2 Immunohistology of Infectious Diseases

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INTRODUCTION

Since the 1980s, surgical pathologists in general and infectious disease pathologists in particular have dealt with an increasing number of surgical specimens from patients in whom one or multiple infectious agents may be responsible for disease.[1] In this context, pathologists have played an important role in recognizing infectious agents. In many cases, when fresh tissue is not available for culture, pathologists can provide a rapid morphologic diagnosis and facilitate clinical decisions in patient treatment.[2] In addition, pathologists have played a central role in the identification of emerging and reemerging infectious agents and describing the pathogenetic processes of emerging diseases, such as hantavirus pulmonary syndrome and other viral hemorrhagic fevers, leptospirosis, and rickettsial and ehrlichial infections as well as the diagnosis of anthrax during the bioterrorist attack of 2001.[3–7]

Conventionally, microbial identification in infectious diseases has been made primarily by using serologic assays and culture techniques. 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.

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. The use of a specific antibody to detect a microbial antigen was first performed by Coons and associates[8] to detect pneumococcal antigen in tissues. The advantages of immunohistochemistry over conventional staining methods (Table 2.1) and the contributions of immunohistochemistry in infectious diseases (Table 2.2) are substantial. It is important to emphasize that both monoclonal and polyclonal antibodies must be tested for possible cross-reactivities with other organisms. The widespread occurrence of common antigens among bacteria and pathogenic fungi is well established.[1,9] Finally it is important to understand 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.[1,10] It is well known that for larger microorganisms such as protozoa, fungi and some bacteria, pretreatment of formalin-fixed, paraffin-embedded tissue is not required. In contrast, for smaller infectious agents, for example, microorganisms such as viruses and chlamydiae, pretreatment of the tissue with proteolytic enzymes or heat-induced epitope retrieval is necessary in order to enhance immunoreactivity.

Table 2.3 lists currently available antibodies for diagnostic use in surgical pathology.

Table 2.1 Advantages of IHC for the diagnosis of infectious diseases

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| Allows rapid results |
| Can be performed on formalin-fixed, paraffin-embedded tissue, reducing the risk of exposure to serious infectious diseases |
| High sensitivity allowing identification of infectious agents even before morphologic changes occur |
| Useful for retrospective diagnosis of individual patients and for in-depth study of the disease |
| Specificity: monoclonal antibodies and some polyclonal antibodies allow for specific identification of infectious agents |

Table 2.2 Contributions of IHC to the diagnosis of infectious diseases

Allows identification of new human pathogens
Allows microbiologic/morphologic correlation establishing the pathogenic significance of microbiological results
Provides a rapid morphologic diagnosis allowing early treatment of serious infectious diseases
Contributes to understanding of the pathogenesis of infectious diseases
Provides a diagnosis when fresh tissue is not available or when culture methods do not exist

Table 2.3 Some commercially available antibodies for immunohistochemical diagnosis of infectious diseases

Microorganism	Antibody/clone	Dilution	Pretreatment	Source
Adenovirus	Mab/20/11 and 2/6	1:2000	Proteinase K	Chemicon
Aspergillus	Mab/WF-AF-1	1:200	HIAR*	Dako
B. henselae	Mab	1:100	HIAR	Biocare Medical
BK virus	Mab/BK T.1	1:8000	Trypsin	Chemicon
C. albicans	Mab/1B12	1:400	HIAR	Chemicon
C. pneumoniae	Mab/RR402	1:200	HIAR	Accurate
Cryptosporidium	Mab/Mabc1	1:100	HIAR	Novocastra
CMV	Mab/DDG9/CCH2	1:50	HIAR	Novocastra
Giardia intestinalis	Mab/9D5.3.1	1:50	HIAR	Novocastra
Hepatitis B core Ag.	Rabbit polyclonal	1:2000	HIAR	Dako
Hepatitis B surface Ag.	Mab/3E7	1:100	HIAR	Dako
Herpes simplex 1 and 2	Rabbit polyclonal	1:3200	HIAR	Dako
H. pylori	Rabbit polyclonal	1:40	Protase 1	Dako
HHV 8	Mab/LNA-1	1:500	HIAR	Novocastra
L. monocytogenes	Rabbit polyclonal	1:5000	Proteinase K	Difco
Parvovirus B19	Mab/R92F6	1:500	HIAR	Novocastra
P. carinii	Mab/3F6	1:20	HIAR	Novocastra
Respiratory syncytial virus	Mab/5H5N	1:200	HIAR	Novocastra
T. gondii	Rabbit polyclonal	1:320	HIAR	Biogenex
West Nile virus	Mab/5H10	1:400	Proteinase K	Bioreliance

* Heat-induced antigen retrieval.
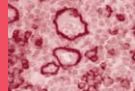
VIRAL INFECTIONS

Immunohistochemistry has played an important role not only in the diagnosis of a large number of viral infections but also in the study of their pathogenesis and epidemiology. Traditionally, the diagnosis of viral infections has relied on cytopathic changes observed on routine histopathology. Several viral pathogens produce characteristic intracellular inclusions, which allow pathologists to make a presumptive diagnosis of viral infection. However, for some viral infections the characteristic cytopathic changes are often subtle and sparse, requiring a meticulous search.[11] Moreover, only 50% of the known viral diseases are associated with characteristic intracellular inclusions.[12] In addition, formalin, which is the most commonly used fixative in histopathology, is a poor fixative for demonstrating the morphologic and tinctorial features of viral inclusions.[12] When viral inclusions are not detected in hematoxylin-eosin stained sections, or when the viral inclusions present cannot be differentiated from those of other viral diseases, immunohistochemical techniques offer a more reliable alternative to reach a specific diagnosis.

Hepatitis B virus

Hepatitis B virus infection constitutes an important cause of chronic hepatitis in a significant proportion of patients. In many instances, the morphologic changes induced by hepatitis B virus on hepatocytes are not typical enough to render a presumptive diagnosis of hepatitis B viral infection. In other instances, there may be so little hepatitis B surface antigen (HBsAg) that it cannot be demonstrated by techniques such as orcein staining. In these cases, immunohistochemical techniques to detect HBsAg are more sensitive than histochemical methods and are helpful in reaching the diagnosis.[13] Immunostaining for HBsAg has been used in the diagnosis of hepatitis B and in the study of carrier states.[14,15] Eighty percent or more of cases with positive serologic results for HBsAg demonstrate cytoplasmic HBsAg using immunohistochemistry (Fig. 2.1).[16] By immunoperoxidase localization, hepatitis B core antigen (HBcAg) can be demonstrated within the nuclei or the cytoplasm of hepatocytes, or both (Fig. 2.2). Predominantly cytoplasmic expression of HBcAg is associated with a higher grade of hepatitis activity.[17]

Hepatitis C virus

The clinical diagnosis of hepatitis C virus (HCV) infection is based on serological demonstration of antibodies against HCV and detection of HCV RNA in serum. However, anti-HCV antibodies may be not detectable in sera of immunocompromised patients.[18] Several polyclonal and monoclonal antibodies directed against HCV
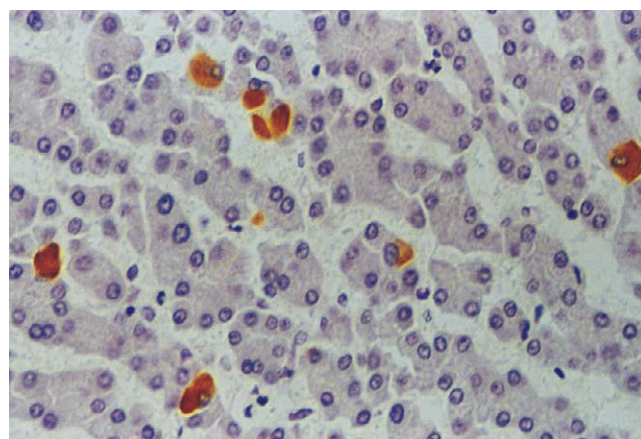

