to 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 hybrid reaction with both nodular disease and alveolar filling.

Aspiration Pneumonia and Lung Abscess

Several pulmonary aspiration scenarios are recognized, including those caused by chemical pneumonitis (so-called Mendelsson syndrome), airway obstruction, exogenous lipid pneumonia, chronic

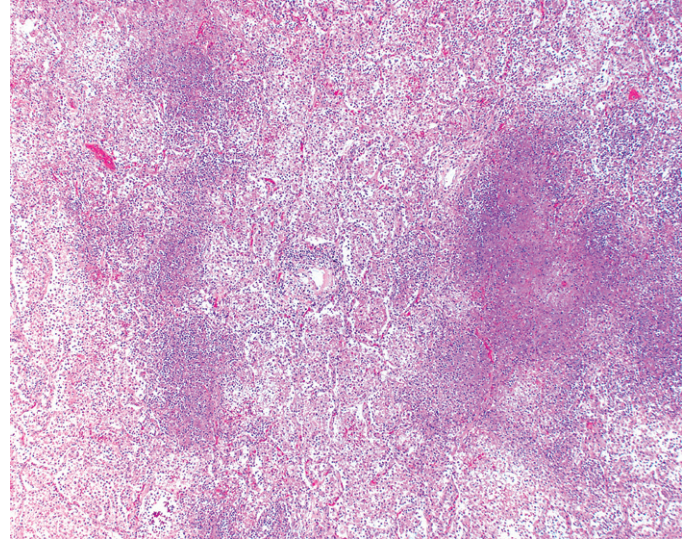


Figure 6-26. Necrotizing pneumonia, miliary pattern.

interstitial fibrosis, diffuse bronchiolar disease, bacterial pneumonia, and lung abscess.^{72,73} *Aspiration pneumonia* refers specifically to aspiration of bacteria in oropharyngeal secretions and is classically a polymicrobial aerobic/anaerobic bacterial infection, 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 key to the diagnosis. These may or may not be invested by giant cells but usually are 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, also may be seen.^{69,74} The pathogens in lung abscess (Fig. 6-27) usually encompass a polymicrobial mixture of aerobic and anaerobic bacteria,⁷⁵ and formation of such abscesses most often is secondary to aspiration (Fig. 6-28). Infections due to *Actinomyces* species (Fig. 6-29) and *Nocardia* species also may manifest this pattern, as can those due to certain pyogenic bacteria, such as *Staphylococcus aureus* and the other organisms listed previously for necrotizing pneumonias. Granulomatous inflammation with foreign bodies may be present if aspiration is the cause (Fig. 6-30).

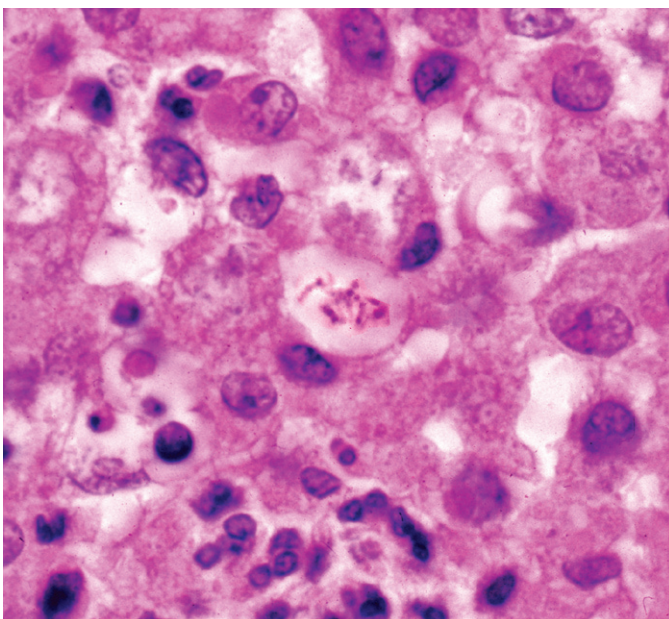


Figure 6-25. *Rhodococcus equi* bacilli in macrophage.



Figure 6-27. Lung abscess showing gross evidence of chronicity with fibrosis in surrounding parenchyma.

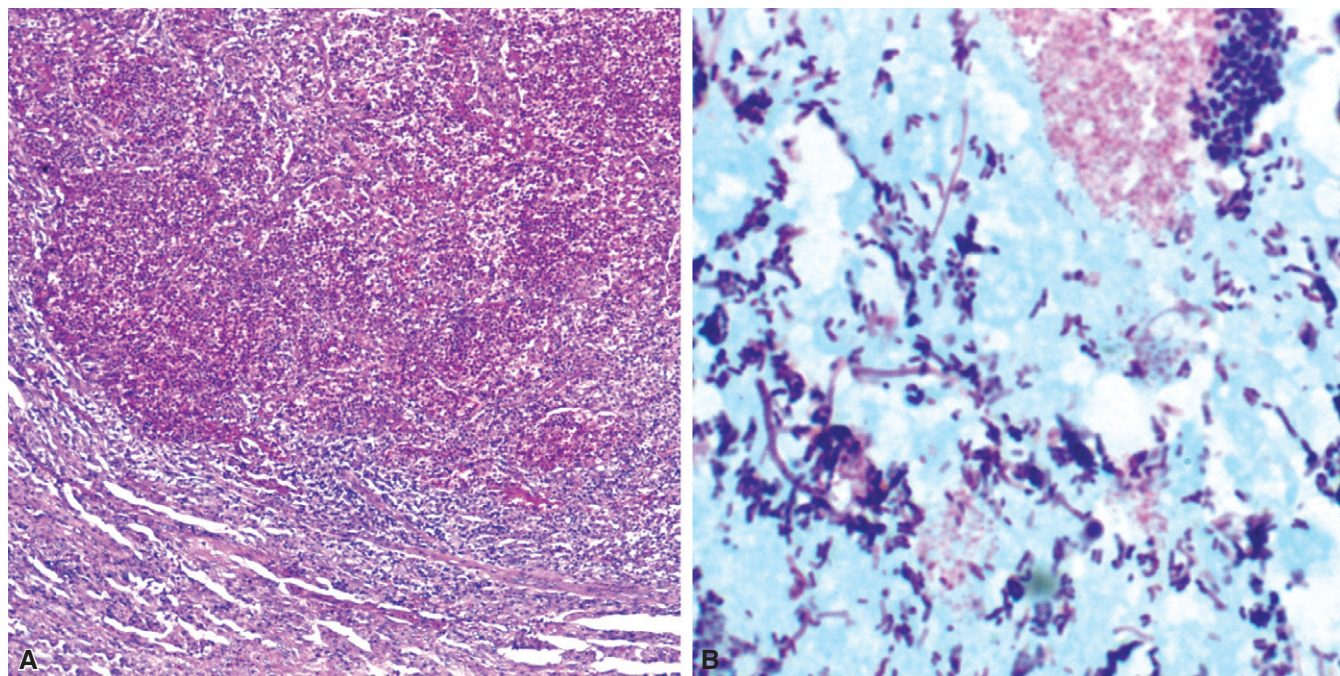


Figure 6-28. A and B, Lung abscess with polymicrobial bacterial population (Gram stain).

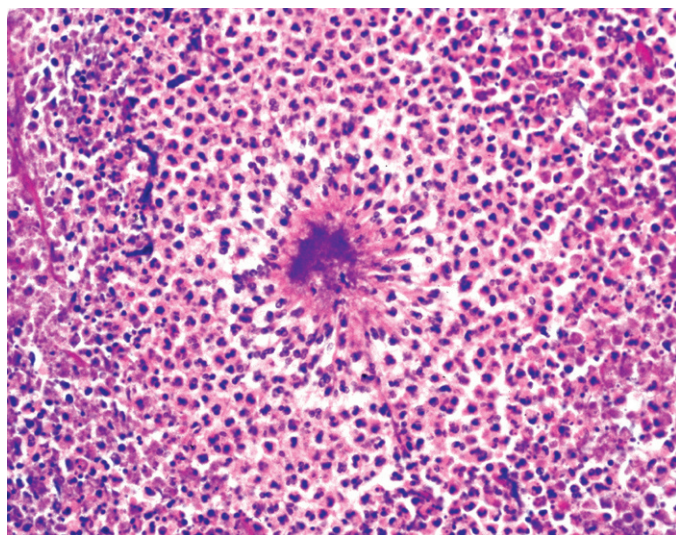


Figure 6-29. Lung abscess with sulfur granule of actinomycosis in purulent exudate.

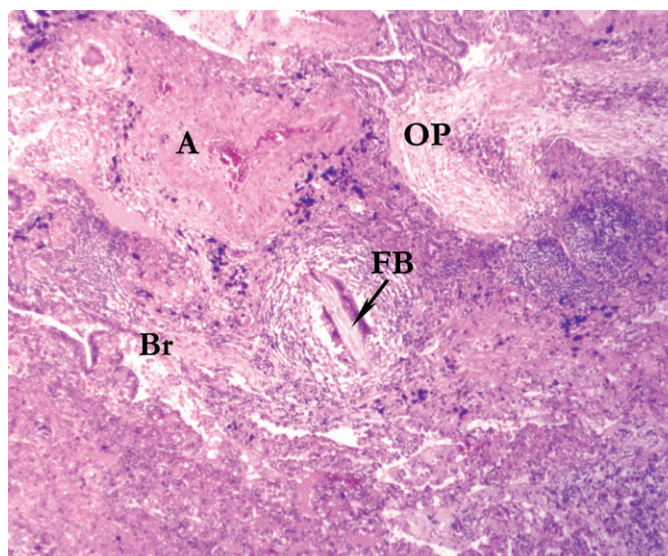


Figure 6-30. Aspiration pneumonia. Giant cells surround vegetable matter (FB) in purulent exudates, organizing pneumonia (OP), bronchiolitis (BR). A, artery.

Chronic Bacterial Pneumonias

Chronic bacterial infections (Fig. 6-31) 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.⁷⁶⁻⁷⁹ If not resorbed, polyps of air space organization may become polyps of intra-alveolar fibrosis, which sometimes ossify (dendriform ossification). Such scarring in chronic pneumonia often is associated with localized interlobular septal and pleural thickening (Fig. 6-32), 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.⁸⁰ Diffuse alveolar damage may coexist with any of the necro-inflammatory patterns described earlier. The initial *exudative* phase of this process is accompanied by hyaline membranes (Fig. 6-33); the later *organizing* phase is attended by air space 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 organisms comprising the atypical group. *Legionella* infection typically results in an intensely neutrophilic acute fibrinopurulent lobular

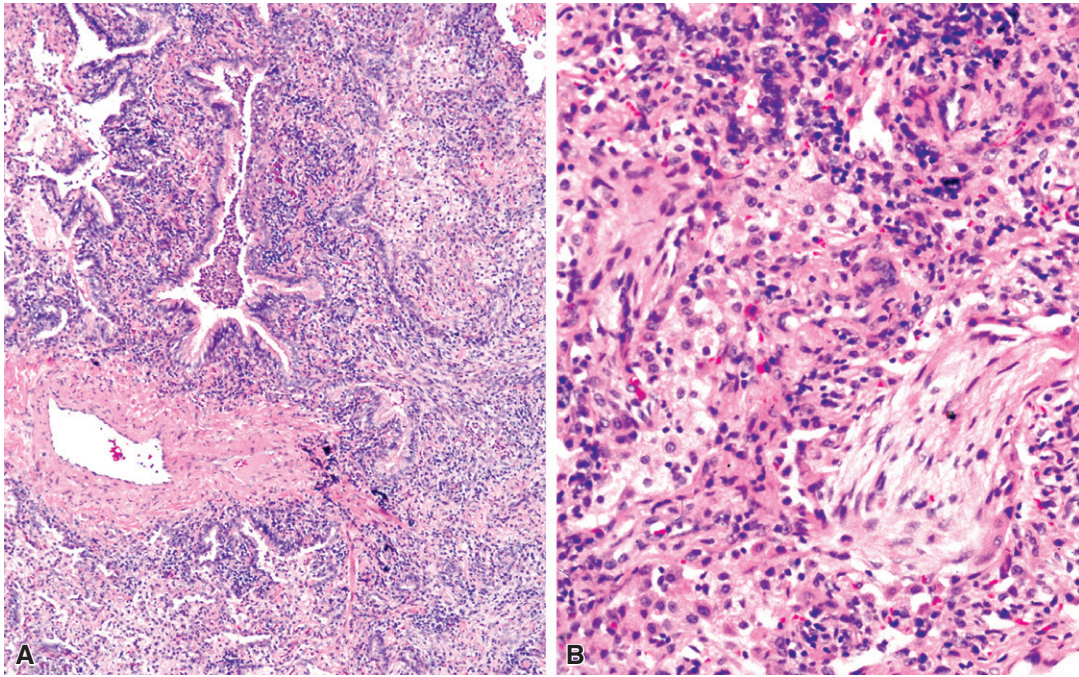


Figure 6-31. Chronic pneumonia. **A**, lymphoplasmacytic infiltrate. **B**, fascicles of fibroblasts in alveolar ducts and spaces.

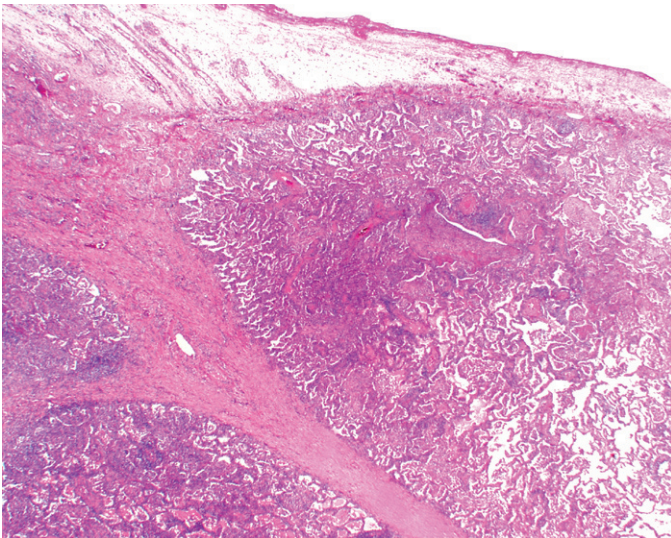


Figure 6-32. Chronic pneumonia with thickened interlobular septum.

pneumonia^{64,67} (Fig. 6-34A). *Legionella* bacilli often can be identified in silver impregnation-stained sections (see Fig. 6-34B) or recovered in culture, but newer diagnostic methods, such as real-time PCR and in situ hybridization (Fig. 6-35) also can be applied when standard approaches fail.⁸¹ 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 that have been described in the literature resemble viral bronchitis or bronchiolitis, with mixed inflammatory infiltrates in airway walls and in the adjacent interstitium^{82,83} (Fig. 6-36). 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.

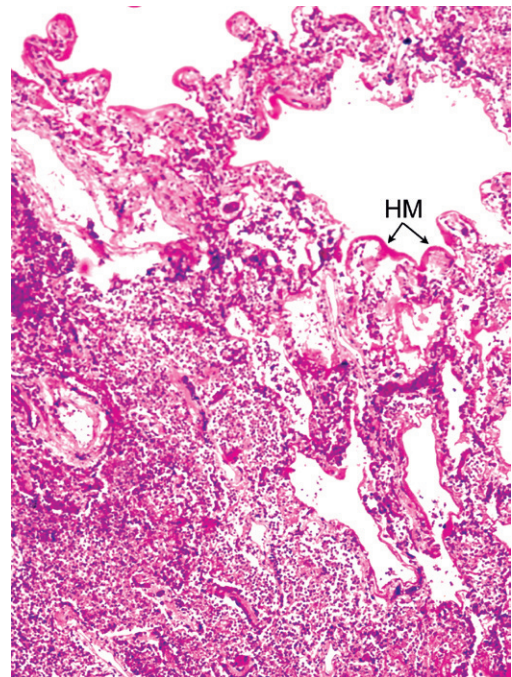


Figure 6-33. Bacterial pneumonia with hyaline membranes (HM) at periphery.

The **grains and granules** formed by the actinomycetes and bacteria of botryomycosis may have a uniform tinctorial hue on routine hematoxylin and eosin (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. 6-37). *Actinomyces* species tend to form similar-appearing granules, and both they and the bacteria of botryomycosis typically are found in the midst

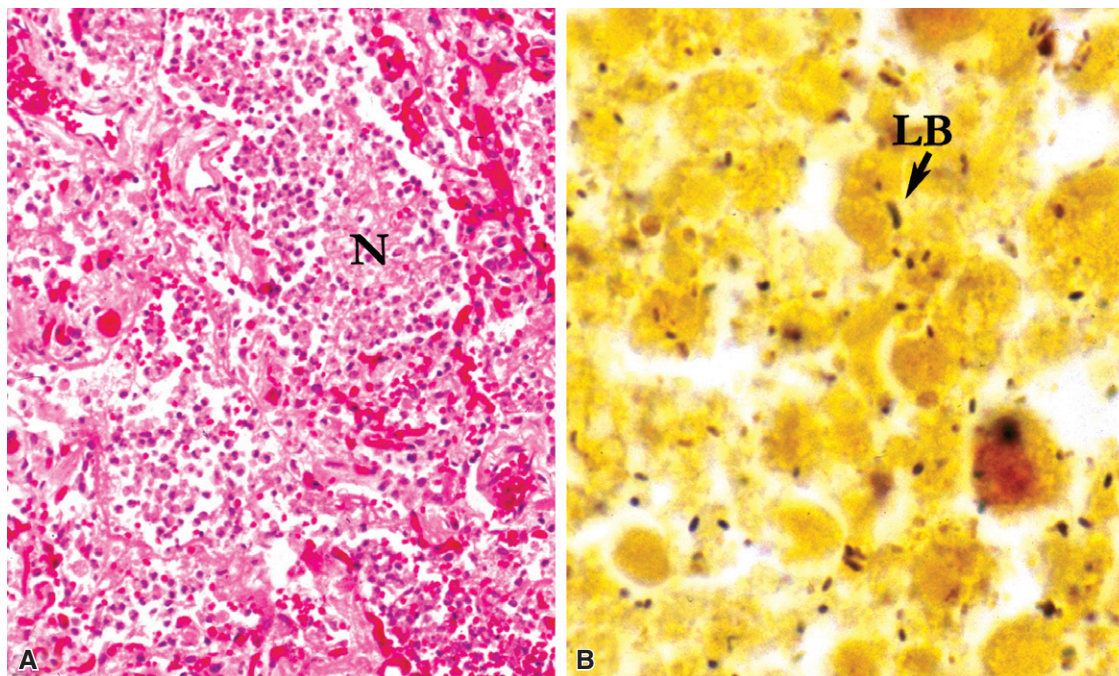


Figure 6-34. A, Legionnaires disease with intra-alveolar necroinflammatory exudates (N) and hemorrhage. B, Enhanced silhouette of *Legionella* bacilli (LB) in alveolar exudate with silver impregnation (Dieterle stain).

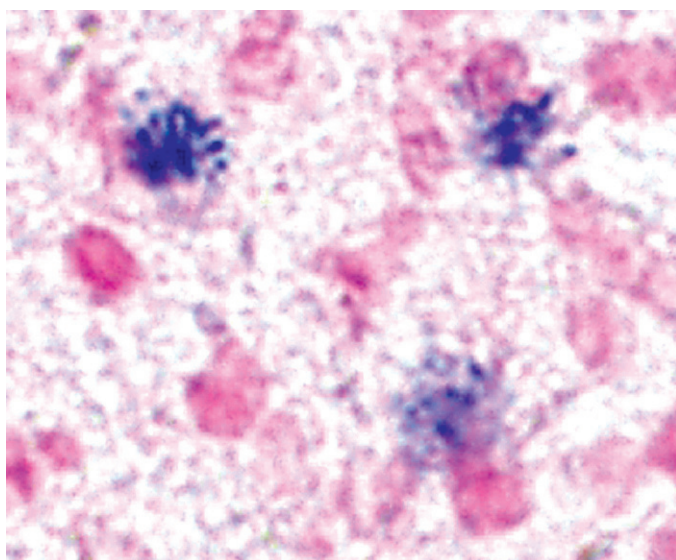


Figure 6-35. Legionnaire's disease. Detection of organisms by in situ DNA hybridization. (Courtesy of R. V. Lloyd, MD, Rochester, MN.)

of purulent exudates.^{65,84–86} The *Nocardia* species may aggregate in colonies simulating granules, but with a much looser texture (Fig. 6-38) and more monochromatic tinctorial properties.⁸⁷ 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.⁸⁸ Respiratory disease caused by the

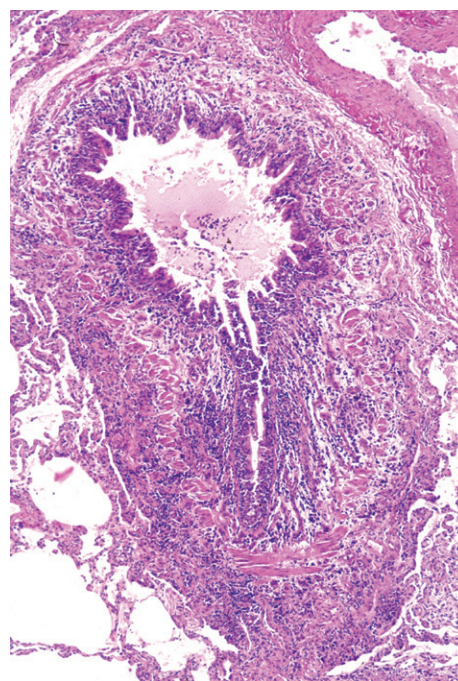


Figure 6-36. *Mycoplasma pneumoniae*. Bronchiolitis with patchy infiltrates in peribronchial interstitium.

inhalation of *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis* is especially pertinent in this context and is discussed next.

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.⁷ Set against its historical importance to medicine, the recent use of anthrax as a bioterrorism agent represents a sad contrast.

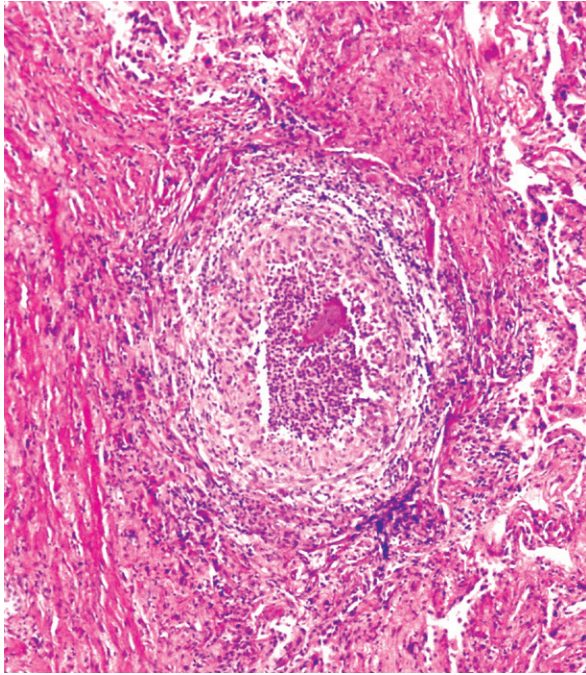


Figure 6-37. Botryomycosis granule with hematoxylinophilic core and eosinophilic investment known as Splendore-Hoeppli effect.

Inhalational anthrax causes a severe hemorrhagic mediastinitis.⁸⁹⁻⁹³ 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 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.⁹² Large gram-positive bacilli (some may appear partially gram-negative) without spores, pervade the alveolar septal vessels, with a few in the alveolar spaces. This distribution suggests hematogenous rather than airway acquisition. Hemorrhagic mediastinitis in a previously healthy adult is essentially pathognomonic for

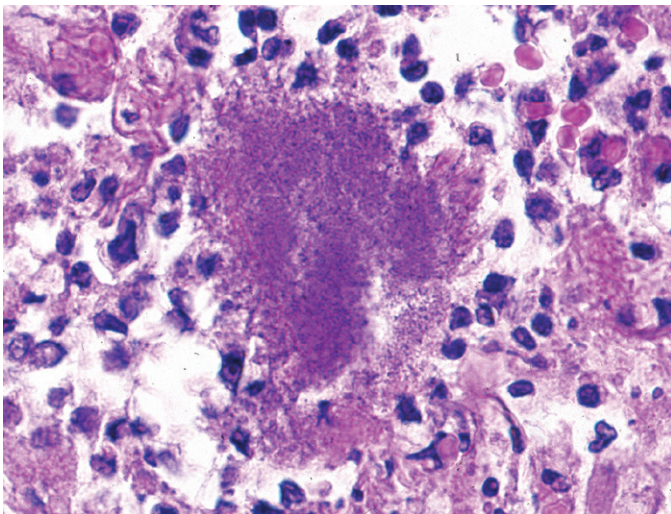


Figure 6-38. Loose-textured aggregate of *Nocardia* filamentous bacteria surrounded by neutrophils.

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.^{91,92} Cultures of blood and pleural fluid, if available, are likely to yield the earliest positive diagnostic results.⁹³ Sputum studies are much less useful in this regard.

Yersinia pestis

Primary pneumonic plague follows inhalation of *Y. pestis* bacilli in a potential bioterrorism scenario.⁹⁴ The infection begins as bronchiolitis and alveolitis that progress to a lobular and eventual lobar consolidation.⁹⁵ The histopathologic features evolve over time, beginning with serosanguineous intra-alveolar fluid accumulation with variable fibrin deposits (Fig. 6-39), progressing through a fibrinopurulent phase, and culminating in a necrotizing lesion.⁹⁶ The presence of myriad bacilli in the intra-alveolar 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.⁹⁷ These bacilli may be obvious in H&E-stained sections (Fig. 6-40) but generally are better visualized

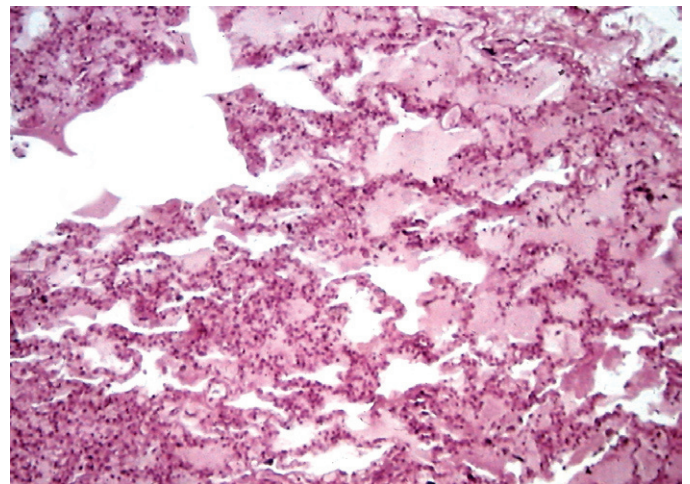


Figure 6-39. Plague pneumonia, early phase. Edema, fibrin, and sparse inflammatory cells are evident.

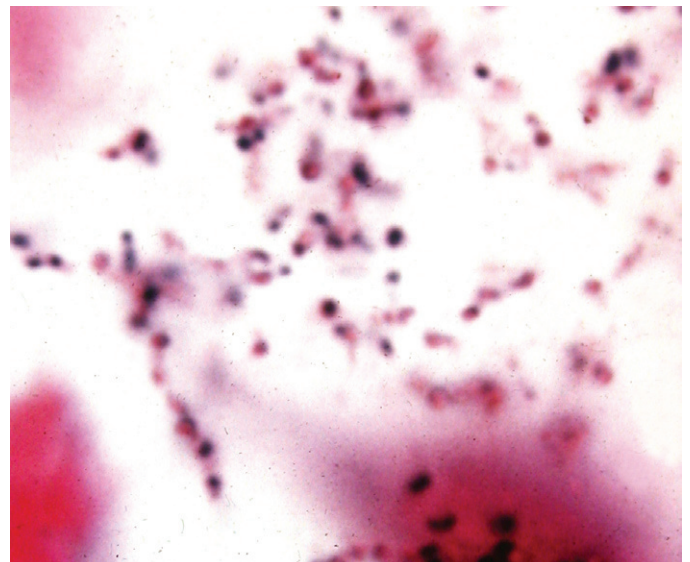


Figure 6-40. *Yersinia pestis* bacilli in alveolar space.

with Giemsa rather than Gram stain. Immunohistochemical staining provides a rapid and specific diagnosis.⁹⁵ Unlike with 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.

Francisella tularensis

Inhalation of *F. tularensis* bacilli, following a bioterrorism aerosol release, generally is expected to result in a slowly progressing pneumonia, with a lower case-fatality rate than with either inhalational anthrax or plague.⁹⁷ Initially, a hemorrhagic and ulcerative bronchiolitis is followed by a fibrinous lobular pneumonia with many macrophages but relatively few neutrophils (Fig. 6-41). 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)

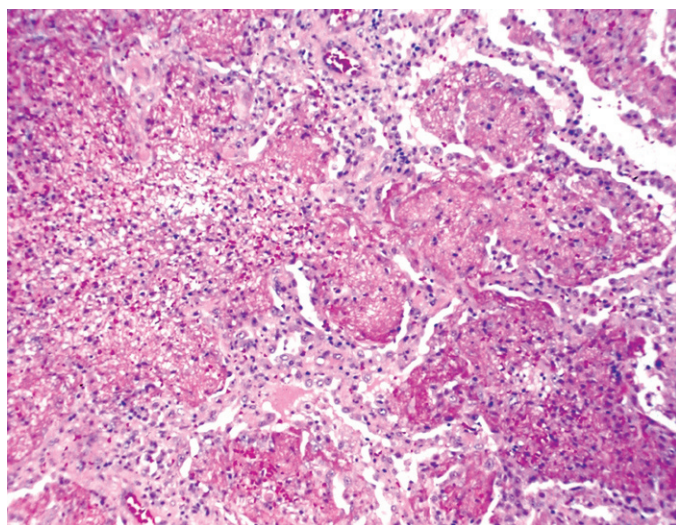


Figure 6-41. Tularemia. Fibrinous lobular pneumonia phase.

is required to enhance their silhouette.⁹⁸ Specific fluorescent antibody testing for formalin-fixed tissue and immunohistochemical studies also are available through public health laboratories. In the microbiology laboratory, Gram stain and culture of respiratory secretions are useful for diagnosis, but blood culture results are not often positive. Antigen detection and molecular techniques, such as PCR amplification, can be used to identify *F. tularensis*. Serologic tests are available but probably would not provide timely information in an outbreak situation.⁹⁷

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.⁴³ The clinical significance is rather limited in these specimens owing to the 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. 6-42A), as would be the case with a typical lobar or lobular consolidation, lung abscess, or other complex pneumonia.^{49,86,99,100} 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.^{47,54,101,102} Proponents consider it an underutilized technique whose potential benefits, 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 (see Fig. 6-42B). A smear also can 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. 6-43), or gram-positive cocci.¹⁰³ Branching filamentous forms suggest actinomycetes or *Nocardia* organisms (Fig. 6-44), with the latter distinguished

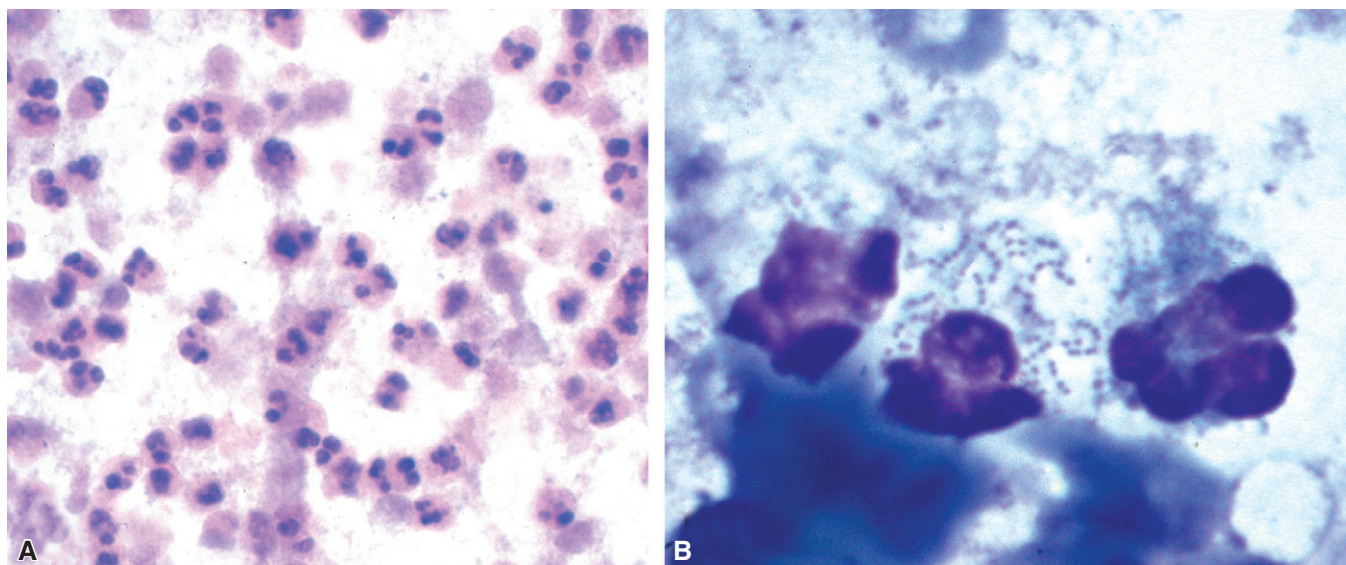


Figure 6-42. **A**, Purulent exudate of nodular pulmonary infiltrate in fine-needle aspirate (alcohol-fixed). **B**, Streptococci (viridans group) in cytoplasm of neutrophil seen in fine-needle aspirate (Diff-Quik preparation).

