

Application of Immunohistochemistry to Infections

Eduardo Eyzaguirre, MD; Abida K. Haque, MD

• **Context.**—Pathologists play an important role in the diagnosis or exclusion of infectious diseases. Traditionally, the diagnosis of infectious diseases rely on serologic assays and cultures. Serologic results may be difficult to interpret in the setting of immunosuppression, fresh tissue is not always available for culture, and culture of fastidious pathogens can be difficult and may take weeks or months to yield a result. Although some microorganisms or their cytopathic effects may be readily identifiable on routine and/or histochemical stains, often these changes are not specific or are sparse in the sample evaluated. In these cases, additional immunohistochemical stains are often needed to establish the diagnosis of infection.

Objective.—To review the current value and limitations

During the last 2 decades, the approach to histopathologic diagnosis has been dramatically transformed by immunohistochemistry specifically in the diagnosis and classification of tumors and more recently in the diagnosis of infectious diseases in tissue samples. Pathologists play an important role in recognizing infectious agents in tissue samples from patients, providing a rapid morphologic diagnosis and facilitating clinical decisions in patient treatment.¹

Traditionally, microbial identification in infectious diseases has been made primarily by using serologic assays and cultures. However, serologic results can be difficult to interpret in the setting of immunosuppression or when only a single sample is available for evaluation. In addition, fresh tissue is not always available for culture, and culture of fastidious pathogens can be difficult and may take weeks or months to yield results. Moreover, culture alone cannot distinguish colonization from tissue invasion. Some microorganisms have distinctive morphologic characteristics that allow their identification in formalin-fixed tissues using routine and special stains. Nevertheless, in several instances it is difficult or even impossible to identify an infectious agent specifically by conventional morphologic methods.

of the use of immunohistochemistry in the diagnosis of infectious diseases in formalin-fixed tissue samples.

Data Sources.—Literature in Medline and the authors' own experience.

Conclusions.—Immunohistochemistry has proven to be a useful tool in the diagnosis of infectious diseases in tissue samples. Immunohistochemistry is especially useful in the identification of microorganisms that are present in low numbers, stain poorly, are fastidious to grow, are noncultivable, or exhibit an atypical morphology. Finally, it is important to remember that there may be widespread occurrence of common antigens among bacteria and pathogenic fungi and both monoclonal and polyclonal antibodies must be tested for possible cross-reactivity with other organisms.

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Immunohistochemistry is one of the most powerful techniques in surgical pathology. There has been an increasing interest in the use of specific antibodies to viral, bacterial, fungal, and parasitic antigens in the detection and identification of the causative agents in many infectious diseases. In many instances, immunohistochemistry has shown high specificity allowing the differentiation of morphologically similar microorganisms.² Immunohistochemistry is especially useful when microorganisms are difficult to identify by routine or special stains, are fastidious to grow, or exhibit an atypical morphology (Table 1).^{3–7} It is important to understand that there may be a widespread occurrence of common antigens among bacteria and pathogenic fungi and both monoclonal and polyclonal antibodies must be tested for possible cross-reactivities with other organisms.⁸ Finally, it is important to emphasize that immunohistochemistry has several steps and all of them can affect the final result; however, in general, the only limitations are the availability of specific antibodies and the preservation of epitopes.⁹

Table 2 lists some commercially available antibodies of frequent use in surgical pathology.

VIRAL INFECTIONS

Traditionally, the diagnosis of viral infections has relied on cytopathic changes observed on routine histopathology. However, in some cases the characteristic cytopathic changes are often subtle and sparse requiring a meticulous search.¹⁰ Moreover, only 50% of the known viral diseases are associated with characteristic intracellular inclusions.¹¹ Immunohistochemistry is highly specific and sensitive in these cases or when the viral inclusions present cannot be identified with confidence and differentiated from those of other viral diseases.^{2,12}

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From the Department of Pathology, The University of Texas Medical Branch at Galveston (Dr Eyzaguirre); and the Departments of Pathology, Weill College of Medicine of Cornell University, New York, NY, and The Methodist Hospital, Houston, Tex (Dr Haque).

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Reprints: Eduardo Eyzaguirre, MD, Division of Surgical Pathology, Department of Pathology, The University of Texas Medical Branch at Galveston, 301 University Blvd, Galveston, TX 77555-0588 (e-mail: jeyzagu@utmb.edu).

