Infectious Disease Pathology

Gary W. Procop[1] and Michael Wilson[2]

[1]Division of Pathology and Laboratory Medicine, Department of Clinical Pathology, Section of Clinical Microbiology, Cleveland Clinic Foundation, Cleveland; [2]Department of Pathology and Laboratory Services, Denver Health, and Department of Pathology, University of Colorado School of Medicine, Denver

The anatomic pathologist performs an important role in the diagnosis or exclusion of infectious diseases. The morphologic interpretation of biopsies and cytologic preparations allows for the definitive establishment or exclusion of a wide variety of diseases. Once the pathologist has determined that a disease is likely to be due to an infection and has characterized the inflammatory response, associated microorganisms or viral-associated cytopathic effects should be recorded. Although some microorganisms or their cytopathic effects may be clearly visible on routine hematoxylin and eosin-stained sections, additional histochemical stains are often needed for their complete characterization. Highly specific molecular techniques, such as immunohistochemistry, in situ hybridization, and nucleic acid amplification, may be needed in certain instances to establish the diagnosis of infection. Through appropriate morphologic diagnoses and interlaboratory communication and collaboration, the anatomic pathologist contributes greatly to the diagnosis and treatment of infectious diseases.

Emerging and reemerging infectious diseases and the threat of bioterrorism call attention to the growing importance of the ability of the anatomic pathologist to recognize infectious diseases [1–5]. Most of the developed world and much of the developing world have been spared from devastating diseases, such as smallpox, diphtheria, and paralytic poliomyelitis, through vaccination and worldwide eradication efforts [6–11]. Nevertheless, new pathogens continuously emerge, old adversaries reappear when suitable conditions such as war or famine exist, and microbes continue to develop resistance, even to the new broad-spectrum antimicrobial agents [9, 12–16]. Furthermore, global environmental changes, such as human encroachment into previously wild ecosystems, deforestation, damming of river systems, expansion of irrigation systems, and possibly changes induced by global warming, are likely to alter the current patterns of infectious diseases, particularly vector-associated or parasitic diseases [5, 17–19]. These factors, combined with the ease of long-distance travel, make it certain that clinicians and laboratory workers alike will encounter diseases exotic to their routine practices [20, 21]. Closer to home, and of more immediate concern, is the diagnosis and treatment of opportunistic infections that affect the ever-growing population of patients with iatrogenic, inherited, or acquired immunodeficiencies [22–24]. In this article, we review methods used in the pathologic diagnosis of infections, emphasizing the critical role of the anatomic pathologist in the diagnosis of both routine and emerging infectious diseases [1–5, 25–30].

The diagnosis of complex diseases, infectious or otherwise, requires the collaborative efforts of clinicians, radiologists, and pathologists. The differential diagnosis generated at the bedside through patient history and physical examination is narrowed through consultation and thoughtfully ordered radiographic and laboratory studies. The anatomic pathologist, by providing the morphologic interpretation of biopsies and cytologic preparations, is an important member of the diagnostic team. Histopathologic and cytopathologic studies often allow for the definitive establishment or exclusion of a wide variety of diseases. In some instances, a microorganism that fails to grow in culture may be detected by means of histopathologic examination of tissue samples or cytopathologic examination of specimens of body fluids or aspirates. Conversely, cultures may yield the causative microorganisms from tissues that demonstrate an inflammatory response strongly indicative of infection, but in which microorganisms are not identified in histologic sections [31].

Once the pathologist has characterized the inflammatory response, associated microorganisms or viral-associated cytopathic effects should be recorded [26–30]. Although some microorganisms or their cytopathic effects may be clearly visible in

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Reprints or correspondence: Dr. Gary W. Procop, Clinical Microbiology/L40, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195 (procopg@ccf.org).

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routine hematoxylin and eosin (HE)–stained sections, additional histochemical stains are often needed for the detection or complete characterization of microorganisms in tissue sections (table 1) [26–30, 32]. The sensitivity of histopathologic testing for the detection of microorganisms is probably similar to that of culture, but some have suggested otherwise [33–36]. In terms of practicality, however, these are complementary methods, and both play a critical role in the diagnosis and optimal treatment of infections. Finally, highly specific molecular techniques, such as immunohistochemistry, in situ hybridization, and nucleic acid amplification, may be needed to establish the diagnosis of certain infections (table 1) [28, 37–43].

ROLE OF THE ANATOMIC PATHOLOGIST IN THE DIAGNOSIS OF INFECTIOUS DISEASES

The anatomic pathologist performs an important role in the diagnosis or exclusion of infectious diseases [1–4, 26–29]. The first task of the anatomic pathologist is to examine the specimen to determine whether normal or abnormal histology (histopathology) is present. If abnormalities are seen, the pathologist then characterizes the disease that is present. Histologic and cytologic criteria are used to separate diseases into a variety of categories, such as reactive or reparative, dysplastic and neoplastic diseases, and inflammatory conditions, which include infectious diseases. The separation of disease into such categories has both therapeutic and prognostic implications.

Carcinoma, for example, may be suspected in a patient with a solitary lung nodule and a history of long-term smoking. However, if the excisional biopsy demonstrates necrotizing granulomatous inflammation, the diagnosis of a malignant tumor is excluded and the search for an inflammatory or infectious etiology would begin. It is not uncommon for the biopsy of a pulmonary nodule, radiographically suspected to represent a malignancy, to reveal an infectious process, such as a coccidioma, a tuberculoma, or remnants of the dog heartworm *Dirofilaria immitis* [36, 44, 45]. In such instances, the diagnosis of an infectious disease may rely entirely on the anatomic pathologist, because, all too frequently, samples are not obtained for culture when the diagnosis of malignancy is suspected. The converse also occurs: pulmonary nodules suspected to be coccidiomas in patients from the southwestern United States, particularly from those with a serologic response to *Coccidioides immitis,* may be revealed to be carcinomas.