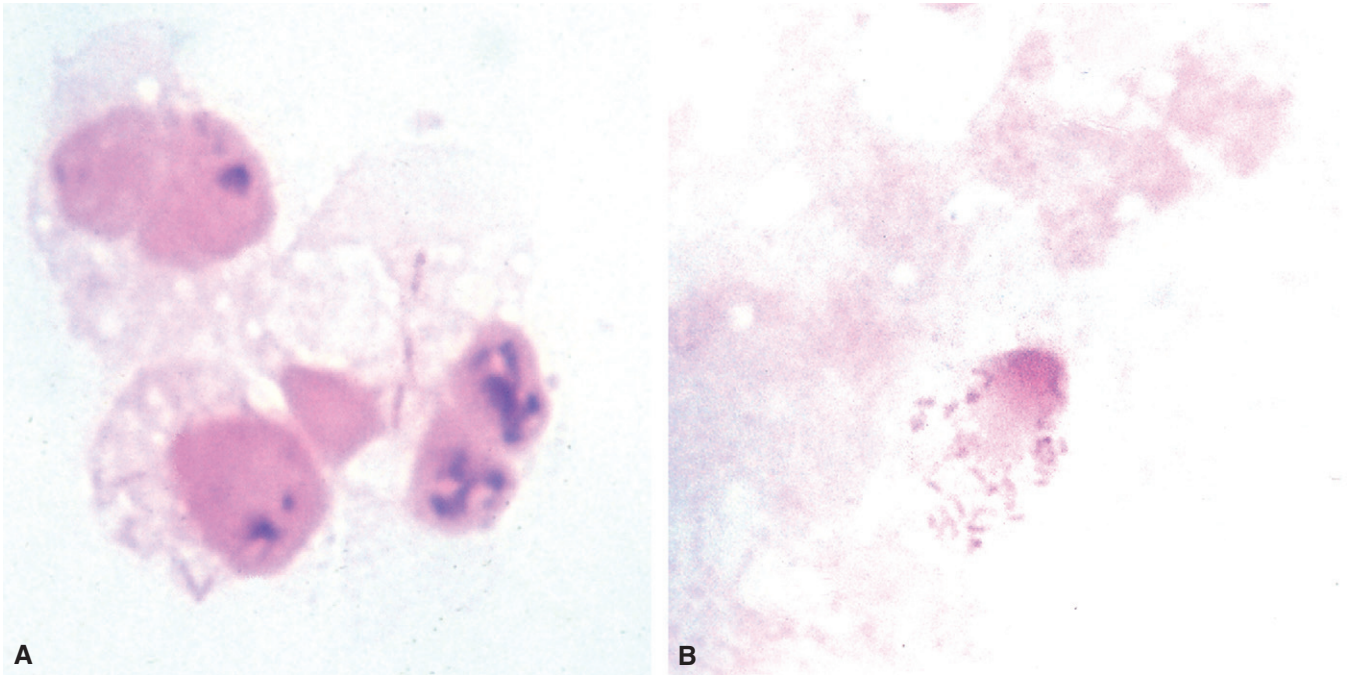


Figure 6-43. **A**, Fusiform bacteria (*Fusobacterium* organisms) in cytoplasm of neutrophil in fine-needle aspirate (Gram stain). **B**, Coccobacilli (*Haemophilus influenzae*) in cytoplasm of leukocyte in fine-needle aspirate (Gram stain).

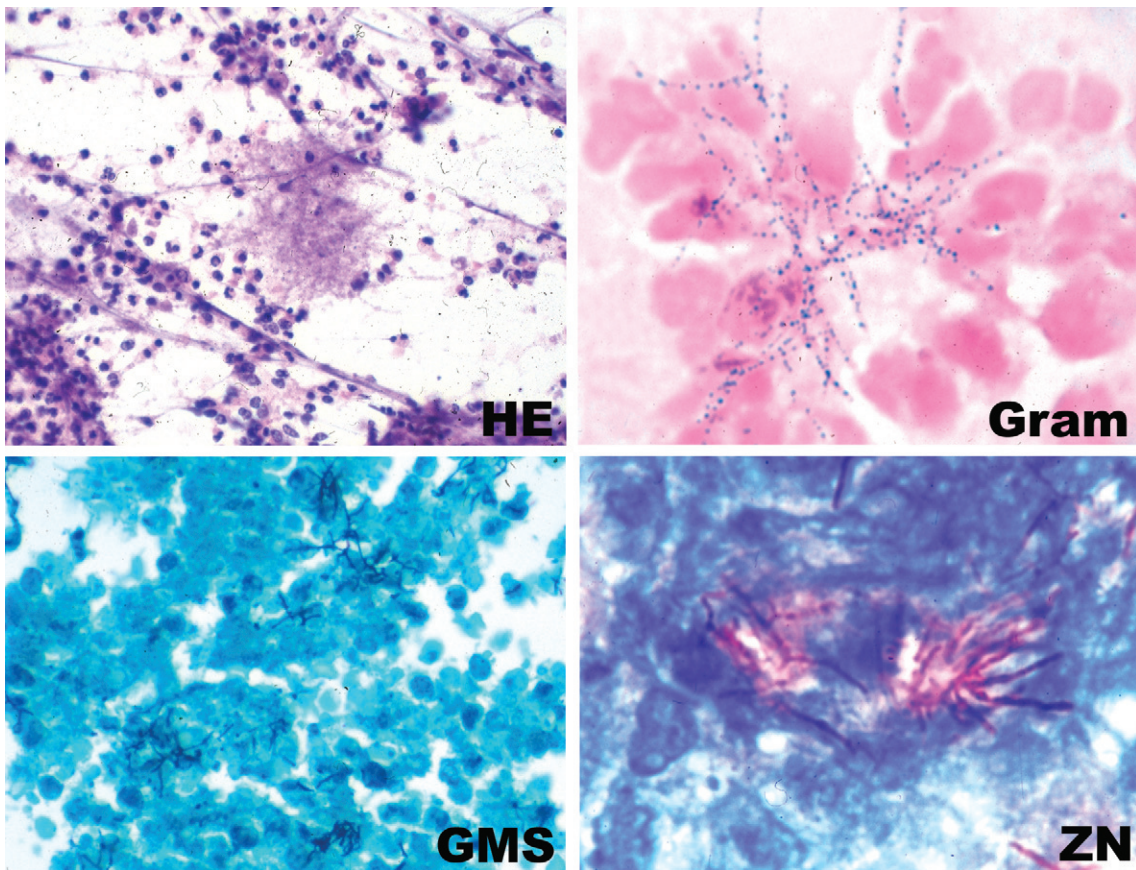


Figure 6-44. Nocardia. Loose, feathery cluster of bacilli in purulent exudate seen in a fine-needle aspirate: alcohol-fixed, H&E stain; Gram stain; Grocott methenamine silver stain; Ziehl-Neelsen stain.

by being partially acid-fast.^{104,105} Extreme care must be exercised in the staining laboratory to prevent contamination of staining solutions, because this can be a cause of false-positive results.

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), Wegener granulomatosis, 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 6-7](#).¹⁰⁶⁻¹⁰⁸ The traditional morphologic and functional approach to microbiologic diagnosis is gradually shifting to molecular methods, but their routine application continues to be a hope for the near future.

The workup 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. Certainly, the value of this workup for community-acquired pneumonias has been questioned for some time, and the guidelines from two specialty societies—the American Thoracic Society and the Infectious Disease Society of America—differ in this regard.¹⁰⁹⁻¹¹¹ Nevertheless, when a carefully collected specimen reveals one or two predominant bacterial morphotypes on a well-prepared Gram stain ([Fig. 6-45](#)), 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.^{112,113} A mixed bacterial population usually is considered nondiagnostic, especially in the absence of inflammation or the presence of many benign oral squamous cells. By contrast, 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 use of invasive techniques beginning with fiberoptic bronchoscopy and BAL with protected catheters should be considered.^{56,58,114} Anaerobic pulmonary infections, typically in the form of a lung abscess, also can be approached in this way or with transthoracic needle aspiration.⁷⁵

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, 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 contamination.

In those cases in which bacteria are visible on H&E-stained sections, the Gram stain is especially 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 a *Pseudomonas* pneumonia ([Fig. 6-46](#)). Other types of gram-negative pneumonias ([Fig. 6-47](#)) also

Box 6-7. 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

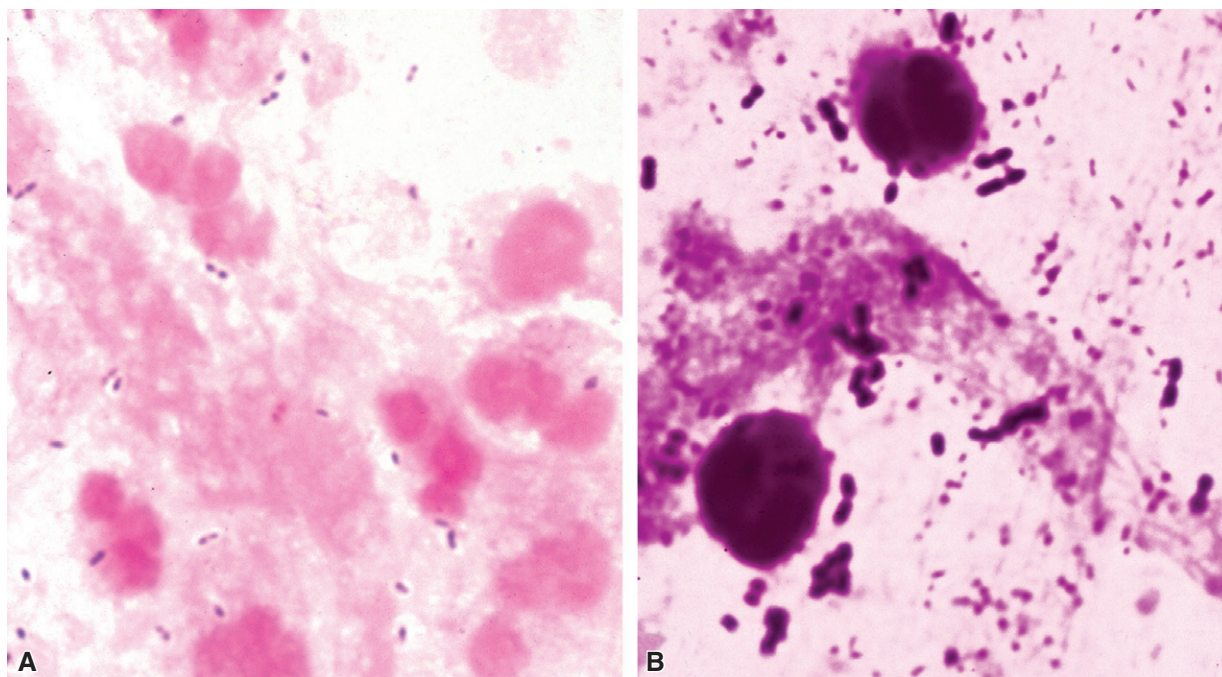


Figure 6-45. Sputum Gram stain. **A**, Gram-positive diplococci (*Streptococcus pneumoniae*) with neutrophils, but no squamous cells. **B**, Gram-positive diplococci (*S. pneumoniae*) and gram-negative coccobacilli (*Haemophilus influenzae*).

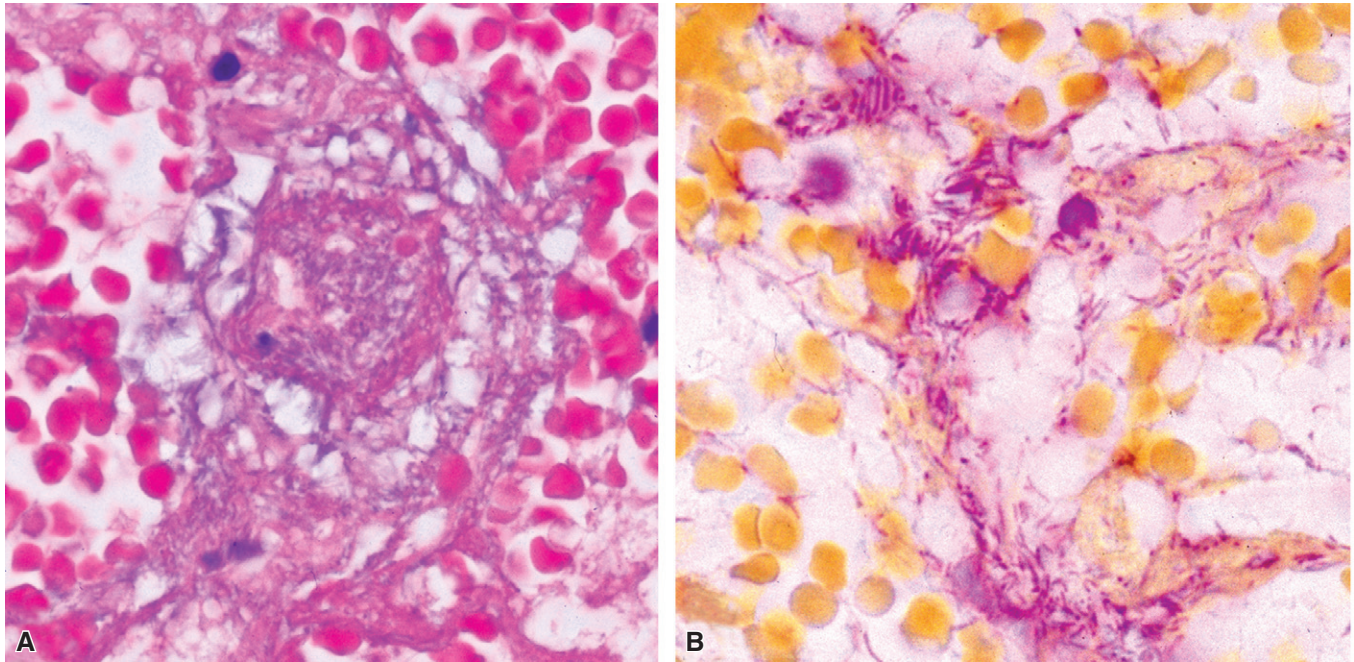


Figure 6-46. A, *Pseudomonas aeruginosa* bacilli investing interstitial vessels (Brown-Hopps stain). B, The slender gram-negative bacilli are nicely demonstrated on Gram stain.

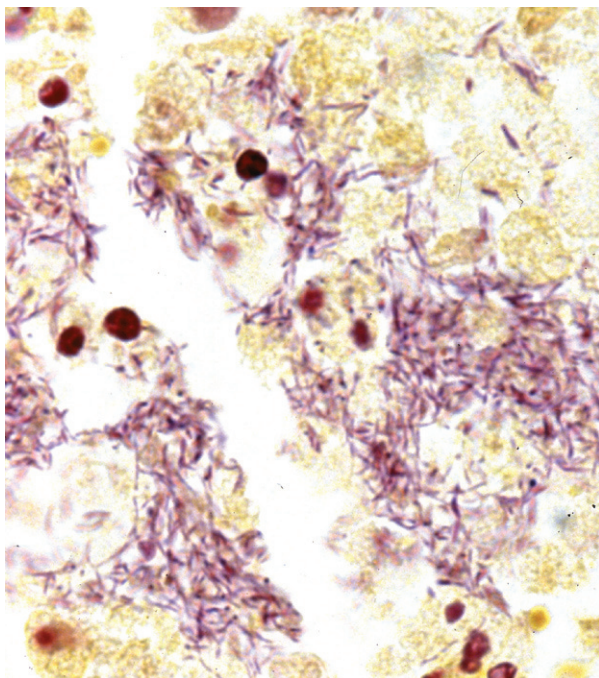


Figure 6-47. *Burkholderia cepacia* bacilli (Brown and Hopps stain).

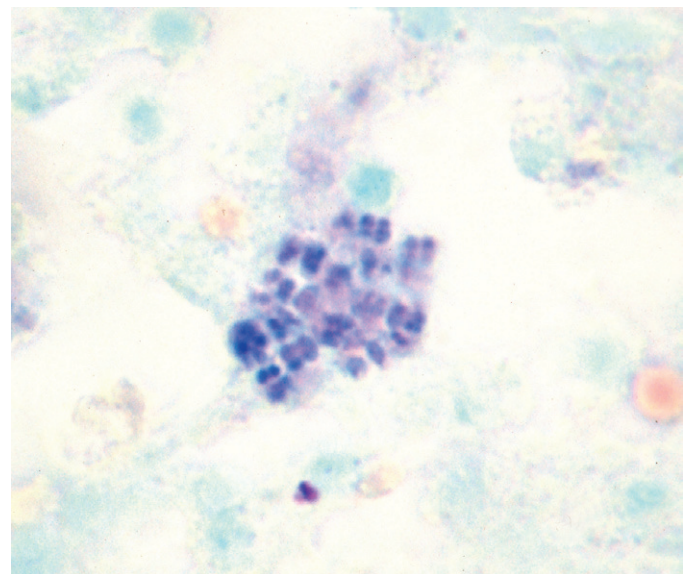


Figure 6-48. Bacterial tetrads in alveolar exudate (Giemsa stain).

can be confirmed with well-prepared Gram stains.⁷⁷ In the case of an abscess, a mixture of gram-positive cocci and gram-negative bacilli in tissue (illustrated earlier in Fig. 6-28) 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. 6-48). The Gram stain also is 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. 6-49).

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).¹⁰⁵

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 Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. It is expected that commercial reagents will become increasingly available for the common etiologic agents in the near future.³²

Culture media that will allow recovery of common bacterial species causing pneumonia from various types of respiratory samples

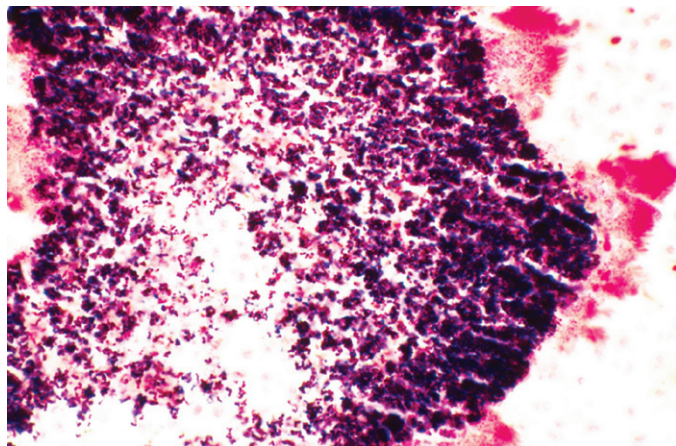


Figure 6-49. Botryomycosis. Cluster of gram-positive cocci (*Staphylococcus aureus*) invested by gram-negative–staining Splendore-Hoeppli material (Brown-Brenn stain). (Courtesy of Dr. Francis Chandler, Augusta, GA.)

(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 transport and immediate plating is essential for recovery. BYE also is a good “all-purpose” medium for growing other fastidious species including *F. tularensis*. However, *F. tularensis* grows best in cysteine-enriched media.¹¹⁵

In addition to respiratory samples, blood can be obtained for cultures in patients sick enough to suspect bacteremias and 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.⁸⁵ 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 to identify these organisms at the genus level. However, genotype rather than phenotype characteristics are required to identify newly emergent species.¹¹⁶

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.^{63,117} Classic cold agglutinin and complement fixation tests for these agents have largely been replaced by enzyme immunoassay and microimmunofluorescence testing.^{83,118,119} Serologic methods also are useful for diagnosis of tularemia because of the difficulty in culturing the fastidious bacterium.

Legionella pneumonia is a common form of severe pneumonia not readily diagnosed for a number of reasons, including the organism's fastidiousness.¹²⁰ 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 *L. 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*, and because the need to collect acute and convalescent sera is obviated, it has become a frequently used diagnostic test.^{120,121} 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.^{122–124}

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.^{81,124,125} Real-time PCR assay appears promising as a sensitive, specific, and rapid diagnostic technique that is likely to find routine clinical application. It provides a platform for the simultaneous amplification and detection of target DNA in a single tube through use of one of several types of fluorescence resonance transfer (FRET) fluorescent probe quencher techniques or melting curve analysis. Furthermore, it obviates the concern for amplicon contamination in the laboratory.¹²⁶ The development of a 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.^{35,127,128}

Differential Diagnosis

The key morphologic and microbiologic features of the bacterial pneumonias are summarized in Table 6-5. The presence of purulent exudates or significant numbers of neutrophils in biopsy or cytologic samples should always trigger a search for bacterial infection. Of note, however, because lung biopsies usually are performed late in the clinical course with respect to an evolving infiltrate, after many procedures have been performed and bacterial infections have been excluded 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 Wegener granulomatosis, 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 air space accumulation of neutrophils, further raising concern for bronchopneumonia. Centrally necrotic or cavitory neoplasms of various types may mimic 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 necro-inflammatory lesion typical of bacterial infection can be produced by viruses, some fungi, and even protozoa (e.g., *Toxoplasma gondii*).

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 a “last resort” effort.^{129,130} In recent years, delays in 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

Table 6-5. 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/gram of tissue) Culture-sterile lung tissue on standard nonselective and selective media (blood, chocolate, MacConkey agars); anaerobic broth and agars for abscesses Urinary antigen for <i>Streptococcus pneumoniae</i>
Atypical Pneumonia Agents	
Surgical pathology	<i>Legionella pneumoniae</i> : fibrinopurulent with bacilli visible in silver-stained (Dieterle; Warthin-Starry) sections DAD often present <i>Chlamydia</i> and <i>Mycoplasma</i> infection: polymorphous bronchiolar and interstitial infiltrate
Cytopathology	Acute inflammation with bacilli stained with silver or by immunofluorescence (<i>Legionella pneumoniae</i>)
Microbiology	DFA for <i>L. pneumophila</i> serotypes Culture on selective (BCYE) agar for <i>Legionella</i> ; urinary antigen for <i>Legionella</i> Serologic testing and/or PCR assay for <i>Mycoplasma</i> and <i>Chlamydia</i>
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: <i>Nocardia</i> (aerobic actinomycete) and <i>Actinomyces</i> (anaerobic actinomycete) <i>Nocardia</i> 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 <i>Actinomyces</i>

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

better methods and technology. In fact, however, because direct acid-fast bacillary smears of respiratory specimens yield negative findings in at least one half of the cases,¹³¹ 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 also can define the organism's relationship to a histopathologic lesion, or host response. This is important in evaluating the significance of a culture result, because 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.¹³²

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 *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microti*. The latter 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 more deaths worldwide than any single microbe. This organism is the etiologic agent of tuberculosis worldwide in its various forms, which are listed in **Box 6-8**.

Box 6-8. Classification of Tuberculosis

- Primary tuberculosis
 - Exogenous first infection
 - Exogenous reinfection
- Progressive primary tuberculosis
- Post-primary 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.

Primary tuberculosis occurs in patients without previous exposure or loss of acquired immunity. Progressive primary tuberculosis occurs in patients with inadequate acquired immunity, that is, impaired cellular immunity. Post-primary 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.^{133,134} Many clinical experts consider that most cases of active tuberculosis in adults with normal immunity arise from reactivation of latent infection (post-primary tuberculosis), whereas reinfection with a new strain derived from the environment (primary or post-primary tuberculosis) can occur in the immunocompromised patient. More recently, DNA fingerprinting methods (genotyping) have challenged this dogma,

however, by showing that exogenous reinfection accounts for a significant percentage of cases in some areas of the world.¹³⁵ Miliary tuberculosis and extrapulmonary disease can occur with any of these forms.^{133,136}

Primary tuberculosis usually is a mild illness that often is not clinically recognized. Of note, however, the bacilemia that occurs during its development can seed extrapulmonary organs and set the stage for subsequent reactivation. Approximately 5% of patients pass through latency to post-primary disease within 2 years of primary infection, and another 5% do so later in their lives.¹³⁷

Non-Tuberculous Mycobacteria

Recognized NTM species number more than 125, many of which were identified during the past decade.^{138,139} However, relatively few cause pulmonary disease.^{132,140–142} 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 non-immunocompromised patients are increasing.^{17,143} 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*, *Mycobacterium fortuitum*, *Mycobacterium szulgai*, *Mycobacterium simiae*, *Mycobacterium xenopi*, *Mycobacterium malmoense*, *Mycobacterium celatum*, *Mycobacterium asiaticum*, and *Mycobacterium shimodii*. These latter 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.¹³²

Histopathology

The histopathologic patterns produced by mycobacteria are listed in Box 6-9. 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.^{144–146}

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 lower lobes).¹⁴⁵ The disease passes through progressive

Box 6-9. 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

phases of exudation, recruitment of macrophages and T lymphocytes, and granuloma formation followed by repair with granulation tissue, fibrosis, and mineralization.^{134,147} 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. 6-50) and an enlarged, sometimes calcified hilar lymph node. In both locations, the histopathologic hallmark is a necrotizing granuloma (Fig. 6-51) composed of epithelioid

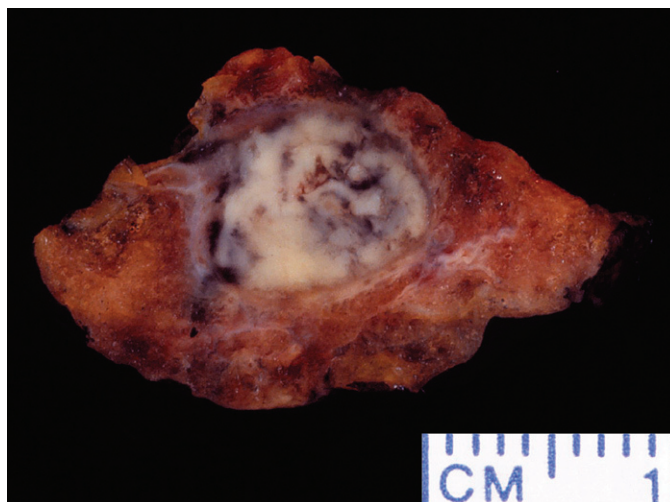


Figure 6-50. Tuberculoma removed from right upper lobe.

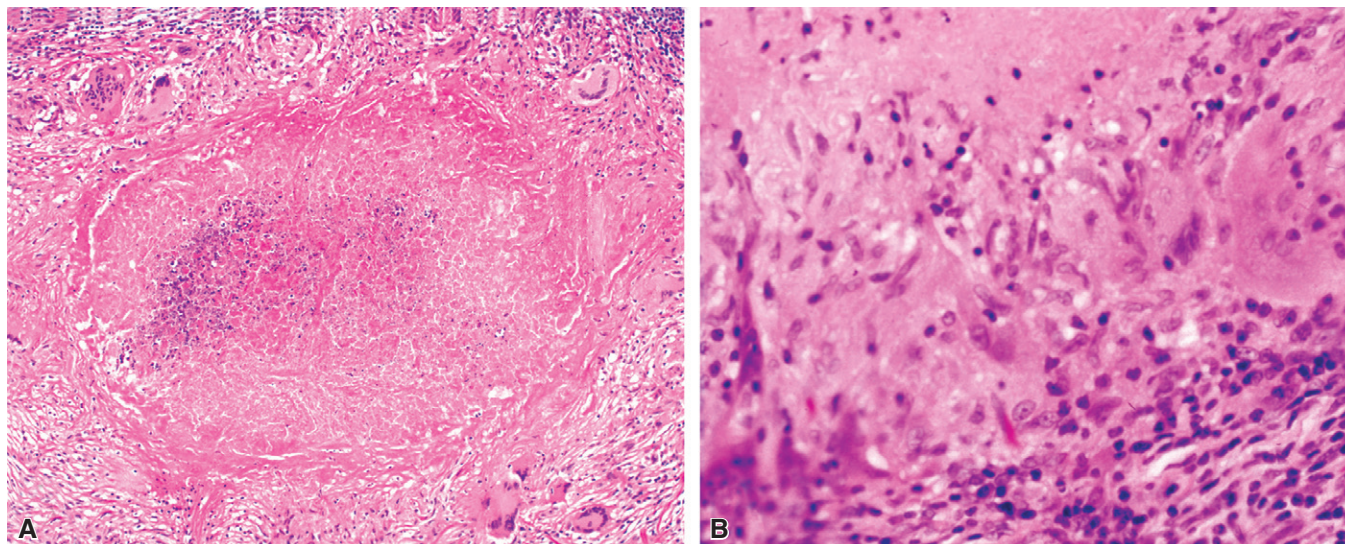


Figure 6-51. **A**, Tuberculoid granuloma with central zone of caseation necrosis surrounded by epithelioid cells, giant cells, and outer investment of lymphocytes. **B**, Palisade of epithelioid histiocytes in giant cells at edge of necrotic zone.

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.^{133,148} A spectrum of lesions may be seen, from the tuberculoid “hard” granuloma without necrosis and rare organisms, to the multibacillary necrotic lesion with scant epithelioid cells.¹⁴⁹ In a minority of patients the lesions enlarge and progress as a result of increased necrosis or liquefaction.

The complications of tuberculosis are listed in **Box 6-10** and illustrated in **Figure 6-52**. Other complications may include extension into blood vessels with miliary (**Fig. 6-53**) or systemic dissemination, lymphatic drainage into the pleura with granulomatous pleuritis and effusions, or to bronchi with bronchocentric granulomatous lesions (**Fig. 6-54**) or tuberculous bronchopneumonia. Granulomas also may encroach upon blood vessels, mimicking a “granulomatous” vasculitis. The hemophagocytic syndrome, which has been implicated in a variety of bacterial, viral, and parasitic infections, also has been associated with tuberculosis.¹⁵⁰

Post-primary Tuberculosis

Post-primary tuberculosis, the most common form in adults, typically involves the apices of the upper lobes, producing granulomatous

Box 6-10. Complications of Tuberculosis

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

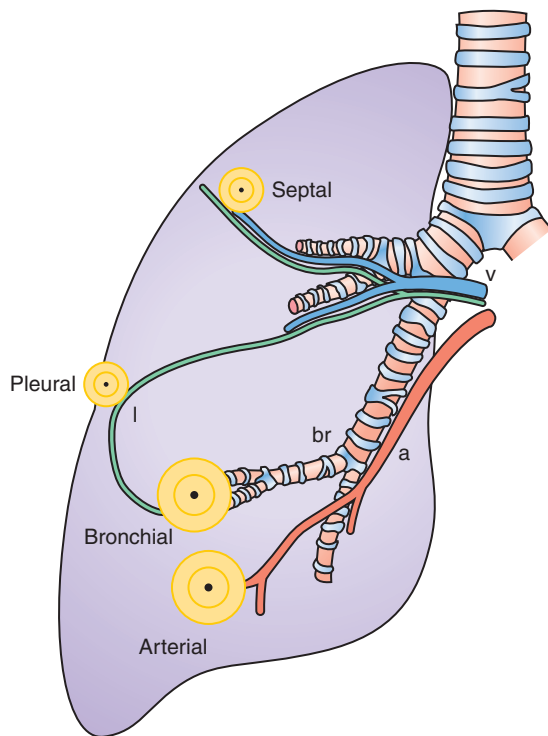


Figure 6-52. Complications of tuberculosis. Invasion of arteries (a) with miliary spread; bronchi (br) with tuberculous bronchopneumonia; lymphatics (l) with granulomatous pleuritis and effusions. Invasion of septal (s) veins (v) leads to extrapulmonary dissemination.

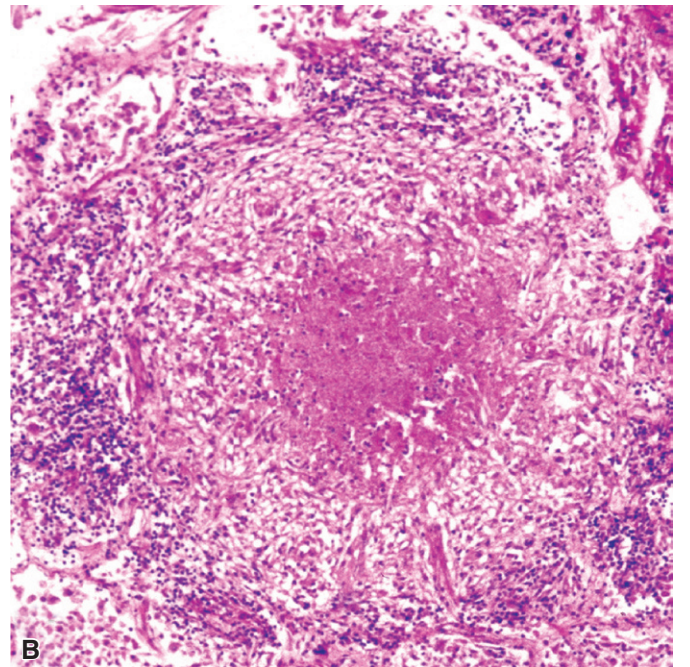
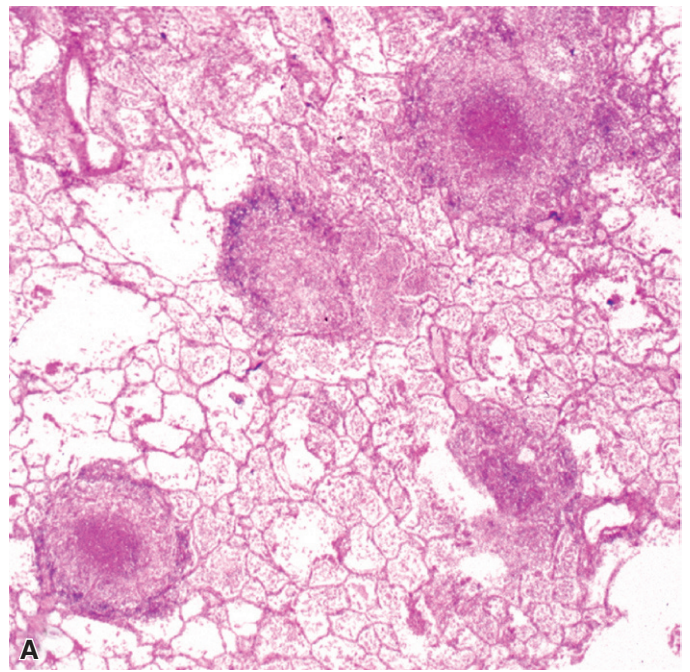


Figure 6-53. Miliary tuberculosis. **A**, Miliary pattern. **B**, Epithelioid granulomas with necrotic central zones.

lesions with greater caseation, often with cavities and variable degrees of fibrosis and retraction of the parenchyma.¹³⁶ Fibrosis and bronchiectasis occurs with the healing of cavities and is the major cause of pulmonary disability in this disease.¹⁵¹ Recent studies have proposed that post-primary disease begins as a form of lipoid pneumonia, with bacilli-laden foamy alveolar macrophages and bronchiolar obstruction progressing to cavitory disease, as a result of caseation, and microvascular occlusion due to delayed-type hypersensitivity.¹⁵² Extension to other lobes, hilar or mediastinal lymph nodes and miliary spread through the lungs and to extrapulmonary sites can complicate this form of disease. Other presentation patterns include acute and organizing diffuse alveolar damage with advanced or miliary disease, acute

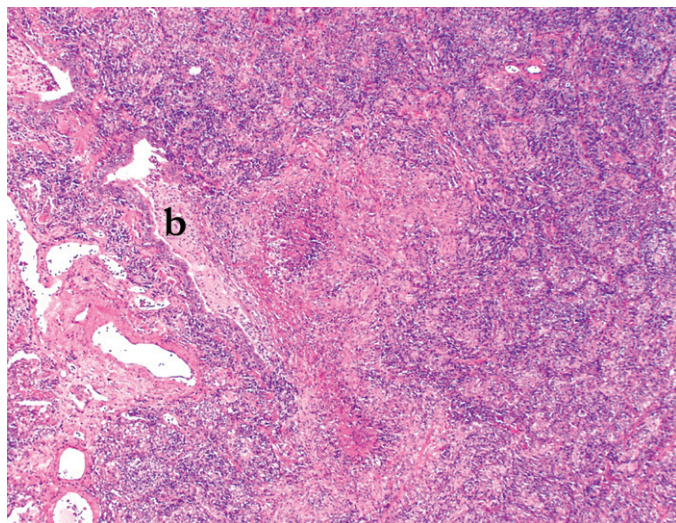


Figure 6-54. Bronchocentric granuloma in mycobacterial infection. Only a small focus of residual bronchial epithelium (b) remains.

tuberculous bronchopneumonia, and the solitary pulmonary nodule (tuberculoma). A proximal endobronchial form may mimic a neoplasm and also is noteworthy for extensive necrosis and often large numbers of bacilli.¹⁵³ Because characteristic granulomatous morphology may not be visible around the necrotic material, stains for mycobacteria should be considered for all necrotic endobronchial samples.