Table 1. Applications of Immunohistochemistry in the Diagnosis of Infectious Diseases

1. To identify microorganisms that are difficult to detect by routine or special stains
2. To identify microorganisms that are present in low numbers
3. To identify microorganisms that stain poorly
4. To identify microorganisms that are fastidious to grow or are noncultivable
5. To identify microorganisms that exhibit atypical morphology

Herpesviruses

The diagnosis of herpes simplex virus (HSV) infection can be difficult when the characteristic intranuclear inclusions or multinucleated cells, or both, are absent or when the amount of tissue in a biopsy specimen is small.¹³ In these cases, immunohistochemistry using either polyclonal or monoclonal antibodies against HSV antigens has proven to be a sensitive and specific technique to diagnose HSV infections¹³⁻¹⁵ (Figure 1). Although polyclonal antibodies are sensitive, they do not allow distinction between HSV-1 and HSV-2 because these 2 viruses are antigenically similar.¹⁶ In addition, the histologic features of HSV infection can also occur in patients with varicella-zoster virus infection. Monoclonal antibodies against the varicella-zoster virus that envelop glycoprotein gp1 are sufficiently sensitive and specific to allow a clear-cut distinction between HSV and varicella-zoster virus infections.^{2,14} Immunohistochemistry has also been useful in demonstrating the association of human herpesvirus 8 with Kaposi sarcoma, primary effusion lymphoma, and multicentric Castlemann disease.^{17,18}

Kaposi Sarcoma

The diagnosis of Kaposi sarcoma may be problematic because of its broad morphologic spectrum and similarity in appearance to other benign and malignant vascular neoplasms. Immunostaining of Kaposi sarcoma latent-associated nuclear antigen 1 is useful to confirm the diagnosis of Kaposi sarcoma, particularly in difficult early le-

sions or when the neoplasm presents in an unusual location.^{19,20} Immunostaining is restricted to the nuclei of spindle cells and endothelial cells of the slitlike vascular spaces (Figure 2).

Cytomegalovirus

Histologic diagnosis of cytomegalovirus (CMV) in fixed tissues usually rests on the identification of characteristic cytopathic effects, including intranuclear or cytoplasmic inclusions or both. However, histologic examination lacks sensitivity, and in some cases atypical cytopathic features can be confused with reactive or degenerative changes.²¹ Monoclonal antibodies against early and late CMV antigens allow the detection of CMV antigens in the nucleus and cytoplasm of infected cells (Figure 3). Also, immunohistochemistry may allow detection of CMV antigens early in the course of the disease when cytopathic changes have not yet developed.²²⁻²⁴ Immunohistochemistry has been useful in the detection of CMV infection in patients with steroid-refractory ulcerative colitis and in the detection of occult CMV infection of the central nervous system in liver transplant patients who develop neurologic complications.^{25,26} The sensitivity of immunohistochemistry is better than light microscopic identification of viral inclusions, compares favorably with culture and in situ hybridization, and can be completed faster than the shell vial technique with immunofluorescence or culture, allowing for rapid results.^{23,27,28}

Epstein-Barr Virus

Immunohistochemistry has also been used to identify Epstein-Barr virus latent membrane protein-1 in cases of Hodgkin lymphoma and posttransplant lymphoproliferative disorder²⁹ (Figure 4).

Adenoviruses

Adenovirus infection has been described in immunocompromised patients secondary to organ transplant, in congenital immunodeficiency, and rarely in human immunodeficiency virus-infected patients.^{12,30,31} The diagno-

Table 2. Commercially Available Antibodies of Frequent Use in Immunohistochemical Diagnosis of Infectious Diseases*