In emergent situations, the histopathologist and cytopathologist may provide a rapid morphologic diagnosis by use of frozen-section analysis and fine-needle aspiration cytology, respectively. The frozen section allows for a rapid histologic assessment of tissue without the delays of fixation and routine processing. Situations that warrant frozen-section analysis for infectious agents often involve rapidly progressive diseases that require emergent surgical debridement, such as rhinocerebral zygomycosis or necrotizing fasciitis. In such diseases, the frozen section is useful not only for diagnosis, but also for the assessment of the resection margins for acute inflammation, tissue viability, and the absence of microorganisms.

Fine-needle aspiration may be used for the rapid examination of aspirate specimens, including aspirate specimens of infectious lesions [46–50]. The pathologist or clinician may aspirate palpable lesions, whereas radiologic guidance is necessary for deeper lesions. An advantage of CT-guided aspiration of deep-seated lesions is that general anesthesia is not necessary. Intraoperative cytologic diagnoses and rapidly stained touch preparations have also been found to be useful [51, 52]. The presence of a pathologist at the aspiration procedure is useful for the immediate assessment of the adequacy of the specimen, often determined by use of air-dried Diff-Quik–stained smears (Dade Behring). This onsite quality assessment reduces the number of nondiagnostic studies, because additional aspirations may be performed if diagnostic material was not obtained. The onsite pathologist may also provide a provisional or definitive diagnosis on the basis of the air-dried smears and can help direct additional studies such as culture [47, 50]. Because the amount of aspirated material may be limited, cytology-based prioritization of cultures, as determined by the type of inflammation present, may be useful [47, 50].

Collaboration between the anatomic pathologist and the microbiologist is necessary to provide optimal patient care, reduce waste, and prevent medical errors [29–30, 53]. The anatomic diagnosis of disease may be used to clarify complex microbiologic cultures, whereas cultures may be used to reveal the identity of microorganisms seen in tissue sections. Information regarding the presence of microorganisms in tissue sections helps the microbiologist to provide clinically relevant information and to minimize misleading reports by not attributing undue significance to contaminants or normal flora. For example, if microorganisms are cultured from specimens of a body site at which they are normal or transient flora, it may not be possible for the microbiologist or the clinician to determine the significance of these microorganisms. If, however, there is a corresponding biopsy that demonstrates invasive disease caused by a particular bacterium or fungus, the cause of disease becomes more readily apparent, and the culture may be handled accordingly. In addition, the notification of the microbiologist of the presence of microorganisms in tissue sections may allow for the culture of fastidious microorganisms that require special media or growth conditions [53].

HOST INFLAMMATORY RESPONSE

Accurate characterization of inflammatory responses is one of the most challenging and important tasks for pathologists. Al-

Table 1. Histopathologic features of selected infections.

Microorganism	Typical histopathologic features	Histochemical stains	Ancillary methods
Viruses			
HSV, VZV	"Glassy," cleared nuclei, often within multinucleate cells with nuclear molding	HE, Papanicolaou, Giemsa, or Wright stains	IHC, ISH, or NAA confirmation possible and useful for differentiation of HSV and VZV
CMV	Acute to chronic inflammation; often with involvement of the endothelium with ischemia or ulceration; intranuclear (figure 2*K*) and sometimes intracytoplasmic inclusions	HE, Papanicolaou stains	IHC (figure 2*L*), ISH, or NAA confirmation possible
HPV	Koilocytosis	HE, Papanicolaou stains	ISH useful to differentiate high- and low-risk types
Adenovirus	Smudge cells present	HE stain	IHC, ISH, or NAA confirmation possible
Bacteria			
Common bacteria	Neutrophilic; occasionally visible in HE-stained sections (figure 1*G*)	Tissue Gram stain (Brown-Brenn stains gram-positive organisms well; Brown-Hopps is preferred for gram-negative organisms)	ISH methods in development for specific identification of certain pathogens, such as *Legionella*
Helicobacter pylori	Neutrophilic and/or chronic inflammation, often with lymphoid follicles; curved bacteria present in apical, mucous layer of gastric epithelial cells; bacteria are often visible in HE-stained sections (figure 1*H*)	A variety of histochemical stains are useful, including Giemsa (figure 1*I*) and Warthin-Starry	Immunohistochemical methods available but often unnecessary; may be useful for the detection of low numbers of microorganisms, possibly after therapy
Bartonella(in cat-scratch disease)	Nonsuppurative (early) to suppurative (later) granulomas in which clusters of bacilli, some of which are curved, may be found	Warthin-Starry or comparable silver stain	NAA methods have been used successfully in research laboratories
Treponema pallidum	Variable depending on stage of disease: primary, neutrophilic inflammation; secondary, nonnecrotizing granulomas; tertiary, plasma cells/chronic inflammation associated with vasculitis; spiral-shaped bacteria	Warthin-Starry or comparable silver stain	NAA methods have been used successfully in research laboratories
Legionella pneumophila	Neutrophilic; bacillary forms are not discernible on HE or tissue Gram stain	Warthin-Starry or comparable silver stain	IHC, ISH, and NAA methods have been used successfully
Mycobacteria	Associated with necrotizing (figure 1*E*) and nonnecrotizing granulomas, as well as acute inflammation	Gram-positive, beaded, nonbranching bacilli; Ziehl-Neelsen (figure 1*F*) or auramine-rhodamine stain; *M. leprae* does not stain with the Ziehl-Neelsen method but stains with Fite's method	ISH and NNA methods have been developed but are not widely available for the identification of mycobacteria in histologic sections
*Nocardia*and *Actinomyces*	Neutrophilic; granules may be present in actinomycosis or mycetoma caused by *Nocardia;* invasive *Nocardia* is usually not associated with granules	Both are beaded, branching, gram-positive filaments; differentiate with Fite's method: *Nocardia* positive, *Actinomyces* negative	Not available
Fungi			
Candida albicans	Neutrophilic, rarely granulomatous; fungi usually visible	GMS or PAS stain	Usually not necessary; not commercially available
Cryptococcus neoformans	Variable; often neutrophilic, but may be granulomatous in the infrequent cryptococcomas that may occur in immunocompetent hosts	GMS or PAS stain to detect microorganisms; mucicarmine (figure 2*J*) or alcian blue stain to detect capsule	IHC stain is commercially available but usually not necessary; may be useful to confirm "acapsular" variants