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 post-primary 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*.¹⁵⁴

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.^{134,155} Solitary pulmonary nodules generally exhibit granulomas resembling those caused by *M. tuberculosis*.

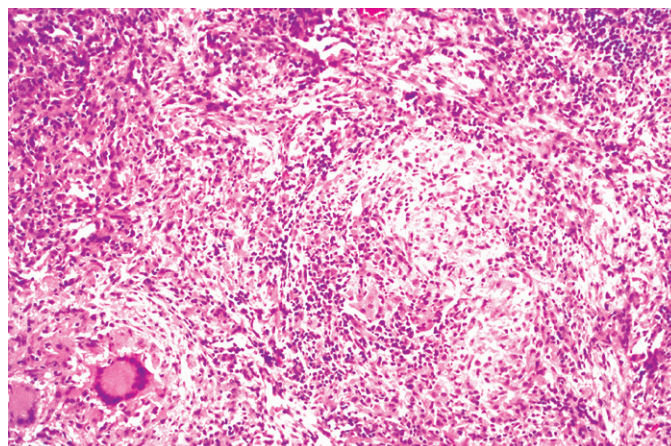


Figure 6-55. Non-necrotizing granuloma in infection due to *Mycobacterium avium* complex (MAC).

Chronic progressive disease also resembles tuberculosis, with upper lobe thin-walled cavities and granulomatous inflammation, with or without caseous necrosis (Fig. 6-55). Multiple confluent granulomas in fibrosis can mimic sarcoidosis. Organisms usually are 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 pre-existing cavitory lung disease of any cause (including old tuberculous cavities).

Disseminated disease typically is associated with the immunocompromise produced by HIV infection, in which the disease tends to target the gastrointestinal tract (the likely portal of entry), and pulmonary and reticuloendothelial disease signifies dissemination.¹⁵⁶ 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. 6-56).

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. 6-57); this has

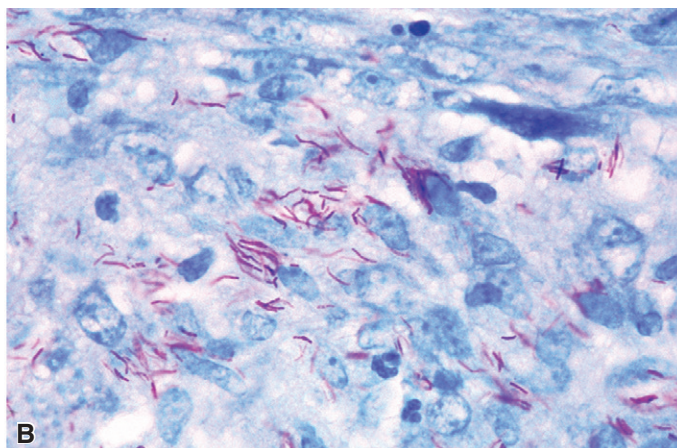
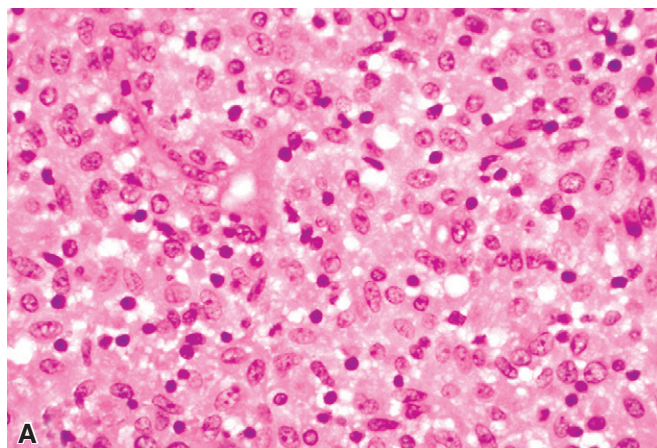


Figure 6-56. **A,** Clusters of macrophages in *Mycobacterium avium* complex (MAC) infection in a patient with AIDS. **B,** Myriad acid-fast bacilli (MAC) in histiocytic infiltrate (Ziehl-Neelsen stain).

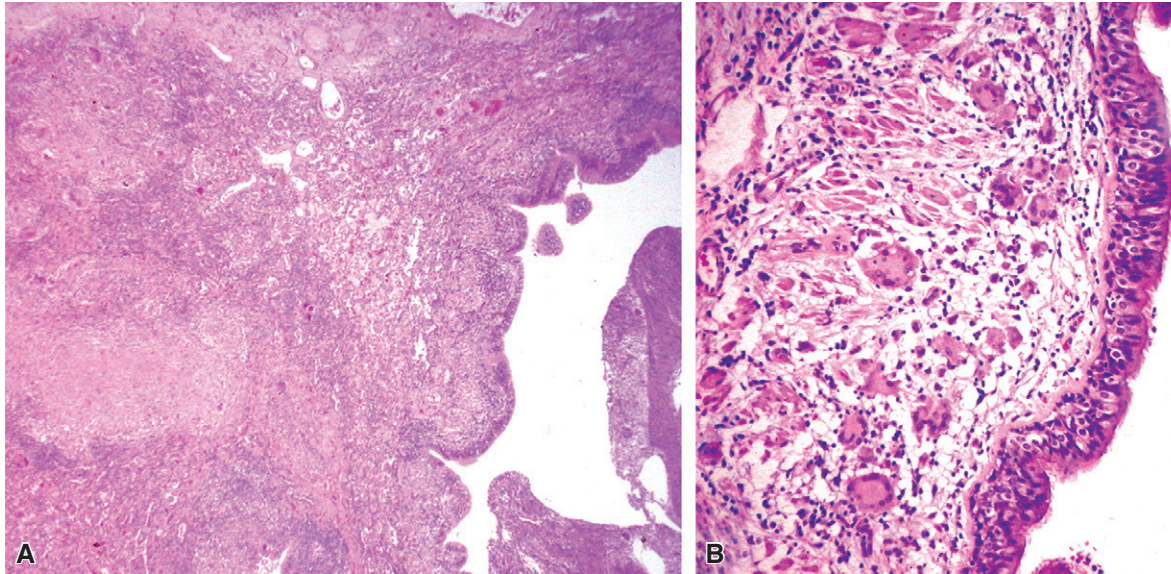


Figure 6-57. Middle lobe syndrome. **A**, Bronchiectasis with peribronchial granulomas containing *Mycobacterium avium* complex. **B**, Airway mucosa with granuloma.

been recognized as one of the patterns of middle lobe syndrome.¹⁵⁷ NTM bacilli also can colonize bronchiectatic lung from any cause, with resultant granulomatous inflammation predominantly affecting the airway walls—presumably a result of localized decreased mucociliary clearance.

Hypersensitivity-like pulmonary disease recently has been associated with contaminated water in hot tubs (“hot tub lung”) and other environmental sources such as humidifiers and air conditioners.¹⁷ Biopsy reveals a miliary bronchiolocentric and interstitial granulomatous pattern, similar to that produced by hypersensitivity pneumonitis (Fig. 6-58). A similar infection-colonization-hypersensitivity syndrome has been described in workers exposed to metal-working fluid aerosols.¹⁵⁸ 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 sometimes can 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. 6-59) which may occur in lung, skin, lymph nodes, and a number of other sites in immunocompromised patients.¹⁵⁹ 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 post-primary tuberculosis. Most cases are due to *M. avium* complex and manifest as polypoid lesions in immunocompromised HIV-infected patients, but this lesion also may be seen in immunocompetent persons.¹⁶⁰

Certain species of rapidly growing mycobacteria (RGM) are capable of producing pulmonary disease, albeit infrequently.^{132,161} Nevertheless, *M. abscessus* is the third most frequently recovered NTM respiratory pathogen in the United States, after *M. avium* complex and *M. kansasii*. It accounts for 80% of respiratory tract isolates, making it the leading rapidly growing mycobacterial species recovered from the lung. *M. abscessus* produces chronic lung infection that has a striking clinical and pathologic similarity to *M. avium* complex infection, including

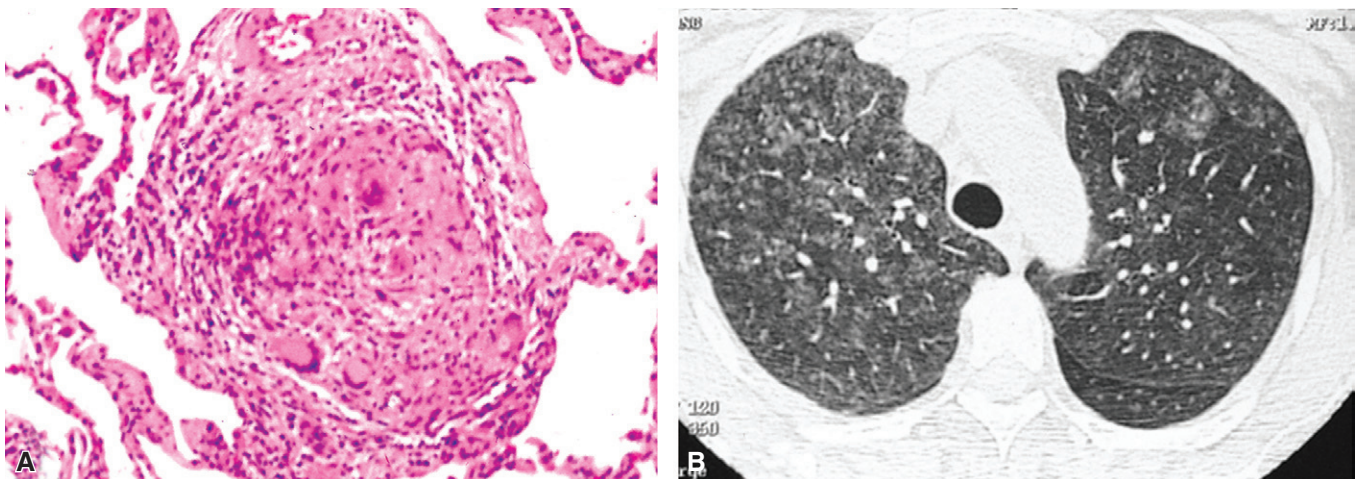


Figure 6-58. “Hot tub lung.” **A**, Non-necrotizing granuloma. **B**, Computed tomographic image with features resembling those of hypersensitivity pneumonitis.

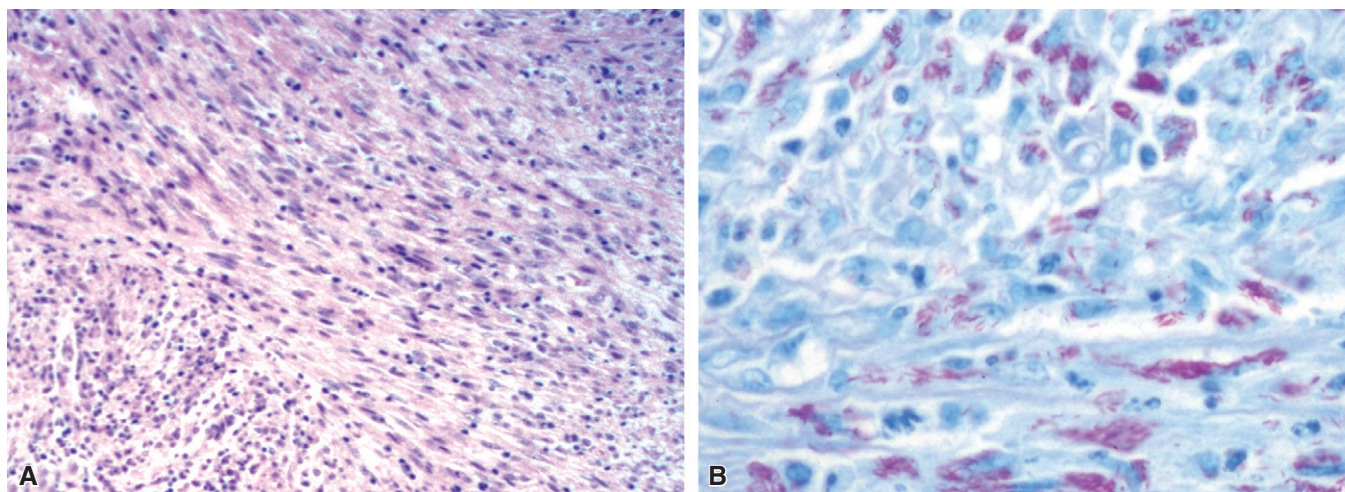


Figure 6-59. Spindle cell pseudotumor. **A**, The fascicles of fibroblasts with scattered lymphocytes. **B**, Myriad acid-fast bacilli (Ziehl-Neelsen stain).

the propensity to involve the lungs of patients with bronchiectasis. The RGM also have been thought to colonize lipoid pneumonia¹⁶²; however, it is more likely that the pathogenesis of the lung injury pattern caused by the RGM 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”).¹⁶³ 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.¹⁶⁴ The finding of finely granular amorphous necrotic debris associated with aggregates of epithelioid histiocytes (with or without multinucleate giant cells) (Fig. 6-60) is suggestive of a mycobacterial or fungal infection.¹⁶⁵ In this setting, necrotic cancers must be excluded by a thorough search for atypical cells.

Special stains for acid-fast bacilli can be applied to aspirate smears, but culture of the aspirate is more likely to yield the etiologic agent

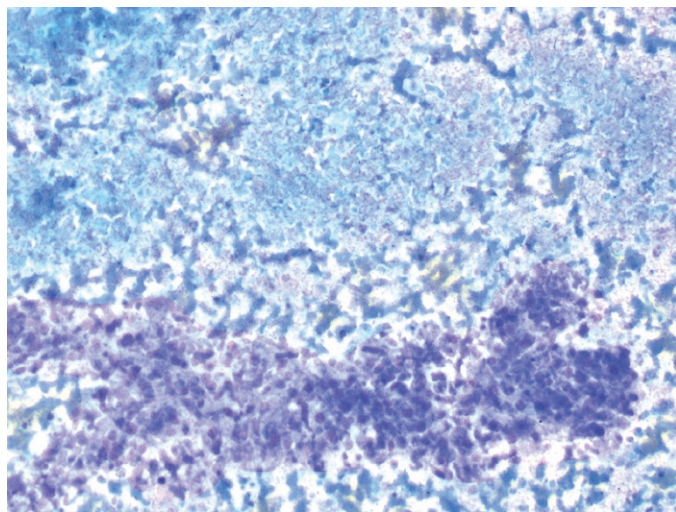


Figure 6-60. Necrotizing granuloma in *Mycobacterium kansasii* infection. Sheets of epithelioid cells in a background of granular necroinflammatory debris are evident in this fine-needle aspirate (Diff-Quik preparation).

when bacilli are sparse. Also, culture is still necessary for species identification and, if necessary, antimicrobial susceptibility testing. 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. 6-61).

Microbiology

The traditional as well as newer molecular approaches to the laboratory diagnosis of mycobacterial lung infection are outlined in Box 6-11. The mycobacterium is a slender but slightly curved bacillus, 4 μm in length, often with a beaded appearance; the length, curvature, and beadedness

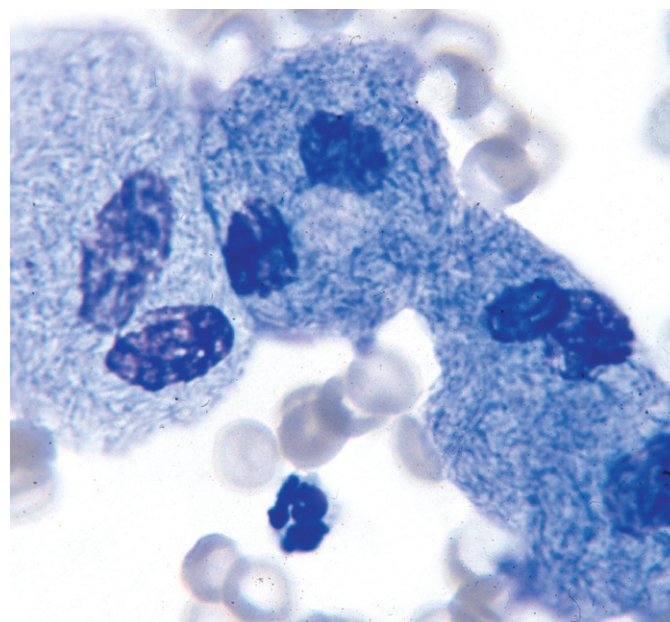


Figure 6-61. Pseudo-Gaucher histiocytes filled with myriad mycobacteria are seen as negative images in this fine-needle aspirate (Diff-Quik preparation).

Box 6-11. 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

sometimes are accentuated in *M. kansasii*.¹⁶⁶ In tissue sections or on smears, the Ziehl-Neelsen acid-fast stain or auramine-rhodamine fluorescent stains are most often recommended for best visualization. Organisms most often are 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 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 sometimes may be identified with the GMS stain. The NTM, especially the RGM, may be more sensitive to acid alcohol decoloration and may not stain well or at all with the auramine-rhodamine method.¹³² A commercial immunohistochemical reagent for mycobacteria is now available but is effective only in cases in which traditional acid-fast stains yield a positive result.³² The differentiation of mycobacterial species in Ziehl-Neelsen–positive, formalin-fixed sections also has been achieved by in situ hybridization techniques with specific nucleic acid probes.^{167–169} 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.¹⁷⁰ 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.^{171,172}

Conventional wisdom states that culture is more sensitive than direct examination; however, the literature clearly documents cases in which 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.¹⁷³ Furthermore, tissue culture is prone to sampling error unless more than one site is sampled.¹⁷⁴ Specimens also may be smear positive and culture negative in patients whose disease has been treated. When only a rare bacillus is found, a 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 also can 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.^{131,175} 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.¹⁷⁶ 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.¹³⁸

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 three weeks in most instances. Direct nucleic acid amplification testing of clinical specimens using commercially available polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) methods can reduce detection and identification times to less than 8 hours.¹⁷⁴ Immunochromatographic techniques based on the detection of secreted mycobacterial proteins have the potential to reduce these times even further.¹⁷⁷ Although NAA is faster, its overall accuracy is higher than that of smears but less than that of culture.¹⁷⁶ In fact, no single test at this time has sufficient sensitivity and specificity to stand alone, and use of a combination of available techniques, depending on the clinical and economic setting, may be the best overall strategy.^{178,179}

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 usually is 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.¹³²

Differential Diagnosis

A synopsis of the key morphologic and microbiologic attributes of mycobacterial lung infections is presented in Table 6-6. 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, Wegener granulomatosis, 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. Wegener granulomatosis can usually be excluded based on the 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 tissue sections containing fungal forms is in a unique position to provide at least a provisional diagnosis at the group or genus level, and to make a judgment as to the significance of the organism in terms of its invasiveness or presence as a saprophyte or allergen. Indeed, often the most effective diagnostic strategy avail-

Table 6-6. Mycobacterial Pneumonias: Summary of Pathologic Findings

Assessment Component	Findings
<i>Mycobacterium tuberculosis</i>	
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 <i>Mycobacterium tuberculosis</i>

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

able is the rapid identification of fungi in tissue sections or cytologic samples.^{43,180,181} This is especially important when opportunistic infection is being considered in the immunocompromised patient. However, optimal performance also requires knowing when the morphologic features of a fungal organism are insufficient to permit group or genus level diagnosis, and when integration of microbiologic data and histopathologic findings is required.

Etiologic Agents

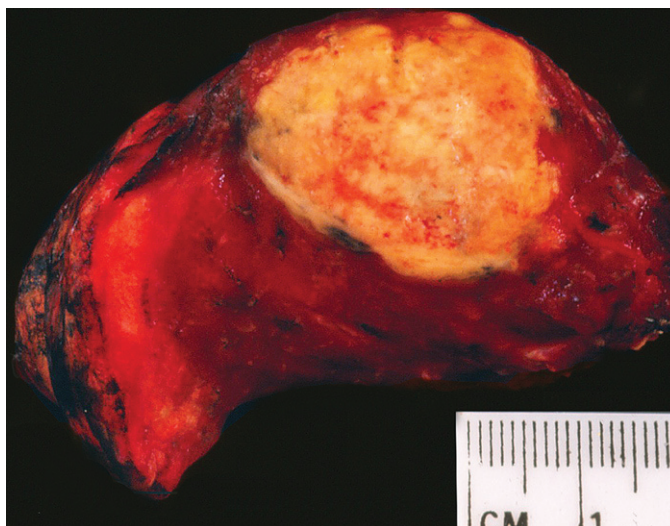
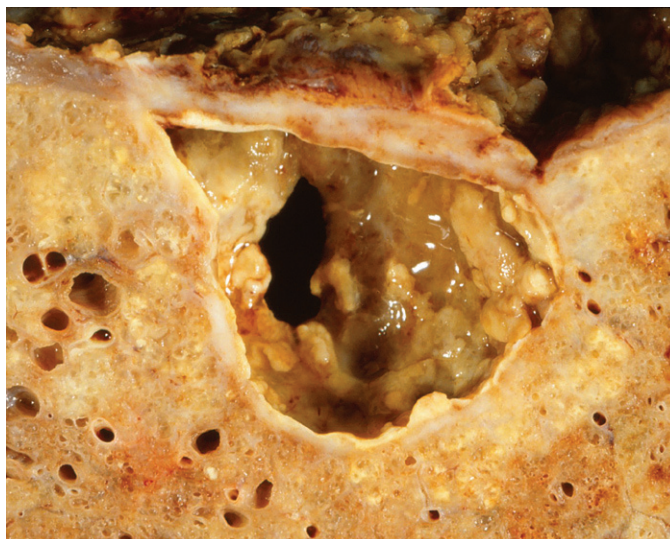
Nearly 70,000 fungi are known, and approximately 100 have been recovered from respiratory infections.¹⁸² Fortunately, only a small number are implicated as pathogenic on a consistent basis, and these are listed in [Box 6-12](#).

Histopathology

Like mycobacterial species, fungal pathogens typically produce one or more nodular lesions in the normal host ([Fig. 6-62](#)) and these may become cavitory as the lesions evolve ([Fig. 6-63](#)). Inflammatory histopathologic patterns that suggest the presence of a fungal infection are summarized in [Box 6-13](#). As is the case for other categories of etiologic agents, there are no absolutely characteristic or diagnostic patterns. Overlap is common and atypical reactions occur, ranging from overwhelming diffuse alveolar damage to little or no reaction in the immunocompromised patient. Proximal endobronchial disease mimicking neoplasm has also been described for various fungal species.¹⁸³ Detection of the etiologic agent in tissue by microscopic examination, ancillary tests, or culture confers specificity and significance to the listed patterns. Large spherules

Box 6-12. Common Fungal Pathogens in the Lung

Dimorphic fungi (mycelia at 25°C to 30°C; yeast at 37°C)
Blastomyces dermatitidis
Coccidioides immitis
Histoplasma capsulatum
Paracoccidioides brasiliensis
Sporothrix schenckii
Penicillium marneffeii
 Yeasts
Cryptococcus neoformans
Candida spp.
 Hyaline (non-pigmented) molds
Aspergillus spp.
 Zygomycetes organisms
 Phaeoid (pigmented; dematiaceous) molds
Bipolaris spp., *Alternaria*, *Curvularia*
Pseudoallescheria boydii/*Scedosporium apiospermum*
 Miscellaneous pathogens
Pneumocystis jirovecii

**Figure 6-62.** *Coccidioides* granuloma.**Figure 6-63.** Cavitory aspergilloma.

Box 6-13. Histopathologic Patterns in Fungal Lung Injury

- Large nodules
 - Non-necrotizing 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

with endospores characteristic of *C. immitis* or yeast with large 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*).³⁸ 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.¹⁸⁴

Mycelial morphology is helpful when it is characteristic of a specific genus or group. For example, broad, sparsely septate, nonparallel, twisted or irregular-diameter, thin-walled mycelia, with variable wide-angle branching, characterize zygomycetes, whereas progressively proliferating, regularly septate, 45-degree angle, dichotomously branching mycelia with parallel walls are typical of *Aspergillus* species (Fig. 6-64). 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*.¹⁸⁴ Sometimes careful examination of tissue with special stains under high magnification or oil emersion will reveal clues, such as in situ sporulation, allowing a more definitive diagnosis.³⁹ However, these clues often are subtle, even for experienced microscopists, and it is important to defer to culture whenever possible.¹⁸⁵ Typical morphologic injury patterns and related etiologic agents are briefly highlighted below. The cited references should be consulted for further details.

Blastomycosis

Blastomycosis, the chronic granulomatous and suppurative infection produced by *B. dermatitidis*, is essentially a North American disease, concentrated in the Ohio and 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.¹⁸⁶ The isolated nodular manifestation can simulate lung cancer, radiologically.¹⁸⁷ 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 damage¹⁸⁶⁻¹⁸⁹ (Box 6-14). Necrotizing granulomas are characteristic and often of the suppurative type (Fig. 6-65A), but non-necrotizing granulomas may be found as well.

Box 6-14. Histopathologic Patterns in Pulmonary Blastomycosis

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

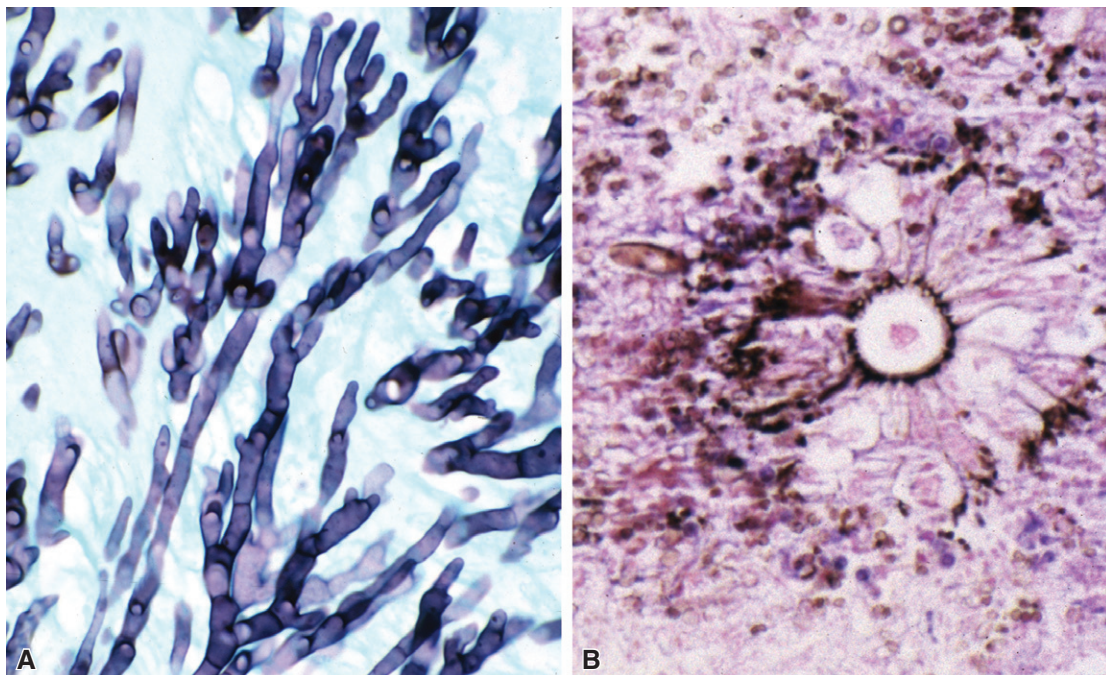


Figure 6-64. *Aspergillus* species. **A**, Septate mycelia with 45-degree angle branching (Grocott methenamine silver stain). **B**, Fruiting body (conidiophore with sterigmata and conidia) (Grocott methenamine silver stain).

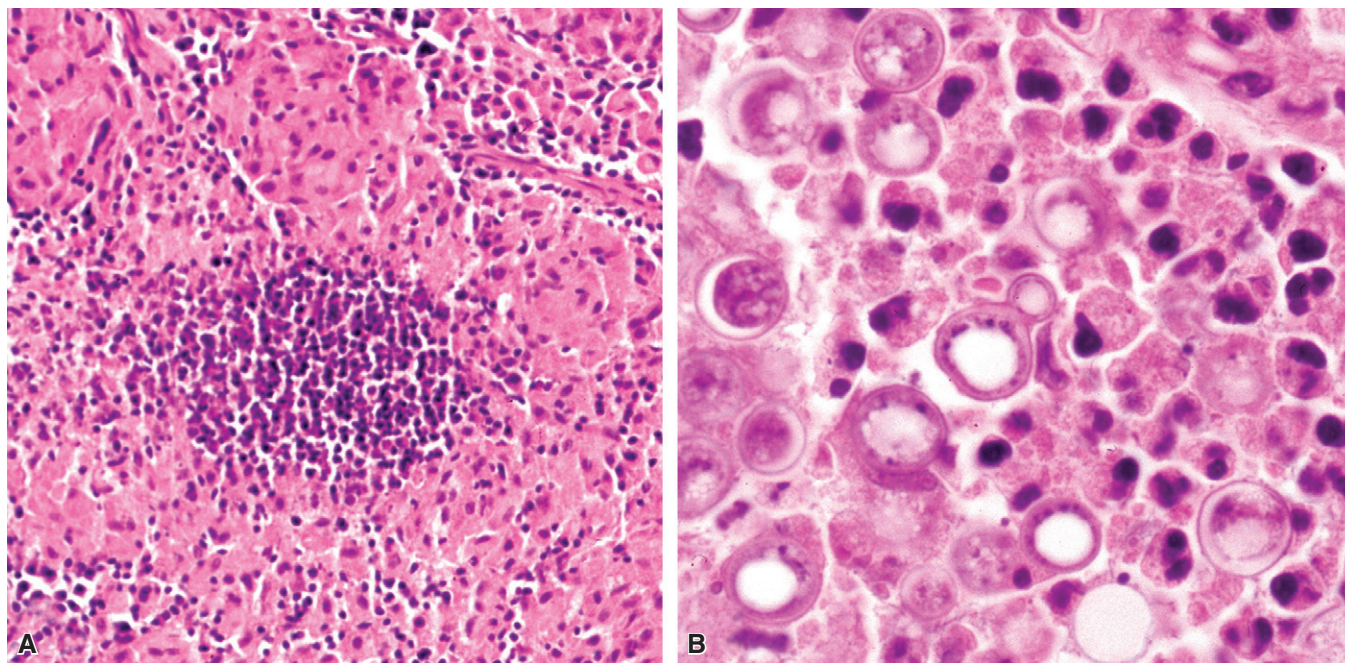


Figure 6-65. Blastomycosis. **A**, The suppurative granuloma is characteristic. **B**, Double-contour-wall yeast with broad-based budding.

The broad-based budding yeast forms of *Blastomyces* are refractile and have double-contoured walls. Multinucleate yeast cells typically are 8 to 15 μm in diameter, with some forms measuring up to 30 μm (see Fig. 6-65B). These large forms can mimic small *Coccidioides* spherules,¹⁹⁰ whereas smaller forms (“microforms”) can mimic *C. neoformans*.¹⁸⁹

Coccidioidomycosis

Endemic in the Lower Sonoran life zone of the southwestern United States, the soil fungus *Coccidioides immitis* and the more recently recognized, morphologically identical and genomically similar species *Coccidioides posadasii*¹⁹¹ 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 6-15. 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

Box 6-15. 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

areas.^{192–195} Granulomas are characteristic and may occur with or without necrosis. Intact spherules induce fibrocavitary granulomas (Fig. 6-66A) whereas ruptured spherules may incite suppurative and bronchocentric granulomatosis (BCG)-like reactions (see Fig. 6-66B).¹⁹²

The large mature spherule (up to 40–60 μm in diameter) has a thick refractile wall lined by or filled with endospores and constitutes the key diagnostic finding (see Fig. 6-66C). 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 the setting of bronchopleural fistula, mycelia resembling various hyaline molds may be seen with or without a variety of mature and immature spherules (see Figs. 6-19 and 6-66D). *Coccidioides* spherule look-alikes include large-variant *B. dermatitidis*, adiasporomycosis, pollen grains, and pulses (legume seeds).