| Antibody Against | Type/Clone | Dilution | Pretreatment | Source | Localization |
|-------------------------------|-------------------|----------|--------------|---------------------------|--|
| Adenovirus | Mab/20/11 and 2/6 | 1:2000 | Proteinase K | Chemicon | Nuclear |
| Aspergillus | Mab/WF-AF-1 | 1:200 | HIER | Dako | Septate hyphae |
| <i>Bartonella henselae</i> | Mab/H2A10 | 1:100 | HIER | Biocare Medical | Intact bacteria |
| BK virus | Rabbit polyclonal | 1:8000 | | Lee Biomolecular Research | Nuclear (tubular cells) |
| <i>Candida albicans</i> | Mab/1B12 | 1:400 | HIER | Chemicon | Yeasts forms |
| CMV | Mab/DDG9/CCH2 | 1:50 | HIER | Novocastra | Nuclear and cytoplasmic |
| Hepatitis B core Ag | Rabbit polyclonal | 1:2000 | HIER | Dako | Nuclear and/or cytoplasmic |
| Hepatitis B surface Ag | Mab/3E7 | 1:100 | HIER | Dako | Cytoplasmic |
| Herpes simplex 1 and 2 | Rabbit polyclonal | 1:3200 | HIER | Dako | Nuclear and cytoplasmic |
| <i>Helicobacter pylori</i> | Rabbit polyclonal | 1:40 | Protase I | Dako | Intact bacteria |
| HHV-8 | Mab/LNA-1 | 1:500 | HIER | Novocastra | Nuclear (spindle and endothelial cells) |
| JC virus | Rabbit polyclonal | 1:20 000 | HIER | Lee Biomolecular Research | Nuclear (oligodendrocytes) |
| <i>Listeria monocytogenes</i> | Rabbit polyclonal | 1:5000 | Proteinase K | Difco | Intact bacteria |
| Parvovirus B19 | Mab/R92F6 | 1:500 | HIER | Novocastra | Nuclear (normoblasts and pronormoblasts) |
| <i>Pneumocystis jirovecii</i> | Mab/3F6 | 1:20 | HIER | Novocastra | Cysts and trophozoites |
| Respiratory syncytial virus | Mab/5H5N | 1:200 | HIER | Novocastra | Cytoplasm (syncytial giant cells) |
| <i>Toxoplasma gondii</i> | Rabbit polyclonal | 1:320 | HIER | BioGenex | Pseudocysts and tachyzoites |
| Varicella-zoster | Mab8612 | 1:8000 | HIER | Chemicon | Cytoplasmic |
| West Nile virus | Mab/5H10 | 1:400 | Proteinase K | Bioreliance | Neuronal cytoplasm and processes |

* Mab, monoclonal antibody; HIER, heat-induced epitope retrieval; CMV, cytomegalovirus; Ag, antigen; and HHV-8, human herpesvirus 8.