(*continued*)

Table 1. (continued)

Microorganism	Typical histopathologic features	Histochemical stains	Ancillary methods
Aspergillus	Neutrophilic-to-granulomatous; tightly associated hyaline septate hyphae with acute angle branching; vascular invasion and thrombosis are common; conidiophores (fruiting bodies) may be seen in *Aspergillus* fungus balls (figure 1*L*).	GMS or PAS stain	IHC and ISH methods developed; useful to differentiate from other hyaline molds, such as *Fusarium* or *Pseudallescheria,* if culture is unavailable
Zygomycetes	Neutrophilic; irregular, broad, ribbonlike hyphae, with rare to no septations apparent; associated with vascular invasion, infarcts, and perineural invasion (figure 2*F*).	PAS stain possibly slightly better than GMS stain	Ancillary methods are not routinely available
Histoplasma capsulatum	Microorganisms often intracellular; granulomatous response in immunocompetent patients; reaction variable in immunocompromised patients and may be predominantly histiocytic, with phagocytosis of small yeast (2–5 μm) with narrow-necked budding	GMS or PAS stain	IHC, ISH, and NAA methods have been developed but are not widely available
Coccidioides immitis	Granulomatous inflammation with necrosis (figure 2*A*) and possibly an eosinophilic infiltrate; spherules visible on HE stain; burst spherules with released endospores may resemble yeast forms (figure 2*B*)	GMS stains endospores, but not outer spherule wall (figure 2*C*); PAS stain is useful; budding is not seen	IHC, ISH, and NAA methods have been developed but are not widely available
Blastomyces dermatitidis	Pyogranulomatous response with yeast with a thick, double-layered cell wall and broad-based budding (figure 2*E*).	GMS or PAS stain	IHC, ISH, and NAA methods have been developed but are not widely available
Parasites			
Intestinal protozoa	Inflammatory response varies from minimal to none with *Giardia lamblia* to neutrophilic with *Entamoeba histolytica*	Trichrome stain may enhance parasites	IHC; immunofluorescence stains are available, but they are usually used on stool specimens
Tissue parasites	Variable, including granulomatous, eosinophilic, and neutrophilic inflammation; migrating larvae of *Strongyloides stercoralis* (figure 2*D*) and eggs of trematodes clearly visible (figure 2*G*)	Giemsa stain useful for the identification of *Leishmania, Trypanosoma cruzi,* and microfilariae	Not routinely available, but some IHC stains have been developed
Blood parasites	*Plasmodium, Babesia,* and microfilaria visible on routine Giemsa-stained thick and thin blood smears	Giemsa stain	NAA methods have been developed
Arthropods	Eosinophilic response; arthropod body parts are visible in HE-stained sections	No special stain required	Not available

NOTE. CMV, cytomegalovirus; GMS, Grocott-Gomori methenamine silver; HE, hematoxylin and eosin; HPV, human papilloma virus; HSV, herpes simplex virus; IHC, immunohistochemistry; ISH, in situ hybridization; NAA, nucleic acid amplification; PAS, periodic acid–Schiff; VZV, varicella-zoster virus.

though inflammation is the hallmark of most infectious diseases, it may also be associated with neoplasia and dysplasia (e.g., inflammatory carcinoma), autoimmune diseases (i.e., rheumatoid nodule; figure 1*D*), allergic responses (i.e., hypersensitivity pneumonitis), metaplastic reactive and reparative changes (i.e., reflux esophagitis and Barrett's metaplasia), and idiopathic disorders such as Wegener's granulomatosis and sarcoidosis. Many of these noninfectious disorders are treated with corticosteroids, the inappropriate use of which may exacerbate infection. Therefore, it is critical for both prognostication and therapy that the anatomic pathologist differentiate, if possible, inflammatory conditions caused by infectious agents from those with noninfectious etiologies.

Once the pathologist has determined that an inflammatory response is likely due to infection, the next step is to determine which etiologic agents are possible causes of the infection. Although the inflammatory response is slightly variable in hosts with intact immune systems, in general, the inflammatory re-