Histoplasmosis

Histoplasmosis, the most common pulmonary fungal infection worldwide, is endemic in the Ohio and Mississippi river valleys of North America and is the most common endemic mycosis in AIDS.¹⁹⁶ The clinical forms of *H. capsulatum* infection^{51,181,197} are presented in Box 6-16. 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 non-necrotizing granulomas occurring as solitary lesions indistinguishable from other granulomatous infections. Other presentations include miliary nodules (Fig. 6-67), cavitary lesions, and laminated fibrous solitary nodules (Fig. 6-68) 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. 6-69A). The exudative lesion resembles acute lobular pneumonia with fibrinopurulent exudates.¹⁹⁸

H. capsulatum organisms are yeasts (2–5 μm), with narrow-based unequal budding (see Fig. 6-69B). They may be seen on H&E-stained sections and, when numerous, appear as small refractile ovoid

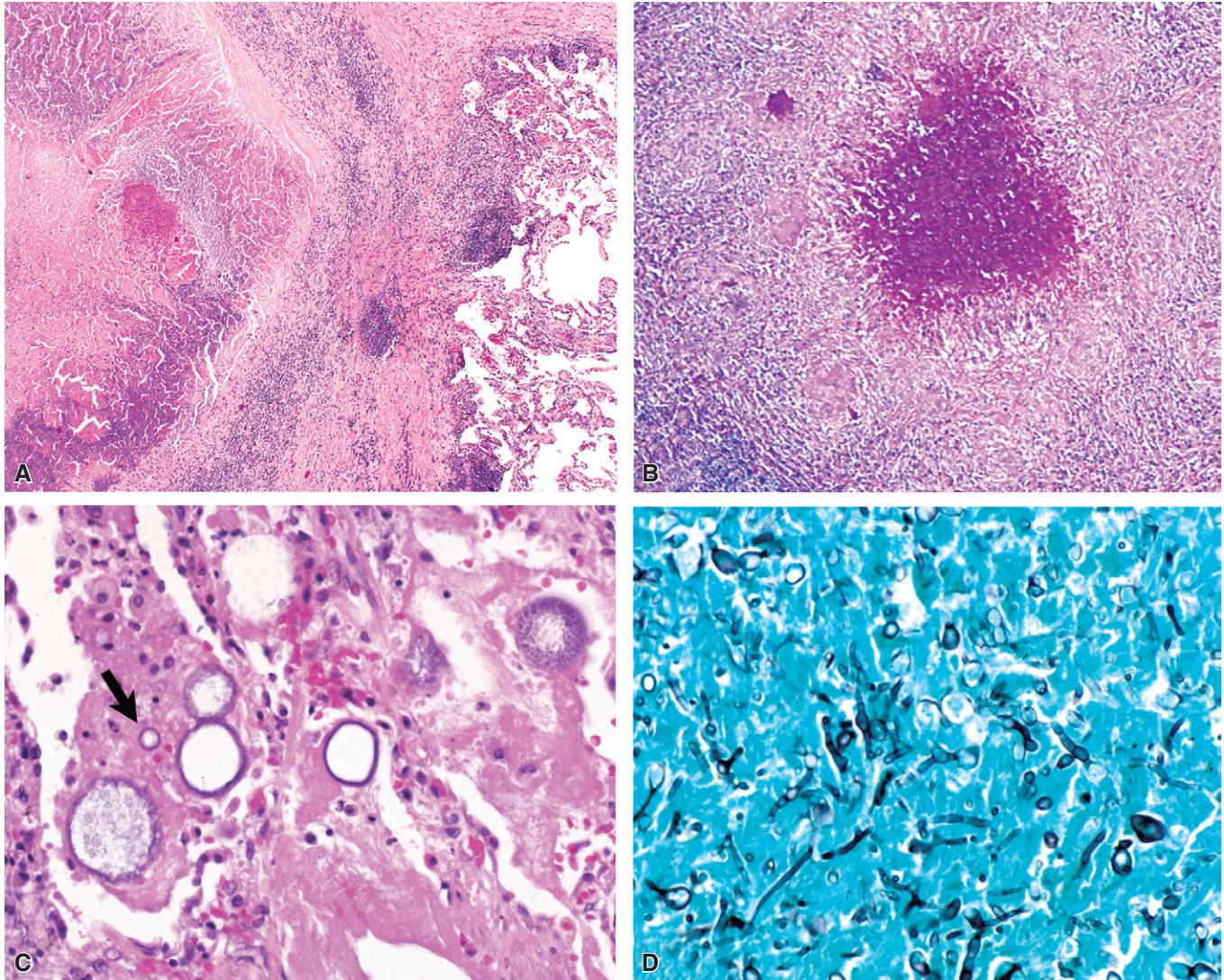


Figure 6-66. Coccidioidomycosis. **A**, Fibrocasseous granuloma. **B**, Bronchocentric granulomatous-like granuloma. **C**, *Coccidioides immitis*. Both small (arrow) and large spherules with and without endospores can be seen. **D**, Biphasic pattern with mycelia and spore-like swellings (Grocott methenamine silver stain).

structures within macrophages. Yeasts typically occur in clusters but may be rare or very localized 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*.⁵¹ Other look-alikes include *Candida* species, *P. marneffeii*, capsule-deficient cryptococci, intracellular *B. dermatitidis*, and Hamazaki-Wesenberg bodies.

Box 6-16. 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 cavitory) pulmonary
- Progressive disseminated
- Mediastinal
 - Lymphadenopathy
 - Middle lobe syndrome
 - Fibrosis

Paracoccidioidomycosis (South American Blastomycosis)

Seven clinical forms occur, but rarely cause lung infections in North America. The histopathology resembles other mycoses and can be exudative or granulomatous. *Paracoccidioides braziliensis* appears as a large spherical yeast (10–60 μm) with multiple buds attached by narrow necks ("steering wheel" or "ship's wheel").¹⁹⁹ When budding is sparse, look-alikes include *H. capsulatum* with small intracellular forms, *B. dermatitidis* and capsule-deficient cryptococci for medium-size forms, and *C. immitis* or *C. posadasii* for large forms.

Sporotrichosis

Infection by *Sporothrix schenckii* usually is confined to the skin, subcutis, and lymphatic pathways, but the organism can disseminate to the lungs. Rarely, *S. schenckii* is a primary pulmonary pathogen. The organism can produce cavitory disease in the form of a single lesion. Infection may be bilateral and apical, progressive and destructive, or may be identified clinically as a solitary pulmonary nodule. Microscopically, caseous

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.

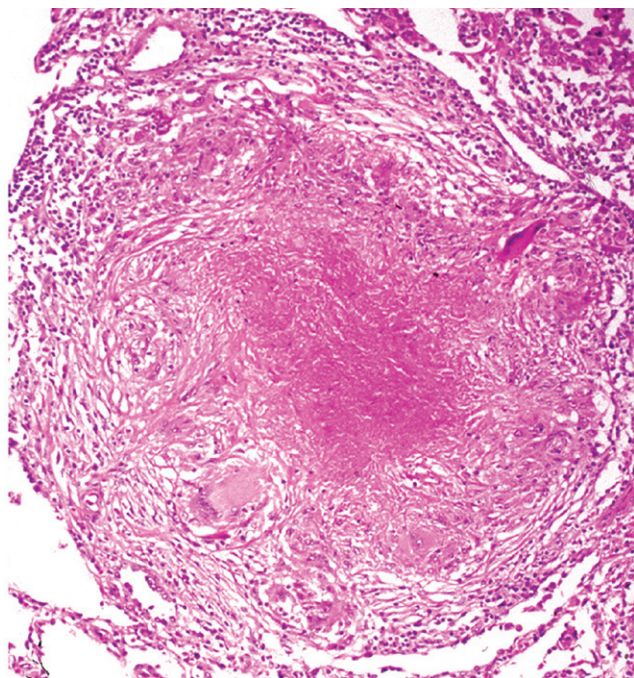


Figure 6-67. Histoplasmosis. Miliary nodule with central zone of necrosis invested by epithelioid histiocytes, multinucleate giant cells, and outer collarette of lymphocytes.

and suppurative type granulomas (Fig 6-70A) occur with variable numbers of round to oval, small (2–3 μm) narrow budding yeast (see Fig 6-70B) or cigar-shaped forms.²⁰⁰ Non-necrotizing 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.

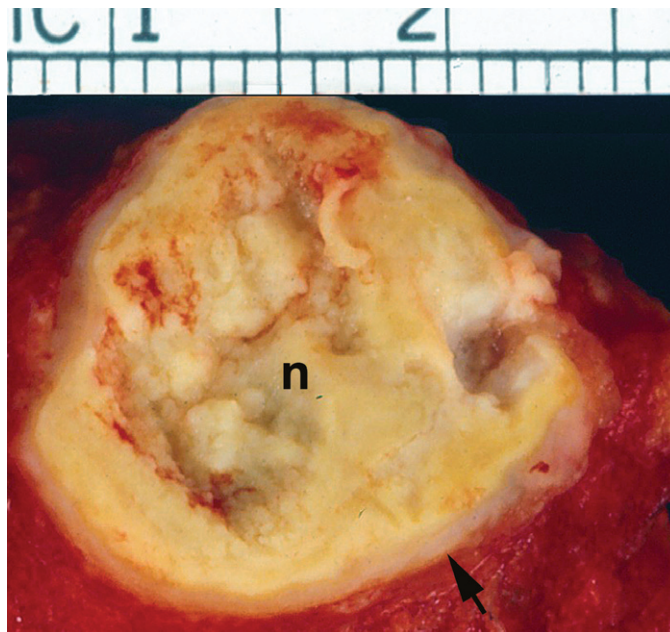


Figure 6-68. Histoplasmosis. Characteristic gross appearance of the persistent granulomatous nodule. Note the fibrous wall (arrow) surrounding caseous necrosis (n).

It is one of the commonest opportunistic infections in AIDS patients in Southeast Asia and a significant clue to the presence of AIDS in that area. The respiratory tract is the portal of entry, with pulmonary infiltrates and disseminated disease, especially to skin. Microscopically, alveolar macrophages stuffed with spherical to oval yeast-like cells (2.5–5 μm) are seen, each with a single transverse septum; short hyphal forms and elongated, curved “sausage” forms may be formed in necrotic and cavitary lesions.^{201,202} The septum distinguishes it from its look-alike, *H. capsulatum*.

Cryptococcosis

C. neoformans is a ubiquitous, facultative intracellular yeast. Pulmonary cryptococcosis occurs worldwide but has a particularly high

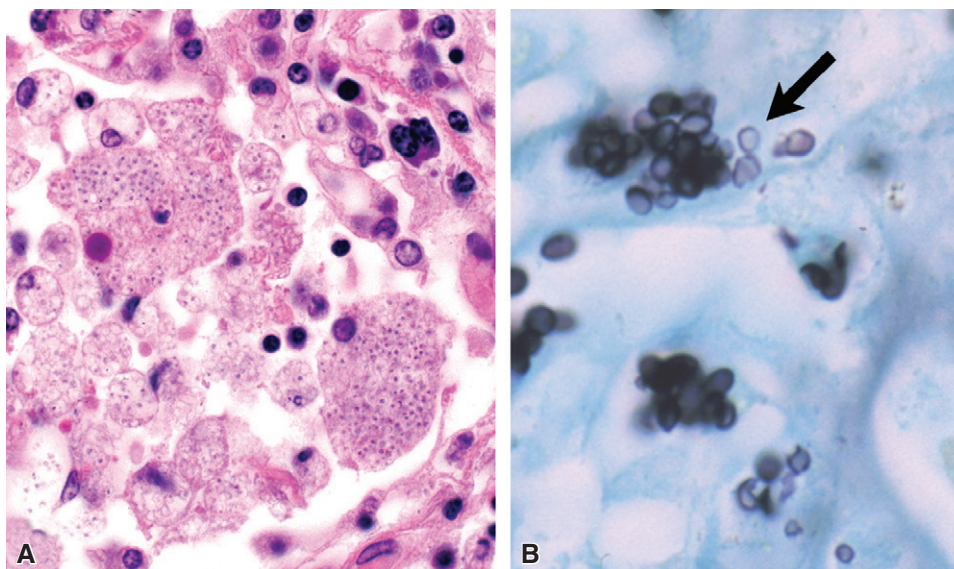


Figure 6-69. Histoplasmosis in an immunocompromised patient. **A**, Numerous *Histoplasma capsulatum* yeast cells in macrophages. **B**, Clusters of *H. capsulatum* yeast cells in macrophages. Note the narrow-based budding (arrow) (Grocott methenamine silver stain).

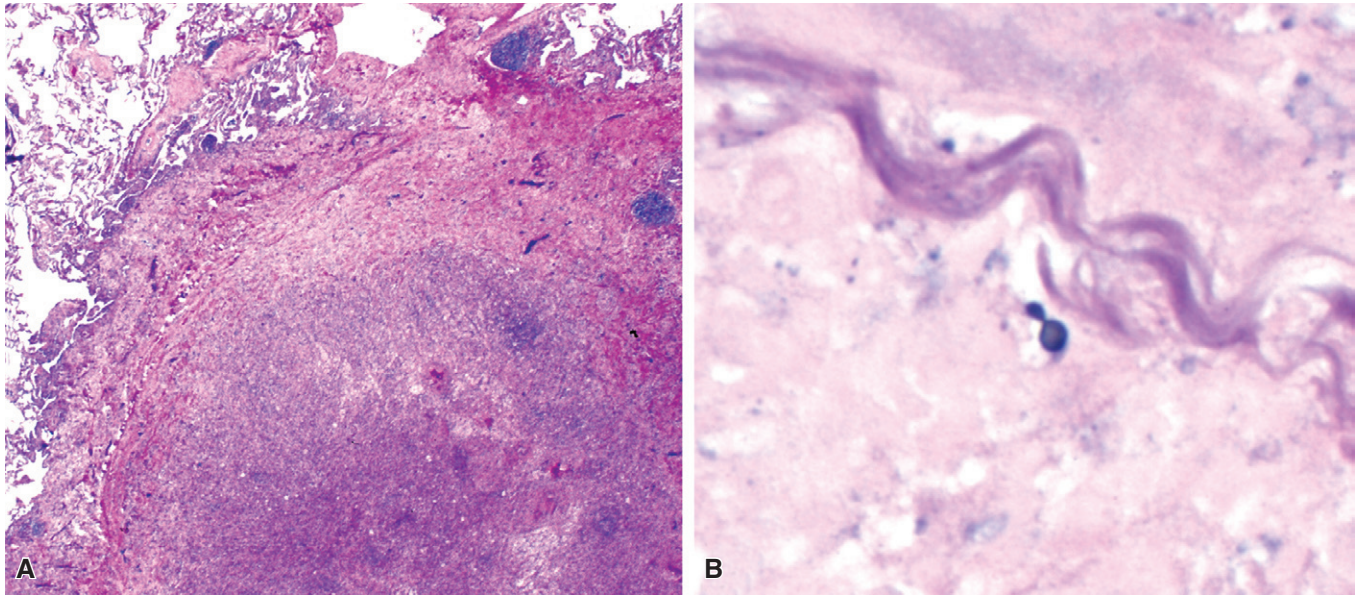


Figure 6-70. Sporotrichosis. **A**, Cavitory granuloma manifesting as a solitary pulmonary nodule. **B**, A rare, oval, narrow, budding yeast (Grocott methenamine silver stain).

incidence in the United States. The pathogenicity and histopathologic features of lung infection depends largely on the patient's immune status, as illustrated earlier in [Figure 6-7](#) and summarized in [Box 6-17](#). In the normal host, a substantial proportion of cryptococcal infections are asymptomatic while the remainders have respiratory symptoms associated 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 comprised of fibrocaceous granulomas ([Fig. 6-71A](#)), or granulomatous pneumonia (see [Fig. 6-71B](#)). Immunocompromised patients are more likely to have histiocytic (see [Fig. 6-71C](#)) or mucoid infiltrates without inflammation (see [Fig. 6-71D](#)).

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 sometimes can be found within giant cells.¹⁸¹ The mucicarmine stain highlights the capsule ([Fig. 6-72A](#)), but with capsule-deficient forms (see [Fig. 6-72B](#)), 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 (see [Fig. 6-72A](#)) with little if any intra-alveolar reaction²⁰³ and this form of the disease also

Box 6-17. Histopathologic Patterns in Cryptococcal Lung Disease

In order of associated decrease in immune function

- Fibrocaceous granuloma
- Granulomatous pneumonia
- Histiocytic pneumonia
- Mucoid pneumonia
- Intracapillary cryptococcosis

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may be associated with mucoid pneumonia.²⁰⁴ The mucoid pneumonia ([Fig. 6-73A](#)) of cryptococcal infection can be confirmed with mucin stains such as Alcian blue (see [Fig. 6-73B](#)). Another microscopic pattern recently described in HIV-infected patients is the so-called “inflammatory spindle cell pseudotumor,” a lesion much more commonly associated with mycobacterial infection.²⁰⁵

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.⁵¹ In general, *C. albicans* is the most frequently isolated of the more than 100 known species which include a few rare and emerging human pathogens. *C. glabrata* and *C. tropicalis*, together with *C. albicans*, account for 95% of bloodstream infections, the principal route for acquisition of *Candida* pneumonia.²⁰⁶ A non-blood-borne route to pneumonia results from aspiration of organisms from a heavily colonized or infected oropharynx. When 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 bronchopneumonia²⁰⁷ ([Fig. 6-74A](#)) or, much less commonly, a bronchocentric granulomatosis pattern.

In tissue sections, oval budding yeast-like cells (blastocidia) 2 to 6 μm in diameter may appear with pseudohyphae, which constrict at points of budding, creating the impression of bulging rather than parallel walls (see [Fig. 6-74B](#)). 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, *C. glabrata* (formerly *Torulopsis glabrata*) and *C. parapsilosis* produce only yeast cells in tissue, in contrast with most other *Candida* species, which produce both yeast and pseudohyphae.¹⁸¹

Other look-alikes include *H. capsulatum*, *Trichosporon beigeli*, 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. beigeli*

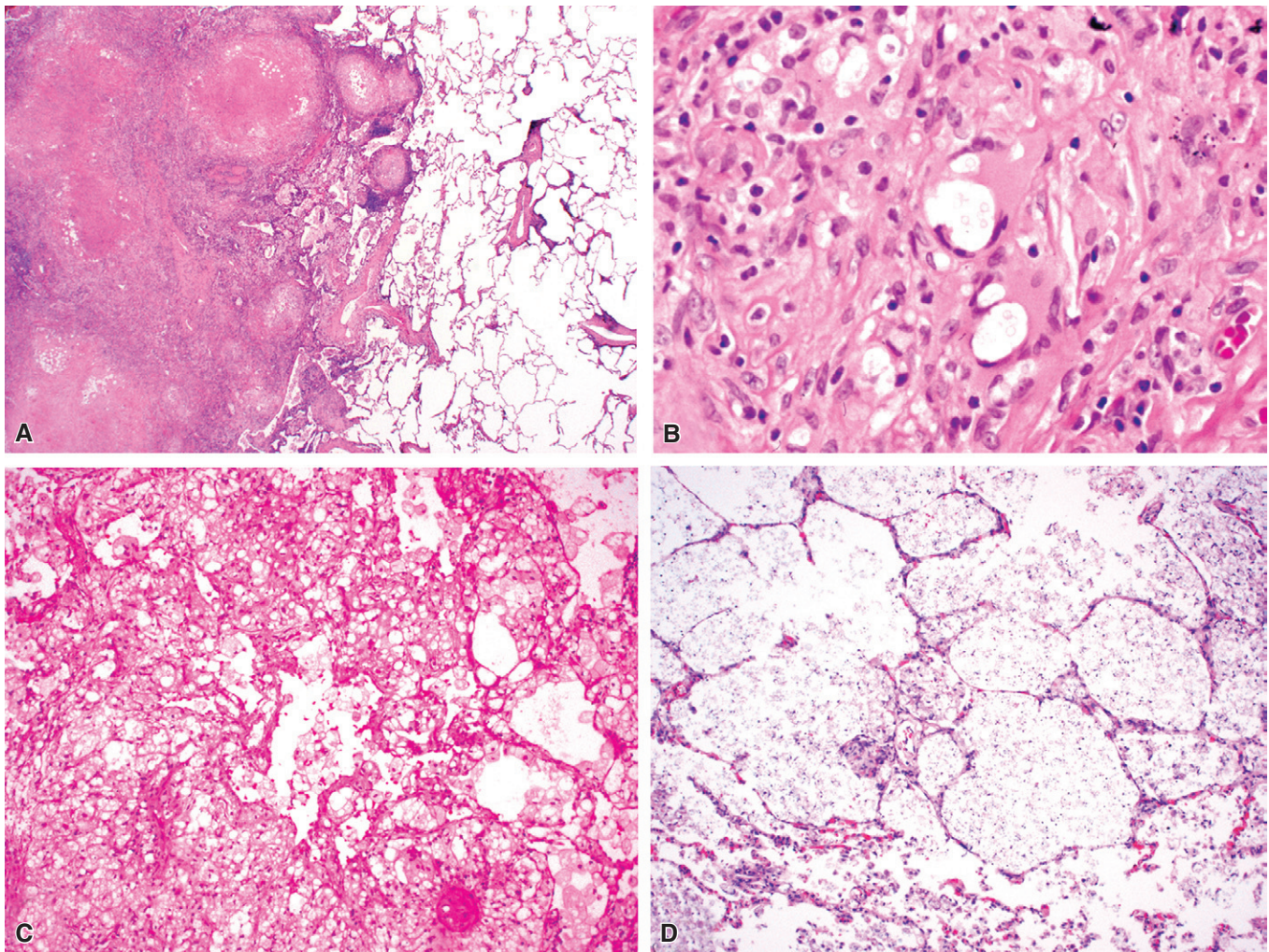


Figure 6-71. Cryptococcosis. **A**, Solitary pulmonary nodule with small satellite granulomas. **B**, Granulomatous pneumonia with clusters of pale staining yeast in clear spaces surrounded by histiocytes and multinucleated giant cells. **C**, Histiocytic pneumonia. **D**, Mucoïd pneumonia with no inflammatory cell reaction.

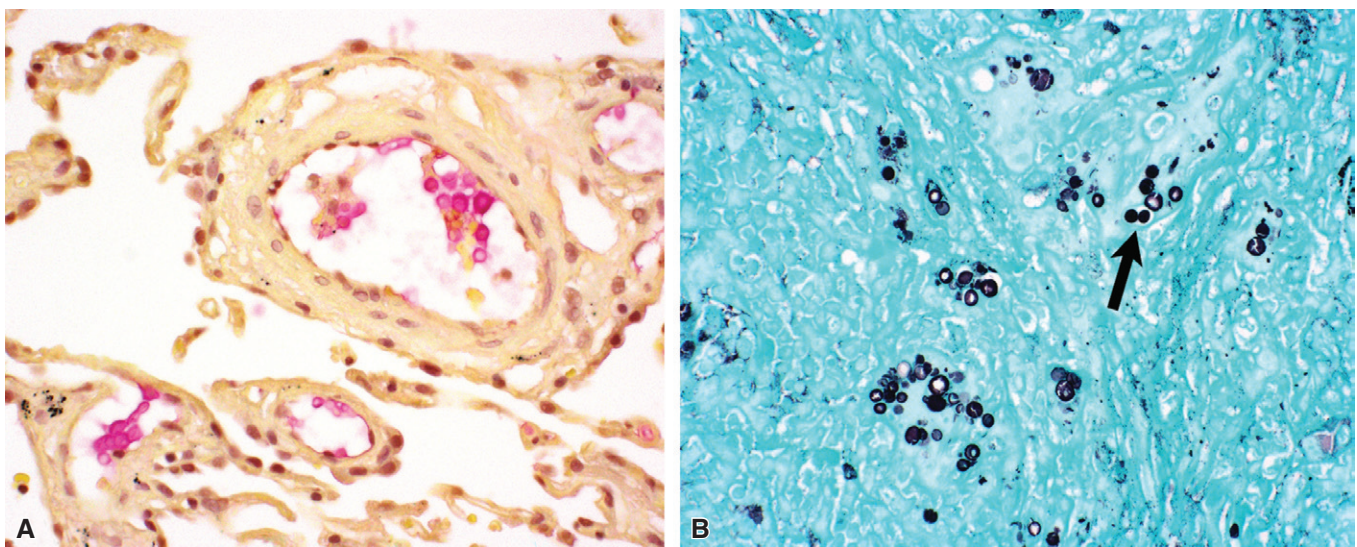


Figure 6-72. **A**, Intravascular cryptococcus. Yeast cells with stained capsules (mucicarmine stain). **B**, Capsule-deficient cryptococcus (Grocott methenamine silver stain).

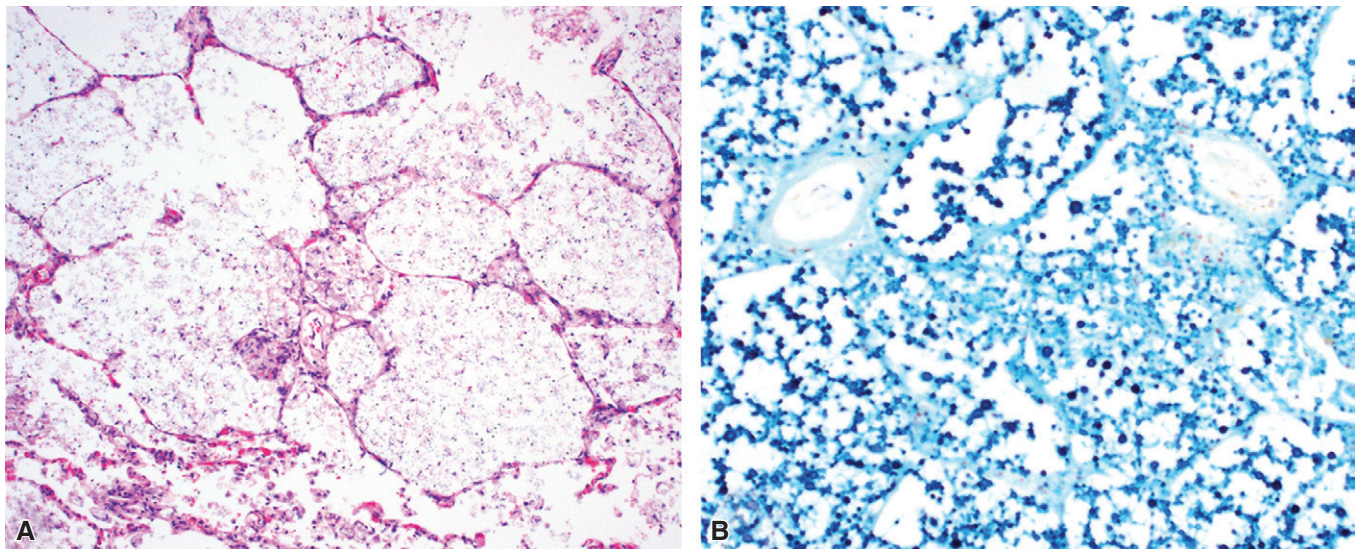


Figure 6-73. Cryptococcal mucoid pneumonia. **A**, Myriad blue-gray yeast in mucoid matrix. **B**, Alcian blue mucin stain accentuates the mucoid matrix.

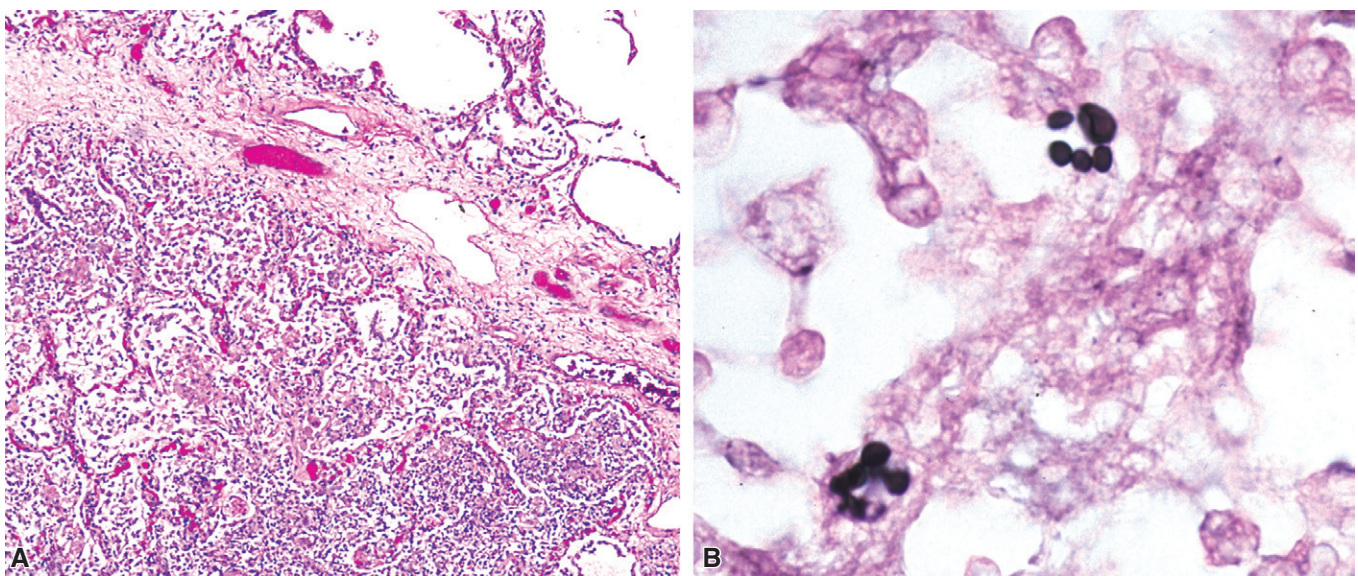


Figure 6-74. **A**, *Candida* bronchopneumonia. **B**, *Candida* yeast cells—blastoconidia (Grocott methenamine silver stain).

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- μ m yeast-like cells. They form distinctive unipolar broad-based buds but no pseudohyphae.⁵¹

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 *A. fumigatus* is the one most often seen in the clinical laboratory and most often isolated from the lungs of immunocompromised patients.²⁰⁸ Respiratory aspergillosis can be classified into a colonizing

or saprophytic form (intra-bronchial and pre-existing cavity fungus ball) (Fig. 6-75A); hypersensitivity forms (allergic bronchopulmonary aspergillosis, including mucoid impaction of bronchi and hypersensitivity pneumonitis) (see Fig. 6-75B); and invasive disease (minimally invasive—chronic necrotizing or angioinvasive—disseminated), as outlined in Box 6-18.^{51,209-211} Invasive disease (Fig. 6-76) 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.²¹² In patients with neutropenia, a characteristic angioinvasive pattern occurs, with intravascular spread resulting in hemorrhagic infarcts (Fig. 6-77). In the non-neutropenic patient, the necroinflammatory pattern tends to lack this angioinvasive feature.²¹³ Some cases defy categorization; are unique, e.g. bronchocentric and miliary patterns (Fig. 6-78); or may be hybrids of infection and hypersensitivity.²¹⁴

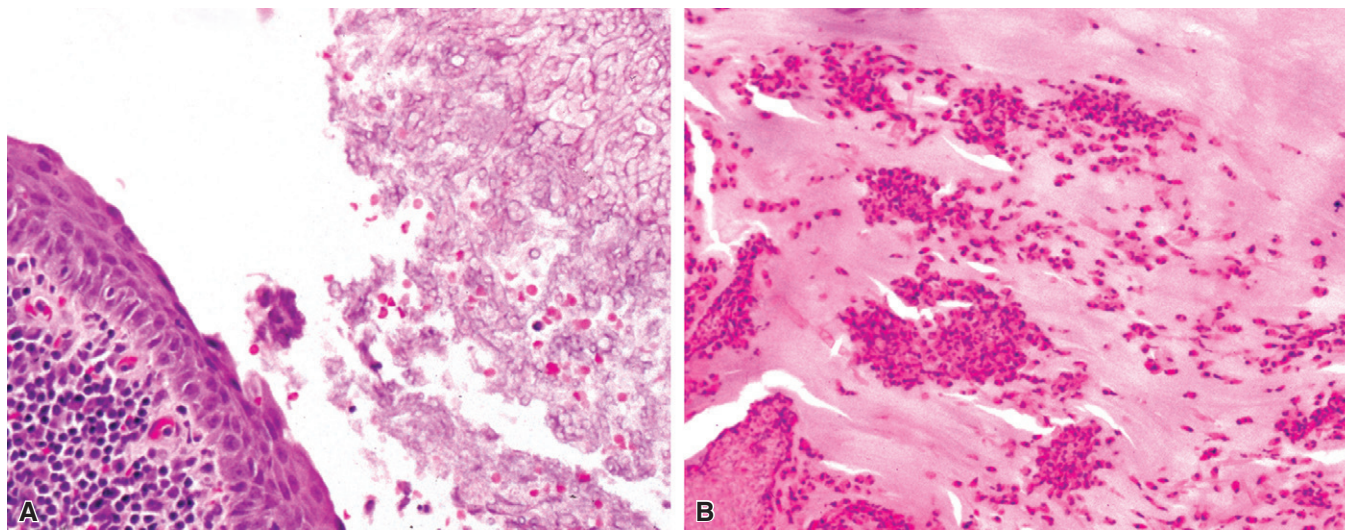


Figure 6-75. **A**, Aspergillosis fungus ball. **B**, Allergic bronchopulmonary aspergillosis. Intraluminal allergic mucin with laminated clusters of eosinophils can be seen in inspissated basophilic mucin with scattered Charcot-Leyden crystals.

Box 6-18. 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

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 12-10.

Microscopically, septate hyphae, dichotomously branched at 45-degree angle, have uniform, consistent width (3–6 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. 6-64) are sometimes formed in cavities. Oxalate crystals, visible in plane-polarized light (Fig. 6-79), 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 *P. boydii*.²¹⁵ Another look-alike is *Fusarium* species. Fusariosis is an emerging mycosis in the immunocompromised host, and *Fusarium* is the second most common opportunist after *Aspergillus* species in immunosuppressed patients with hematologic malignancies.²¹⁶ The clinical and pathological features in the lung and at sites of dissemination mimic those of aspergillosis, and the mycelia are essentially indistinguishable. Isolation in culture or by immunohistochemistry or molecular techniques, such as in situ hybridization or PCR amplification, is required for definitive diagnosis. Other previously uncommon but newly



Figure 6-76. Resected lung specimen from an immunocompromised patient with necrotizing *Aspergillus* pneumonia.

emerging hyaline molds that may be difficult to distinguish from *Aspergillus* in tissue are *Paecilomyces*, *Acremonium*, *Scedosporium*, and *Basidiobolus*.^{206,217,218}

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.²¹⁹ 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.²²⁰ The zygomycete species share clinical and pathologic features with invasive

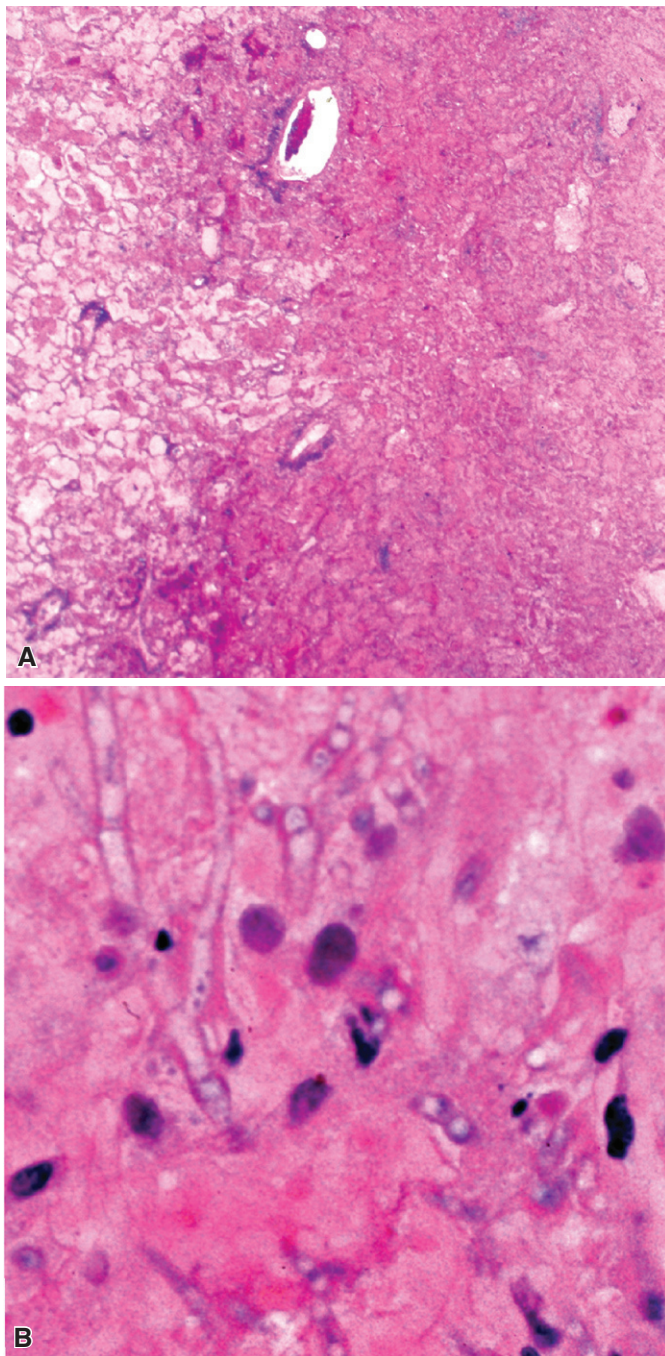


Figure 6-77. Invasive aspergillosis. **A**, Hemorrhagic infarct. **B**, 45-degree angle branching septate hyphae.