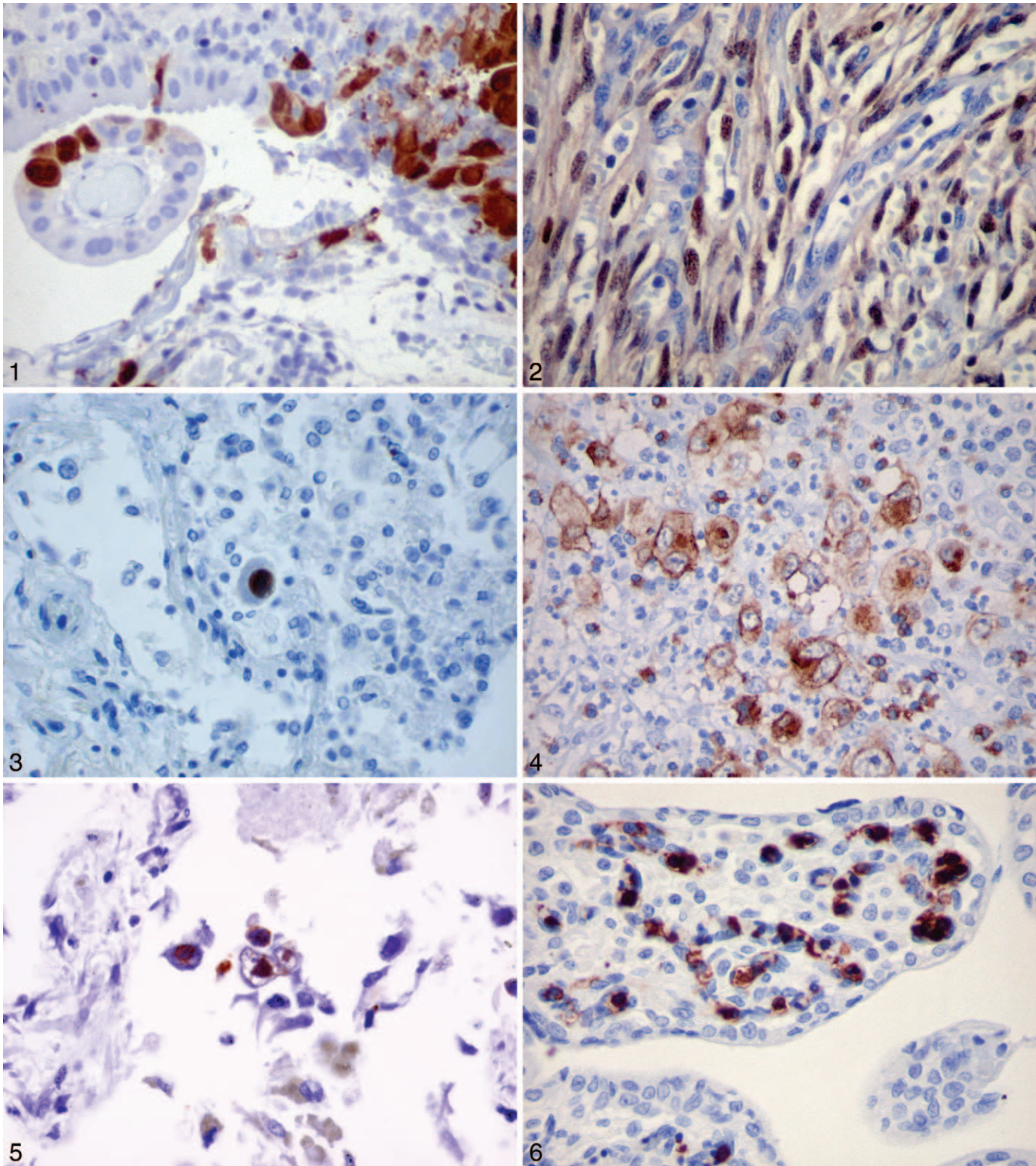


Figure 1. Photomicrograph of cervical biopsy from a patient with herpes simplex virus infection showing abundant nuclear and cytoplasmic antigen (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 2. Lymph node biopsy from a patient with Kaposi sarcoma. The spindle cells show strong nuclear staining for human herpesvirus 8 latent-associated nuclear antigen 1. Endothelial cells of well-formed vascular spaces are negative (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 3. Cytomegalovirus (CMV) pneumonitis in an immunodeficient patient. Rare macrophages show intranuclear CMV antigen (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 4. Epstein-Barr virus LMP-1 within cytoplasm of characteristic Reed-Sternberg cells in a case of Hodgkin lymphoma (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