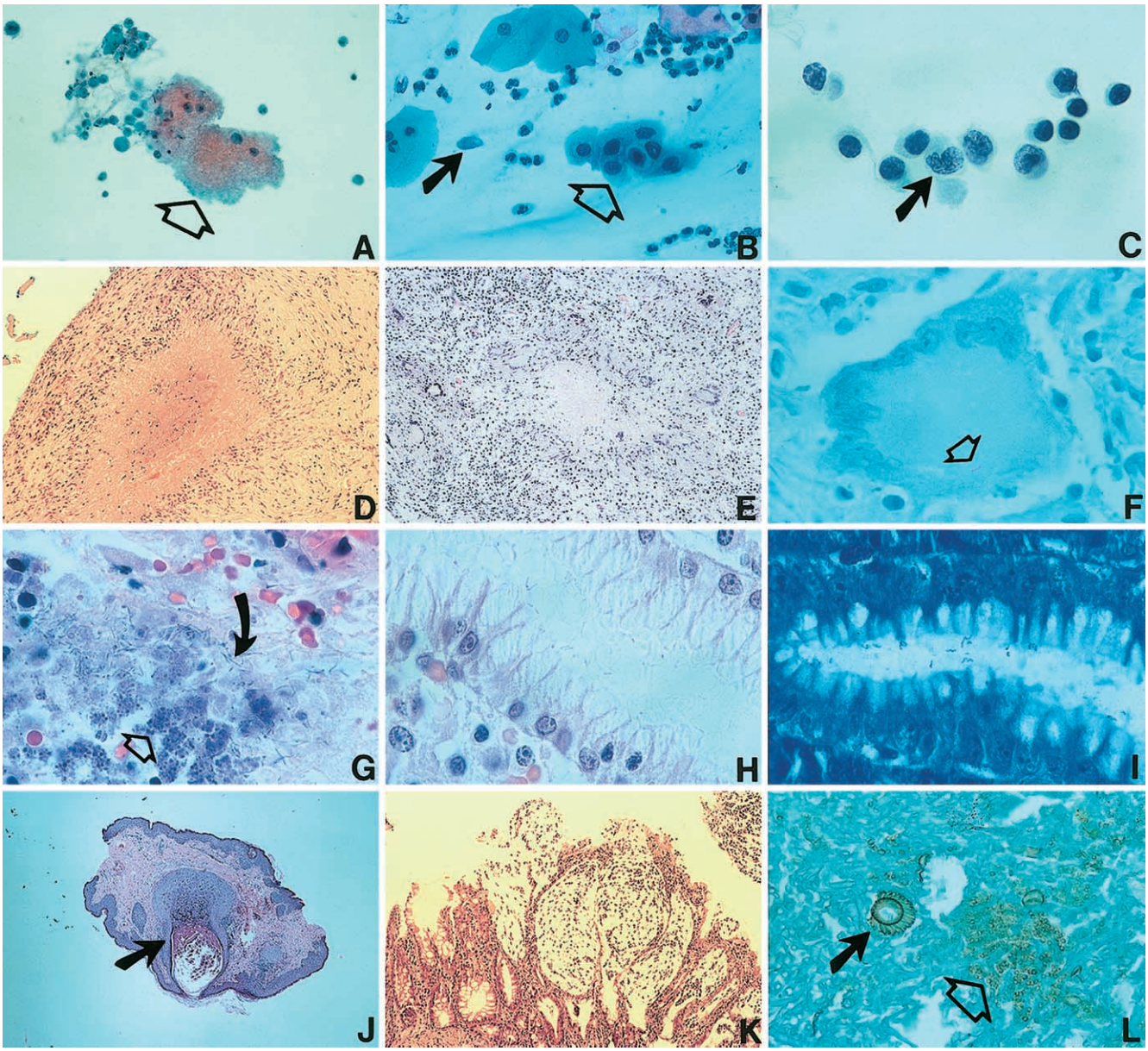

Figure 1. *A,* An amorphous, partially eosinophilic alveolar cast *(arrow)* in a bronchoalveolar lavage specimen that is highly suggestive of *Pneumocystis carinii*. Papanicolaou stain; magnification, ×200. *B,* A cervical smear in which both *Trichomonas vaginalis (solid arrow)* and atypical squamous cells *(open arrow)* are present. Elsewhere in the smear, human papilloma virus–associated moderate dysplasia was seen. Papanicolaou stain; magnification, ×200. *C,* Mollaret cells *(arrow),* activated monocytes with cerebriform nuclei, and lymphocytes in the CSF of a patient with recurrent, aseptic, or Mollaret's meningitis. Papanicolaou stain; magnification, ×1000. *D,* A rheumatoid nodule, consisting of central necrosis surrounded by granulomatous inflammation with palisading histiocytes, which demonstrates that not all inflammation has an infectious etiology. Hematoxylin and eosin (HE) stain; magnification, ×100. *E,* A necrotizing granuloma with giant cells in a patient with tuberculosis. HE stain; magnification, ×100. *F,* A rare acid-fast bacillus *(arrow)* is seen in a giant cell in the biopsy from the patient represented in panel *E.* Ziehl-Neelsen stain; magnification, ×1000. *G,* Cocci *(open arrow)* and bacilli *(closed arrow)* visible in a routine histologic section from a patient with pneumonia caused by methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. HE stain; magnification, ×1000. *H, Helicobacter pylori* visible in the mucus layer in a gastric pit. HE stain; magnification, ×1000. *I, H. pylori* are more readily apparent in a special stained section. Giemsa stain; magnification, ×1000. *J,* The lobular, craterform indentation of keratinocytes characteristic of *Molluscum contagiosum* infection *(arrow).* HE stain; magnification, ×400. *K,* The superficial enterocytes are necrotic and the colonic gland is distended by inflammation, mucus, and necrotic debris in pseudomembranous colitis. HE stain; magnification, ×100. *L,* An aspergillum, the fruiting body of *Aspergillus (solid arrow)* and conidia (spores; *open arrow*) disclose the etiology of this fungus ball. Methenamine silver stain; magnification, ×500.

sponse elicited by a given pathogen occurs in a relatively predictable manner [26, 32]. Therefore, the character of the inflammatory response provides clues to the type of infecting agent and guides the ordering of histochemical stains for microorganisms [32]. Inflammatory responses may consist of a wide variety of permutations of acute, chronic, and/or granulomatous inflammation.

A chronic inflammatory infiltrate, which consists primarily of lymphocytes and plasma cells, is usually nonspecific, such as that which is present in the base of an ulcer or fistula tract. In some instances, however, it may suggest a particular pathogen. For instance, *Helicobacter pylori* is associated with chronic gastritis with lymphoid follicles [54]. In the same way, hepatitis C virus infection is associated with lymphoid follicles in a liver with hepatitis [55, 56]. Follicular hyperplasia within lymph nodes may be seen as a reaction to many infections, but if present with monocytoid B cells and loose aggregates of histiocytes, the findings suggest toxoplasmosis [57]. Finally, syphilitic aortitis is characteristically associated with a lymphocytic and plasma cell periarteritis and endarteritis [58].

Microorganisms associated with a predominantly neutrophilic inflammatory response include most bacteria, especially the pyogenic bacteria *Staphylococcus* and *Streptococcus* species [32]. *Actinomycetes* species also elicit a neutrophilic response, as do invasive protozoal parasites, such as *Entamoeba histolytica* and *Balantidium coli,* and some fungal pathogens, such as *Candida albicans* and *Aspergillus* species [32]. The initial host response to mycobacterial infections is neutrophilic, only later evolving into the more familiar granulomatous response [59, 60].