Aspergillus species, being angiotropic and capable of inducing hemorrhagic infarcts with sparse inflammation.

Clinical syndromes produced by these fungi include rhinocerebral, pulmonary, cutaneous, and gastrointestinal infections, with a predilection for neonates. Hematopoietic malignancies and diabetes mellitus with acidosis underlie most cases of pulmonary infection in children and adults.²²¹ Box 6-19 lists a broad spectrum of pulmonary diseases that includes solitary or multiple and bilateral nodular lesions, segmental or lobar consolidation, cavitory lesions, fistulas, infarcts (Figs. 6-80 and 6-81); direct extension into mediastinal, thoracic soft tissue, chest wall and diaphragm; chronic tracheal and endobronchial infection; and fungus ball similar to

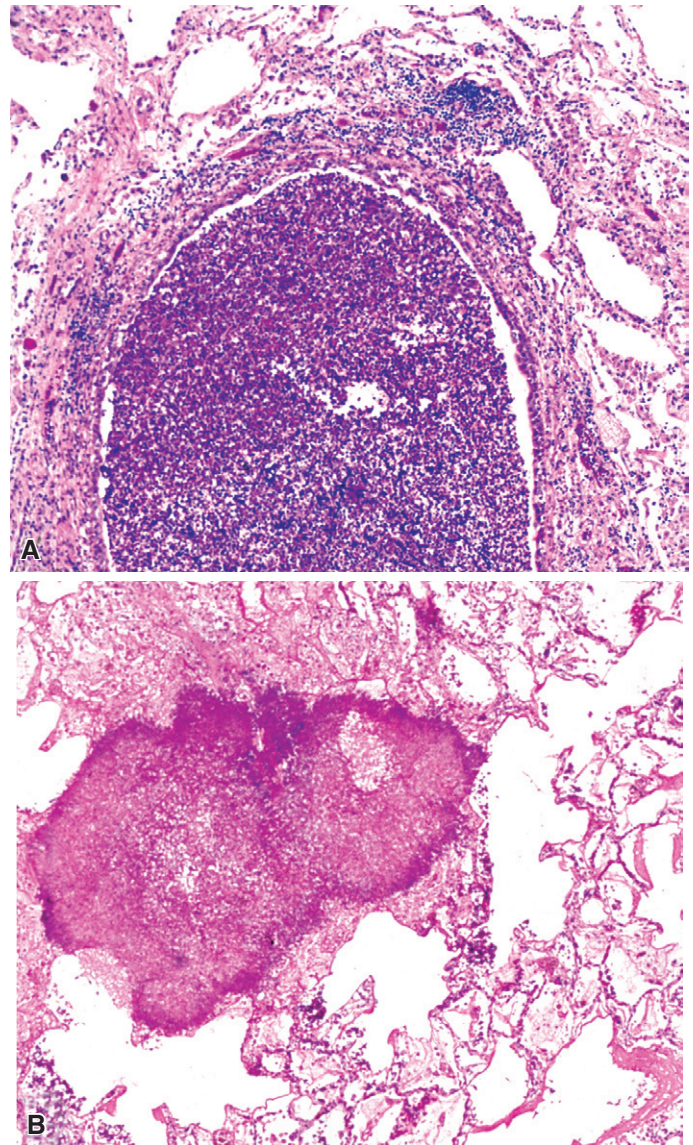


Figure 6-78. Bronchocentric aspergillosis. **A**, Bronchiole expanded and filled with purulent exudate. **B**, Miliary aspergillosis. Colony of organisms with hyaline membranes evident at periphery of the image (lower right).

aspergilloma.²²² An endobronchial syndrome with a propensity for blood vessel erosion also has been described, sometimes resulting in fatal hemoptysis.²²³

Hyphae are broad (6–25 μm), thin-walled, and pauciseptate (Fig. 6-82A). They display considerable variation in width, with twisted, nonparallel contours and random wide-angle branching, nearing 90 degrees.¹⁸¹ They also have a tendency to fragment more commonly than *Aspergillus* organisms, which tend to retain their elongate 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.²²² In addition to being angiotropic, they are neurotropic.²²⁴ In lesions exposed to air, the hyphae may form ovoid or spherical thick-walled chlamydoconidia, within or at the terminal ends (see Fig. 6-82B).²²⁵ Look-alikes at the lower-width range include *Aspergillus* and other *Aspergillus*-like hyaline molds. The pseudohyphae of *Candida* species sometimes can be closely simulated.

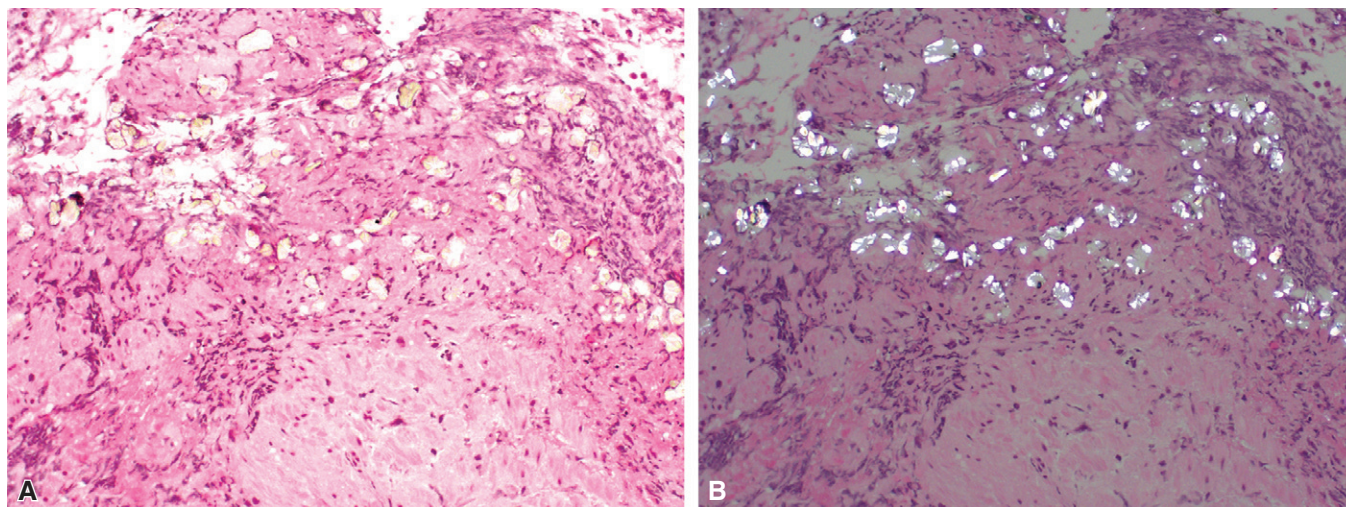


Figure 6-79. A, Pale yellow oxalate crystal sheaths in necroinflammatory debris. B, Birefringent oxalates seen under polarized light.

Box 6-19. Histopathologic Patterns in Pulmonary Zygomycosis

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

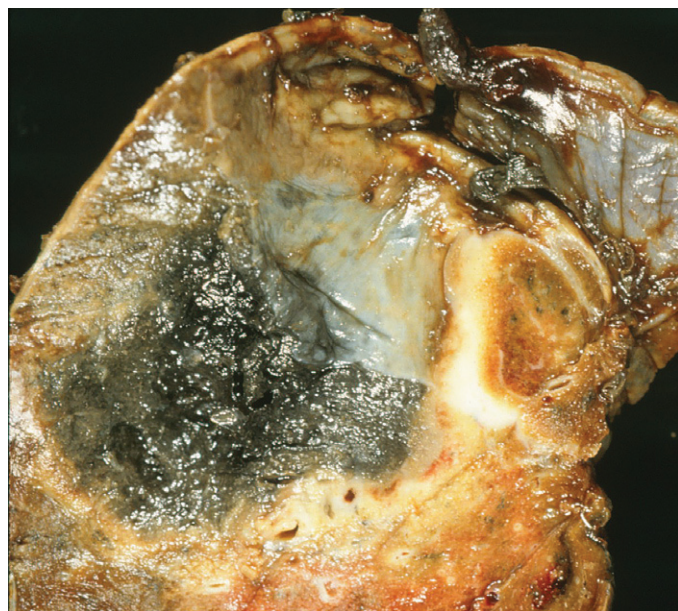


Figure 6-80. Resected lung specimen from patient with necrotizing pneumonia caused by zygomycosis.

Phaeohyphomycosis

A few genera of dematiaceous molds produce infections resembling those of *Aspergillus*, including allergic bronchopulmonary disease (Fig. 6-83A) and bronchocentric granulomatosis patterns.^{226,227} 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.¹⁸¹ 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 (2–6 μm in diameter) generally are 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 (see Fig. 6-83B). 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 often is refractory to treatment.²²⁸

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 jiroveci* (formerly *Pneumocystis carinii*).²²⁹ Once a disease primarily of malnourished children and occasionally occurring in the setting of treatment for childhood leukemia, 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.²³⁰ The classic pattern during the HIV epidemic was the foamy alveolar cast (Fig. 6-84) with moderate to numerous organisms, type II pneumocyte hyperplasia, and a scant to moderate interstitial lymphoplasmacytic infiltrate.^{231,232}

In recent years a number of atypical and unusual patterns have been described that are worth recognizing.^{51,233,234} These are listed in Box 6-20. *Pneumocystis jiroveci* infection can mimic any lung injury pattern, ranging from acute diffuse alveolar damage with hyaline membranes (Fig. 6-85) and minimal or no foamy exudates to an organizing phase with sparse organisms. There is also a spectrum of granulomatous infection, both non-necrotizing and necrotizing, that may overlap morphologically with mycobacterial or other fungal infections, particularly histoplasmosis (Fig. 6-86). Cavitory disease, solitary pulmonary

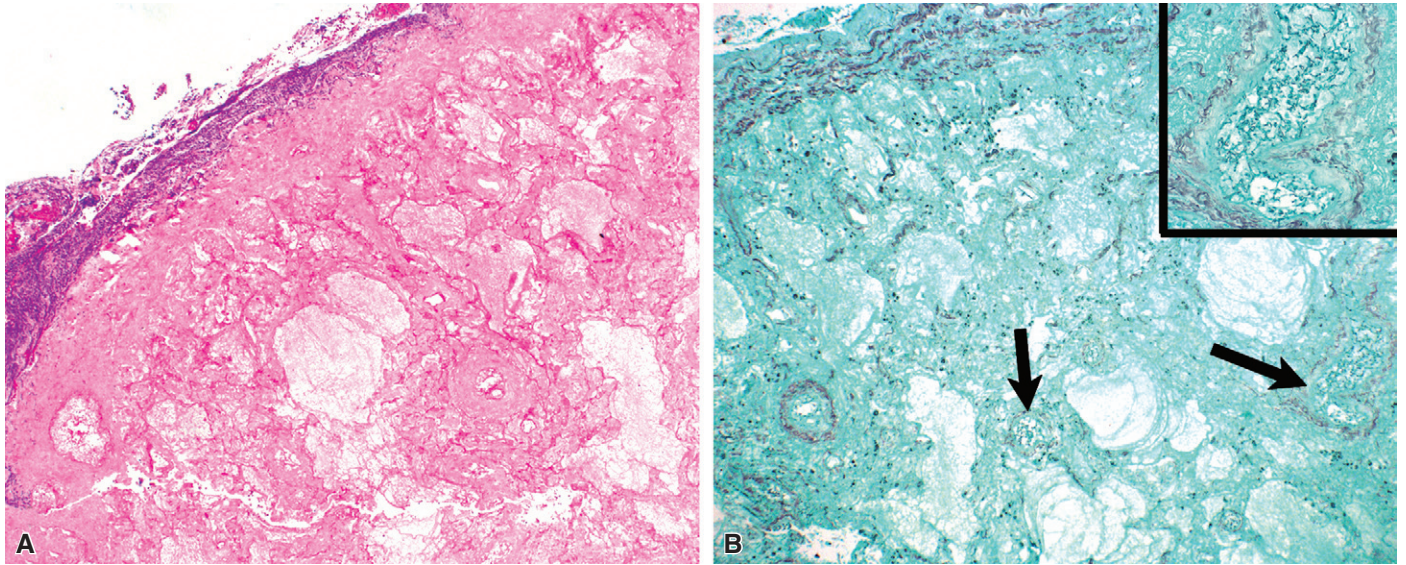


Figure 6-81. Zygomyces. **A**, Nodular infarct. **B**, Intravascular organisms (arrows). Vessel at right arrow is shown at high magnification (inset) (Grocott methenamine silver stain).

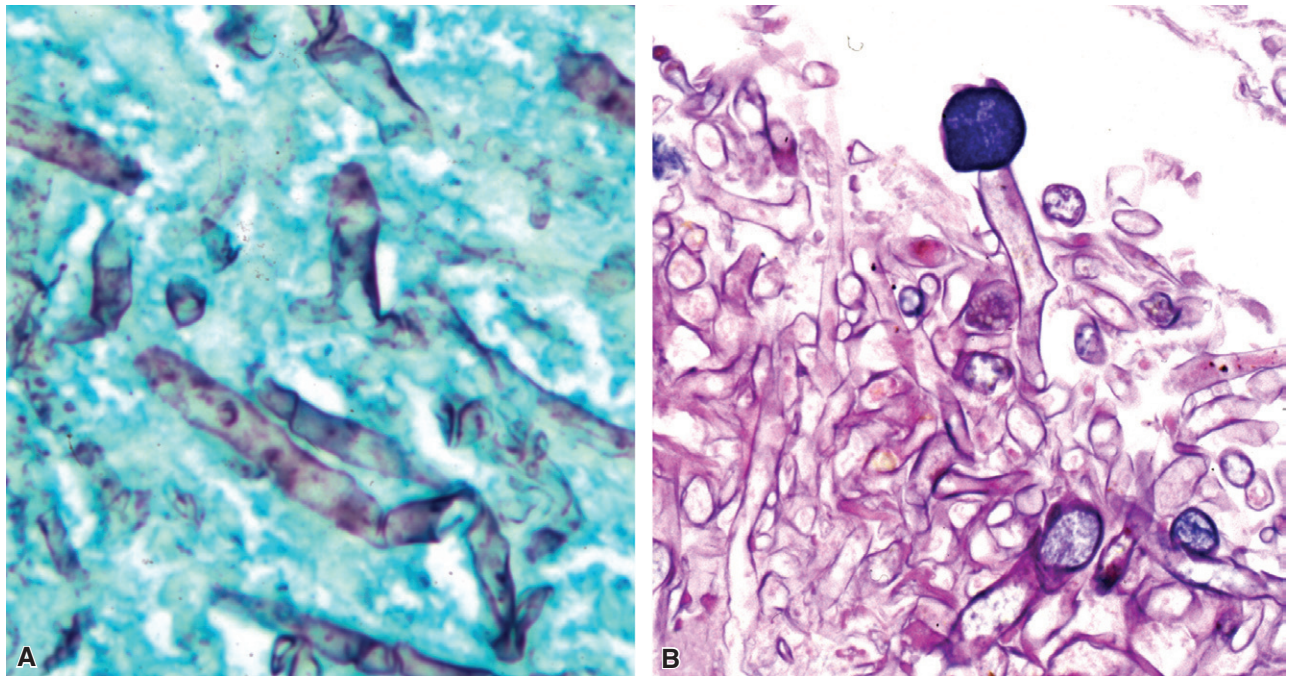


Figure 6-82. Zygomyces. **A**, Twisted pauciseptate, broad mycelia characteristic of zygomyces (Grocott methenamine silver stain). **B**, Endobronchial zygomyces with chlamydozoospores.

nodules that may be relatively fibrotic, cysts, and dystrophic calcification also are described.^{234–236}

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 (4–7 μm) yeast-like cell that may be collapsed, helmet-shaped, or variably crescentic. The intracystic dot or paired-comma structures are important keys to distinguish *P. jiroveci* cysts from look-alikes such as *Histoplasma*, the capsule-deficient form 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.⁴⁴ The aspirates and other samples also can be submitted for culture and ancillary studies.²³⁷ 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. jiroveci* also can enter

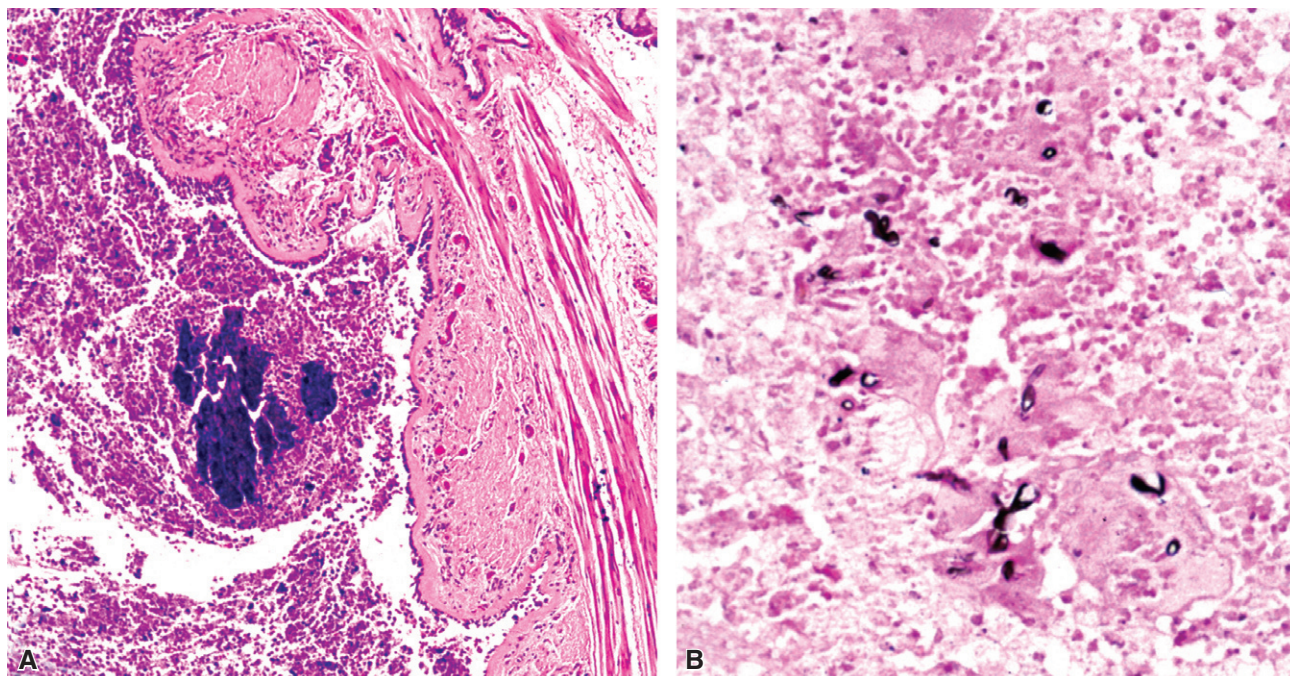


Figure 6-83. Allergic bronchopulmonary fungal disease. **A**, Ectatic bronchus with thick eosinophilic basement membrane and intraluminal necroinflammatory debris. **B**, Mycelial fragments of *Bipolaris* organisms (Grocott methenamine silver stain).

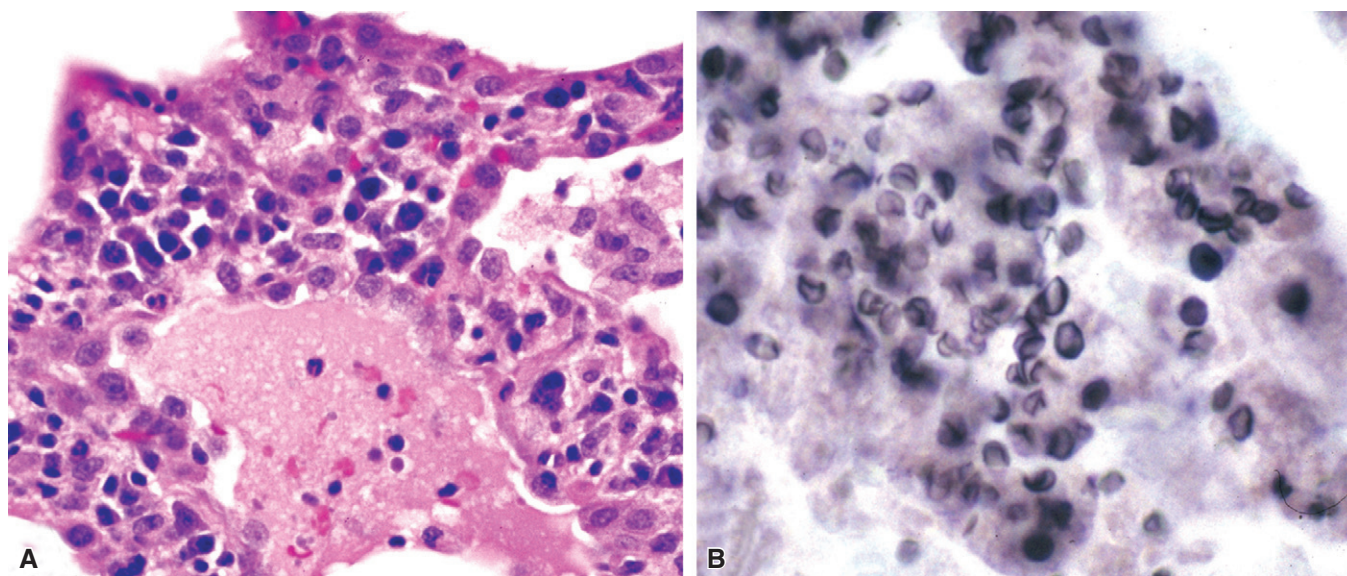


Figure 6-84. *Pneumocystis* pneumonia. **A**, Lymphoplasmacytic interstitial infiltrate and intra-alveolar foamy alveolar cast. **B**, Numerous yeast-like cells of *Pneumocystis jiroveci* of various shapes (Grocott methenamine silver stain).

Box 6-20. 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

the differential diagnosis.⁴³ 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 (Grocott methenamine silver, Gridley, and Fontana-Masson) often can 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

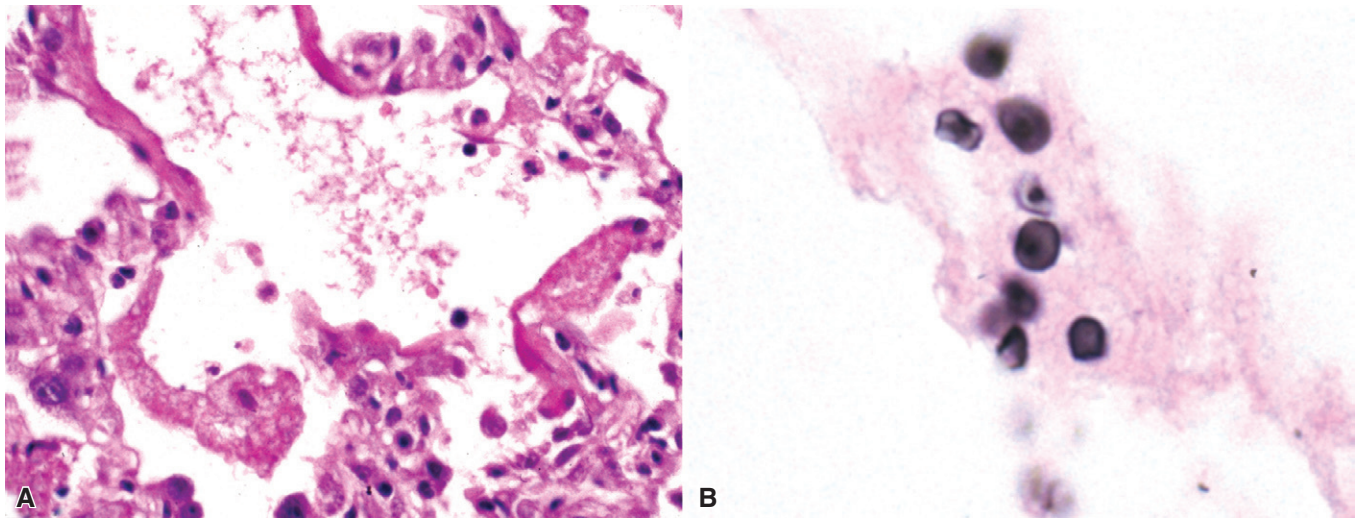


Figure 6-85. *Pneumocystis pneumonia*. **A**, Diffuse alveolar damage pattern with hyaline membranes. **B**, Cysts in hyaline membrane (Grocott methenamine silver stain).

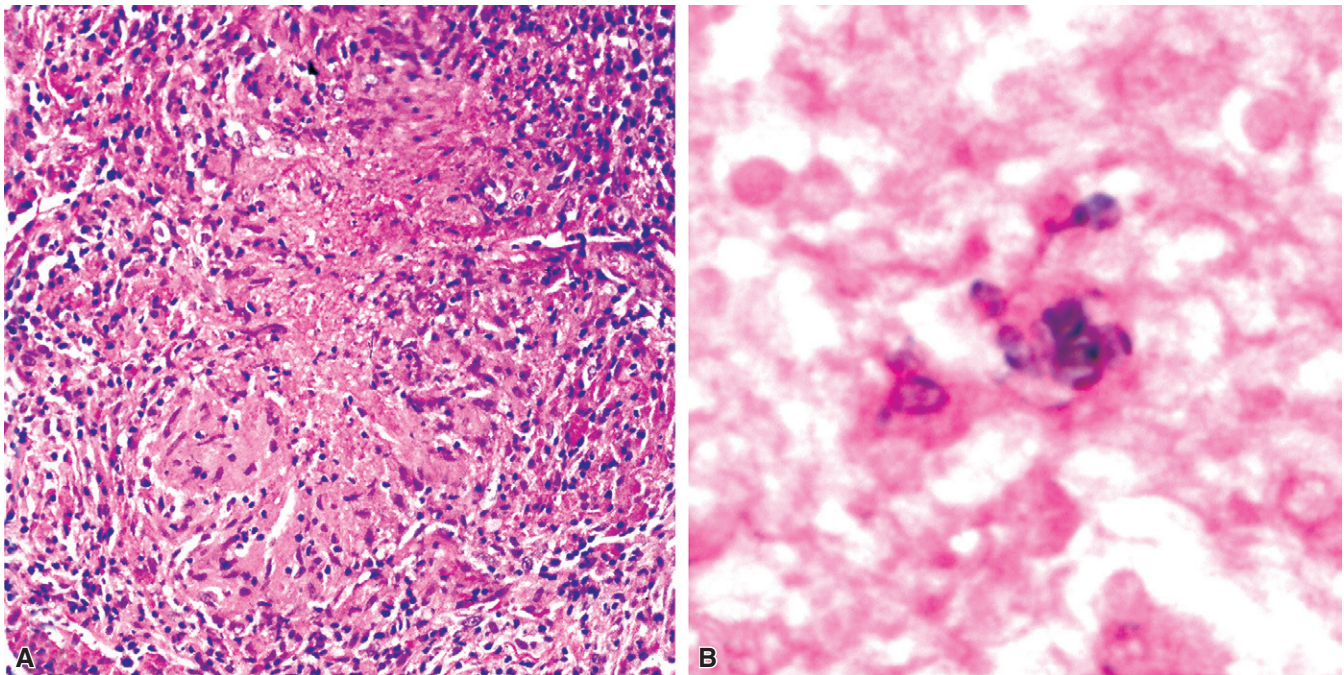


Figure 6-86. *Pneumocystis pneumonia*. **A**, Miliary granuloma with central necrosis. **B**, Sparse organisms in granuloma (Grocott methenamine silver stain).

associated with little or no accompanying inflammation in the immunocompromised patient.

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 6-7.

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. 6-87).

B. dermatitidis organisms are refractile, double-contoured yeast forms and range in diameter from 8 to 15 μm with broad-based budding (Fig. 6-88). 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. 6-89A) to empty collapsed spheres and small immature spherules.²³⁸ 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 (see Fig. 6-89B).

H. capsulatum yeast cells are small (2–5 μm) and stain poorly in routine smears, but 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. 6-90) are discernible, and they are outlined entirely in GMS-stained smears.

Table 6-7. Morphologic Features of Selective Yeast Forms

Feature	Small			Intermediate		Large
	<i>Candida</i>	<i>Pneumocystis</i>	<i>Histoplasma</i>	<i>Cryptococcus</i>	<i>Blastomyces</i>	<i>Coccidioides</i>
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*. ASCP Press: Chicago; 1987:87.

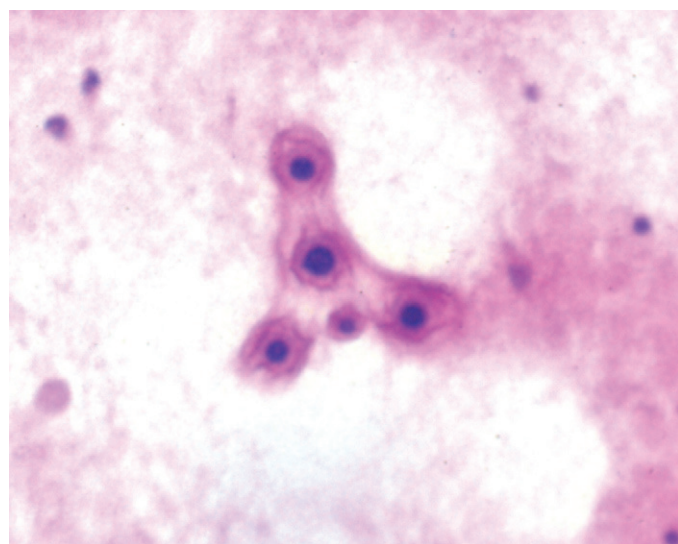


Figure 6-87. *Cryptococcus neoformans*. In this fine-needle aspirate, clusters of yeast cells resembling “spare tires” are invested by capsule in sparse inflammatory background (alcohol-fixed).

P. jiroveci most commonly is 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. 6-91A). These organisms rarely occur singly. The GMS stain outlines the characteristic cysts (see Fig. 6-91B).

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—zygomycete and *Candida* hyphae. The morphologic features of some of the more common agents are compared in Table 6-8.

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 cavitory infiltrates, provides strong morphologic evidence of infection.

Aspergillus species are characterized by septate mycelia that branch at angles approaching 45 degrees (Fig. 6-92). *Aspergillus* hyphae lack constrictions at points of septation. However, *Aspergillus* organisms cannot be differentiated from one of their mimics by morphology

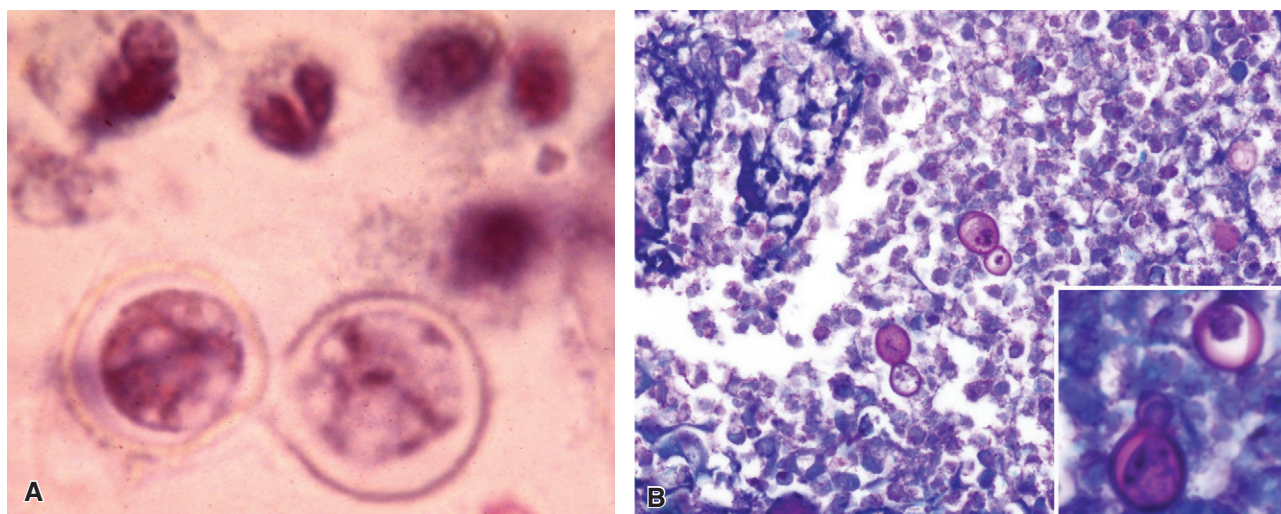


Figure 6-88. *Blastomyces dermatitidis*. **A**, Necroinflammatory infiltrate with refractile yeast forms. **B**, Periodic acid/Schiff staining highlights the double-contoured yeast with broad-based budding (see inset for greater detail).