Fig. 2.1 Liver biopsy specimen from a patient with chronic hepatitis B. Scattered hepatocytes show cytoplasmic reactivity with monoclonal antibody to HBsAg. (Immunoperoxidase staining with diaminobenzidine [DAB] and hematoxylin counterstain, ×400)
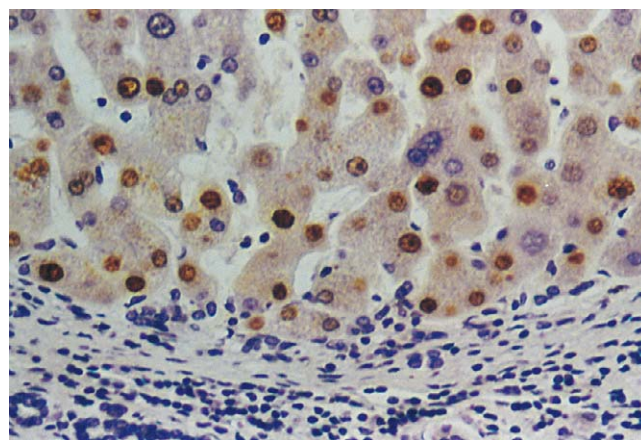

Fig. 2.2 Chronic active hepatitis B. Numerous hepatocytes display intranuclear reactivity with polyclonal antibody to hepatitis B core antigen (HBcAg). (Immunoperoxidase staining with DAB and hematoxylin counterstain, ×400)

nonstructural proteins have been produced for use in immunohistochemistry. Nevertheless, most of the antibodies are not clinically useful because of low sensitivities compared with HCV RNA detection by RT-PCR.[18–21] Moreover, cross-reactivity with non-HCV epitopes has been found with the monoclonal antibody TORDJI-22.[21,22] Diffuse or coarse granular cytoplasmic staining is usually seen in a variable number of hepatocytes in patients with chronic HCV hepatitis,[19,23,24] and within rare biliary epithelial cells, lymphocytes, and sinusoidal endothelial cells.

More recently, a monoclonal antibody against HCV E2 envelope glycoprotein has been demonstrated to be a highly sensitive antibody for the diagnosis and clinical follow-up of chronic HCV hepatitis with an overall accuracy of 95% when used with the EnVision technique.[25] This antibody is useful for the early detection of graft reinfection in patients with liver transplant for

HCV-related cirrhosis, and to differentiate reinfection from graft rejection.[25]

Herpesviruses

Histologically, the diagnosis of herpes simplex virus (HSV) infection involves the detection of multinucleated giant cells containing characteristic molded, ground glass-appearing nuclei and Cowdry's type A intranuclear inclusions. When there are abundant viral inclusions within infected cells, the diagnosis is usually straightforward. However, the diagnosis of 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.[26] In these cases, the use of immunohistochemistry to detect HSV antigens is advantageous.[27,28]

Immunohistochemistry using either polyclonal or monoclonal antibodies against HSV antigens has proven to be a sensitive and specific technique to diagnose HSV infections (Fig. 2.3).[29,30] Although polyclonal antibodies against the major HSV glycoprotein antigens are sensitive, they do not allow distinction between HSV-1 and HSV-2 because these two viruses are antigenically similar.[31] In addition, the histologic features of HSV infection are not specific and can also occur in patients with varicella-zoster (VZV) infection. Monoclonal antibodies against the VZV envelope glycoprotein gp1 are sufficiently sensitive and specific to allow a clear-cut distinction between HSV and VZV infections.[27,32,33]

Immunohistochemistry has also been useful in demonstrating the association of human herpesvirus 8 (HHV-8) with Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease.[34–38] The diagnosis of Kaposi's sarcoma may be problematic due to its broad morphologic spectrum and similar appear-

ance to other benign and malignant neoplastic vascular lesions. Immunostaining of Kaposi's sarcoma latent associated nuclear antigen-1 (LANA-1) is useful to confirm the diagnosis of Kaposi's sarcoma, particularly in difficult early lesions or when the neoplasm presents in an unusual location, and allows distinction of Kaposi's sarcoma from several morphologically similar vasoproliferative lesions (see Chapter 12).[39,40] Immunostaining is restricted to the nuclei of spindle cells and endothelial cells of the slit-like vascular spaces. Immunohistochemistry has also demonstrated expression of HHV-8 LANA-1 in mesothelial cells of HIV-associated recurrent pleural effusions[41] and in the cells of the plexiform lesions of primary pulmonary hypertension.[42]

Cytomegalovirus (CMV) is an important opportunistic pathogen in immunocompromised patients. Histologic diagnosis of 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.[43] In these cases, immunohistochemistry using monoclonal antibodies against early and late CMV antigens allows the detection of CMV antigens in the nucleus and cytoplasm of infected cells (Fig. 2.4). In addition, immunohistochemistry may allow detection of CMV antigens early in the course of the disease when cytopathic changes have not yet developed.[44–49] For example, CMV early nuclear antigen is expressed 9 to 96 hours after cellular infection and indicates early active viral replication. Immunohistochemistry has been useful in the detection of CMV infection in patients with steroid refractory ulcerative colitis, and in detecting occult CMV infection of the central nervous system in liver transplant patients who develop neurological
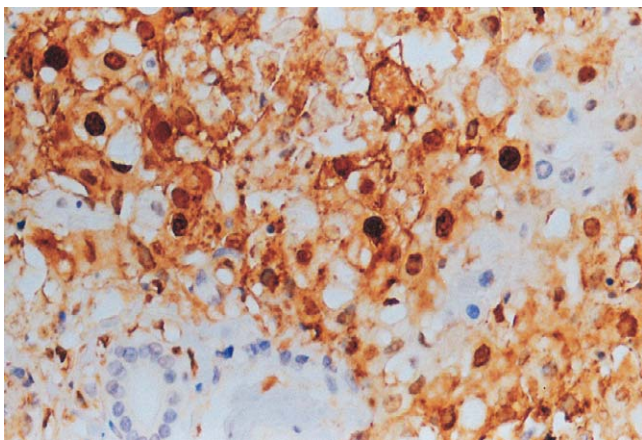

Fig. 2.3 Herpes simplex hepatitis. The nuclei and cytoplasm of many hepatocytes and Kupffer cells are strongly immunostained for herpes simplex antigen. (Immunoperoxidase staining with DAB and hematoxylin counterstain, ×400)
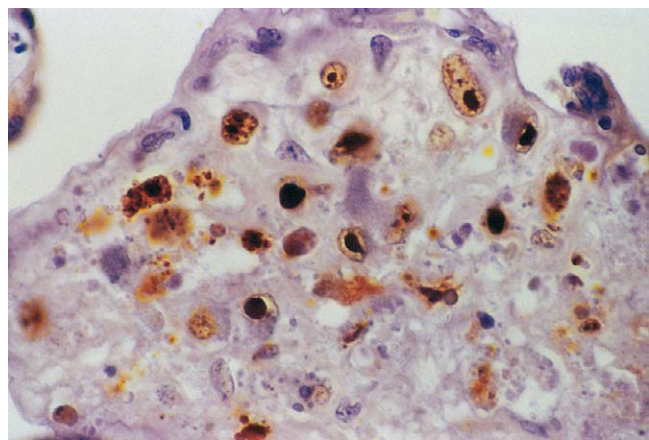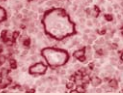

Fig. 2.4 Cytomegalovirus (CMV) villitis in a case of congenital CMV infection. Stromal cells and Hofbauer cells show intranuclear and cytoplasmic CMV antigen. (Immunoperoxidase staining with diaminobenzidine [DAB] and hematoxylin counterstain, ×400)

complications.[50,51] It has also been used to demonstrate a high frequency of CMV antigens in tissues from first trimester abortions.[52] The sensitivity of immunohistochemistry is better than light microscopic identification of viral inclusions and compares favorably with culture and in situ hybridization.[44,46,47,49,53] Additionally, immunohistochemical assays can be completed faster than the shell vial technique, with immunofluorescence, or culture allowing for rapid results that are important for early anti-CMV therapy.[49]