Granulomas are usually separated into those with and those without caseous necrosis. Other types include pyogranulomatous inflammation, suppurative granulomas, and palisading granulomas. Granulomas without necrosis may be caused by infections with mycobacteria, *Brucella* species, and fungi such as *Histoplasma capsulatum* [32]. The eggs of trematode worms (*Schistosoma* and *Paragonimus* species), which become entrapped in tissues, also generate a granulomatous response that may be nonnecrotizing and rich in eosinophils. These eggs become embedded in dense fibrous tissue later in the course of disease, after much of the inflammatory response has subsided (figure 2*G*) [61]. Nonnecrotizing granulomas are also present in the tissues of patients with sarcoidosis [62]. Granulomas with central caseous necrosis are characteristic of *Mycobacterium tuberculosis,* but are also elicited by other mycobacterial species (figure 1*E*). Necrotizing granulomas may also be caused by systemic fungal pathogens, such as *H. capsulatum* and *Coccidioides immitis* (figure 2*A*). Of interest, an increased number of eosinophils admixed with the granulomatous inflammation may also be seen in response to *C. immitis* infections (figure 2*A*). Granulomatous inflammation, usually mixed with neutrophilic and eosinophilic components, may also be seen in response to the remnants of helminth or trematode worms in tissue sections. A roughly equal amount of neutrophilic and granulomatous inflammation or a pyogranulomatous response may be encountered in infections caused by *Blastomyces dermatitidis* or mycobacteria. Suppurative granulomas, which contain central stellate abscesses, may be seen in the lymph nodes of patients with cat-scratch disease (*Bartonella* species) or in infections caused by *Mycobacterium abscessus* [63, 64]. Finally, although palisading granulomas are characteristically associated with Wegener's granulomatosis, these may also be caused by infectious agents [65, 66].

Some infectious agents have a predilection for vascular invasion, which may result in tissue infarction. These agents include the angiotrophic fungi *Aspergillus* and members of *Zygomycetes. Zygomycetes* has also recently been shown to cause perineural and neural invasion [67] (figure 2*F*). The dog heartworm, *D. immitis,* causes pulmonary infarcts through obstruction of branches of pulmonary artery. *Bartonella* organisms, although not associated with tissue infarcts, are often found in clusters closely associated with the microvasculature and are best visualized in this location with the Warthin-Starry stain [63].

Examination of tissue sections from patients with HIV has challenged every practicing surgical and cytopathologist to recognize the wide variety of atypical inflammatory responses that may be seen in immunocompromised patients. Early in the AIDS epidemic, before the advent of highly active antiretroviral therapy, we learned how patients with a complete lack of T cell–mediated immunity responded to a variety of infectious agents. The histopathologist was then challenged to recognize both typical and atypical inflammatory responses and to become familiar with the associated etiologic agents. Today, the challenge continues. Patients with HIV now receive a variety of antiretroviral drugs that are highly active against the virus, which results in substantial immune reconstitution. Many of these therapies, however, have untoward side effects, so therapeutic compliance becomes an issue and optimal immune reconstitution may not be achieved. On a similar note, patients who are treated for cancer or autoimmune disease or to avert transplant rejection have a variable immune response, depending on the degree of immunosuppression. The pathologist examining tissues from these patients, therefore, must be able to recognize the entire range of the host responses, from entirely normal to a complete lack of T cell immunity, as well as the various infecting agents [68].

TISSUE STAINS

The HE stain is the standard stain for the histopathologic evaluation of tissue sections. Many microorganisms may be detected or definitively identified in HE-stained sections (figure 1*G, H, J,* and *K;* figure 2*D, E, F, G, H,* and *K*). These include