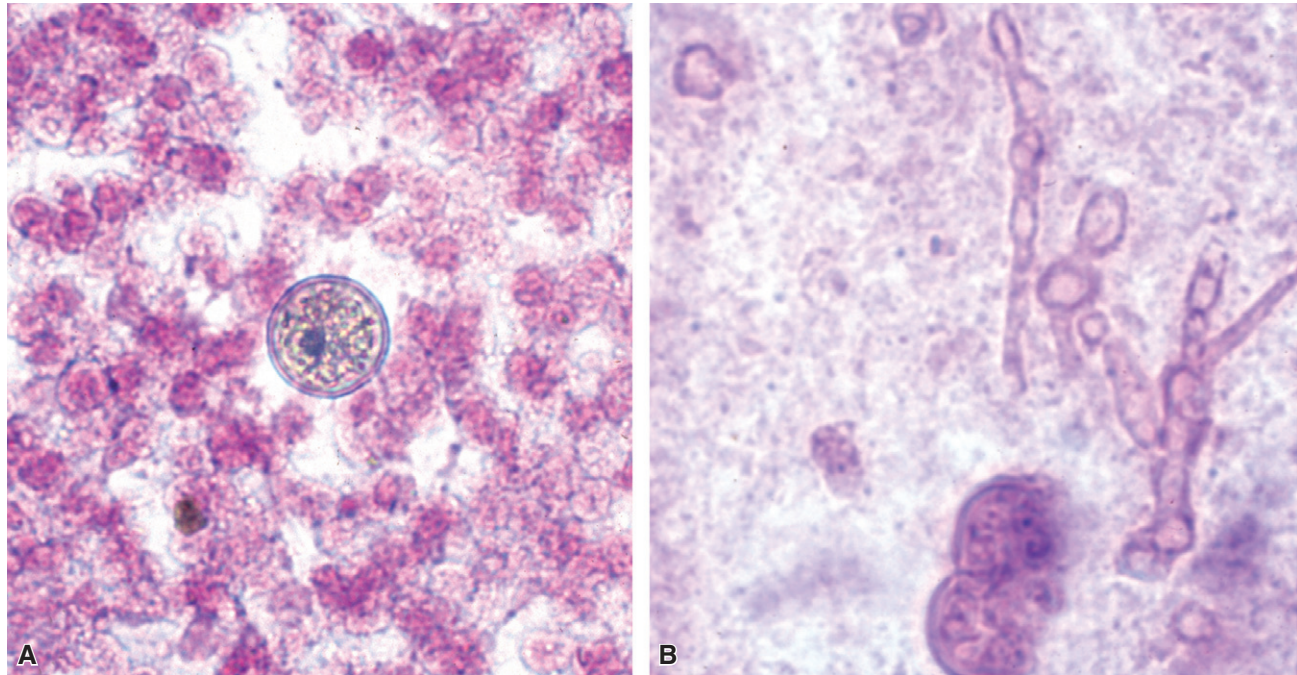


Figure 6-89. *Coccidioides* species. **A**, Negative-staining spherule in suppurative inflammatory background in a fine-needle aspirate (alcohol-fixed). **B**, Ruptured spherules and mycelia with arthrospores in granular necrotic background in another fine-needle aspirate (alcohol-fixed).

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.²³⁹ An additional advantage is that this technique may assist in this otherwise difficult differential diagnosis.

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 also may 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.⁴⁶ 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.²⁴⁰

Microbiology

Complementary laboratory methods are often required for diagnosis of fungal infection, and these are listed in [Box 6-21](#).¹⁸² 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 co-evaluation 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 also will stain with this reagent.^{225,241} 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 also can 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

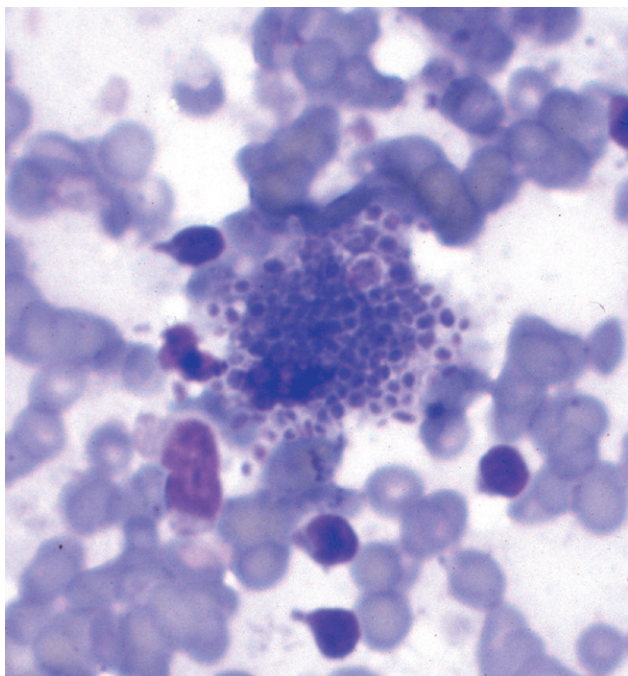


Figure 6-90. *Histoplasma capsulatum*. Clusters of purple polarized yeast cells are readily seen in this fine-needle aspirate (Diff-Quik preparation).

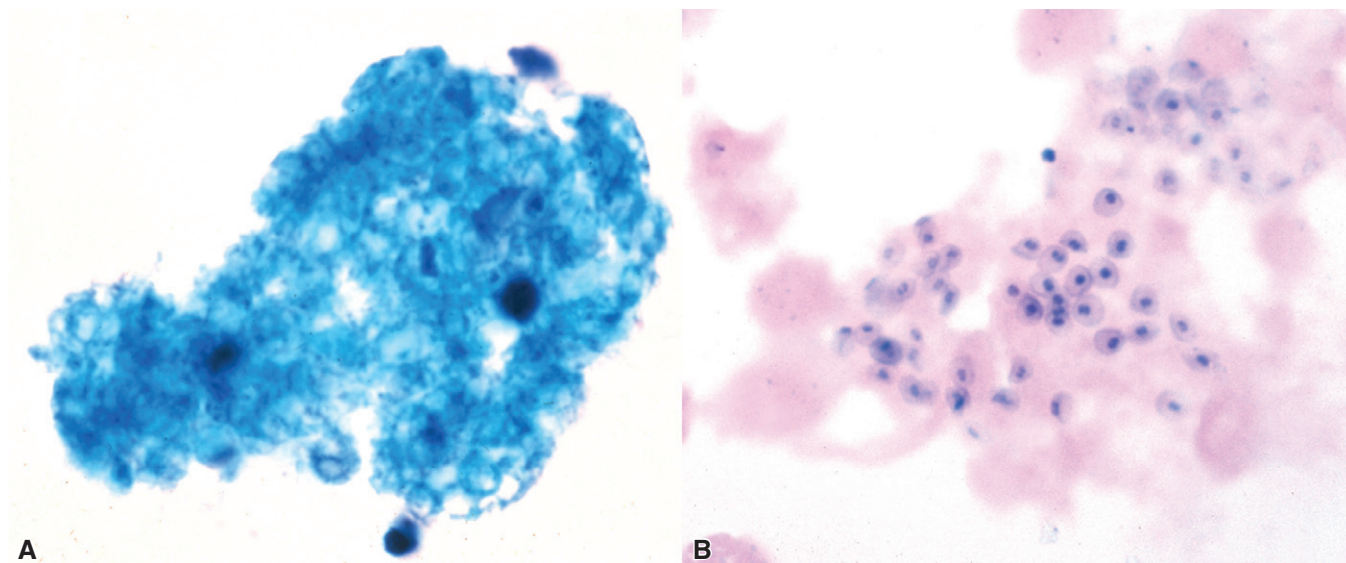


Figure 6-91. *Pneumocystis jiroveci*. **A**, Foamy alveolar cast in bronchial washing (ThinPrep Papanicolaou stain). **B**, Cysts with intracystic dot in bronchial washing (ThinPrep, Grocott methenamine silver stain).

Table 6-8. Morphologic Features of Selected Fungal Mycelia

Feature	<i>Aspergillus</i>	<i>Bipolaris</i>	Zygomycetes	<i>Pseudallescheria boydii</i>	<i>Fusarium</i>
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	<i>Aspergillus</i> "look-alikes"	<i>Aspergillus</i> "look-alikes"

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

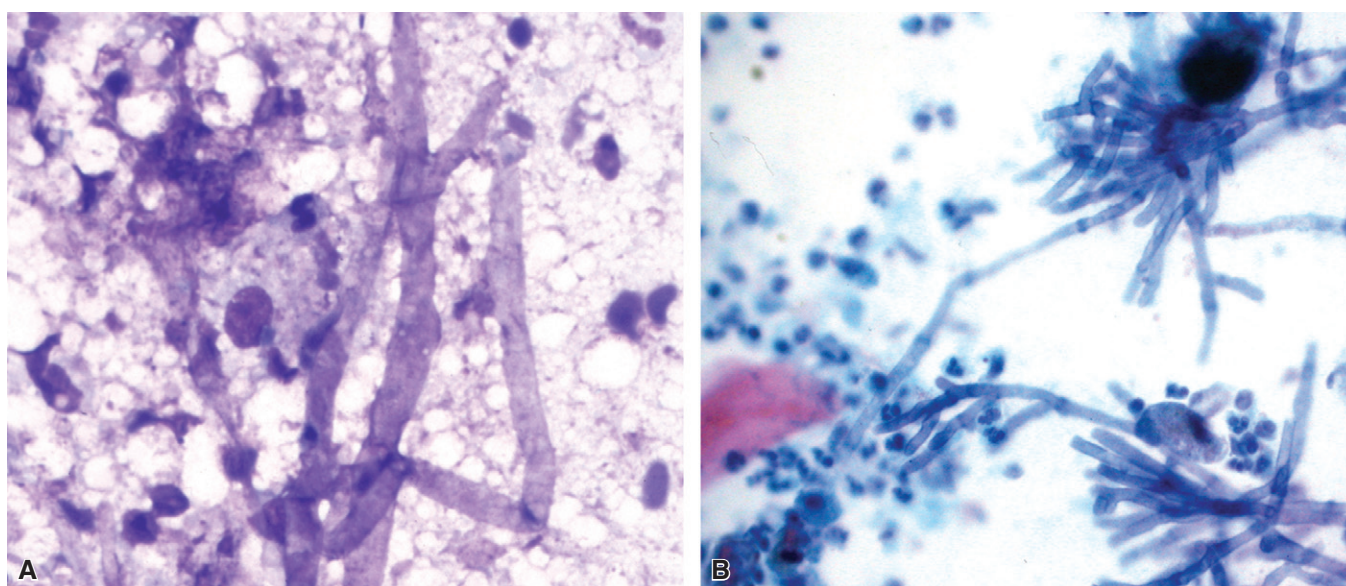


Figure 6-92. *Aspergillus* species. **A**, Twisted, sparsely septate mycelia are difficult to differentiate from mimics, including zygomycetes, in this fine-needle aspirate (Diff-Quik preparation). **B**, Characteristic mycelia in a bronchial washing (Papanicolaou stain).

Box 6-21. 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

blood cell nuclei, reticulin and elastic fibers, calcium deposits, and even Hamazaki-Wesenberg bodies.⁴⁰

In the microbiology laboratory, the age-old technique of direct light microscopic visualization of fluids, exudates, and tissue homogenates treated with potassium hydroxide (KOH) is being replaced by chemofluorescent cotton-brightening agents (such as calcofluor white and fungiquil). Fluorescence microscopy with these reagents can detect a wide variety of fungi in wet mounts as well as frozen sections and paraffin-embedded tissue.^{242,243}

The time-honored laboratory techniques for the identification of fungi (gross colonial and microscopic morphologic analysis after isolation on fungal media, followed by biochemical testing) may be the principal means to an 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.²⁴⁴ 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 cyclohexamide 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 *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 judgement. This is because 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 HIV infection, organ transplantation, or immunocompromising drug therapy.²⁴⁵

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 Ringers 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.^{246,247} 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.^{47,248} Immunofluorescence testing using specific monoclonal antibodies can achieve 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.^{32,249,250}

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 which may be present in small numbers or manifest overlapping histologic features with one another.^{243,251-253} 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.²⁵⁴

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.²⁵⁵ 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.^{256,257}

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.²⁴⁶ For these two fungi, useful antigen detection techniques are available using serum, urine, cerebrospinal and BAL fluids. They are especially sensitive in patients with defective immunity.^{237,257} 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 1,3 β -D-glucan, a cell wall component of several fungi such as *Aspergillus*, *Candida*, *Fusarium*, and others, and for galactomannin, a polysaccharide antigen in the cell wall of *Aspergillus*, have shown fair sensitivity and specificity.^{181,258}

Differential Diagnosis

A synopsis of the key morphologic and mycologic features of the fungal pneumonias is presented in Table 6-9. When H&E and GMS stains fail to detect or clearly identify fungal elements in a suspected fungal infection, 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 non-necrotizing granulomatous disorders also enter the differential diagnosis. These include Wegener granulomatosis, idiopathic bronchocentric granulomatosis, aspiration, sarcoidosis, rheumatoid nodules, pyoderma gangrenosum-like lung lesions in patients with inflammatory bowel disease, and Churg-Strauss syndrome.

Table 6-9. 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	Fibrocaceous 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
Sporotrichosis	
Surgical pathology	Necrotizing granuloma, often cavitary with small, usually round, sometimes cigar-shape 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 yield 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 <i>Histoplasma</i> species, but with septum reflecting binary fission, not budding reproduction
Cytopathology	Macrophage with intracellular characteristic yeast forms

Continued

Table 6-9. Fungal Pneumonias: Summary of Pathologic Findings—cont'd

Assessment Component	Findings
Penicilliosis—cont'd	
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; acapsular types sometimes
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 <i>C. glabrata</i> 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, cavitory 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

Table 6-9. Fungal Pneumonias: Summary of Pathologic Findings—cont'd

Assessment Component	Findings
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 <i>Pneumocystis carinii</i> , classified as a fungus and renamed <i>Pneumocystis jiroveci</i> ; 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; KOH, potassium hydroxide.

Viral Pneumonia

Viruses cause more infections than all other types of microorganisms combined, and involve the respiratory tract more commonly than other organ systems.²⁵⁹ Fortunately, the lung diseases produced by viruses usually are mild and self-limited. Nevertheless, viruses cause major public health illnesses and account for many of the new and emerging diseases in today's headlines. At times, viruses also are capable of producing serious and life-threatening infections that come to the attention of pathologists in both immunocompromised patients and young, healthy persons.²⁶⁰ The viruses that commonly infect the lung are listed in Table 6-10.

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 those patients with chronic diseases, these pathogens can cause serious pneumonias. Pneumonia in immunocompromised persons usually is attributed to the herpesviruses (herpes simplex virus and CMV). Less appreciated is that the conventional respiratory viruses also are frequent causes of respiratory illness in these patients, and that such infections result in high rates of morbidity and mortality.²⁶¹

Newly recognized respiratory viruses^{262,263} include a highly pathogenic strain of influenza, H5N1. 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.²⁶⁴ An acute cardiopulmonary syndrome in the southwest United States was etiologically linked to a new hantavirus referred to as *Sin Nombre* (“without a name”). The severe acute respiratory syndrome (SARS), which began in southern China and was carried by travelers to 33 other countries and 5 continents, was caused by a newly recognized coronavirus, SARS-CoV. Four other coronaviruses linked to respiratory illnesses (HCoV-229E; HCoV-NL63; HCoV-OC43;

HCoV-HKU1) have since been reported.²⁶⁵ 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 also can cause illness in adults and immunocompromised patients. Human bocavirus (hobv) has been isolated in several countries from children with wheezing.²⁶⁶ Other viruses such as the picornavirus group (rhinovirus and enterovirus) can cause pneumonia, as can BK polyoma virus.²⁶⁷ Other unusual viral lung infections have been attributed to henipah and hemorrhagic fever viruses.²⁶⁸ 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 also has been described.²⁶⁹ 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. Box 6-22 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.²⁷⁰

The histopathologic diagnosis of viral infection is impossible without identification of the characteristic CPE. The term *cytopathic effect* traditionally has been used by virologists to describe cellular changes in unstained cell culture monolayers seen by light microscopy,^{271,272} 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.^{273,274} 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

Table 6-10. 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 6-22. Histopathologic Patterns in Viral Lung Injury

Diffuse alveolar damage
Bronchitis and bronchiolitis
Diffuse interstitial pneumonia
Perivascular lymphoid infiltrates
Miliary small nodules
Air space organization—bronchiolitis obliterans—organizing pneumonia (BOOP) pattern
Calcified nodules

Table 6-11. 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

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 6-11. Finally, it is worth mentioning that most clinically significant viral pneumonias that have CPE also show necrosis somewhere in the biopsy.

Influenza virus

Influzaviruses 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

panemics 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 versus late), outcome (fatal versus 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 are those of active tracheobronchitis.²⁷⁵ 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. 6-93). This constellation of findings comprises

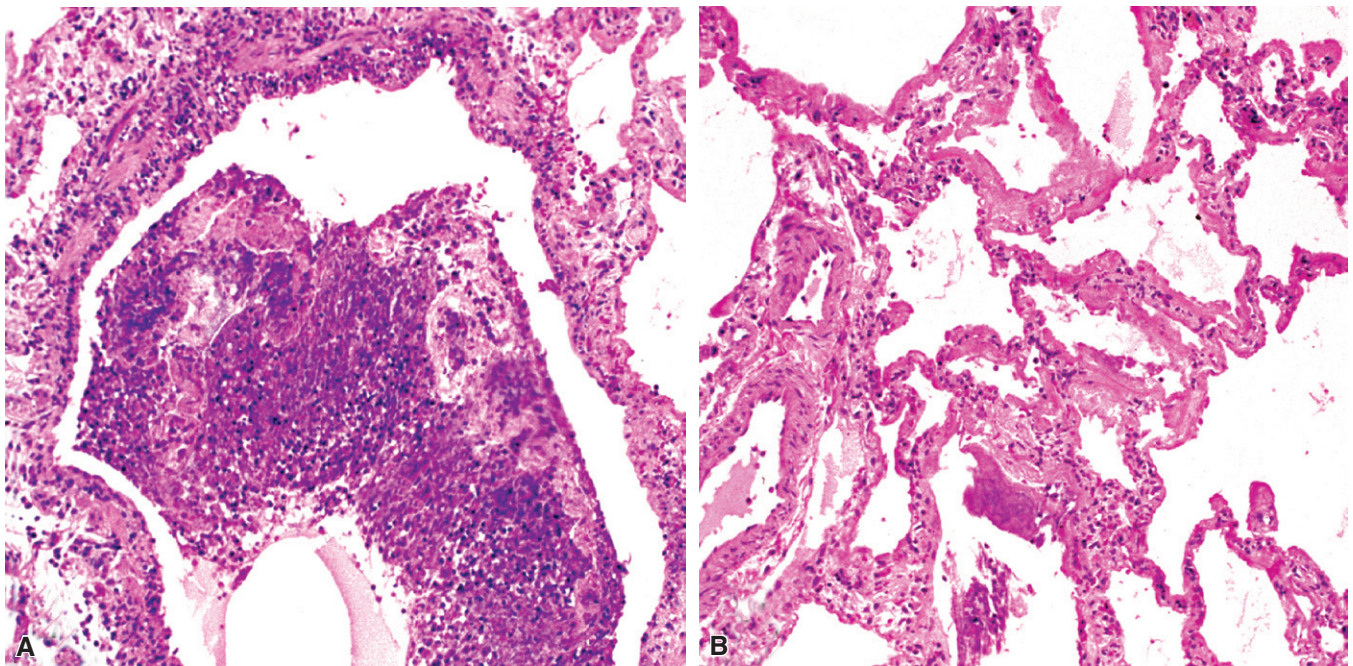


Figure 6-93. Influenza virus. **A,** Bronchiolitis with intraluminal necroinflammatory debris. **B,** Acute diffuse alveolar damage pattern with hyaline membranes.

the “lesion of characterization.”²⁷⁶ 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.^{277,278} 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 which may obscure the features of primary viral pneumonia.

From 2003 through 2008, 391 human cases of highly pathogenic avian influenza involving the H5N1 strain were recorded, with 247 deaths.²⁷⁹ 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 inter pandemic (seasonal) influenza.²⁷⁸ A characteristic feature of the the H5N1 and 1918 cases is the high mortality rate, especially among previously healthy older children and young adults. Excessively high levels of cytokine 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.²⁸⁰ 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.²⁸¹

Parainfluenza Virus

Parainfluenza virus comprises four serotypes (I to IV) that typically target the upper respiratory tract, classically in the form of croup.²⁸² 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 respiratory syncytial virus infection. The giant cells of parainfluenza tend to be larger and have more intracytoplasmic inclusions.⁵¹ Parainfluenza virus is a potential opportunist in immunocompromised patients, especially children with congenital immunodeficiency disorders²⁸³ in whom fatal pneumonitis with disseminated disease may occur.²⁸⁴

Respiratory Syncytial Virus

RSV causes more significant respiratory infections in early childhood than those attributable to either influenza viruses or parainfluenza viruses.²⁸⁵ 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.²⁸² 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.^{283,286,287} Also, RSV is often an unsuspected opportunistic pathogen in immunocompromised patients.^{261,288} RSV targets the epithelium of the distal airway, producing bronchiolitis with disorganization of the epithelium and epithelial cell sloughing²⁶⁸ (Fig. 6-94A). In fatal cases, airway obstruction due to sloughed cell detritus, mucus, and fibrin is compounded by airway lymphoid hyperplasia.²⁸⁹ 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 air spaces around areas of bronchiolitis (see Fig. 6-94B). 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.²⁹⁰

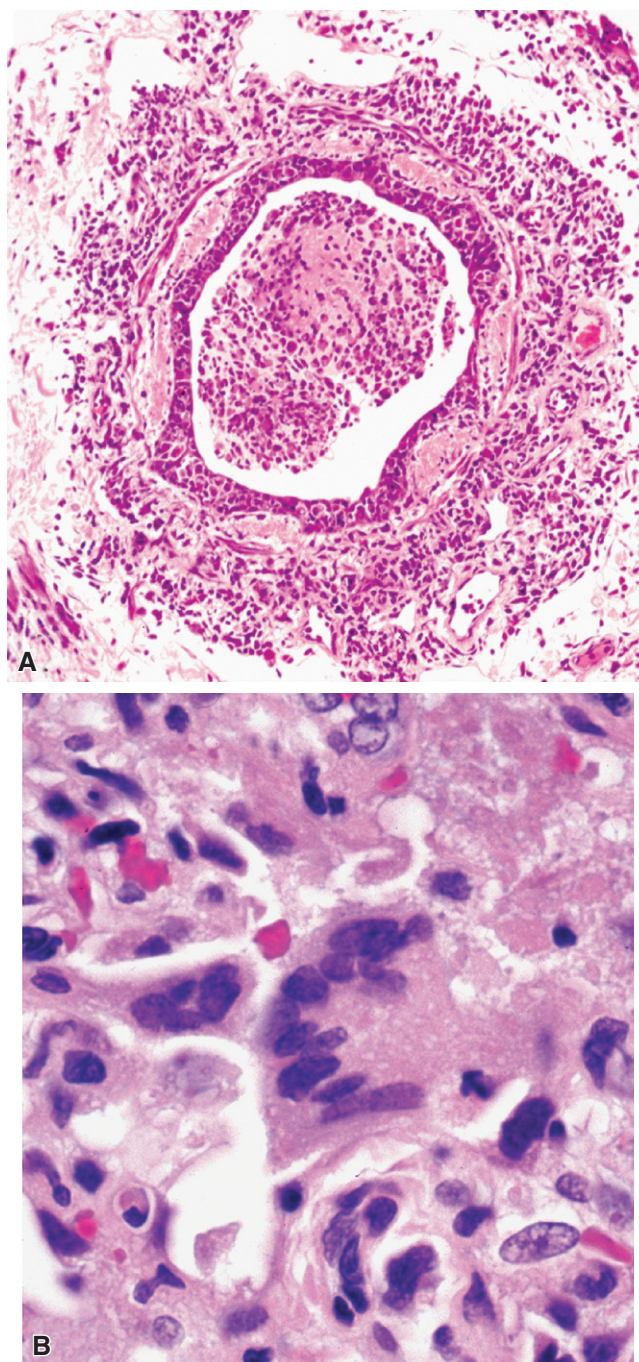


Figure 6-94. Respiratory syncytial virus. **A**, Bronchiolitis with intraluminal sloughing. **B**, Bronchiolitis with giant cell syncytia.

The virus also causes disease in immunocompromised patients 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.^{291,292} 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 exanthema worldwide that, unlike varicella (chickenpox), leads to complications that are common and serious.²⁹³ 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. 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.²⁹⁴ Characteristic giant cells show distinctive intranuclear eosinophilic inclusions surrounded by halos (Fig. 6-95). This is the

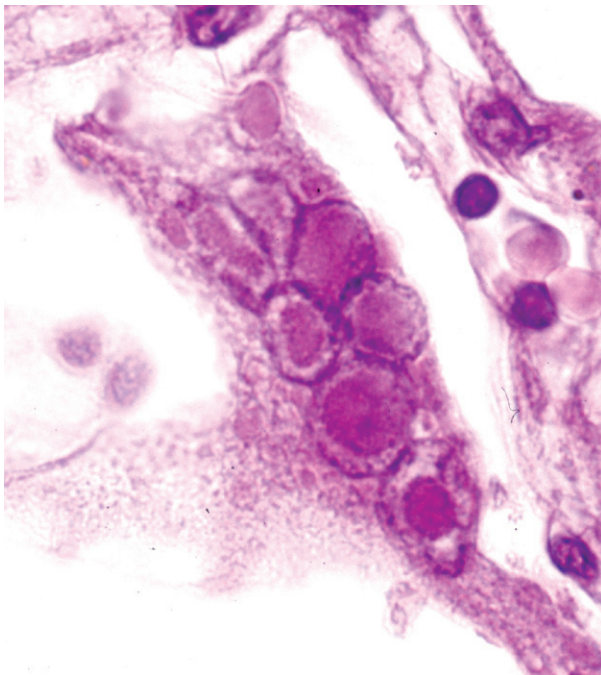


Figure 6-95. Measles virus pneumonia with characteristic eosinophilic intranuclear inclusions in giant cell.

classic measles injury pattern²⁶⁸ 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, but similar changes can be seen in RSV and parainfluenza pneumonias, and not all cases of measles pneumonia have these giant cells.²⁶⁸ 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 (NSIP).

Hantavirus

The recently identified hantavirus produces a rapidly evolving cardio-pulmonary 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 that 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, designated *Sin Nombre* (“without a name”), 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 membranes²⁹⁵ (Fig. 6-96A). Immunoblast-like cells are present in vascular spaces and in the peripheral blood (see Fig. 6-96B). Morphologic diagnosis is presumptive, because hantaviral antigen in endothelial cells, detected by immunohistochemistry, is required for definitive diagnosis.²⁹⁶ 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 detection of hantavirus-specific immunoglobulin M (IgM) antibodies, or by detection of hantavirus RNA by PCR assay in peripheral blood leukocytes.^{297,298}

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.²⁶⁵ They are responsible for a majority of common colds, along with the rhinoviruses. Co-infections with other respiratory viruses occur in infants and children presenting with more severe respiratory disease. In certain

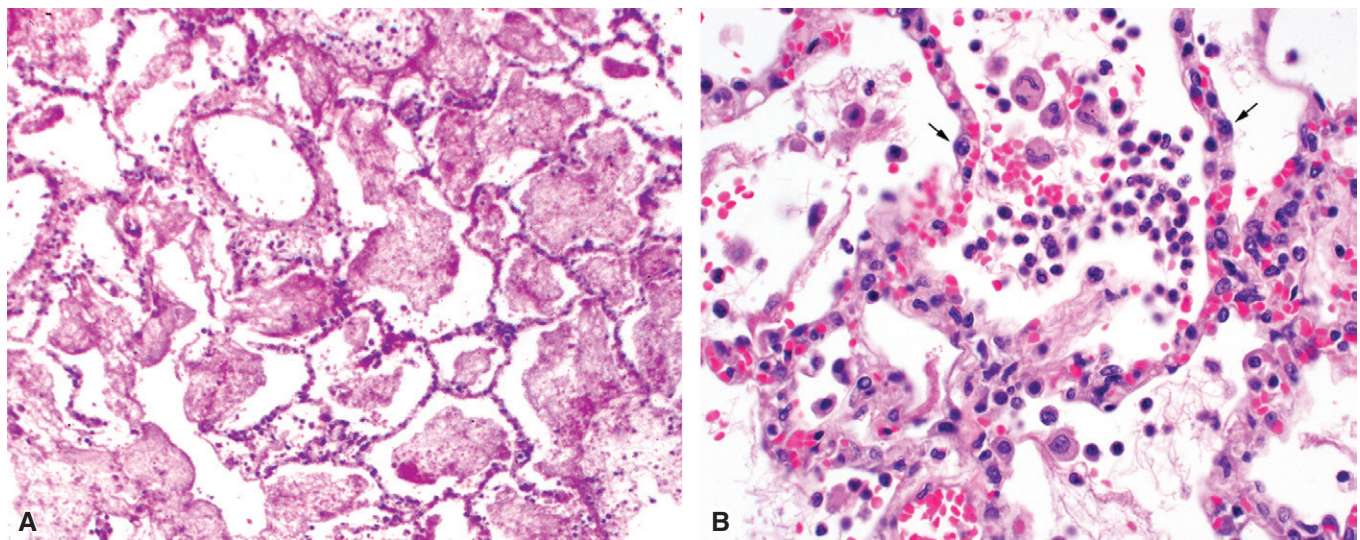


Figure 6-96. Hantavirus. **A,** Pulmonary edema with fibrin deposits. **B,** Immunoblast-like cells in alveolar capillaries at arrows.

epidemiologic situations, they can cause pneumonia in children, frail elderly individuals, and immunocompromised adults.^{299,300}

In November 2002, the appearance of an atypical pneumonia in China, subsequently labeled *severe acute respiratory syndrome* (SARS), became an alarming global health problem in the period of a few months.³⁰¹ 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.³⁰²

Clinically, the disease ranges from a nonhypoxemic febrile respiratory disease (with minimal symptoms in some patients) to one of severe pulmonary dysfunction, manifesting as acute respiratory distress syndrome and eventuating in death for approximately 5% of the patients affected.³⁰³ In the reported cases, either the chest x-ray appearance on presentation was normal or the chest film showed unilateral, predominantly peripheral areas of 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 normal x-ray appearance, CT scans often revealed bilateral ground-glass consolidation resembling that in bronchiolitis obliterans with organizing pneumonia (cryptogenic organizing pneumonia). Laboratory abnormalities in some but not all patients included leukopenia with lymphopenia and thrombocytopenia. The partial thromboplastin time and D-dimer levels were increased. Biochemical abnormalities included elevated lactate dehydrogenase (LDH), alanine aminotransferase, and creatinine levels. 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.^{304,305} Lung biopsy specimens in milder cases showed relatively scant intra-alveolar fibrin deposits with some congestion and edema (Fig. 6-97). 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.³⁰⁶ 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

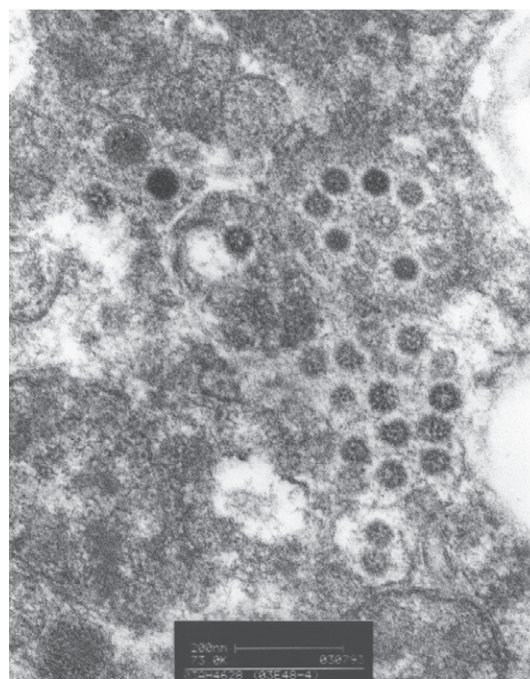


Figure 6-98. Coronavirus-infected cell can be seen in this electron photomicrograph. (Courtesy of Dr. Oi-Yee Cheung, Queen Elizabeth Hospital, Hong Kong, China.)

(predominantly type II pneumocytes) and alveolar macrophages using immunohistochemical staining, in situ hybridization, RT-PCR methods, and electron microscopy. A unique coronavirus (Fig. 6-98) was finally implicated as the etiologic agent.^{306,307} Comparative histopathologic studies in fatal cases of SARS and H5N1 avian influenza reveal similarities and differences.³⁰⁸ Both infections feature acute and organizing diffuse alveolar damage, but SARS appears to be more frequently associated with subacute injury with intra-alveolar organization, whereas H5N1 virus causes a more fulminant diffuse alveolar damage pattern with patchy interstitial inflammation and paucicellular fibrosis.