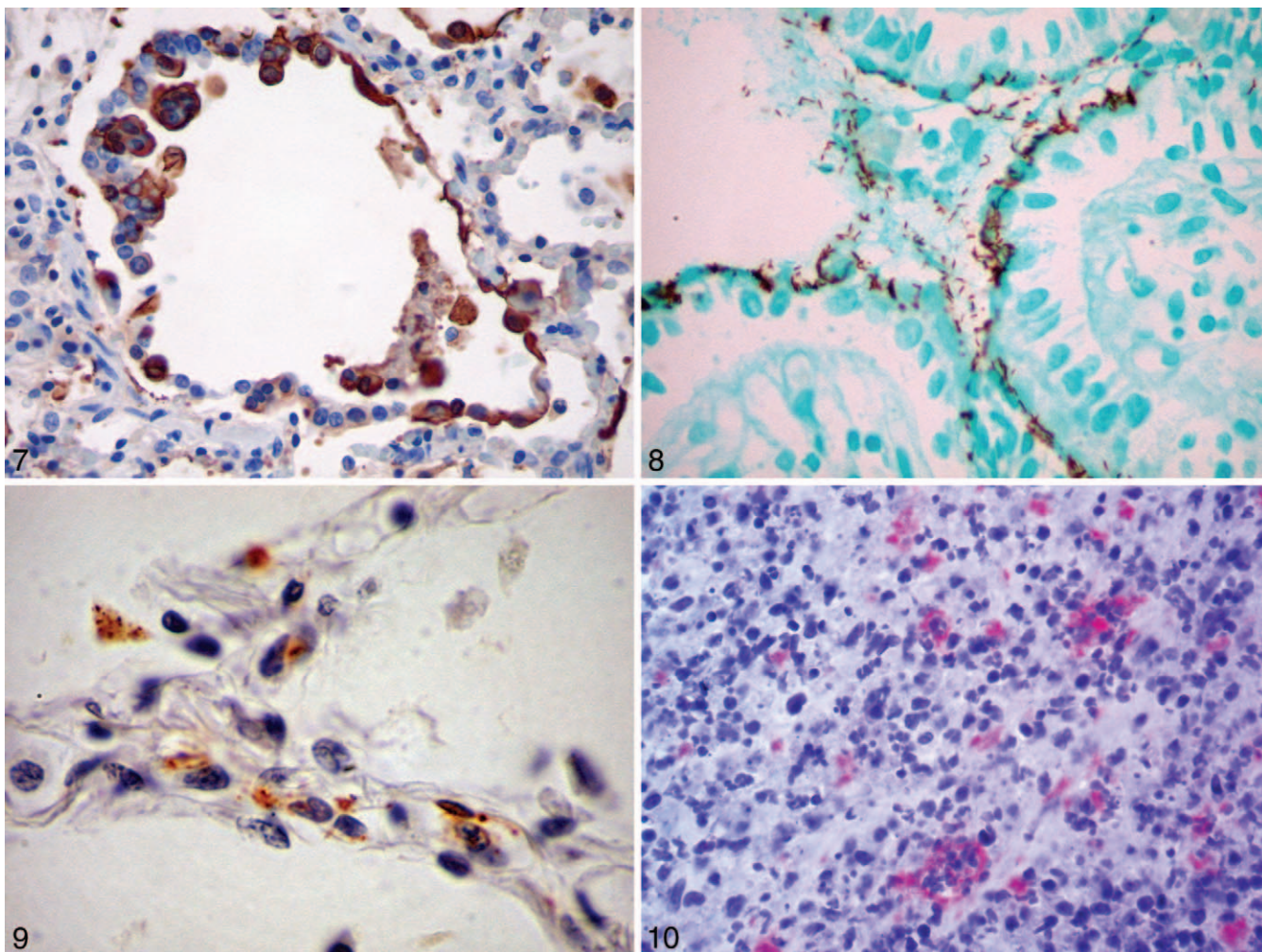


Figure 7. Immunostaining of respiratory syncytial virus antigens in desquamated bronchial cells using a monoclonal antibody (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 8. Numerous curved *Helicobacter pylori* in the superficial gastric mucus are clearly demonstrated by immunoperoxidase staining in this patient with chronic active gastritis (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 9. Immunohistologic demonstration of *Rickettsia rickettsii* within vascular endothelium in the lung of a patient with fatal Rocky Mountain spotted fever (immunoperoxidase with aminoethyl carbazole and hematoxylin counterstain, original magnification $\times 400$).

Figure 10. Photomicrograph of a lymph node biopsy from a patient with cat-scratch disease showing abundant extracellular, clumped coccobacilli of *Bartonella henselae* in necrotic foci (immunoalkaline phosphatase with monoclonal antibody against *B. henselae*, naphthol fast red substrate and hematoxylin counterstain, original magnification $\times 200$).

sis of adenovirus infection may be difficult when only rare cells show the characteristic cytopathic effect.¹² In addition, other viral inclusions, including CMV, human papillomavirus, HSV, and varicella-zoster virus, can be mistaken for adenovirus inclusions and vice versa. A monoclonal antibody reactive with all 41 serotypes of adenovirus has been used to demonstrate intranuclear adenoviral antigen in these cases and to differentiate adenovirus colitis from CMV colitis (Figure 5).^{12,32-34}

Parvovirus B19

Parvovirus B19 has been associated with asymptomatic infections, erythema infectiosum, acute arthropathy, aplastic crisis, hydrops fetalis, and chronic anemia and red cell aplasia. The diagnosis of parvovirus infection can be achieved by identifying eosinophilic or amphophilic intranuclear inclusions in erythroid precursor cells.^{35,36} Because intravenous immunoglobulin therapy is effective, a rapid

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Figure 5. Adenovirus pneumonia in a heart transplant patient who developed acute respiratory distress syndrome and respiratory failure. Infected cells within necrotizing exudate show intranuclear reactivity with antibody to adenovirus antigen. The inclusion in 1 cell has a clear halo around it, making a differential diagnosis from cytomegalovirus difficult on hematoxylin-eosin stain (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 6. Hydrops fetalis caused by parvovirus B19 infection. Normoblasts within the villous capillaries show intranuclear viral antigen (immunoperoxidase with aminoethyl carbazole and hematoxylin counterstain, original magnification $\times 400$).

and accurate diagnosis is important. A monoclonal antibody against VP1 and VP2 capsid proteins is a rapid and sensitive method to establish the diagnosis of parvovirus B19 infection in formalin-fixed tissues^{37,38} (Figure 6). Immunohistochemistry has been of particular help in cases with sparse inclusions and in cases of hydrops fetalis in which there is advanced cytolysis, and it has shown good correlation with morphologic, immunohistochemical, in situ hybridization, and polymerase chain reaction findings.³⁶⁻³⁹

Other Viruses

Immunohistochemistry has also been used to confirm the diagnosis of respiratory viral diseases such as influenza A virus and respiratory syncytial virus infections when cultures were not available^{40,41} (Figure 7).