Adenoviruses

Adenovirus is increasingly recognized as a cause of morbidity and mortality among immunocompromised patients owing to transplant and congenital immunodeficiency.[54,55] Rarely, adenovirus infection has been described in HIV-infected patients.[56–58] Characteristic adenovirus inclusions are amphophilic, intranuclear, homogeneous, and glassy. However, in some cases, the infection may contain only rare cells showing the characteristic cytopathic effect.[57] In addition, other viral inclusions, including CMV, human papillomavirus, HSV, and VZV, can be mistaken for adenovirus inclusions and vice versa. Moreover, in immunosuppressed patients the incidence of coinfection with other viruses is high. In these circumstances immunohistochemical assay may be necessary for a definitive diagnosis. A monoclonal antibody that is reactive with all 41 serotypes of adenovirus has been used in an immunohistochemical technique to demonstrate intranuclear adenoviral antigen in immunocompromised patients (Fig. 2.5).[57–61] Histologic diagnosis of adenovirus colitis is difficult, and it is usually underdiagnosed. Immunohistochemical staining has been of value in differentiating adenovirus colitis from CMV colitis.[57,62]

Other herpesviruses infections that have been diagnosed using immunohistochemical methods include Epstein-Barr viral infection[63] and human herpesvirusus 6 infection.[64]

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 typical findings in bone marrow specimens, including decreased or absent red cell precursors, giant pronormoblasts, and eosinophilic or amphophilic intranuclear inclusions in erythroid cells.[65,66] Because intravenous immunoglobulin therapy is effective, a rapid and accurate diagnostic method is important. Immunohistochemistry with a monoclonal antibody against VP1 and VP2 capsid proteins has been used as a rapid and sensitive method to establish the diagnosis of parvovirus B19 infection in formalin-fixed, paraffin-embedded tissues.[67–70] Immunohistochemistry is of particular help in detecting parvovirus B19 antigen in cases with sparse inclusions, to study cases not initially identified by examination of routinely stained tissue sections, or in cases of hydrops fetalis where there is advanced cytolysis (Fig. 2.6).[67,71,72] Several studies have found a good correlation between morphologic, immunohistochemical, in situ hybridization and polymerase chain reaction (PCR).[66,67,70,72]

Viral hemorrhagic fevers

Since the 1980s, numerous emerging and reemerging agents of viral hemorrhagic fevers have attracted the attention of pathologists.[3–5] These investigators have
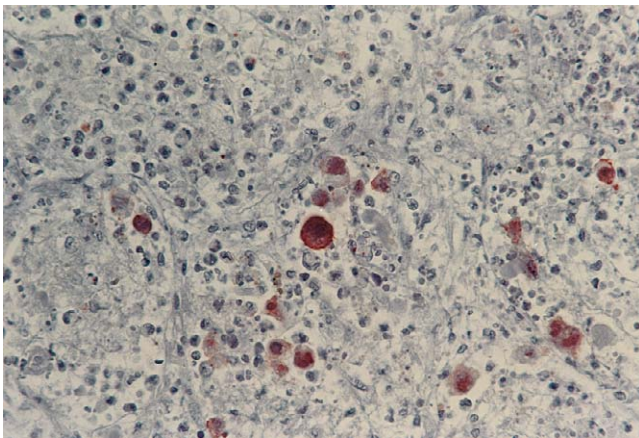

Fig. 2.5 Adenovirus pneumonia. Infected cells within a necrotizing exudate show intranuclear reactivity with antibody to adenovirus antigen. (Immunoperoxidase staining with aminoethylcarbazole [AEC] and hematoxylin counterstain, ×400)
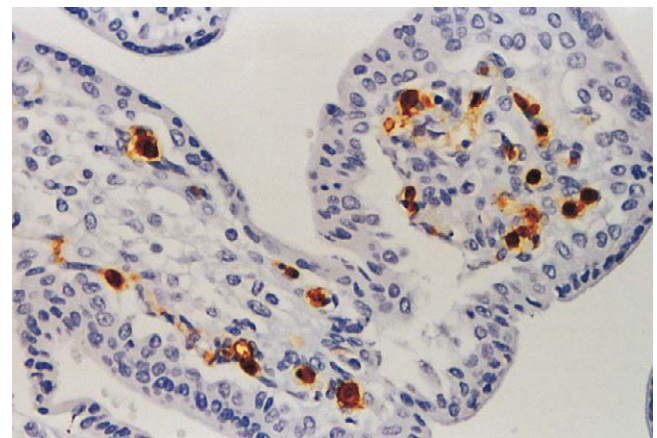

Fig. 2.6 Hydrops fetalis caused by parvovirus B19 infection. Normoblasts within the villous capillaries show intranuclear viral antigen. (Immunoperoxidase staining with DAB and hematoxylin counterstain, ×600)

played an important role in the identification of these agents and supporting epidemiologic, clinical, and pathogenetic studies of the emerging viral hemorrhagic fevers.[4,5,7] Viral hemorrhagic fevers are often fatal, and in the absence of bleeding or organ manifestations these diseases are clinically difficult to diagnose and frequently require handling and testing of potentially dangerous biological specimens. In addition, histopathologic features are not pathognomonic, and they can resemble other viral, rickettsial and bacterial (e.g., leptospirosis) infections. Immunohistochemistry is essential and has been successfully and safely applied to the diagnosis and study of the pathogenesis of these diseases.

Several studies have established the utility of immunohistochemistry as a sensitive, safe, and rapid diagnostic method for the diagnosis of viral hemorrhagic fevers such as yellow fever (Fig. 2.7),[73–75] dengue hemorrhagic fever,[75,76] Crimean-Congo hemorrhagic fever,[77] Argentine hemorrhagic fever,[78] Venezuelan hemorrhagic fever,[79] and Marburg disease.[80] Additionally, a sensitive, specific, and safe immunostaining method has been developed to diagnose Ebola hemorrhagic fever in formalin-fixed skin biopsies (Fig. 2.8).[81] Immunohistochemistry demonstrated that Lassa virus targets primarily endothelial cells, mononuclear inflammatory cells, and hepatocytes (Fig. 2.9).[81–83]

Papovaviruses

Immunohistochemistry for the detection of human papillomavirus in formalin-fixed tissue has been replaced by more sensitive diagnostic molecular techniques such in situ hybridization.[84–87] In addition to low sensitivity compared with molecular techniques,
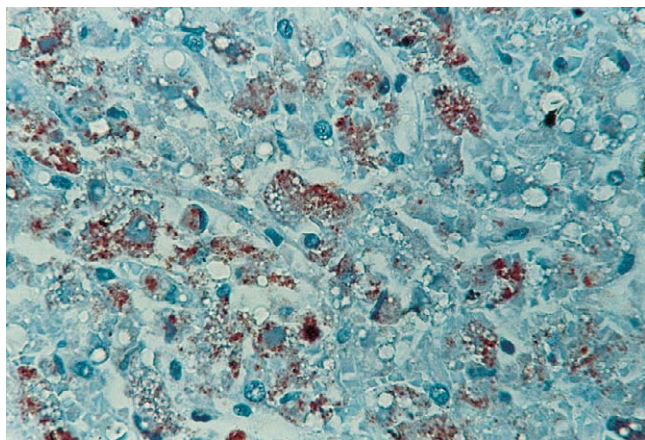