Adenovirus

Adenovirus comprises several genera, with multiple serotypes that cause infections of the upper and lower respiratory tract, conjunctiva,

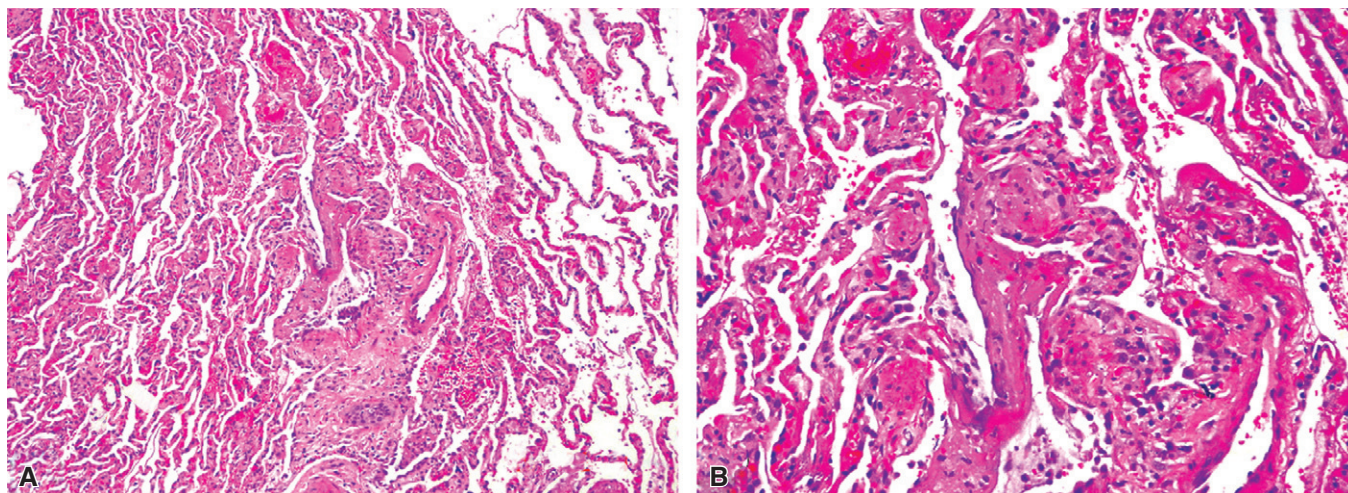


Figure 6-97. Coronavirus pneumonia: Severe acute respiratory syndrome (SARS). **A** and **B**, Acute fibrinous lung injury is evident. (Courtesy of Dr. Oi-Yee Cheung, Queen Elizabeth Hospital, Hong Kong, China.)

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 and children and in immunocompromised persons.^{268,309} 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 necrosis³¹⁰ (Fig. 6-99). These patterns may coexist in some cases and the pneumonia may be accompanied by hemorrhage secondary to adenovirus-induced endothelial cell damage.³¹¹ 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 herpes simplex virus (Fig. 6-100A). This later enlarges and becomes

amphiphilic and then more basophilic, obliterating the nuclear membrane, producing the characteristic “smudged cell” (see Fig. 6-100B).²⁶⁸

Herpes Simplex Viruses

Herpes simplex virus (HSV) type I and type II have had traditional assigned roles as etiologic agents of mucocutaneous disease of the head and neck (type I) and 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

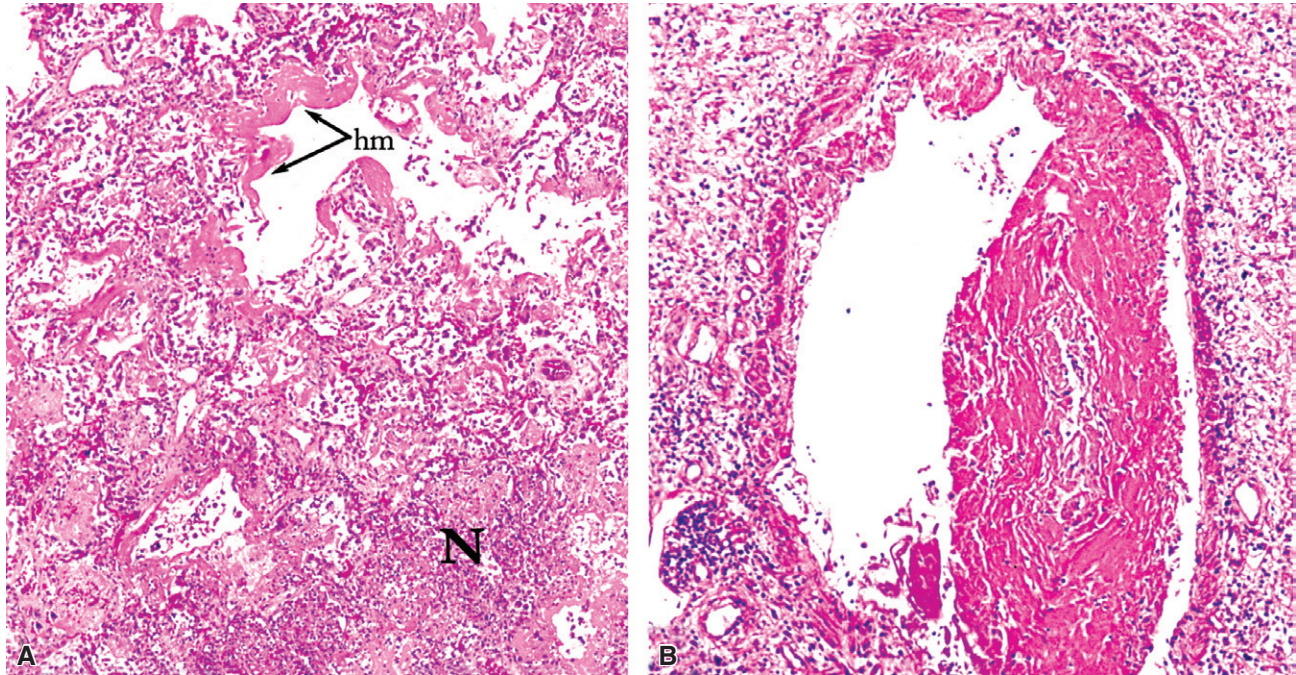


Figure 6-99. Adenoviral pneumonia. A, Necrosis (N) and diffuse alveolar damage (hm). B, Necrotizing bronchiolitis. hm, hyaline membrane.

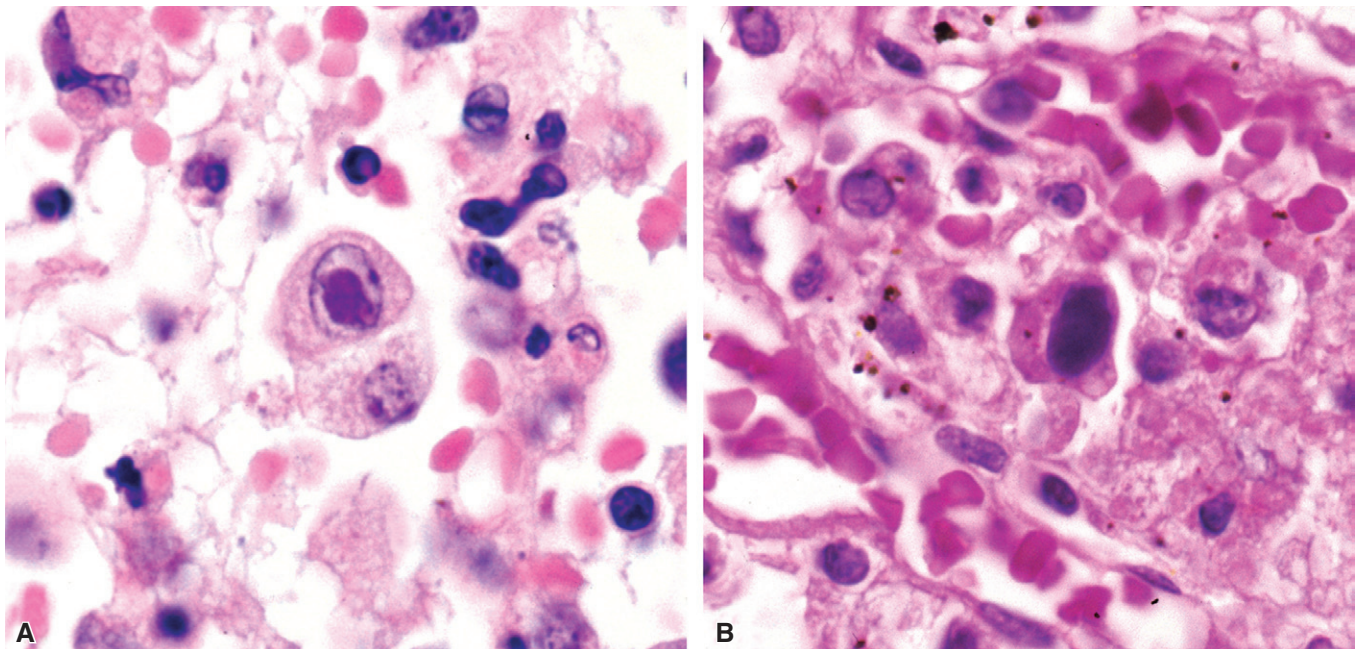


Figure 6-100. Adenovirus. A, Cowdry A intranuclear inclusions. B, Smudged cell.

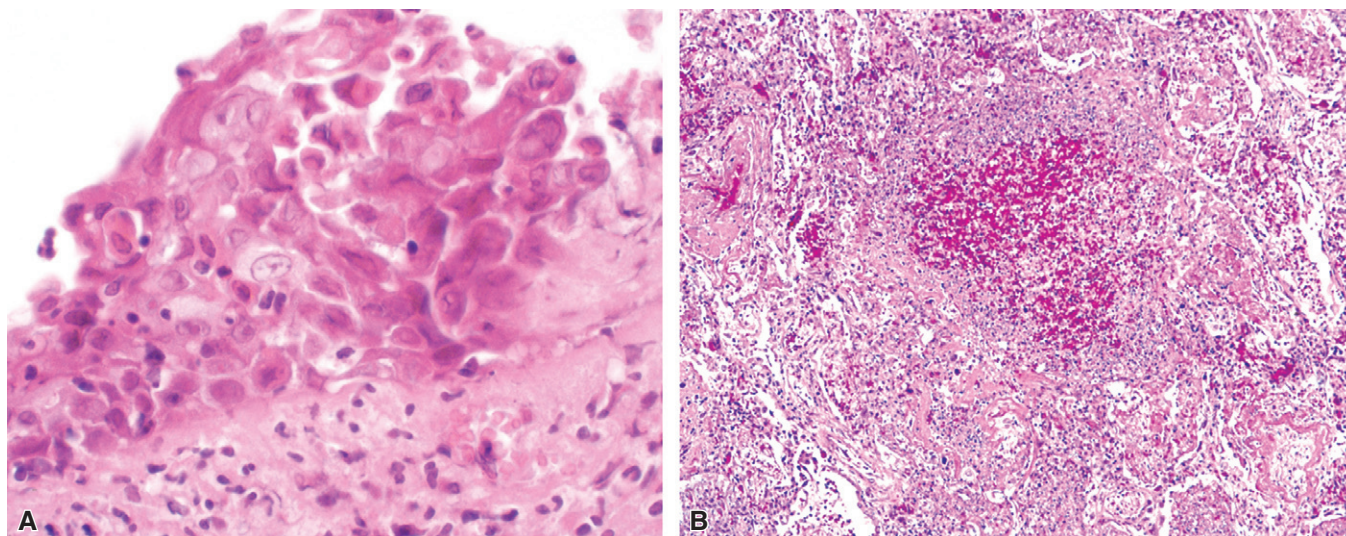


Figure 6-101. Herpes simplex virus pneumonia. **A**, Tracheobronchitis. Note cells with ground-glass inclusion. **B**, Miliary nodular pattern of hemorrhagic necrosis.

in neonates and in patients who are immunosuppressed or compromised by various chronic diseases. Characteristic lesions include tracheobronchitis (Fig. 6-101A) 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 CPE⁶⁹ (see Fig. 6-101B). 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. 6-102) 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.²⁶⁸ 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.

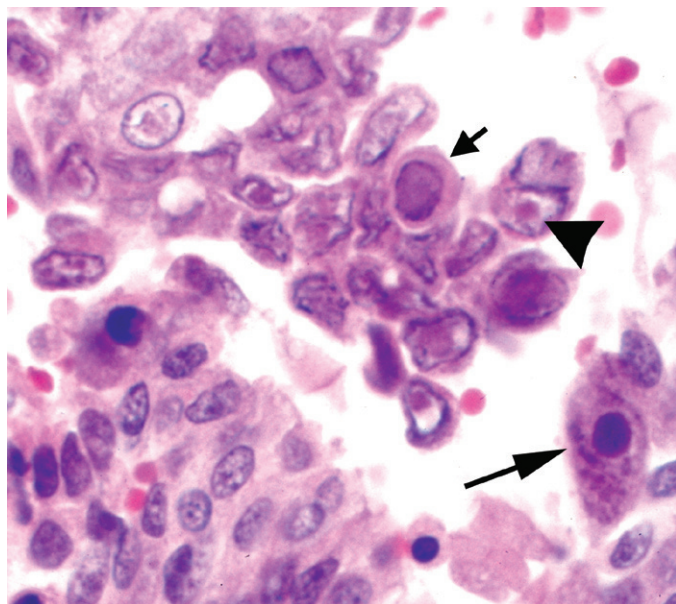


Figure 6-102. Herpes simplex virus pneumonia. Note two types of nuclear cytopathic effect: Cowdry A ground-glass type (*short arrow*) and Cowdry B eosinophilic inclusion (*arrowhead*). Compare with cytomegalovirus intranuclear and cytoplasmic inclusion at *long arrow*.

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 generally is mild and self-limited. Nevertheless, fatality rates of up to 10% have been reported.²⁶⁸ 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. 6-103A). HSV-like intranuclear inclusions are present but may be sparse and difficult to identify. A miliary pattern of calcified nodules (see Fig. 6-103B) may be present in the healed phase.³¹²

Cytomegalovirus

CMV infections are acquired throughout life. This virus can cause considerable morbidity and even death in the neonate, but infection generally is asymptomatic in older healthy children and adults. Like 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 viroplasmic 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. 6-104A).

Later, multiple eosinophilic cytoplasmic inclusions develop that may be positive on staining with PAS and GMS (see Fig. 6-104A, 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 usually will be highlighted with immunohistochemical stains.³¹³ Another typical pattern that suggests viral infection is the presence of miliary small nodules with central

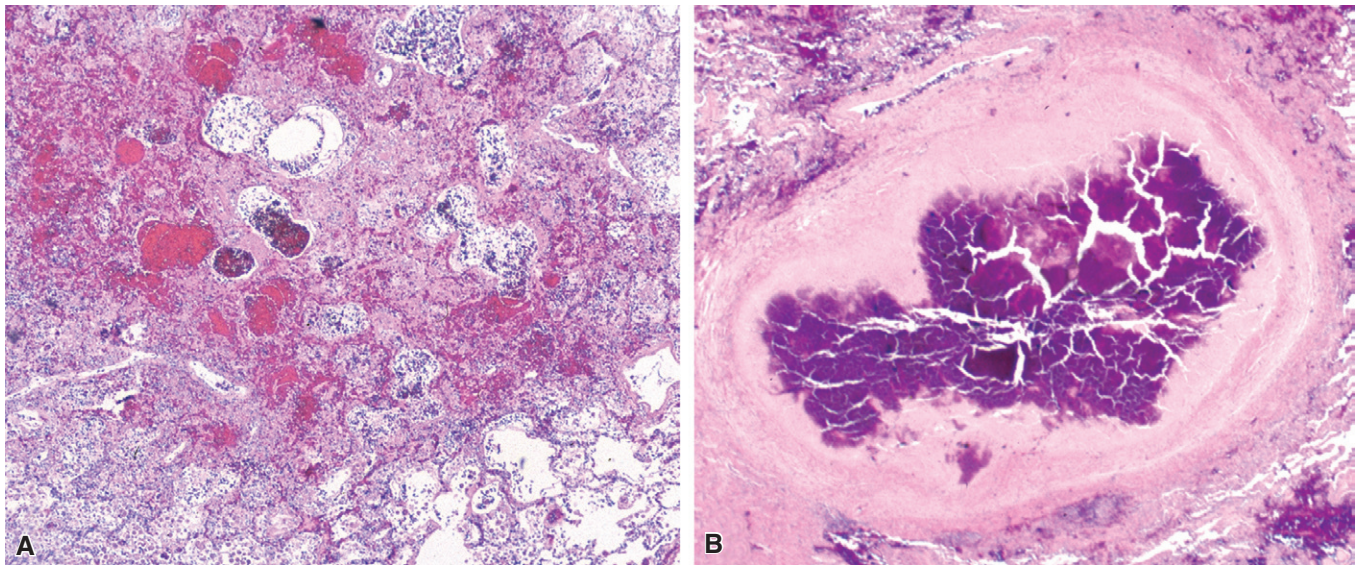


Figure 6-103. Varicella pneumonia. **A**, A hemorrhagic miliary nodule. **B**, Late phase with calcified nodules.

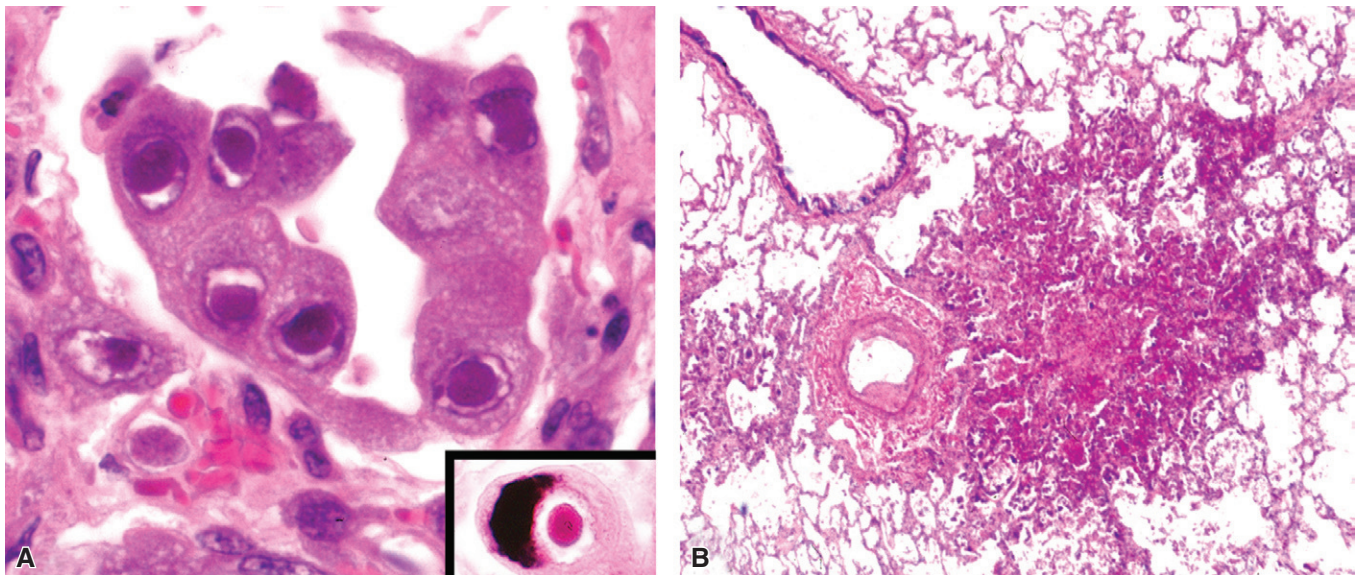


Figure 6-104. Cytomegalovirus (CMV) pneumonia. **A**, Multiple characteristic intranuclear and intracytoplasmic inclusions in alveolar lining cells. Note Grocott methenamine silver-positive staining of inclusions (*inset*). **B**, Miliary nodule pattern of CMV pneumonia. (**A**, Courtesy of Dr. Francis Chandler, Augusta, GA.)

hemorrhage surrounded by necrotic alveolar walls⁶⁹ (see Fig. 6-104B). Interstitial pneumonitis is the least common pattern of CMV infection. Ulcers may be seen in the trachea and bronchi, but occur less often than in herpetic infections. In CMV pneumonias, it is advisable to look for other pathogens, typically *P. jiroveci* (Fig. 6-105), but bacteria, fungi, protozoa, and other viruses all are possible co-infecting organisms.³¹⁴

Epstein-Barr Virus

EBV infections usually are acquired in childhood and generally are 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 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^{315,316} (Fig. 6-106). CPE is absent, and although serologic studies can be supportive of a clinicopathologic diagnosis, etiologic proof of EBV infection requires 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.^{317,318} 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. 6-107), and adenovirus are the most commonly identified viral pathogens in respiratory cytologic

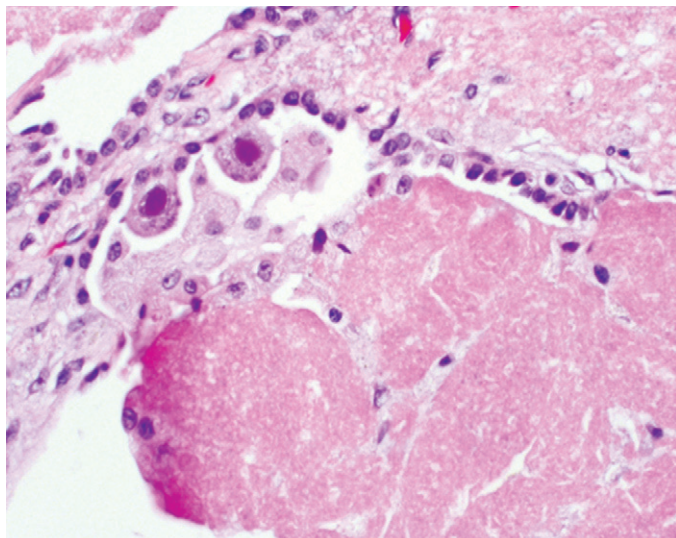


Figure 6-105. Cytomegalovirus-infected alveolar lining cells associated with the foamy alveolar casts of *Pneumocystis jiroveci*.

specimens, but varicella virus, parainfluenza virus, RSV, human metapneumovirus, and measles virus also have been detected.

Characteristic CPE produced by these viruses often is 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.⁴⁴

Microbiology

Diagnostic virology is the newest of the microbiology and infectious disease specialities 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.^{260,319,320} 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 a majority of viral assays in laboratories of all sizes. As a result, the pathologist who

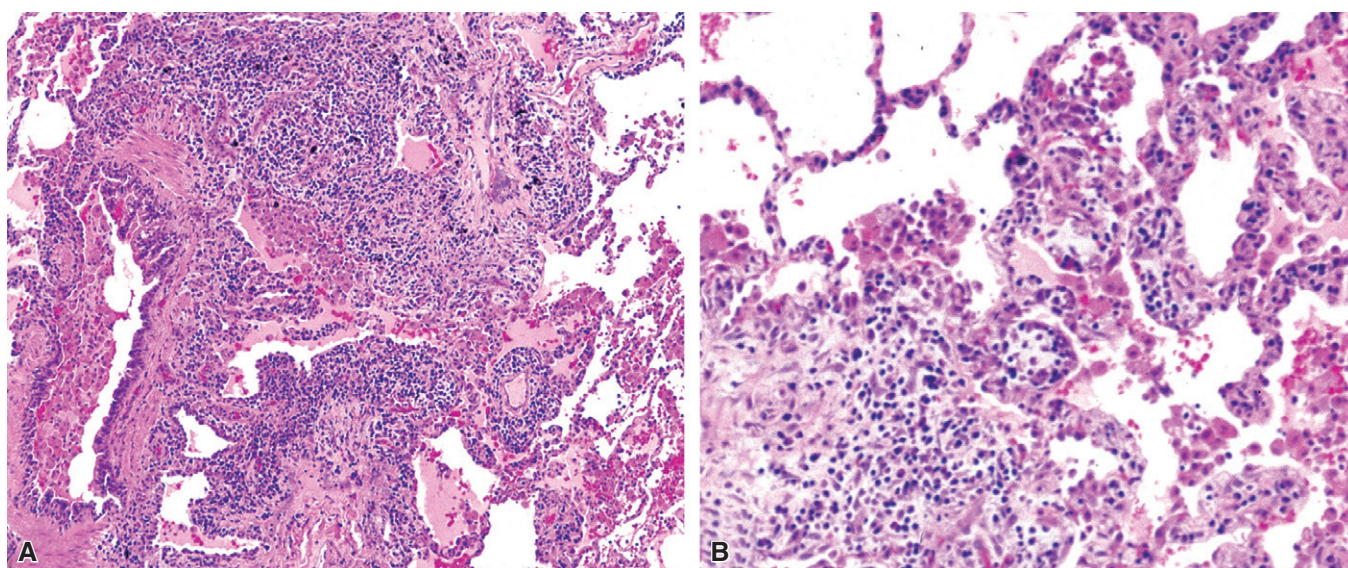


Figure 6-106. Epstein-Barr virus pneumonitis. **A**, Nonspecific cellular interstitial pneumonitis. **B**, Patchy interstitial infiltrate.

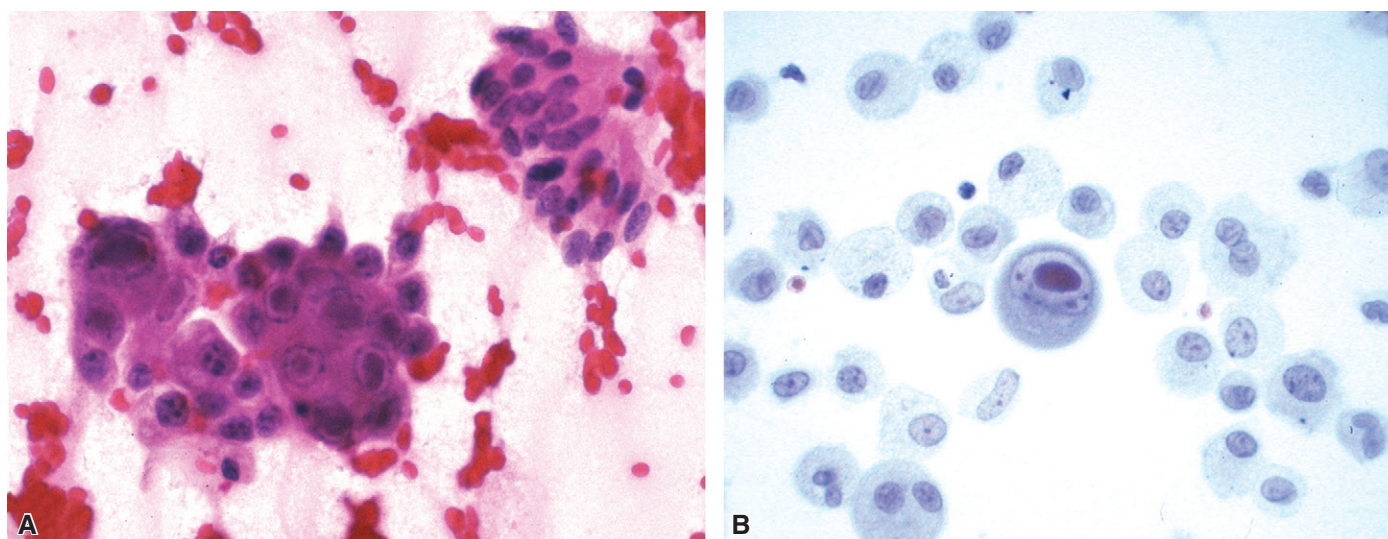


Figure 6-107. Cytomegalovirus pneumonitis with characteristic cytopathic effect. **A**, Fine-needle aspirate. **B**, Bronchoalveolar lavage specimen.

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 6-23**. In questionable cases, confirmation by immunohistochemical studies (**Fig. 6-108A**), in situ hybridization (see **Fig. 6-108B**), or electron microscopy may be helpful.^{31,321}

In the microbiology laboratory, the diagnosis of viral respiratory infections is based primarily on antigen detection and culture (**Fig. 6-109**). 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,

Box 6-23. 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

while negative specimens can be submitted for culture.³²² Enzyme immunoassay includes methods that offer speed and convenience at the point of care. However, they are less sensitive than standard virologic methods, which still must 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. Recently, PCR methodology has 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.^{323–327} These systems have the potential to more rapidly and accurately diagnose acute infections and also may allow the study of complex coinfections and the active monitoring of outbreaks of influenza and other viral illnesses.³²⁸ Current molecular diagnostic approaches are more technically demanding than culture, antigen detection by immunofluorescence, or enzyme immunoassay; and few are approved by the U.S. Food and Drug Administration (FDA). At present, isolation still remains useful for many respiratory viral infections, and antigen detection methods offer the speed and immediacy of reporting that many molecular methods lack.

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 (1–2 days) and antigen detection.³¹⁹ It is important therefore to preserve a portion of tissue from a bronchial or transbronchial biopsy or thoracotomy specimen in viral transport medium, especially in the immunocompromised patient, who may not have had BAL fluid submitted for culture.

Viral serologic testing commonly has been used for diagnosis but may be the least sensitive approach. A positive serodiagnosis typically is based on a fourfold rise in titer between acute and convalescent sera and therefore cannot be achieved by this means in the acutely ill

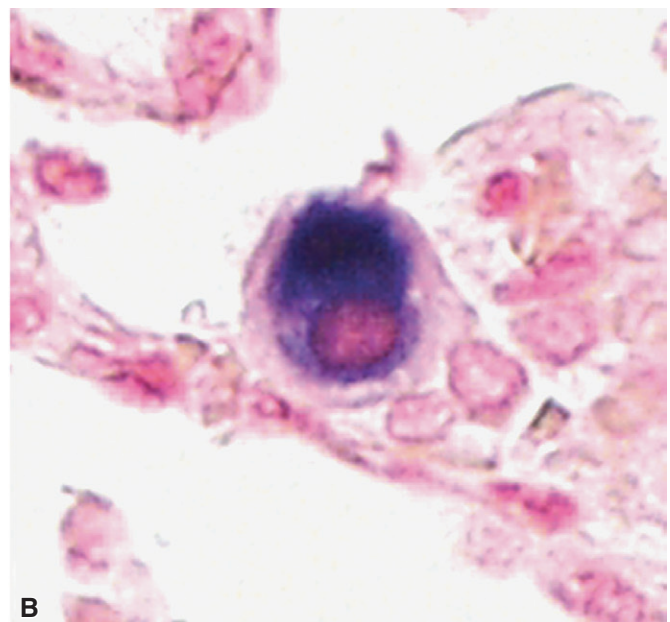
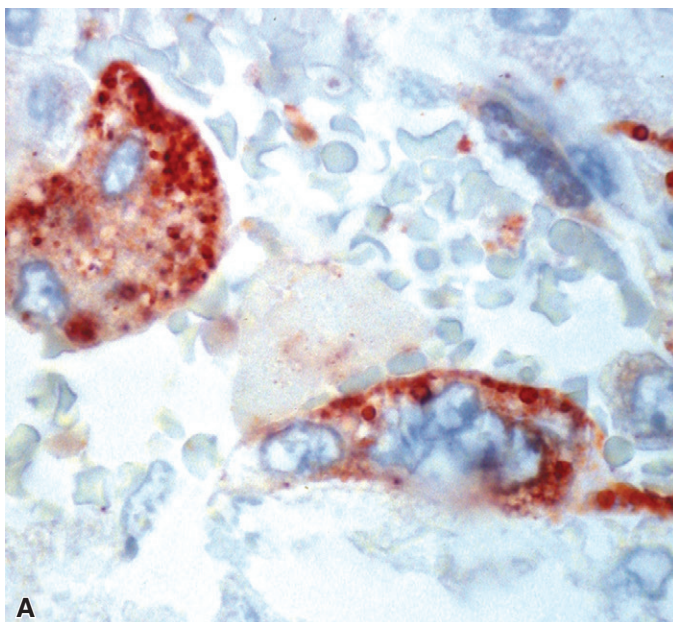


Figure 6-108. **A**, Respiratory syncytial virus cytoplasmic inclusions detected by immunohistochemical staining. **B**, Cytomegalovirus-infected cell with cytoplasmic inclusions detected by in situ hybridization. (Courtesy of R. V. Lloyd, MD, Rochester, MN.)

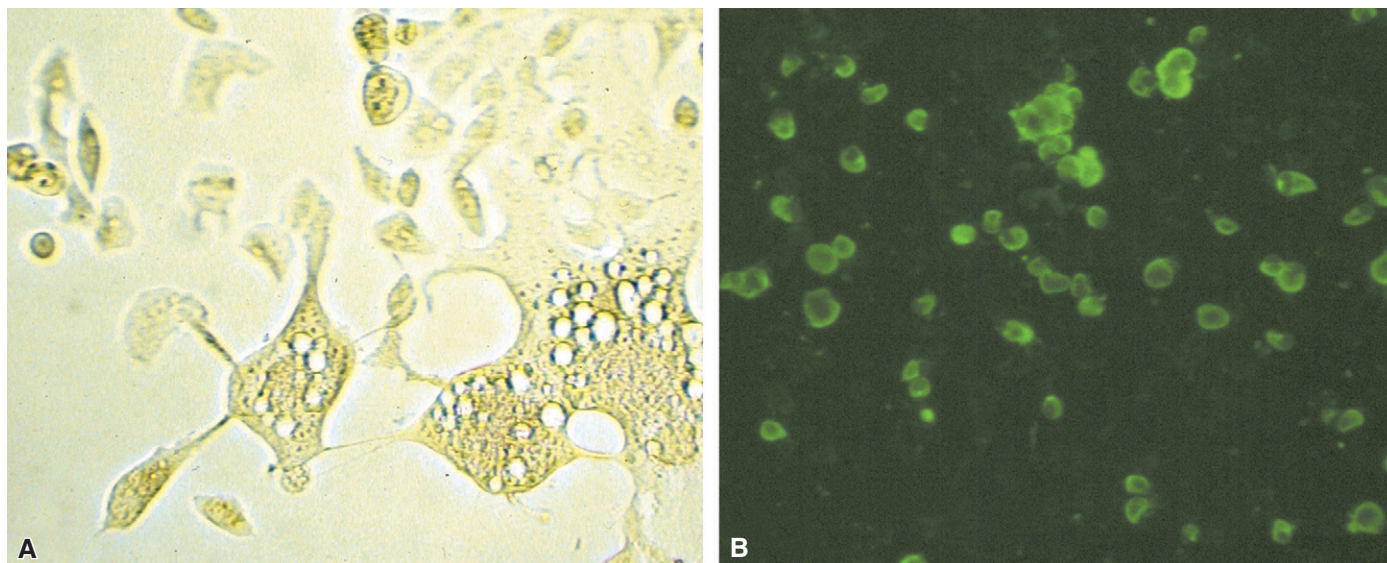


Figure 6-109. Respiratory syncytial virus (RSV) infection. **A**, RSV cytopathic effect in tissue culture. **B**, RSV antigen in nasopharyngeal swab specimen detected by direct immunofluorescence microscopy.

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.³²⁹

A case also can be made for the benefit of CMV serologic testing for assessment of the antibody status of organ donors and recipients for predicting risk of post-transplantation 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).³³⁰ These assays may eventually replace culture of BAL fluid for surveillance of CMV infection in such patients.³³¹ 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.³³²

Differential Diagnosis

A synopsis of the key morphologic and microbiologic features of the viral pneumonias is presented in Table 6-12. 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, herpes simplex virus, 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 also can be simulated by aggregated altered protein and particulate matter.