Immunohistochemistry has been useful to demonstrate BK virus infection in renal transplant specimens in which the infection is usually associated with mononuclear interstitial inflammatory infiltrates and tubular atrophy, findings that can be difficult to distinguish from acute cellular rejection.^{42,43}

Other viral infections in which immunohistochemistry has shown to be a useful diagnostic tool include viral hemorrhagic fevers such as Ebola virus infection,⁴⁴ yellow fever,⁴⁵ and dengue hemorrhagic fever.⁴⁶ Immunohistochemical staining is a very sensitive, safe, and specific diagnostic tool for the diagnosis of rabies when the characteristic Negri bodies are inconspicuous.⁴⁷

Immunohistochemistry has also been used in the histopathologic diagnosis of viral hepatitis B and C. However, immunohistochemistry for these viruses is not superior to serologic assays.

BACTERIAL INFECTIONS

Because of the frequent occurrence of common antigens among bacteria, interpretation of the results can be complicated, and testing of antibodies for cross-reactivity is always recommended.

Helicobacter pylori Infection

Gastric infection by *H pylori* results in chronic active gastritis and is strongly associated with lymphoid hyperplasia and gastric lymphomas. Heavy infections with numerous organisms are easily detected on hematoxylin-eosin-stained tissues; however, the detection rate is only 66% with many false-positive and false-negative results.^{48,49} Conventional histochemical methods are more sensitive than hematoxylin-eosin in detecting *H pylori*. However, treatment for chronic active gastritis and *H pylori* infection can change the shape and decrease the number of the microorganisms making difficult their identification and differentiation from extracellular debris or mucin globules. In these cases, immunohistochemistry improves the rate of successful identification of the bacteria even when histologic examination and cultures are false-negative and is superior and in some cases less expensive than cultures^{48,50-52} (Figure 8).

Whipple Disease

Whipple disease affects primarily the small bowel and mesenteric lymph nodes and less commonly other organs such as heart and central nervous system. Numerous foamy, periodic acid-Schiff-positive macrophages characterize the disease. Nevertheless, the presence of periodic

acid-Schiff-positive macrophages is not pathognomonic because they can be observed in other diseases such as *Mycobacterium avium* complex infections, histoplasmosis, *Rhodococcus equi* infection, and macroglobulinemia. A sensitive and specific rabbit polyclonal antibody has been used for the rapid diagnosis of intestinal and extraintestinal Whipple disease and for follow-up of treatment response.^{7,53-55}

Rocky Mountain Spotted Fever

Several studies have illustrated the value of immunohistochemistry in the diagnosis of suspected cases of Rocky Mountain spotted fever using skin biopsies and in confirming fatal cases of seronegative Rocky Mountain spotted fever^{3,56,57} (Figure 9).

Bartonella Infections

Bartonella are associated with bacillary angiomatosis, peliosis hepatis, cat-scratch disease, trench fever, relapsing bacteremia, disseminated granulomatous lesions of liver and spleen, and blood culture-negative endocarditis.⁵⁸ Immunohistochemistry has been used successfully to identify *Bartonella henselae* and *Bartonella quintana* in heart valves from patients with blood culture-negative endocarditis, cat-scratch disease, spontaneous splenic rupture, bacillary angiomatosis, and peliosis hepatis⁵⁹⁻⁶³ (Figure 10). The polyclonal rabbit antibody does not allow differentiation between *B henselae* and *B quintana*; however, a monoclonal antibody specific for *B henselae* is commercially available.

Other Bacterial Infections

Q fever is a zoonosis caused by *Coxiella burnetii* characterized by protean and nonspecific manifestations that usually delay the diagnosis resulting in increased mortality. This microorganism has been recognized as one of the agents causing blood culture-negative endocarditis.⁶⁴ A monoclonal antibody has been used to specifically identify *C burnetii* in cardiac valves of patients with Q fever endocarditis.^{5,65}

Other bacterial diseases that can be identified by immunohistochemistry in formalin-fixed tissue include leptospirosis,^{66,67} spirochetes in patients with syphilis,⁶⁸ and soft tissue infections associated with group A *Streptococcus*, *Staphylococcus aureus*, and *Clostridium* sp.^{69,70}

FUNGAL INFECTIONS

The great majority of fungi are readily identified by hematoxylin-eosin staining alone or in combination with special stains such as Gomori methenamine silver stain and periodic acid-Schiff stain used in routine histopathology. However, sometimes fungal elements may appear atypical in tissue sections, and histochemical stains cannot distinguish morphologically similar fungi with potential differences in susceptibility to antimycotic drugs.⁷¹ The final identification of fungi relies on culture; however, these may take several days or longer to yield a definitive result, and many times surgical pathologists have no access to fresh tissue.