Fig. 2.7 Yellow fever. Abundant yellow fever viral antigen is seen within hepatocytes and Kupffer cells. (Immunoperoxidase staining with AEC and hematoxylin counterstain, ×400) (Courtesy of Dr. JF Aronson, University of Texas Medical Branch.)
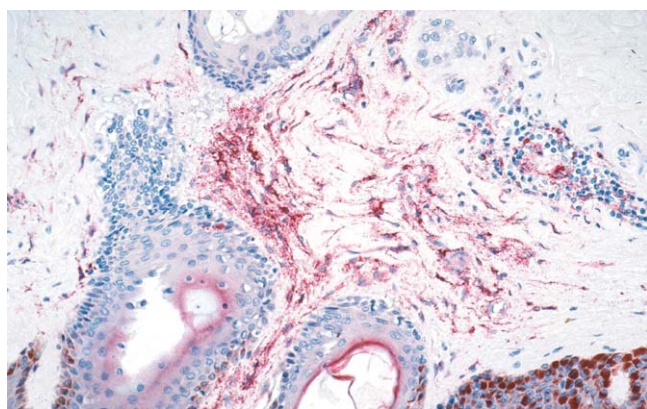
Fig. 2.8 Ebola virus. Extensive Ebola viral antigens are seen primarily within fibroblasts in dermis of a skin specimen from a fatal case of Ebola hemorrhagic fever. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×20)
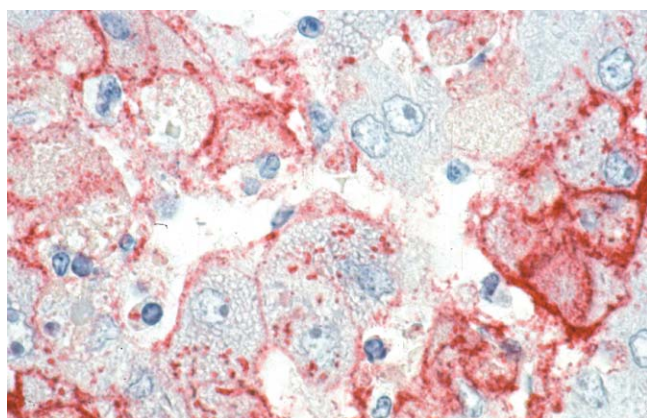

Fig. 2.9 Lassa fever. Liver from a patient with Lassa fever. Scattered hepatocytes and reticuloendothelial cells show reactivity with monoclonal antibody to Lassa virus. (Naphthol fast red substrate and hematoxylin counterstain, original magnification ×100)

immunohistochemistry detects only productive and not latent infections and cannot be used to determine the type of virus present (Fig. 2.10).

BK virus infections are frequent during infancy; in immunocompetent individuals the virus remains latent in the kidneys, central nervous system and B-lymphocytes. In immunocompromised patients, the infection reactivates and spreads to other organs. In the kidney, the infection is associated with mononuclear interstitial inflammatory infiltrates and tubular atrophy, findings that can be difficult to distinguish from acute transplant rejection. Besides, the cytopathic changes observed in BK virus infection are not pathognomonic and can be observed in other viral infections. In this setting, immunohistochemistry has been useful to demonstrate BK virus infection.[88–91]

The human polyomavirus JC is a double-stranded DNA virus that causes progressive multifocal leukoencephalopathy (PML). This fatal demyelinating disease
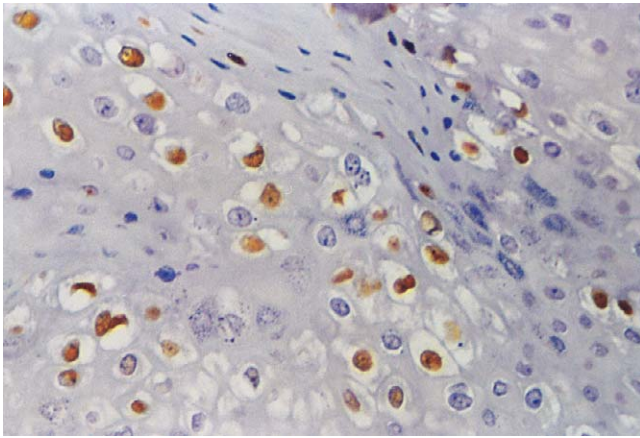
Fig. 2.10 Immunoperoxidase staining for human papillomavirus (HPV) in a patient with mild squamous dysplasia. HPV viral antigen localizes within the nuclei of koilocytotic cells. (DAB with hematoxylin counterstain, ×600)

Fig. 2.11 Rabies. Immunostaining of rabies viral antigens in neurons of CNS using a rabbit polyclonal antibody. Red precipitate corresponds to Negri inclusions on H&E. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×40)

is characterized by cytopathic changes in oligodendrocytes and bizarre giant astrocytes. Immunohistochemical technique using a polyclonal rabbit antiserum against the protein VP1 is a specific, sensitive, and rapid method for confirming the diagnosis of PML.[92–95] JC virus antigen is usually seen within oligodendrocytes and occasional astrocytes, and antigen-bearing cells are more commonly seen in early lesions.

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.[96–99]

The diagnosis of rabies relies heavily on histopathologic examination of tissues to demonstrate the characteristic cytoplasmic inclusions (Negri bodies). In an important percentage of cases, Negri bodies may be inconspicuous and so few that confirming the diagnosis of rabies may be extremely difficult.[100] Furthermore, in nonendemic areas the diagnosis of rabies is usually not suspected clinically or the patient can present with an ascending type of paralysis. In these settings, immunohistochemical staining is a very sensitive, safe, and specific diagnostic tool for rabies (Fig. 2.11).[100–104] Other viral agents that can be diagnosed using immunohistochemical methods include enterovirus,[105–108] Eastern equine encephalitis,[109–111] and rotavirus.[112–114]

BACTERIAL INFECTIONS

Among bacterial infections, the greatest number of immunohistochemical studies have been performed in the investigation of *Helicobacter pylori*. A few studies
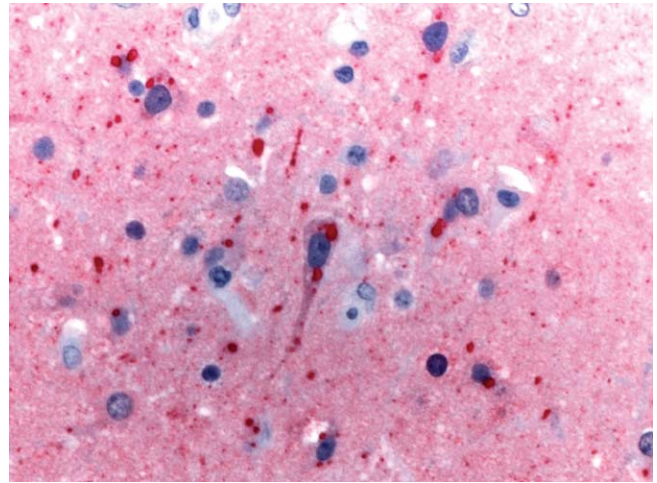
have evaluated the use of immunohistochemistry in other bacterial, mycobacterial, rickettsial, and spirochetal infections.

Antigen retrieval is generally not required for the immunohistochemical demonstration of bacteria in fixed tissue. However, interpretation of the results can be complicated by the fact that many of these antibodies will cross-react with other bacteria. Moreover, antibodies may react with only portions of the bacteria, and they may label remnants of bacteria or spirochetes when viable organisms are no longer present.

Helicobacter pylori infection

Gastric infection by *H. pylori* results in chronic active gastritis and is strongly associated with lymphoid hyperplasia, gastric lymphomas, and gastric adenocarcinoma. Heavy infections with numerous organisms are easily detected on routine hematoxylin and eosin-stained tissues; however, the detection rate is only 66% with many false-positive and false-negative results.[115–117] Conventional histochemical methods such as silver stains are more sensitive than hematoxylin and eosin in detecting *H. pylori*. Nonetheless, for the detection of scant numbers of organisms, immunohistochemistry has proved to be highly specific and sensitive, less expensive when all factors are considered, and superior to conventional histochemical methods (Fig. 2.12).[116,117] Treatment for chronic active gastritis and *H. pylori* infection can change the shape of the microorganism, making difficult its identification and differentiation from extracellular debris or mucin globules. In these cases, immunohistochemistry improves the rate of successful
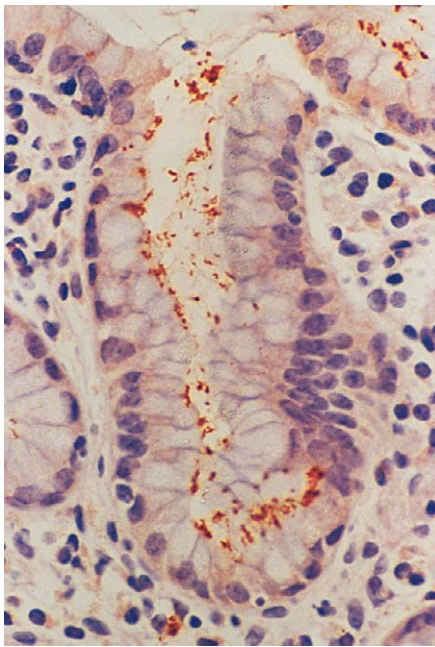
Fig. 2.12 Numerous curved *Helicobacter pylori* in the superficial mucus are clearly demonstrated by immunoperoxidase staining in this patient with chronic active gastritis. (DAB with hematoxylin counterstain, ×600)

identification of the bacteria even when histologic examination and cultures are falsely negative.[118–121]