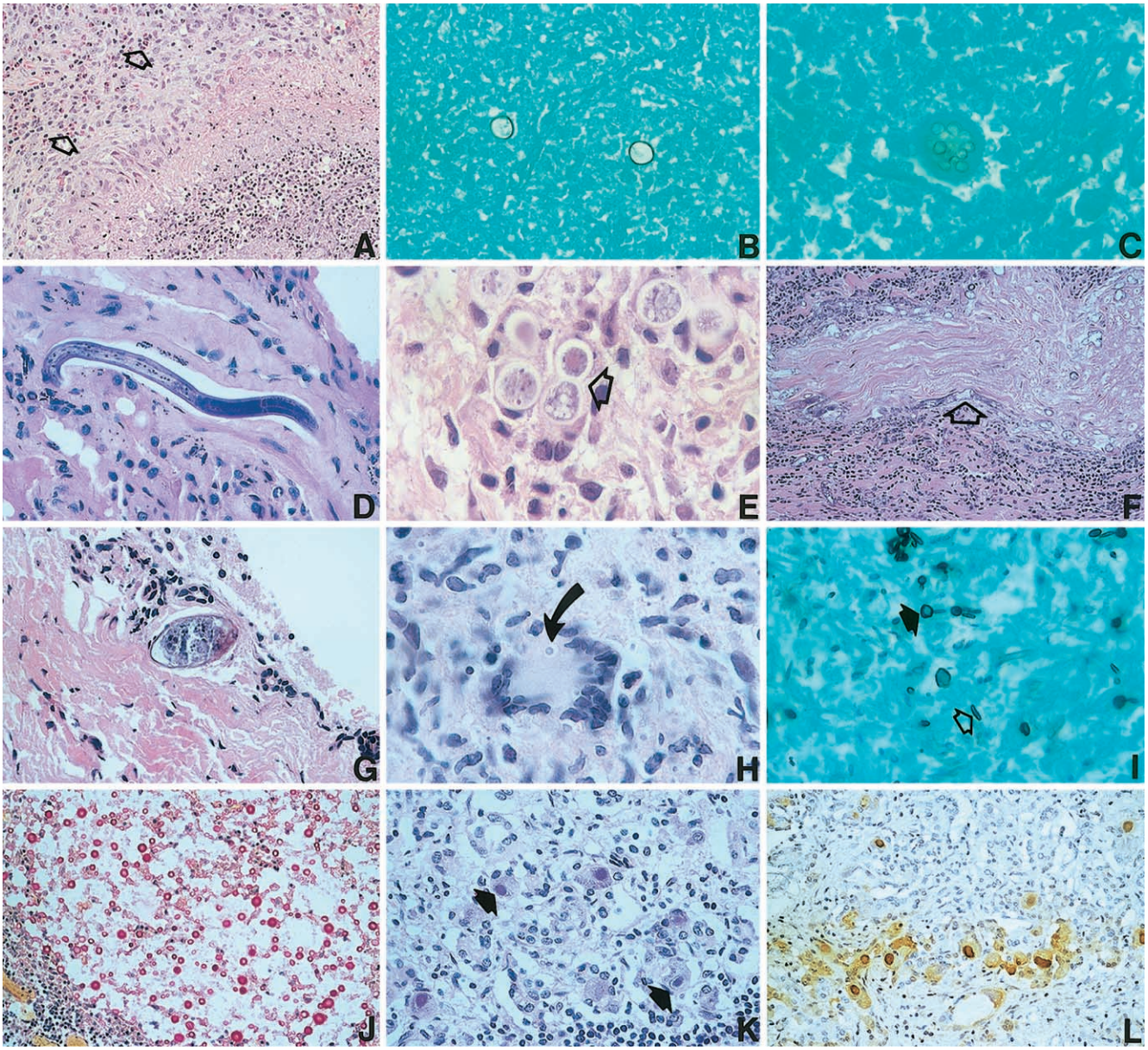

Figure 2. *A,* The inflammatory response to *Coccidioides immitis* consists of granulomatous and acute inflammation with necrosis. A prominent eosinophilic infiltrate *(open arrows)* is also present. Hematoxylin and eosin (HE) stain; magnification, ×200. *B,* Endospores and immature spherules (pictured here) of *C. immitis* may be mistaken for nonbudding yeast forms. Methenamine silver stain; magnification, ×500. *C,* An intact spherule of *C. immitis*. Note that the endospores, but not the wall of the spherule, stain with the silver stain. Methenamine silver stain; magnification, ×1000. *D,* A migrating filariform larvae of *Strongyloides stercoralis* is seen in a transbronchial biopsy of a patient with *Strongyloides* hyperinfection. The 1:1 ratio of esophagus to intestine is evident here—the criterion used to differentiate the filariform larvae of *Strongyloides* from that of a hookworm. HE stain; magnification, ×500. *E, Blastomyces dermatitidis* demonstrates a thick cell wall and the characteristic broad-based bud *(arrow).* HE stain; magnification, ×1000. *F, Mucor* demonstrates perineural *(arrow),* neural, and angioinvasion (not shown) in this patient with rhinocerebral zygomycosis. HE stain; magnification, ×200. *G,* An egg of *Schistosoma japonicum* embedded in fibrosis in a liver biopsy. Note the absence of a lateral spine. HE stain; magnification, ×400. *H,* Rare yeast forms *(arrow)* are present in this giant cell in this patient with sporotrichosis. HE stain; magnification, ×1000. *I,* Yeast forms of *Sporothrix schenckii* are more readily seen in the methenamine silver stain. Note that both "cigar" bodies *(open arrow)* and round yeast forms *(solid arrow)* are present. Methenamine silver stain; magnification, ×1000. *J,* Mucicarmine stains the capsule of *Cryptococcus neoformans* red. Mucicarmine stain; magnification, ×200. *K,* Typical Cowdry type A intranuclear inclusions *(arrows)* of cytomegalovirus (CMV) are seen in this patient with CMV pancreatitis. HE stain; magnification, ×400. *L,* Immunohistochemical staining confirms that CMV is the etiologic agent of the inclusions in panel *J.* CMV immunohistochemistry, DAB (3,3′-diaminobenzidine tetrahydrochloride) stain; magnification, ×200.

some bacteria, most parasites, some viral cytopathic effects, and most fungi. For bacteria, the HE stain may reveal the sulfur granules of actinomycosis, the filaments of *Nocardia* species, and in some instances, pyogenic bacteria such as *Staphylococcus* and *Streptococcus* species (figure 1*G*). For the parasites, the cestodes, trematodes, nematodes, pentostomids, and arthropods are readily detected in tissue sections stained with HE, as are many of the protozoa (figure 2*D* and *G*). Some parasites, such as the microsporidia, stain poorly, if at all, with HE stain. Viruses that produce cytopathic effects that can be identified in HE-stained sections include cytomegalovirus (CMV; figure 2*K*), herpes simplex virus (HSV), varicella-zoster virus (VZV), adenovirus, human papilloma virus (HPV), molluscum bodies of *Molluscum contagiosum* (figure 1*J*), and the JC polyoma virus [32, 69]. Activated monocytes with cerebriform nuclei, Mollaret cells, may be seen in the CSF of patients with recurrent aseptic or Mollaret's meningitis; this disease is thought by some to be caused by HSV [70–72] (figure 1*C*). The respiratory viruses influenza, parainfluenza, and respiratory syncytial virus generally do not produce a demonstrable cytopathic effect in HE-stained tissue sections but may produce ciliocytophthoria, or detached ciliary tufts of the respiratory epithelium, that can be seen in cytology preparations [73, 74]. Many yeasts and hyphae stain with the HE stain, although all are more readily visualized with a methenamine silver stain or the periodic acid–Schiff (PAS) stain. Furthermore, subtleties of fungal morphology are better visualized with a silver stain than with the HE stain (figure 2*B, C, H,* and *I*).

Microorganisms that may be routinely identified in Papanicolaou-stained smears include fungal elements, from organisms such as *Aspergillus* or *Candida* species; *Pneumocystis carinii; Trichomonas vaginalis;* and the viral cytopathic effects of HPV, HSV, CMV and the BK polyoma virus [75, 76] (figure 1*A–C*).

The sensitivity of special stains for the detection of microorganisms is influenced by a variety of factors. These include the choice of the special stain used, which is usually made on the basis of the inflammatory response present, the quantity of microorganisms present, the ability of the pathologist to recognize the microorganism, the magnification used, and, not surprisingly, the amount of time devoted to searching for the microorganism [32]. Once a microorganism is detected, other factors affect the diagnostic accuracy. These include technical factors such as the quality of the stain, the observer's familiarity with the morphology of the microorganism, and the availability of specific confirmatory methods such as immunohistochemistry or in situ hybridization (figure 2*L*).