Immunohistochemistry has been used to identify various fungal elements in formalin-fixed tissue providing rapid and specific identification of several fungi, allowing the identification of unusual filamentous hyphal and yeast infections and distinguishing true infection from harmless colonization.⁷²⁻⁷⁴ Immunohistochemistry can also be help-

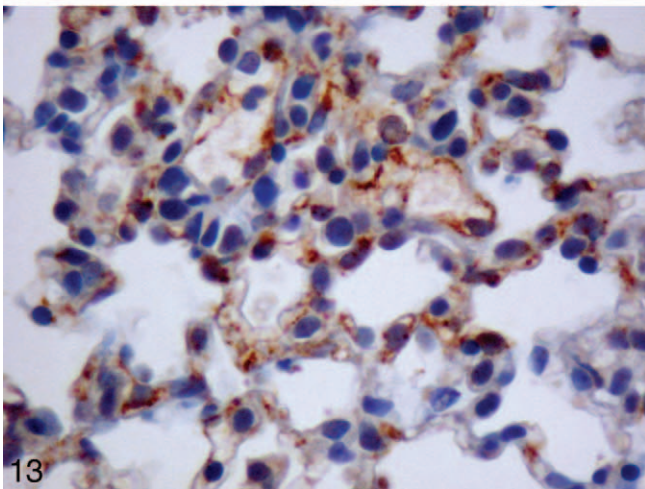
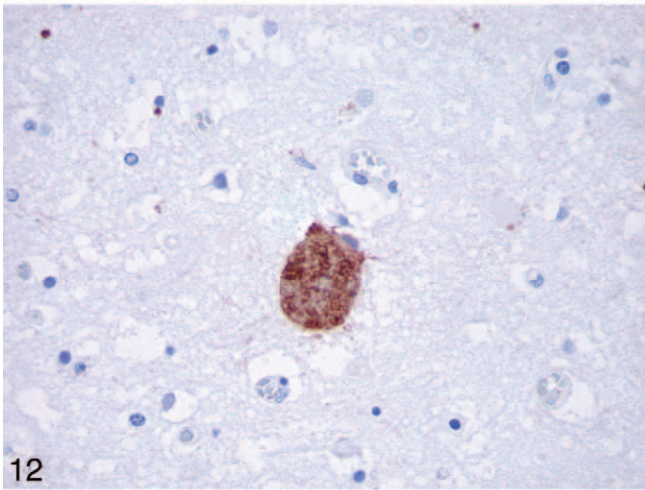
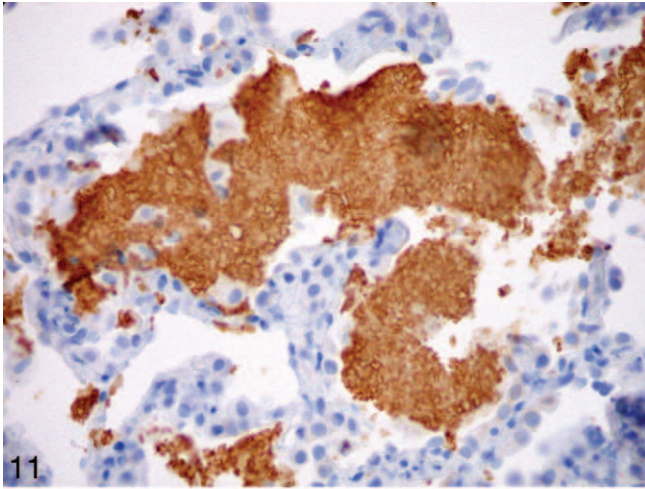


Figure 11. Immunodeficient patient with *Pneumocystis jiroveci* pneumonia. Cohesive aggregates of cyst forms and trophozoites within alveolar spaces are demonstrated by a monoclonal antibody against *P jiroveci* with an immunoperoxidase technique (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

Figure 12. Human immunodeficiency virus-infected patient with toxoplasmic encephalitis. Immunoperoxidase staining highlights cyst forms and scattered tachyzoites (diaminobenzidine substrate with hematoxylin counterstain, original magnification $\times 400$).