Whipple's disease

Whipple's disease affects primarily the small bowel and mesenteric lymph nodes and less commonly other organs such as heart and central nervous system. Numerous foamy macrophages characterize the disease, and the diagnosis usually relies on the demonstration of PAS-positive intracytoplasmic bacteria. Nevertheless, the presence of PAS-positive macrophages is not pathognomonic; they can be observed in other diseases such as *Mycobacterium avium* complex infections, histoplasmosis, infections due to *Rhodococcus equi*, and macroglobulinemia. Immunohistochemical staining with a rabbit polyclonal antibody provides a sensitive and specific method for the rapid diagnosis of intestinal and extraintestinal Whipple's disease and for follow-up of treatment response.[122–124]

Rocky Mountain spotted fever

Confirmation of Rocky Mountain spotted fever (RMSF) usually requires the use of serologic methods to detect antibodies to spotted fever group (SFG) rickettsiae; yet a significant percentage of patients with RMSF lack diagnostic titers during the first week of disease. Immunohistochemistry has been successfully used to

detect SFG rickettsiae in formalin-fixed tissue sections (Fig. 2.13).[125,126] Several studies have illustrated the value of immunohistochemistry in the diagnosis of suspected cases of RMSF using skin biopsies, and in confirming fatal cases of seronegative RMSF.[127,128]

Bartonella infections

Bartonella are slow growing, fastidious Gram-negative, Warthin-Starry-stained bacteria associated with bacillary angiomatosis, peliosis hepatis, cat-scratch disease, and blood culture-negative endocarditis. Immunostaining has been successfully used to identify *Bartonella henselae* and *B. quintana* in heart valves from patients with blood culture-negative endocarditis (Fig. 2.14).[129,130] This polyclonal rabbit antibody that does not allow
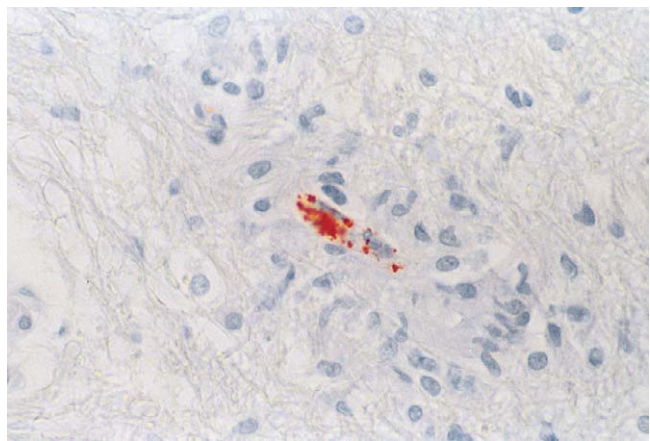
Fig. 2.13 Immunohistologic demonstration of *Rickettsia rickettsii* within endothelial cells surrounded by a small glial nodule in the brainstem of this patient with fatal Rocky Mountain spotted fever. (Immunoperoxidase staining with AEC and hematoxylin counterstain, ×600)

Fig. 2.14 *Bartonella*. Immunohistologic demonstration of *Bartonella henselae* within heart valve of patient with culture-negative endocarditis. Mouse monoclonal anti-*B. henselae* antibody. (Naphthol fast red substrate and hematoxylin counterstain, original magnification ×40)

differentiation between *B. henselae* and *B. quintana* has also been used in the detection of these microorganisms in cat-scratch disease, bacillary angiomatosis, and peliosis hepatis.[131,132] A commercially available monoclonal antibody specific for *B. henselae* is also available and has been used to demonstrate the organism in a case of spontaneous splenic rupture caused by this bacterium.[133]

Other bacterial infections

Other bacterial diseases that can be identified by immunohistochemistry in formalin-fixed tissue including leptospirosis, which is a zoonosis that usually presents as an acute febrile syndrome but occasionally can have unusual manifestations such as pulmonary hemorrhage with respiratory failure or abdominal pain.[134–136] Rabbit polyclonal antibodies have been used in immunohistochemistry to detect leptospiral antigens in the gallbladder and lungs from patients with unusual presentations (Fig. 2.15).[134–137]

Lyme disease has protean clinical manifestations, and *Borrelia burgdoferi* is difficult to culture from tissues and fluids. In addition, cultures are rarely positive before 2–4 weeks of incubation. *Borrelia burgdoferi* can be identified in tissues by immunostaining with polyclonal or monoclonal antibodies. Although immunohistochemistry is more specific than silver impregnation stains, the sensitivity of immunostaining is poor, and the microorganisms are difficult to detect due to the low numbers present in tissue sections.[138,139] Immunohistochemistry is useful in identifying *Haemophilus influenzae*,[140–142] *Chlamydia* species,[143–145] *Legionella pneumophila* and *L. dumoffii*,[146–148] *Listeria monocytogenes*,[149–151] *Salmonella*,[152,153] mycobacteria,[154–159] rickettsial infections other than Rocky Mountain spotted fever such as boutonneuse
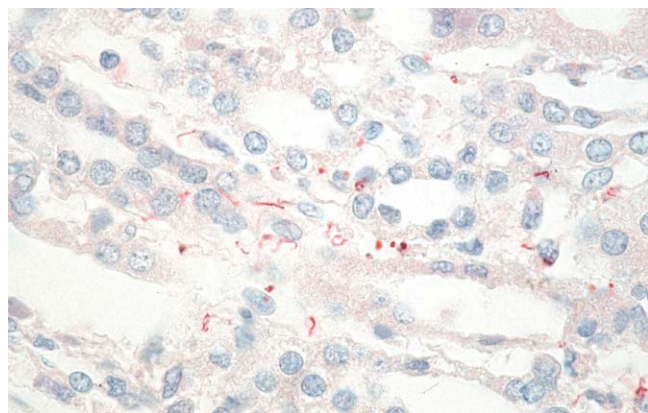

Fig. 2.15 *Leptospira*. Immunostaining of intact leptospires and granular forms of leptospiral antigens in kidney of patient who died of pulmonary hemorrhage. (Immunoalkaline phosphatase with rabbit polyclonal antisera with naphthol fast red substrate and hematoxylin counterstain, original magnification ×63)

fever, typhus fever,[160] rickettsialpox,[161,162] African tick bite fever,[125] scrub typhus,[163] and spirochetes in patients with syphilis.[164–166]

FUNGAL INFECTIONS

The great majority of fungi are readily identified by hematoxylin and eosin staining alone or in combination with histochemical stains (periodic acid–Schiff [PAS], and Gomori's methenamine silver [GMS]). However, these stains cannot distinguish morphologically similar fungi with potential differences in susceptibility to antimycotic drugs. In addition, fungal elements may appear atypical in tissue sections because of several factors including steric orientation, age of the fungal lesion, effects of antifungal chemotherapy, type of infected tissue, and host immune response.[167] Currently, the final identification of fungi relies on culture techniques; however, culture may take several days or longer to yield a definitive result, and often surgical pathologists have no access to fresh tissue.