Table 6-12 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

Table 6-12 Viral Pneumonias: Summary of Pathologic Findings—cont'd

Assessment Component	Findings	Assessment Component	Findings
Measles Virus—cont'd		Herpesvirus—cont'd	
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	Microbiology	Antigen detection by immunofluorescence Culture on diploid fibroblasts produces characteristic cytopathic effect, sometimes within 24 hours Serologic testing less useful
Hantavirus		Varicella-Zoster Virus	
Surgical pathology	Pulmonary edema pattern with variable fibrin deposits Immunoblast-like cells in vascular spaces Confirm by immunohistochemistry	Surgical pathology	Miliary necroinflammatory lesions; calcified nodules in healed phase
Cytopathology	Noncytopathic	Cytopathology	Intranuclear Cowdry A inclusions sparse and less well-defined than with herpes simplex
Microbiology	Serology: Hantavirus-specific IgM or detection of specific RNA by PCR assay in peripheral blood leukocytes	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
Adenovirus		Cytomegalovirus	
Surgical pathology	Diffuse alveolar damage with or without necrotizing bronchiolitis and/or pneumonitis with necrosis and karyorrhexis	Surgical pathology	Minimal changes with scattered cytomegalic cells; miliary necroinflammatory lesions; interstitial pneumonitis
Cytopathology	Early Cowdry A intranuclear inclusions, later smudge cell Reactive and reparative-type atypia in background	Cytopathology	Large “owl eye” Cowdry A inclusions with halo; cytoplasmic inclusions stained with GMS
Microbiology	Antigen detection by EIA and DFA Culture on continuous epithelial cell lines produces characteristic grape-like clustered cytopathic effect	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
Herpesvirus		Epstein-Barr Virus	
Surgical pathology	Tracheobronchitis; diffuse alveolar damage; miliary necroinflammatory lesions	Surgical pathology	Polymorphous lymphoid interstitial pneumonitis Confirm by in situ hybridization
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	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-eosin; IgG, IgM, immunoglobulins G and M; Pap, Papanicolaou; PCR, polymerase chain reaction; VCA, viral capsid antigen.

Parasitic Infections

It is estimated that approximately 300 species of helminth worms and 70 species of protozoa have been acquired by humans during our short history on Earth.³³³ Most of these are rare, but approximately 90 are relatively common, and some of them have been found in the lung.³³⁴ A world made smaller by globalization and travel to endemic areas, in combination with the emergence (and re-emergence) of parasitic pathogens in immunocompromised patients, guarantees that pathologists will be increasingly challenged by diagnostic problems associated with these organisms.³³⁵ Nevertheless, pulmonary parasitic infections are relatively rare and continue to be exotic diseases for surgical pathologists and cytopathologists in the United States.

Etiologic Agents

Several parasite species migrate through the lungs as part of their normal life cycle, but few preferentially infect the human lung.³³⁶ 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 etiologic listing in [Box 6-24](#) is selective, based on the more common pathogens known to be associated with pulmonary involvement. The reader is encouraged to consult the References for a more comprehensive compilation.

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 6-25](#) 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.

Box 6-24. 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 6-25. Histopathologic Patterns in Parasitic Lung Injury

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

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.³³⁴ 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 history can help to further narrow the scope of the differential diagnosis. 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.³³⁷

Toxoplasmosis

Toxoplasma 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 also may 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.³³⁸ Free forms (crescent-shaped tachyzoites) and cysts may be identified (Fig. 6-110). Pseudocysts packed with tachyzoites can be distinguished from true cysts with bradyzoites by staining of the latter with PAS and GMS.³³⁹

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

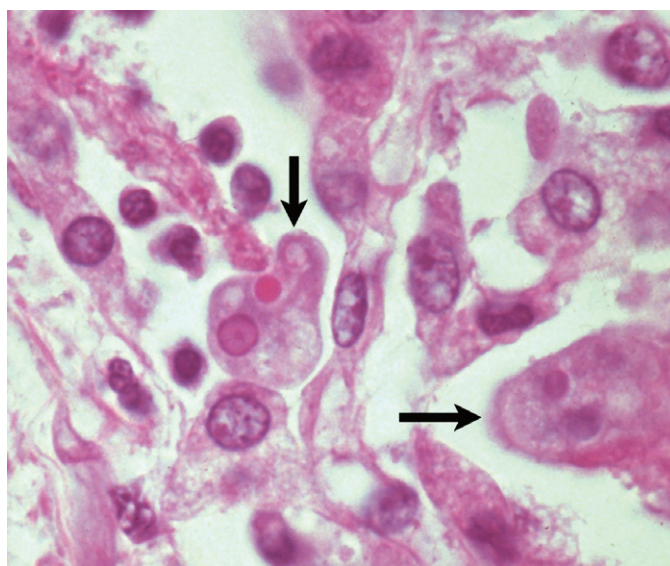


Figure 6-111. Amoebic trophozoite in lung tissue (arrows). Note delicate marginal nuclear chromatin with small central karyosome and small red blood cell in cytoplasm. (Courtesy of Ronald Neafi, Armed Forces Institute of Pathology, Washington, DC.)

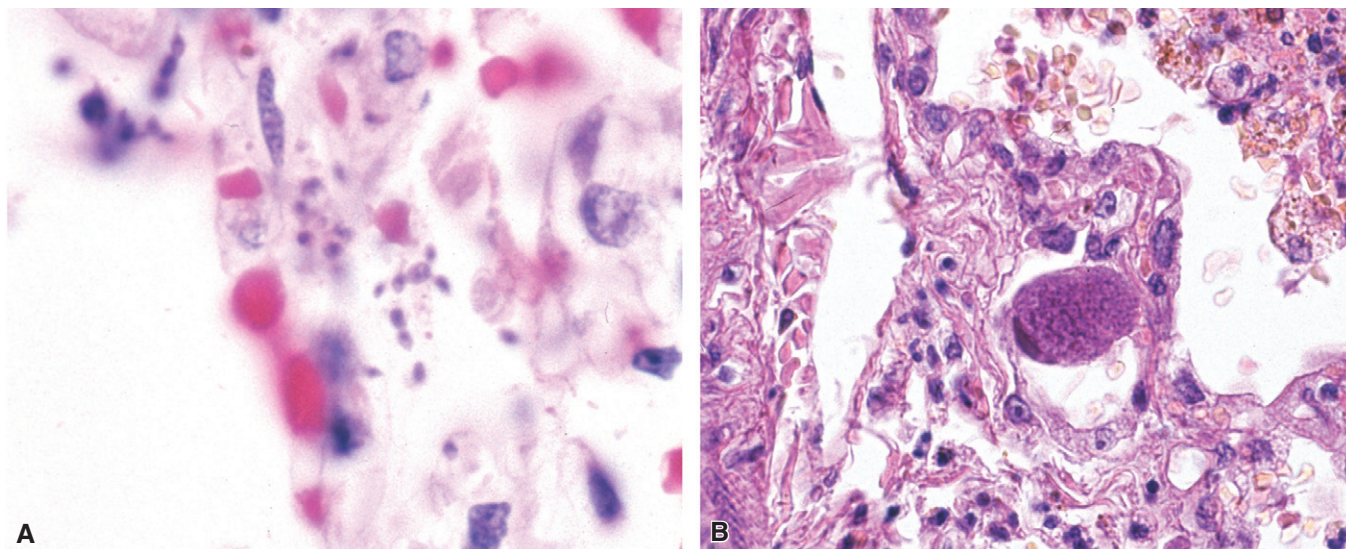


Figure 6-110. Toxoplasmosis. **A,** Tachyzoites. **B,** Pseudocysts packed with tachyzoites.

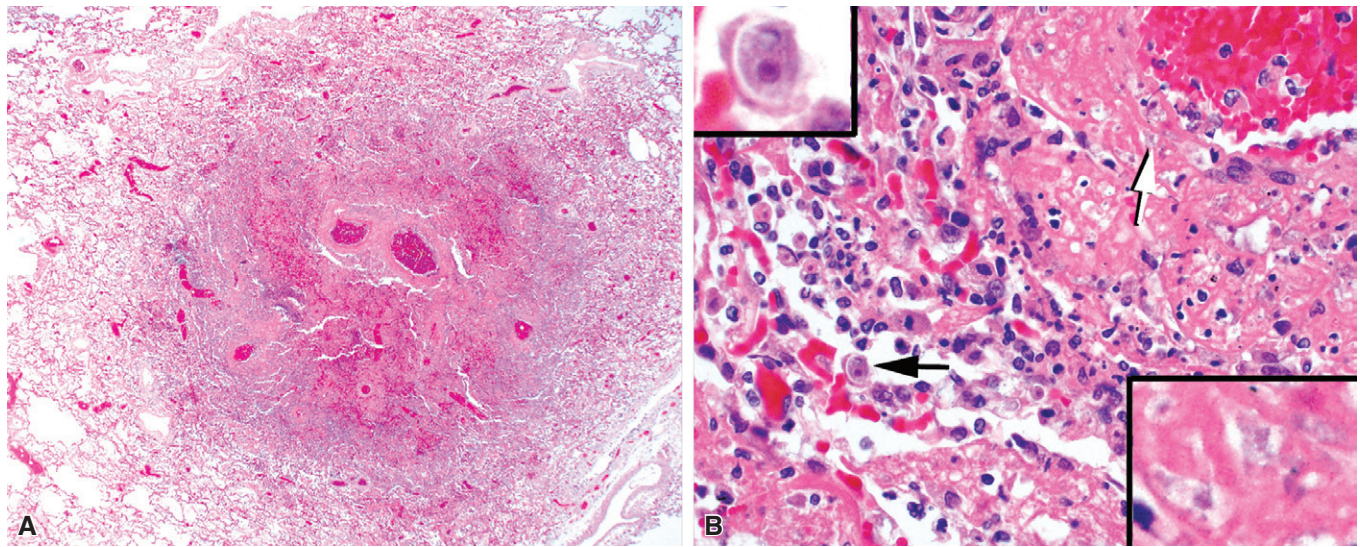


Figure 6-112. Free-living ameba in lung tissue from an immunocompromised patient. **A**, Necroinflammatory nodule. **B**, Encysted form, black arrow and left upper inset; trophozoite, white arrow and right lower inset.

from bacterial abscess where neutrophils are dominant—may be seen, most often in the right lower lobe adjacent to the liver.^{340,341} Trophozoites can be best seen at the margin of viable tissue (Fig. 6-111). They resemble histiocytes but usually are larger, with a lower nucleocytoplasmic ratio. A tiny central karyosome within a round nucleus having vesicular chromatin is characteristic.^{342,343} Bronchial fistula formation and empyema can occur as complications; amebae 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. 6-112) may occur in certain epidemiologic situations, especially those involving compromised immune status.³⁴⁴

Cryptosporidiosis

Ten species of the intracellular coccidian protozoa are currently recognized, but one of them, *Cryptosporidium parvum*, causes most human infections.³⁴⁵ 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.³⁴⁵ 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.³⁴⁶ In H&E sections, cryptosporidia appear as small (4–6 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 usually is present in the submucosa. Recognition of this disease in patients with AIDS can be challenging because the findings may be subtle and coexistent pneumonias caused by other pathogens can divert the pathologist's attention.

Microsporidiosis

The microsporidia are obligate intracellular, spore-forming protozoa. More than 140 genera and 1200 species are recognized, but only seven genera and a few species have been confirmed as human pathogens.³⁴⁷ They are opportunists that have recently emerged in severely immunocompromised patients, especially people with AIDS and transplant recipients. They are found less often in persons with intact immunity. 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 infection or disease in other sites, especially the biliary tract.³⁴⁸ The predominant pathologic changes are in the airways, which show a mixed inflammatory cell infiltrate of mononuclear and polymorphonuclear leukocytes.³⁴⁹ The organisms are found within vacuoles in the apical portion of epithelial cells lining the airways. They appear as very small (1–1.5 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 often is 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.³⁵⁰

Leishmaniasis

Leishmaniasis (*Leishmania donovani* infection) is transmitted to humans by several species of the *Phlebotomus* sand fly.³⁵¹ Pulmonary leishmaniasis has been reported in HIV-infected patients and transplant recipients.³³⁴ The organisms (*L. donovani* amastigotes) can be found in the alveoli and alveolar septa and may be recovered in BAL fluid from these patients.³⁵² They also can be found in bronchoscopic biopsies. (Fig. 6-113). Serologic testing for leishmaniasis has been suggested as part of the pre-transplantation workup in endemic areas.³⁵³ A rapid PCR-amplified diagnostic method has been described.³⁵⁴

Dirofilariasis

The zoonosis caused by *Dirofilaria immitis*, a parasite of dogs and other mammals, is transmitted by mosquitos and black flies to humans. 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 periphery (Fig. 6-114) that may be visualized on a positron emission tomography (PET) scan.^{355–357} 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 (Fig. 6-115). 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.³⁵⁸

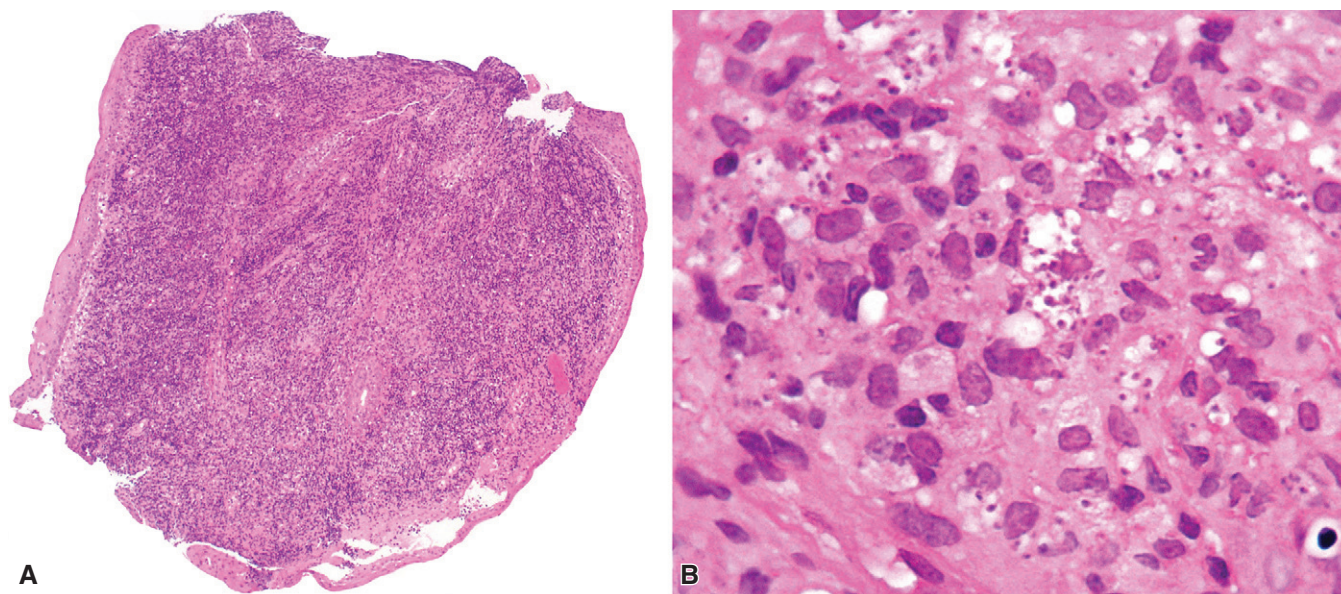


Figure 6-113. *Leishmania donovani* in bronchoscopic biopsy specimens obtained from a North African immigrant to Sicily. **A**, Lower-power view of cellular infiltrate. **B**, High-power view of dot-like organisms. (Courtesy of Dr. Francesca Guddo, Palermo, Italy.)

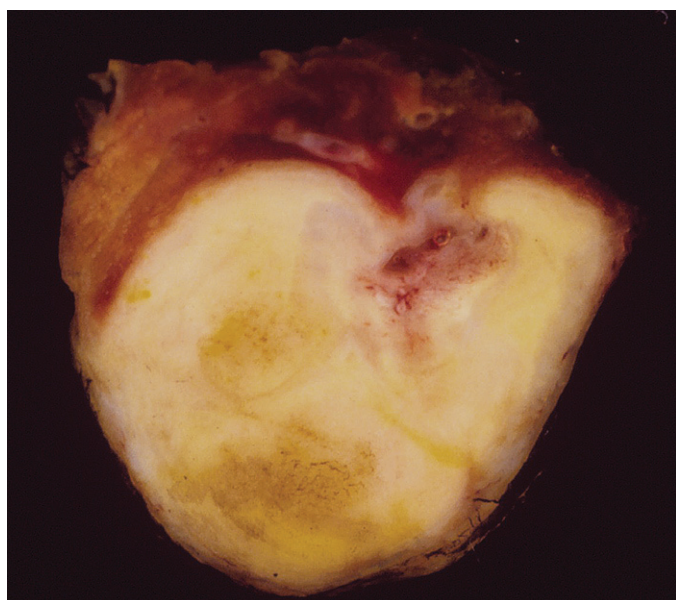


Figure 6-114. Dirofilarial nodule, gross specimen.

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. Rabditiform larvae of the nematode *Strongyloides stercoralis*, after hatching from ingested eggs, 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. 6-116), they provoke hemorrhage and inflammation.³⁵⁹ 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 rabditiform larvae. Larvae identified in the sputum indicate

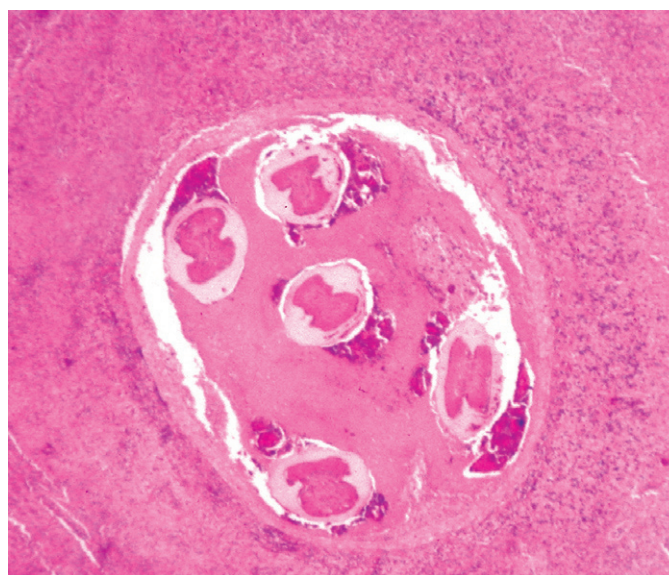


Figure 6-115. Dirofilarial nodule, with worm remnants in organizing thrombosed vessel.

hyperinfection.³⁶⁰ 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 area harboring pathogens 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. In the lung, unilocular slow-growing cysts are produced by *Echinococcus granulosus*.³⁶¹ *Echinococcus multilocularis* proliferates by budding, producing an alveolar pattern

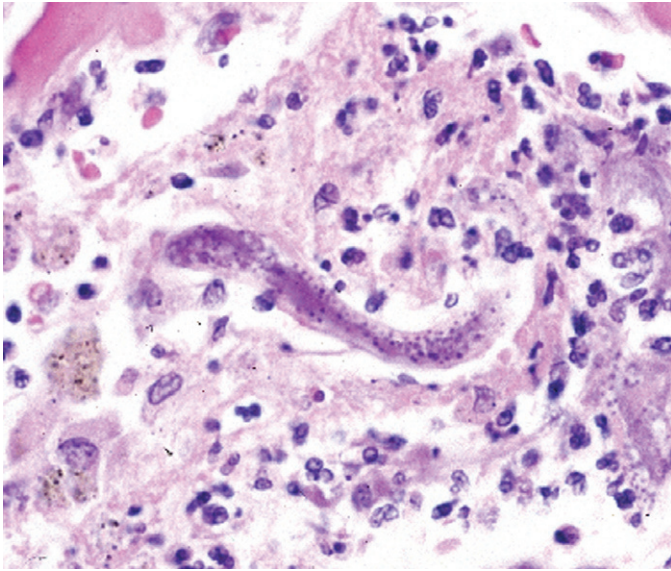


Figure 6-116. Filariform larva of *Strongyloides stercoralis* penetrating into alveolar space with associated inflammation.

of microvesicles.³⁴³ The cyst of *E. granulosus* has a trilayered membrane (Fig. 6-117A) with an outer fibrous, middle-laminated hyaline, and inner germinal layer that gives rise to brood capsules containing infective protoscolices with hooklets and suckers (see Fig. 6-117B). The layers usually become separated in tissue, with the outer fibrous layer containing chronic inflammatory cells forming 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.³⁶²

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. Most cases worldwide are due to *P. westermani* but several other species exist in Asia, Africa, South and Latin America. In the United States, infections due to *P. kellicotti* have been reported.³³⁶ 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. 6-118A). 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 (see Fig. 6-118B) that progress to scars.³⁶³ 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.³³⁶

Schistosomiasis

The public health burden of schistosomiasis is enormous: This parasitic infection affects 200 million people in 74 countries while continuing to expand its geographic range.^{364,365} 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 pneumonitis and a Loeffler-like eosinophilic pneumonia may be seen in this setting.^{364,366} 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

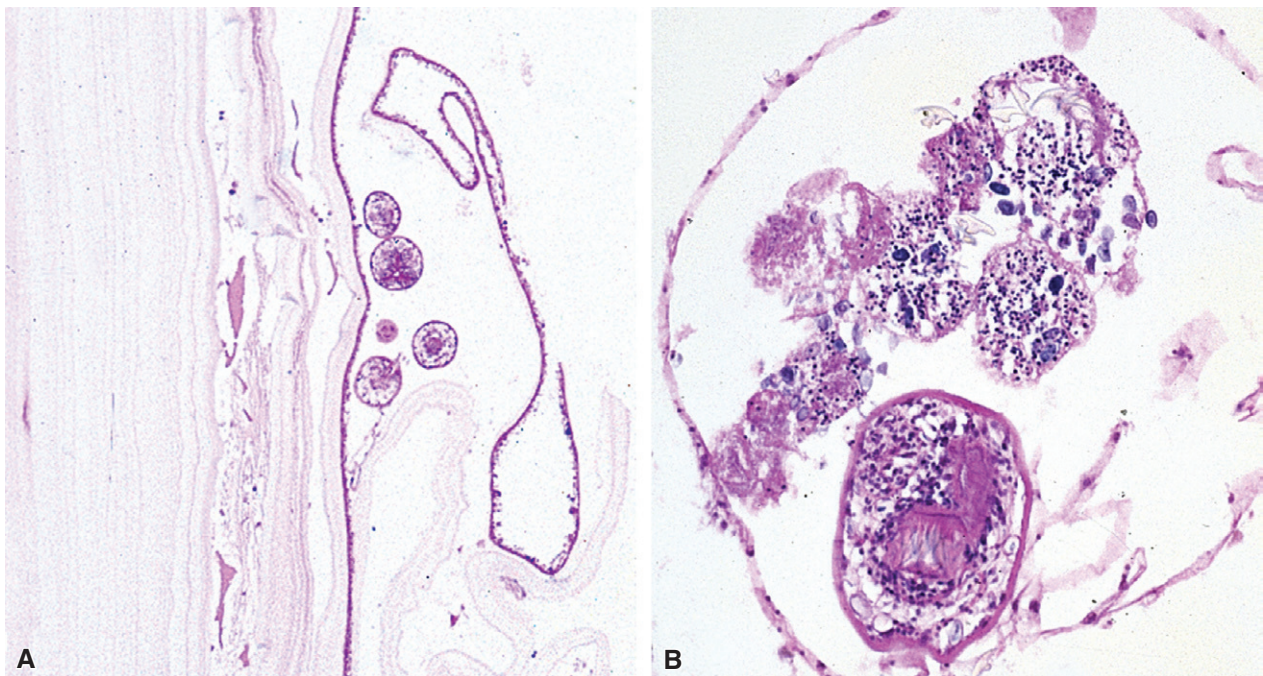


Figure 6-117. *Echinococcus granulosus*. **A**, Cyst with trilayered membrane. **B**, Brood capsules.

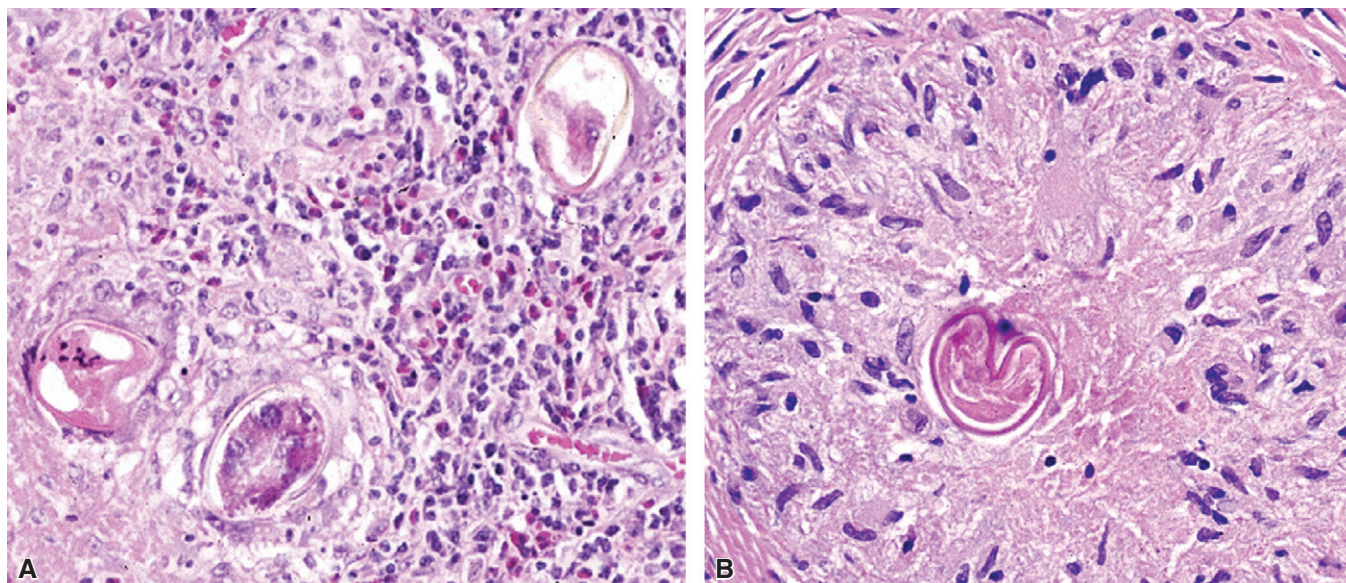


Figure 6-118. **A,** *Paragonimus westermani* with yellowish refractile eggs in eosinophil-rich exudates. **B,** Distorted egg of *Paragonimus kellicotti* in granuloma.

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.^{367,368} When the endarteritis is accompanied by angiomatoid changes, the lesion is considered pathognomonic for pulmonary schistosomiasis.³³⁶

Eggs typically are surrounded by epithelioid cells and collagen (Fig. 6-119). 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.

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 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.³⁶⁹

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 6-26.^{352,355-357,362,370-381}

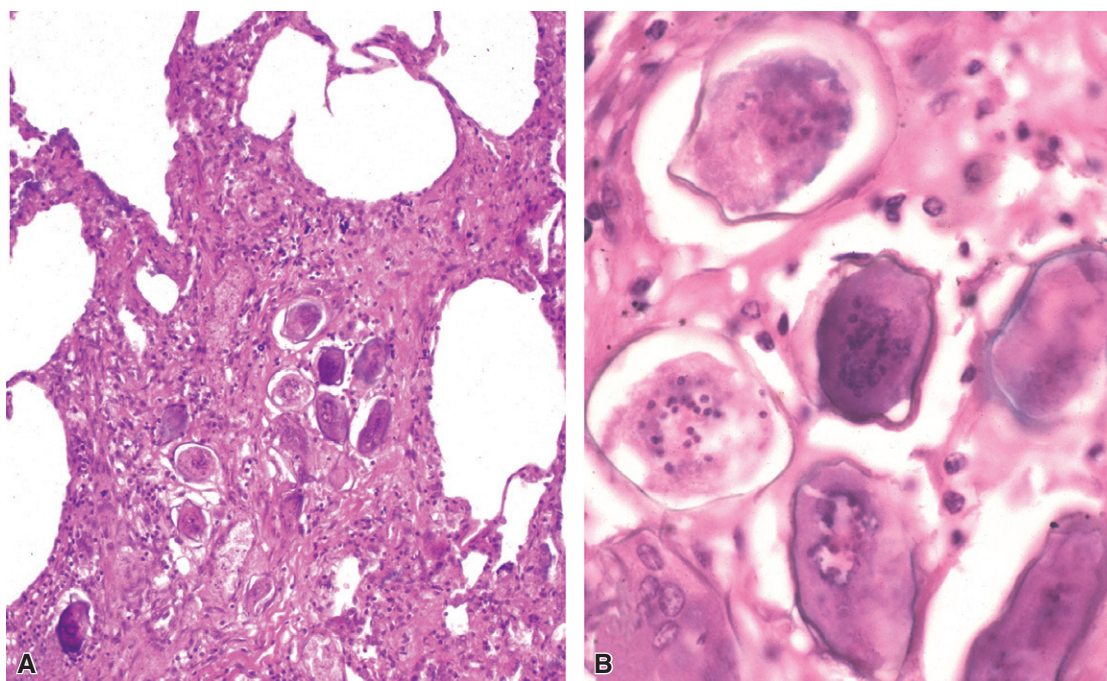


Figure 6-119. **A,** Schistosome eggs in lung parenchyma. **B,** Eggs of *Schistosoma japonicum*. (**A** and **B,** Courtesy of Ronald Neafi, Armed Forces Institute of Pathology, Washington, DC.)

Box 6-26. Parasites Reported in Respiratory Cytology Specimens

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

Commonly cited in textbooks and reviews is the finding of *Strongyloides stercoralis* larvae in expectorated sputum or bronchial washings of patients with hyperinfections (Fig. 6-120). Also common are reports of *Echinococcus* protoscolices and hooklets in needle aspirates from patients with pleuropulmonary disease.^{43,44} Use of large-bore and cutting needle biopsies traditionally has been contraindicated in the setting of suspected *Echinococcus* infections; reports of success with fine-needle aspiration, without untoward reactions, suggest that this latter technique is a relatively safe procedure in which the benefits outweigh the risks.³⁷²

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 usually are better defined in cytologic preparations than in tissue biopsy specimens, provided that obscuring background debris is limited and that 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.

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.³³⁵ As in the case for cytologic samples, these specimens often reveal the characteristic micro-anatomic 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.³⁴¹ 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.³³⁵ 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 commercially available, and diagnostic services are available from the CDC and other reference laboratories.³⁸²

With protozoal infections, serologic testing is especially useful for the diagnosis of toxoplasmosis. Several commercial kits are available for detection of immunoglobulin G (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.³⁸³ Real-time PCR analysis has been used for the diagnosis of toxoplasmosis in the immunocompromised patient.^{384,385} 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

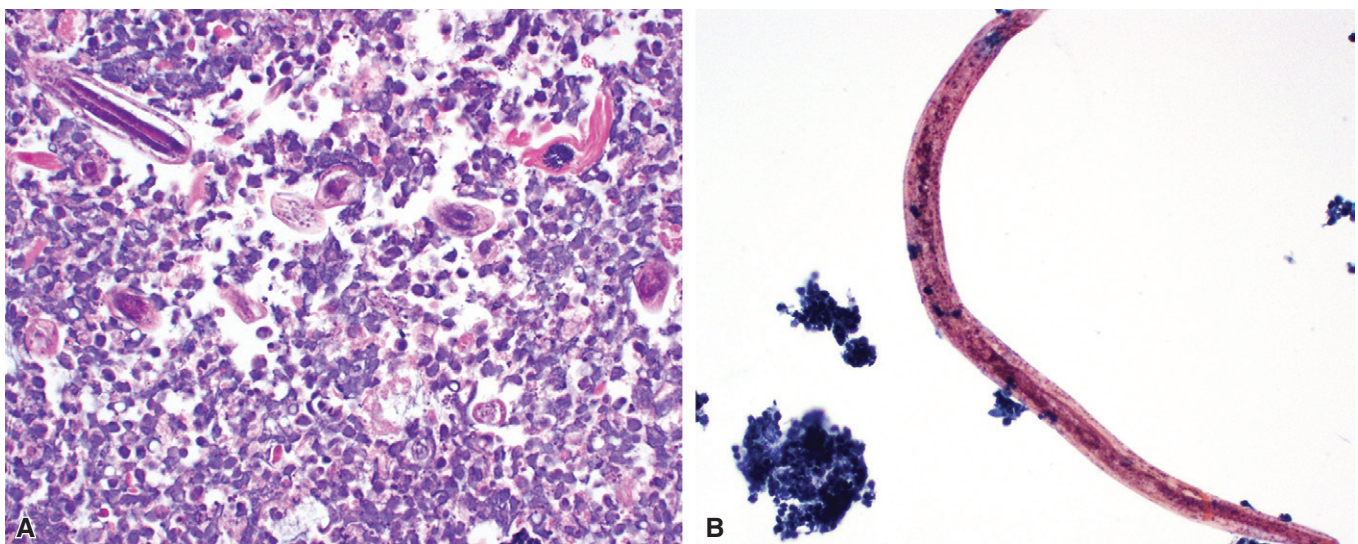


Figure 6-120. *Strongyloides stercoralis* larvae in bronchial washing. **A**, Larval fragments in cell block. **B**, ThinPrep smear.

to invasive disease may be the use of serologic testing, antigen detection, and PCR methods, in various combinations.³⁸⁶ For 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 at this time. For the helminths, serodiagnosis is possible for *Echinococcus*, *Paragonimus*,

Strongyloides, and *Schistosoma* species using enzyme immunoassay methods, which have fair sensitivity and specificity.^{334,382} 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 6-13. In the absence of eggs,

Table 6-13. Parasitic Pneumonias: Summary of Pathologic Findings

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

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.³⁸⁷ Acute and chronic forms of eosinophilic pneumonia, as previously emphasized, have a varied etiology that includes parasitic infections.³⁸⁸ False-positive morphologic diagnosis of a parasitic infection may be based on presence of objects resembling parasites^{337,389} such as lentils in aspiration pneumonia, pollen grains, or Liesegang rings. These ring-like structures can simulate various types of nematodes.³⁹⁰ Careful attention to the microanatomy of an apparent foreign body and comparison with parasites illustrated in atlases often can resolve such diagnostic dilemmas. Some cases, however, may require referral to pathologists with specialized training and experience in parasitic diseases.

Self-assessment questions related to this chapter can be found online on the Expert Consult site for this title.

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