Although many microorganisms can be visualized in HE-stained sections, others can be visualized only with certain histochemical stains [27, 28]. These histochemical stains remain the most used ancillary method for the histopathologic diagnosis of infectious diseases. The most common special stains for microorganisms are the tissue Gram stain (of which there are several common variants), Grocott-Gomori methenamine silver stain (GMS), and the Ziehl-Neelsen and Kenyoun acid-fast stains (figure 1*F*) [26–28]. Other histochemical stains for detecting or identifying microorganisms include the PAS stain for fungi, mucicarmine and alcian blue stains to demonstrate the capsule of *Cryptococcus neoformans* (figure 2*J*), modified acid-fast stains (e.g., Fite's) for *Nocardia*, the Giemsa stain for *Helicobacter pylori* (figure 1*I*), and silver stains such as the Warthin-Starry, Steiner, and Dieterle stains for bacteria [26–28, 32]. These histochemical stains are useful for detecting the majority of human pathogens, but limitations in specificity exist, and special conditions warrant additional diagnostic techniques. Woods and Walker [32] have written a comprehensive review of the use of cytologic and histologic stains for the detection of microorganisms.

Bacteria are the most difficult microorganisms to detect in routine HE-stained histologic sections [32]. Several modifications of Gram stains can be used for the detection of bacteria in tissue sections. Gram-negative bacteria are particularly difficult to visualize, mainly because of a lack of contrast between the bacteria and the counter stain. The Brown-Brenn tissue Gram stain is preferred for the detection of gram-positive bacteria, whereas the Brown-Hopps modification is superior for the detection of gram-negative bacteria [28, 32]. Some bacteria, such as *Legionella* species, *Bartonella* species, and the spirochetes, stain weakly or not at all with tissue Gram stains. Silver precipitation stains, such as the Warthin-Starry, Steiner, or Dieterle stains, may be used to detect these bacteria. Tissue sections stained by use of silver precipitation techniques should be correlated with the tissue Gram stain, because the silver precipitation techniques are nonspecific and will stain any bacterium that is present. It is technically challenging to produce high-quality and reproducible silver stains, but these stains are important for diagnostic purposes, and histology laboratories should make an effort to remain proficient with these stains. For the histopathologist, any excess silver precipitate makes interpretation difficult or impossible because collagen, fragmented elastin fibers, mucin, neutrophil granules, and cytolytic debris all stain with the silver precipitation techniques; such artifacts can be misleading to even the experienced infectious disease pathologist.

The GMS and PAS stains are used to stain fungi [26, 32, 77]. These stain the cell walls of fungi, but they also stain other substances in human tissues. The GMS stain, like the Warthin-Starry and other silver precipitation stains, stains elements such as collagen and elastin. Excessive stain precipitation may lead to difficulties in differentiating microorganisms, such as *P. carinii,* from yeasts (e.g., *Histoplasma capsulatum*). The PAS stain also stains glycogen, which may be so abundant in some tissues, such as the liver, that it makes interpretation difficult or im-

possible. For this reason, digestion of glycogen with the enzyme diastase is often applied before application of the PAS stain. Mucicarmine and alcian blue both stain the polysaccharide capsule of *C. neoformans* (figure 2*J*). The mucin stains may also weakly stain the cell walls of *B. dermatitidis* and *Rhinosporidium seeberi* [78].

Both the Ziehl-Neelsen and Kenyoun acid-fast stains will stain acid-fast microorganisms red and the surrounding tissue and other microorganisms blue (figure 1*F*). *Nocardia* species and *Mycobacterium leprae* do not stain with the Ziehl-Neelsen stain, which uses a more concentrated acid decolorizor. Hence, these bacteria are considered partially acid fast, retaining stain only when a less concentrated acid decolorizor is used. Fite's method and modifications thereof are common partial acid-fast stains used in tissue sections [32, 79]. These stains are useful for differentiating *Nocardia* species from the *Actinomycetes* species, both of which are gram-positive, filamentous bacteria with a beaded, branching morphology, but most *Nocardia* species are partially acid fast [32, 80]. *Rhodococcus equi,* another partially acid-fast bacteria, causes pneumonia in patients with AIDS; a pulmonary biopsy from these patients demonstrates pulmonary malakoplakia [81, 82]. Finally, the modified acid-fast stain has been suggested as a means of differentiating the eggs of certain schistosomes in tissue [83].

SPECIAL TECHNIQUES: IMMUNOHISTOCHEMISTRY, IN SITU HYBRIDIZATION, AND PCR

Molecular assays increase the specificity and in some instances may increase the sensitivity of histopathologic tests [27, 38, 39, 84, 85]. Molecular methods of detection may be particularly useful when microorganisms are undetectable by means of histochemical methods, are present in low numbers, stain poorly, are uncultivable, or exhibit an atypical morphology [38, 86, 87]. In some cases, molecular methods are important for the rapid, specific, and in some instances quantitative detection of microorganisms [28, 39, 40]. After use of traditional histochemical stains, these are the next step in the diagnosis of infectious agents by means of histopathologic testing.

Immunohistochemistry has revolutionized histopathology, particularly for the categorization of solid tumors and hematopoietic neoplasms and the identification of infectious agents. After histochemical staining, it is the most commonly used ancillary diagnostic technique for the detection of microorganisms in histologic sections. This technique uses monoclonal or polyclonal antibodies directed against specific microbial antigens. Once bound, the antibodies are detected by use of either fluorescent or chromogenic signal amplification. The specificity of this method is dependent on the specificity of the antigen binding (Fab) portion of the immunoglobulin molecule used. Immunofluorescent immunohistochemistry is usually performed on fresh, frozen tissue, whereas immunoperoxidase methods are usually performed on formalin-fixed, paraffin-embedded tissues.