Figure 13. Hantavirus antigen-containing endothelial cells of pulmonary microvasculature in the lung of a hantavirus cardiopulmonary syndrome patient as detected by immunohistochemistry using anti-

ful when more than one fungus is present; in these cases, dual immunostaining techniques can highlight the different fungal species present in the tissue.⁷⁵

Polyclonal and monoclonal antibodies against *Candida* genus antigens are sensitive and strongly reactive and do not show cross-reactivity with other fungi tested.^{72,73,76,77}

Invasive aspergillosis is a frequent cause of fungal infection with high morbidity and mortality rates in immunocompromised patients. The diagnosis is often difficult and relies heavily on histologic identification of invasive septate hyphae and culture confirmation. Nevertheless, several filamentous fungi such as *Fusarium* species, *Pseudallescheria boydii*, and *Scedosporium* species share similar morphology with *Aspergillus* species in hematoxylin-eosin-stained tissues. In addition, the yield of cultures in histologically proven cases is low, ranging from 30% to 50%.^{78,79} Several polyclonal and monoclonal antibodies against *Aspergillus* antigens have been tested in formalin-fixed tissues with variable sensitivities, and most of them cross-react with other fungi.⁸⁰ More recently, monoclonal antibodies (WF-AF-1, 164G, and 611F) against *Aspergillus* galactomannan have shown high sensitivity and specificity in identifying *A fumigatus*, *A flavus*, and *A niger* in formalin-fixed tissues without cross-reactivity with other filamentous fungi.^{79,81}

Cysts and trophozoites of *Pneumocystis jiroveci* (*Pneumocystis carinii*) can be detected using monoclonal antibodies that yield results that are slightly more sensitive than Gomori methenamine silver, Giemsa, or Papanicolaou staining^{73,82,83} (Figure 11). Antibodies are most helpful when atypical pathologic features are present such as granulomatous *P jiroveci* pneumonia and hyaline membranes or in cases of extrapulmonary pneumocystosis.

Penicillium marneffeii usually causes a disseminated infection in immunocompromised patients that clinically resembles histoplasmosis or leishmaniasis.⁸⁴ Morphologically, the organisms must be differentiated from *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Candida albicans*. The monoclonal antibody EBA-1 against the galactomannan of *Penicillium* species cross-reacts with and detects *P marneffeii* in tissue sections.⁸⁵

PROTOZOAL INFECTIONS

Protozoa usually can be identified in tissue sections stained with hematoxylin-eosin or Giemsa stain; however, because of the small size of the organisms and the subtle distinguishing features, an unequivocal diagnosis cannot always be made. Immunohistochemistry has been helpful in cases in which the morphology of the parasite is distorted by tissue necrosis or autolysis or in cases with unusual presentation of the disease⁸⁶ (Figure 12).

The diagnosis of leishmaniasis may be challenging in cases of chronic granulomatous leishmaniasis with small numbers of parasites, when the microorganism presents in unusual locations, or when necrosis distorts the morphologic appearance of the disease.⁸⁷ A highly sensitive and specific monoclonal antibody that recognizes different species of *Leishmania* has been a valuable diagnostic tool and allows differentiation from morphologically sim-

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Andes virus antibody (immunoperoxidase with diaminobenzidine and hematoxylin counterstain, original magnification $\times 400$).

ilar microorganisms (*Toxoplasma*, *Trypanosoma cruzi*, and *P. marneffei*).^{87,88}

IMMUNOHISTOCHEMISTRY, EMERGING INFECTIONS, AND BIOTERRORISM

Pathologists have played an important role in the diagnosis and characterization of emerging infectious agents and in the investigation of bioterrorism-related cases.

Immunohistochemistry provides a simple, safe, sensitive, and specific method for the rapid detection, either at the time of investigation or retrospectively, of biologic threats, facilitating the rapid implementation of effective public health responses. Immunohistochemical methods using polyclonal or monoclonal antibodies have been applied to the identification of several potential biologic terrorism agents, including antibodies to the causative agents of anthrax,⁸⁹ tularemia,⁹⁰ plague,⁹¹ brucellosis,⁹² viral encephalitis (Eastern equine encephalitis),⁹³ and rickettsioses (typhus and Rocky Mountain spotted fever).⁹⁴ In addition, immunohistochemistry has been a very valuable tool for the identification and study of several emerging infectious diseases such as hantavirus cardiopulmonary syndrome⁹⁵ (Figure 13), West Nile virus encephalitis,⁹⁶ enterovirus 71 encephalomyelitis,⁹⁷ Nipah virus infection,⁹⁸ Ebola hemorrhagic fever,⁴⁴ and hendra virus encephalitis⁹⁹ and more recently in identification of a new coronavirus associated with severe acute respiratory syndrome.¹⁰⁰

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