In past years, immunohistochemistry has been used to identify various fungal elements in paraffin-embedded, formalin-fixed tissue.[168–170] Immunohistochemical methods have the advantage of providing rapid and specific identification of several fungi and allowing pathologists to be able to identify unusual filamentous hyphal and yeast infections and accurately distinguish them from confounding artifacts.[169,172] In addition, immunohistochemistry allows pathologists to correlate microbiological and histological findings of fungal infections and to distinguish them from harmless colonization. Immunohistochemistry can also be helpful when more than one fungus is present; in these cases dual immunostaining techniques can highlight the different fungal species present in the tissue.[173] An important limitation of immunohistochemistry in the identification of fungi is the well-known, widespread occurrence of common antigens among pathogenic fungi that frequently results in cross-reactivity with polyclonal antibodies and even with some monoclonal antibodies.[169,171–174] Therefore, assessment of cross-reactivity using a panel of fungi is a very important step in the evaluation of immunohistochemical methods.[169,170]

Candida species are often stained weakly with hematoxylin and eosin, and sometimes the yeast form may be difficult to differentiate from *Histoplasma capsulatum*, *Cryptococcus neoformans*, and even *Pneumocystis carinii*. Polyclonal and monoclonal antibodies against *Candida* genus antigens are sensitive and strongly reactive and do not show cross-reactivity with other fungi tested.[169,170,175,176] In particular, two monoclonal antibodies against *Candida albicans* mannoproteins show high sensitivity and specificity. Monoclonal antibody 3H8 recognizes primarily filamentous forms of *C. albicans*,

whereas monoclonal antibody 1B12 highlights yeast forms.[176,177]

Identification of *Cryptococcus neoformans* usually is not a problem when the fungus produces a mucicarmine-positive capsule. However, infections by capsule-negative strains are more difficult to diagnose, and the disease can be confused with histoplasmosis, blastomycosis, or torulopsis. Also, in longstanding infections the yeast often appear atypical and fragmented. Polyclonal antibodies raised against *C. neoformans* yeast cells are sensitive and specific.[169,170] More recently, monoclonal antibodies have been produced that allow identification and differentiation of varieties of *C. neoformans* in formalin-fixed tissue. The antibodies are highly sensitive (97%) and specific (100%) to differentiate *C. neoformans* var. *neoformans* from *C. neoformans* var. *gattii*.[178,179]

Sporothrix schenckii may be confused in tissue sections with *Blastomyces dermatitidis* and fungal agents of pheohyphomycosis. In addition, yeast cells of *S. schenckii* may be sparsely present in tissues. Specific antibodies against yeast cells of *S. schenckii* are sensitive but demonstrate cross-reactivity with *Candida* species; however, after specific adsorption of the antibody with *Candida* yeast cells, the cross-reactivity of the antibodies is eliminated.[169,170]

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 and eosin-stained tissues. In addition, the yield of cultures in histologically proven cases is low, ranging from 30% to 50%.[180,181] 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.[174,182,183] 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.[181,184]

Cysts and trophozoites of *Pneumocystis carinii* can be detected in bronchoalveolar lavage specimens using monoclonal antibodies that yield results that are slightly more sensitive than GMS, Giemsa or Papanicolaou staining for detecting cysts (Fig. 2.16).[170,185,186] Antibodies are most helpful in the diagnosis of *P. carinii* pneumonia (PCP) when atypical pathologic features are present such as granulomatous PCP or the presence of hyaline membranes or in cases of extrapulmonary pneumocystosis.

Penicillium marneffei usually causes a disseminated infection in immunocompromised patients that clini-
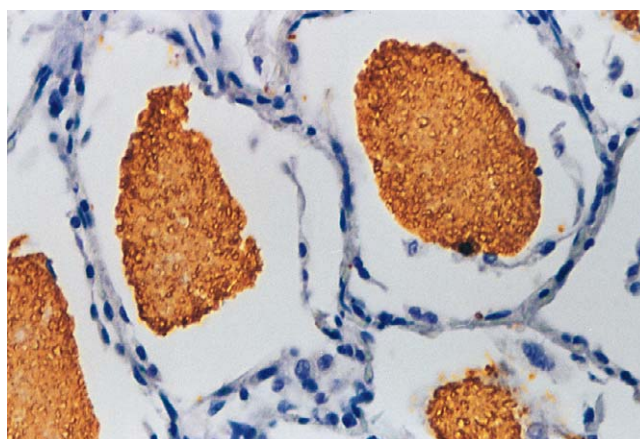

Fig. 2.16 Human immunodeficiency virus (HIV)-infected immunodeficient patient with *Pneumocystis carinii* pneumonia. Cohesive aggregates of cyst forms and trophozoites within alveolar spaces are demonstrated with a monoclonal antibody against *P. carinii* in an immunoperoxidase technique. (DAB with hematoxylin counterstain, ×400)

cally resembles histoplasmosis or leishmaniasis.[171,187] Morphologically, the organisms must be differentiated from *H. capsulatum*, *C. neoformans*, and *C. albicans*. The monoclonal antibody EBA-1 against the galactomannan of *Aspergillus* species cross-reacts with and detects *P. marneffei* in tissue sections.[182,188] Immunohistochemistry has also been used to detect *Blastomyces*, *Coccidioides*, and *Histoplasma*.[169,170,189] However, the antibodies have significant cross-reactivity with several other fungi.

PROTOZOAL INFECTIONS

Protozoa usually can be identified in tissue sections stained with hematoxylin and 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. The role of immunohistochemistry in the detection of protozoal infections has been limited to cases in which the morphology of the parasite is distorted by tissue necrosis or autolysis. In addition, in immunocompromised patients, toxoplasmosis can have an unusual disseminated presentation with numerous tachyzoites without bradyzoites (Fig. 2.17).[190,191] Immunohistochemistry has also been useful in cases with unusual presentation of the disease.[192]

The diagnosis of leishmaniasis in routine practice usually is not difficult; however, in certain circumstances the pathologic diagnosis may be more problematic as is the case in 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.[193] In these cases, immunohistochemical staining has been a valuable diagnostic tool.[193–196] The highly sensitive and
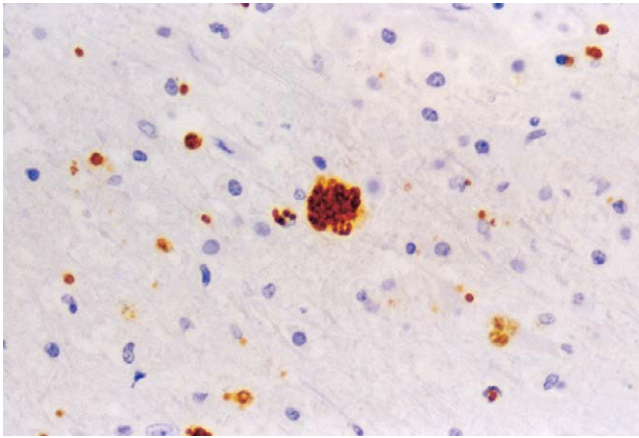
Fig. 2.17 HIV-infected patient with toxoplasmic encephalitis. Immunoperoxidase highlights pseudocysts and scattered tachyzoites. (DAB with hematoxylin counterstain, ×400).
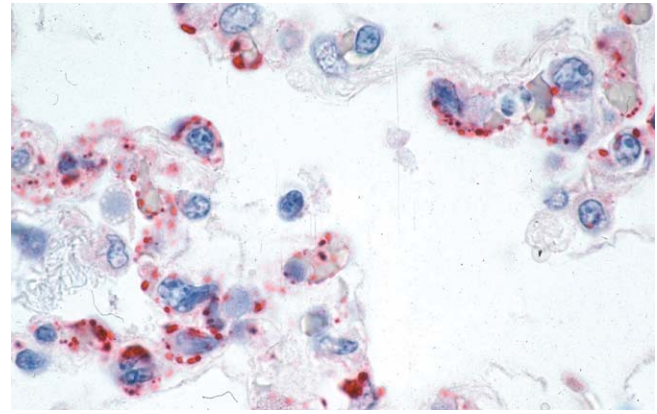

Fig. 2.18 Hantavirus antigen-positive endothelial cells of pulmonary microvasculature in lung of an HPS patient as determined by immunohistochemistry using a mouse monoclonal antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×100)

specific monoclonal antibody p19-11 recognizes different species of *Leishmania* and allows differentiation from morphologically similar microorganisms (*Toxoplasma*, *Trypanosoma cruzi*, and *P. marneffei*).[193]

Immunohistochemistry has also been used to identify *Cryptosporidium*,[197] *Entamoeba histolytica*,[198] *Trypanosoma cruzi*,[199–201] babesia,[202] and *Giardia lamblia*[203] in formalin-fixed, paraffin-embedded tissue samples.