These methods are useful for the detection of fastidious or noncultivable microorganisms, for differentiating between morphologically similar microorganisms or cytopathic effects, and for the detection of highly infectious microorganisms involved in outbreaks of infection [88–90]. Detection of fastidious microorganisms by use of ancillary methods is particularly important because these may go undetected in the microbiology laboratory. For example, *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, is usually not cultured, but it can be readily detected in biopsies of skin samples from infected patients by use of either immunofluorescence or immunoperoxidase methods [91]. The specificity imparted by immunohistochemical stains has been used to differentiate morphologically similar microorganisms such as *Histoplasma, Trypanosoma,* and *Leishmania* species, and such strains have been developed as an adjunct to the histopathologic diagnosis of Chagas' disease [37]. Similarly, these immunohistochemical methods have been used to differentiate morphologically indistinguishable cytopathic effects, such as those produced by HSV and VZV [92]. Immunohistochemistry may also be more sensitive for detection of microorganisms that are difficult to locate in histologic sections. In fact, with automation, this may prove to be more cost-effective than manual, histochemical methods [93]. Finally, immunohistochemical methods may be useful when examining tissues from patients involved in outbreaks of infection. In outbreak situations that involve a highly infectious agent that produces a high mortality rate, the immunohistochemical examination of formalin-fixed, noninfectious tissues would be preferred to culture, given the risk to laboratory personnel who handle live virus. For example, these methods have been used to document patients in the outbreak of epidemic leptospirosis infection in Nicaragua in 1998 and to detect the Ebola virus [89, 90]. The antibodies necessary to detect agents of exotic infectious diseases are usually not available commercially, but they are used in tests performed at the Centers for Disease Control and Prevention, National Center for Infectious Diseases, or other specialized laboratories.

In situ hybridization has many of the same advantages as immunohistochemistry. This method uses the complementary nature of nucleic acids, rather than an antibody, to impart specificity [94]. The nucleic acid probe, which may be labeled by a variety of methods, anneals to a specific target sequence in the microbial DNA or RNA [95]. A signal is generated by methods similar to those used in immunohistochemistry [95]. In situ hybridization is gaining popularity, and with the development of automated and more standardized methods, it is becoming more widely available and more cost effective. This method has been used to detect the presence of a variety of microorganisms

in tissue sections [96]. Although it is most commonly used to detect viral targets, in situ hybridization has also been used to detect common bacteria and fungi as well as intracellular bacteria such as *Chlamydia pneumonia* and *Rickettsia* species [84, 85, 97–100]. The viruses that are most commonly detected by in situ hybridization methods are HPV, Epstein-Barr virus, and CMV [96]. The high specificity that is possible through the selection of unique target sequences has been used to detect the high-risk HPV subtypes in biopsies of cervical specimens and to distinguish HSV type 1 and type 2 [92, 96]. Although in situ hybridization is useful in these regards, competitive molecular methods exist that may be less labor intensive. The fact that in situ hybridization does not require animals or cell lines for the production of antibody is a distinct advantage. Furthermore, the nucleic acid probes may be synthesized quickly and relatively inexpensively, which allows for the detection of relatively rare microorganisms for which immunohistochemical stains are not commercially available.

Nucleic acid amplification technologies (mainly PCR, but also ligase chain reaction, strand-displacement technology, and others) have revolutionized the detection of infectious agents [39, 41, 101, 102]. These allow for the detection of microorganisms that are difficult to culture or are not cultivable, and they may be used to quantify the amount (load) of microorganisms present [101–103]. Nucleic acid amplification methods that use tissue sections include in situ PCR and conventional amplification using tissue extracts.

In situ PCR is a method that may be used to amplify the nucleic acid of a specific target by use of the PCR in an intact tissue section to detect and localize the amplified product. This method has been used to detect viruses in tissue sections [104]. It is, however, technically difficult to perform and prone to contamination because amplification occurs on a slide rather than in a closed tube, and diffusion of amplified product into the supernatant does occur [105]. Although in situ PCR is attractive and the problems with this technology may be ultimately resolved, any convincing advantages that this technology may have over immunohistochemistry, in situ hybridization, or conventional PCR have yet to be demonstrated [106, 107].

Nucleic acid amplification tests for the detection of microorganisms are now commonplace in many microbiology and molecular pathology laboratories [41, 101, 108, 109]. The methods discussed thus far each rely on the visual interpretation of a reaction in histologic sections. Molecular assays from tissue extracts may seem separate and distant from the histopathologic diagnosis of disease, but this need not be so. These very different assays are, in fact, complementary in nature. For example, if a cavitary lung lesion is thought to have an infectious etiology and the histopathologic examination demonstrates the cause to be fungal, then a nucleic acid amplification test for *M. tuberculosis* would be unnecessary. Molecular methods may be used to provide definitive identification of an organism and in some instances could be used to provide susceptibility information through the detection of resistance genes [110, 111]. Molecular hematopathologists have used these methods to detect Epstein-Barr virus in tissues from patients with posttransplant lymphoproliferative disorders [112, 113]. These molecular methods are powerful and appropriate for many applications in infectious disease pathology [28, 114]. For example, prompted by serologic results, we have used a Warthin-Starry stain to locate the portion of tissue that contained microorganisms and specifically identified the agent of a culture-negative endocarditis as *Bartonella quintana* by use of PCR and sequence-based identification [103]. The use of molecular methods on tissue extracts to reveal the specific identify of the infectious agent is possible, but it awaits further development, and it needs to be embraced by traditional morphologic pathologists.

CONCLUSION

Diseases from decades ago have reemerged, and emerging infectious diseases, such as hantavirus pulmonary syndrome and Ebola virus hemorrhagic fever, have only recently been described [115, 116]. The ease of world travel allows for the introduction of unusual infectious diseases from one part of the globe to another. Undoubtedly, the anatomic pathologist plays a critical role in the diagnosis of emerging and reemerging, as well as common, infectious diseases [1–4]. The task of the pathologist is to characterize all pathologic findings appropriately and to identify those lesions that are likely to have an infectious etiology. The successful characterization of the infectious disease pathology requires the proper characterization of the inflammatory response, a knowledge of associated pathogens, the use of special histochemical stains, and in some instances, the use of highly specific molecular technologies.

The histopathology of infectious diseases is particularly useful when cultures have not been obtained, the infectious agent is slow-growing or fastidious, or the agent does not grow in culture. In addition, histopathologic information may be useful to corroborate and clarify complex microbiologic results. Through appropriate morphologic diagnoses and interlaboratory communication and collaboration, the anatomic pathologist contributes greatly to the diagnosis and treatment of infectious diseases.

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