EMERGING INFECTIOUS DISEASES

In 1992, the Institute of Medicine defined emerging infectious diseases (EID) as caused by new, previously unidentified microorganisms or those whose incidence in humans has increased within the past two decades or threatens to increase in the near future.[204] The list of pathogens newly recognized since 1973 is long and continues to increase, and recognizing emerging infections is a challenge with many new infectious agents remaining undetected for years before emerging as identified public health problems.[205] EID are global phenomena that require a global response. The Centers for Disease Control (CDC) has defined the strategy to prevent and detect EID.[205] The anatomic pathology laboratory plays a critical role in the initial and rapid detection of EID.[206,207] Immunohistochemistry, besides assisting in the identification of new infectious agents, has contributed to the understanding of the pathogenesis and epidemiology of EID.

Hantavirus pulmonary syndrome

In 1993, several previously healthy individuals died of rapidly progressive pulmonary edema, respiratory insufficiency and shock in southwestern United States.[208,209] Immunohistochemistry was central in the

identification of viral antigens of a previously unknown hantavirus.[210,211] Immunohistochemical analysis was also important in identifying the occurrence of unrecognized cases of hantavirus pulmonary syndrome prior to 1993 and in showing the distribution of viral antigen in endothelial cells of the microcirculation, particularly in the lung (Fig. 2.18).[210,212]

West Nile virus encephalitis

West Nile virus (WNV) was originally identified in Africa in 1937, and the first cases of WNV encephalitis in the US were described in 1999. The clinical picture is variable and non-specific ranging from subclinical to flaccid paralysis and encephalitis characterized morphologically by perivascular mononuclear cell inflammatory infiltrates, neuronal necrosis, edema, and microglial nodules, particularly prominent in the brainstem, cerebellum, and spinal cord.[213–217] The diagnosis of WNV is usually established by identification of virus-specific IgM in CSF and/or serum, and demonstration of viral RNA in serum, CSF, or other tissue.[218] Immunostaining with either monoclonal or polyclonal antibodies has been successfully employed to diagnose WNV infection in immunocompromised patients who lacked an adequate antibody response (Fig. 2.19).[214]

Enterovirus 71 encephalomyelitis

Enterovirus 71 (EV71) has been associated with hand, foot, and mouth disease, herpangina, aseptic meningitis, and poliomyelitis-like flaccid paralysis. More recently, EV71 has been associated with unusual cases of fulminant encephalitis, pulmonary edema and
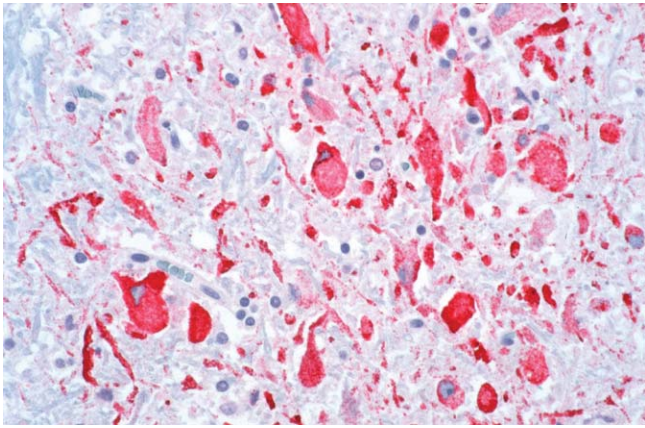
Fig. 2.19 West Nile virus. Immunostaining of flaviviral antigens in neurons and neuronal processes in CNS tissue from an immunosuppressed patient who died of WNV encephalitis. (Flavivirus-hyperimmune mouse ascitic fluid, naphthol fast red substrate and hematoxylin counterstain, original magnification ×40)
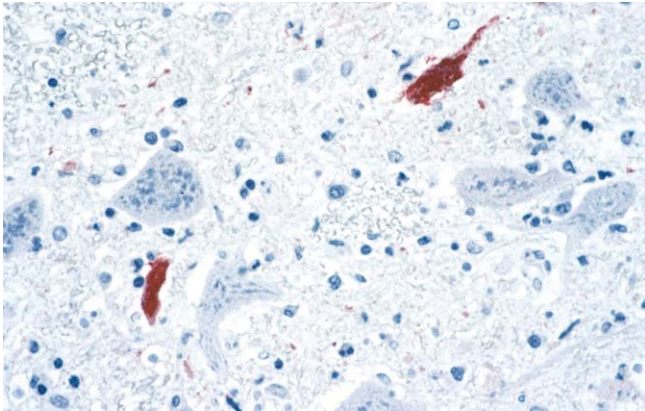

Fig. 2.20 Enterovirus 71. Positive staining of EV71 viral antigens in neurons and neuronal processes of a fatal case of enterovirus encephalitis. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×40)

hemorrhage, and heart failure.[219,220] Severe and extensive encephalomyelitis of the cerebral cortex, brainstem, and spinal cord has been described. Immunohistochemical staining with monoclonal antibody against EV71 has played a pivotal role in the linking of EV71 infection to fulminant encephalitis (Fig. 2.20). Viral antigen is observed within neurons, neuronal processes, and mononuclear inflammatory cells.[221–223]

Nipah virus infection

Nipah virus is a recently described paramyxovirus that causes an acute febrile encephalitic syndrome with high mortality rates.[224–226] Pathology played a key role in identifying the causative agent. Histopathologic findings include vasculitis with thrombosis, microinfarctions,
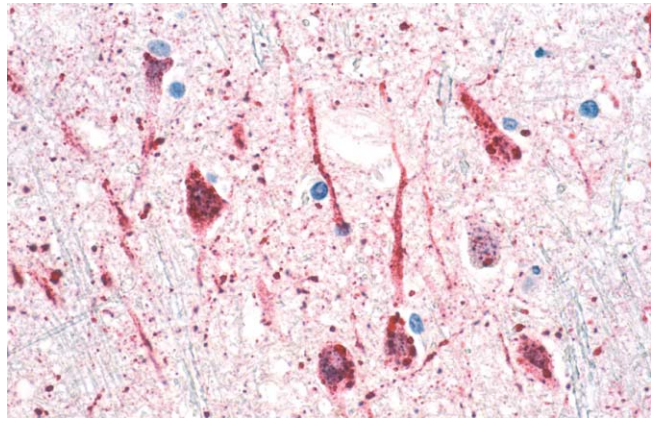

Fig. 2.21 Nipah virus. Immunostaining of Nipah virus antigens in neurons and neuronal processes in CNS of a fatal case of Nipah virus encephalitis. (Naphthol fast red substrate and hematoxylin counterstain, original magnification ×63)

syncytial giant cells, and viral inclusions.[224,226] Syncytial giant endothelial cells, albeit characteristic of this disease, are seen only in 25% of cases,[224] and viral inclusions of similar morphology can be seen in other paramyxoviral infections. Immunostaining provides a useful tool for unequivocal diagnosis of the disease, demonstrating viral antigen within neurons and endothelial cells of most organs (Fig. 2.21).[5,224]

Ehrlichioses

Bacteria belonging to the genera *Ehrlichia* and *Anaplasma* are the agents of human monocytotropic ehrlichiosis, and human granulocytotropic anaplasmosis, respectively. The acute febrile illnesses usually present with cytopenias, myalgias, and mild to moderate hepatitis.[227–230]

Diagnosis of ehrlichiosis depends upon finding the characteristic monocytic and/or granulocytic cytoplasmic inclusions (morulae), PCR analysis of blood, and detection of specific antibodies in blood. However, morulae are rare and often missed on initial evaluation, hematoxylin and eosin-stained sections often fail to show organisms even when immunohistochemistry reveals abundant ehrlichial antigen, and antibody titers may take several weeks to rise to diagnostic levels.[227] Additionally, immunocompromised patients may not develop anti-ehrlichial antibodies prior to death.[227,229] In these cases, immunostaining for *Ehrlichia* or *Anaplasma* has been demonstrated to be a sensitive and specific diagnostic method.[227,229–231]

Immunohistochemistry has been a very valuable approach for the identification and study of several other EID such as Ebola hemorrhagic fever,[81–83] hendra virus encephalitis,[5,232,233] leptospirosis,[135–137] and more recently to identify a new coronavirus associated with
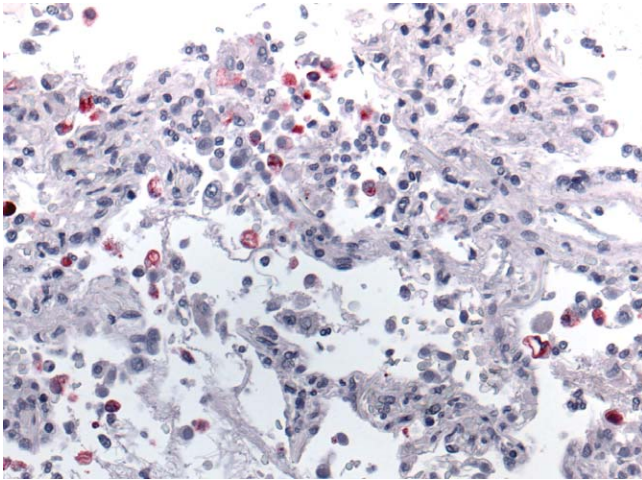
Fig. 2.22 SARS. Coronavirus antigen-positive pneumocytes and macrophages in lung of a SARS case. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×63)

severe acute respiratory syndrome (SARS).[234,235] SARS was first recognized during a global outbreak of severe pneumonia that first occurred in late 2002 in Guangdong Province, China, and then erupted in February 2003 with cases in more than two dozen countries in Asia, Europe, North America, and South America. Early in the investigation the clinical, pathologic, and laboratory studies focused on previously known agents of respiratory illness. Subsequently, a virus was isolated from the oropharynx of a SARS patient and identified by ultrastructural characteristics as belonging to the family Coronaviridae.[234,235] Various reports have described diffuse alveolar damage as the main histopathologic findings in SARS patients, and SARS-associated coronavirus (SARS-CoV) has been demonstrated in human and experimental animal tissues by immunohistochemical (Fig. 2.22) or in situ hybridization (ISH) assays.[236–245]

PATHOLOGISTS, IMMUNOHISTOCHEMISTRY, AND BIOTERRORISM

Currently, there is increasing concern about the use of infectious agents as potential biological weapons. Biological warfare agents vary from rare, exotic viruses to common bacterial agents, and the intentional use of biologic agents to cause disease can simulate naturally occurring outbreaks or may have unusual characteristics.[246] The CDC has issued the recommendations for a complete public health response to a biological attack.[247–249] Two important components of this response plan include the rapid diagnosis and characterization of biological agents. Pathologists using newer diagnostic techniques such as immunohistochemistry, in situ hybridization, and PCR will have a direct impact on the

rapid detection and control of emerging infectious diseases from natural or intentional causes. Immunohistochemistry provides a simple, safe, sensitive, and specific method for the rapid detection, either at the time of investigation or retrospectively, of biological threats, facilitating the rapid implementation of effective public health responses.

Anthrax

Immunohistochemical staining of *Bacillus anthracis* with monoclonal antibodies against cell wall and capsule antigens has been successfully used in the identification of bioterrorism-related anthrax cases, being an important step in the early diagnosis and treatment of these cases.[5,250–254] Gram's staining and culture isolation of *B. anthracis* are the usual methods to diagnose anthrax; nevertheless, previous antibiotic treatment affects culture yield and Gram's staining identification of the bacteria.[252] Immunohistochemistry has demonstrated high sensitivity and specificity for the detection of *B. anthracis* in skin biopsies, pleural biopsies, transbronchial biopsies, and pleural fluids (Fig. 2.23).[251–253]

In addition, immunostaining has been very useful for determining the route of entry of the bacteria and identification of the mode of spread of the disease.[252,255]

Tularemia

Immunohistochemical staining is also valuable in the rapid identification of *Francisella tularensis* in formalin-fixed tissue sections. Tularemia can have a variable clinical and pathologic presentation that can simulate other infectious diseases such as anthrax, plague, cat-scratch disease, or lymphogranuloma venereum. Moreover, the microorganisms are difficult to demonstrate in tissue sections even with Gram's stain or silver staining methods. A mouse monoclonal antibody against the lipopolysaccharide of *F. tularensis* has been used to demonstrate intact bacteria and granular bacterial antigen in the lungs, spleen, lymph nodes, and liver with high sensitivity and specificity (Fig. 2.24).[256,257]

Plague

A mouse monoclonal antibody directed against the fraction 1 antigen of *Yersinia pestis* has been used to detect intracellular and extracellular bacteria in dermal blood vessels, lungs, lymph nodes, spleen, and liver (Fig. 2.25).[258–262] This technique is potentially useful for the rapid diagnosis of plague in formalin-fixed skin biopsies. In addition, immunohistochemistry may allow distinction of primary and secondary pneumonic plague by
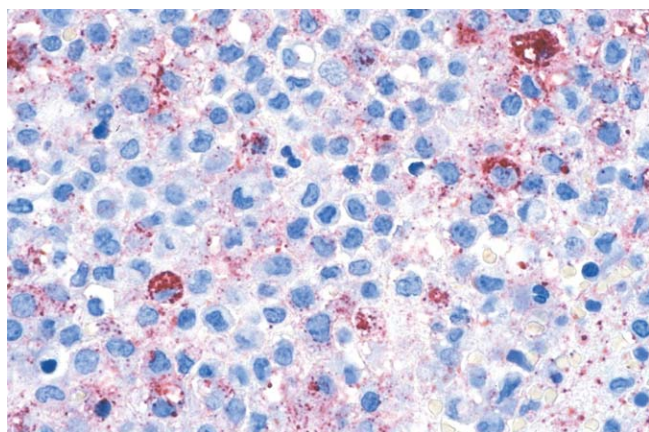
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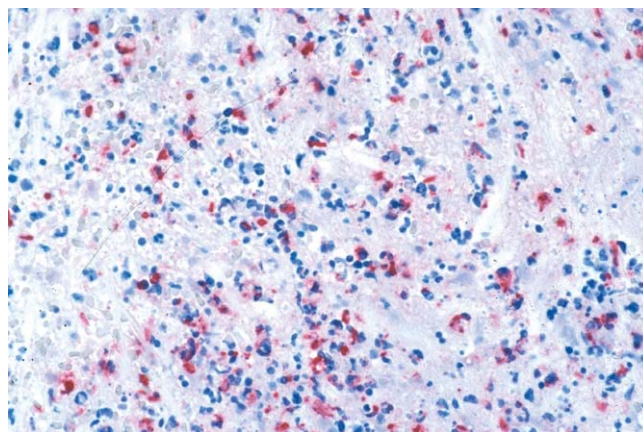
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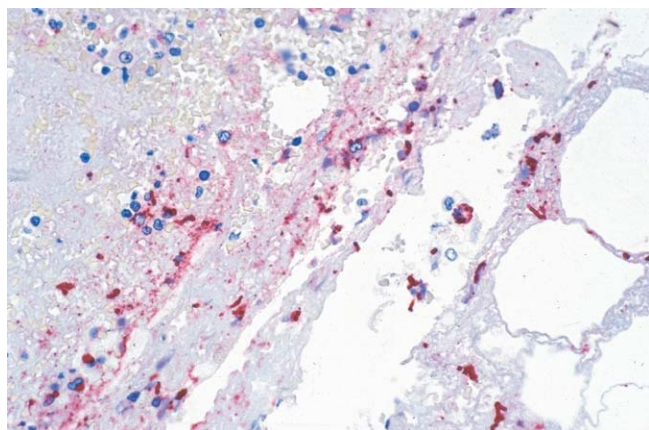
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Fig. 2.23 Anthrax. **(A)** Photomicrograph of pleural effusion cell block showing bacillary fragments and granular antigen-staining using the *B. anthracis* capsule antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×63) **(B)** Skin biopsy from a patient with cutaneous anthrax showing abundant granular antigen-staining and bacillary fragments using *B. anthracis* cell wall antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×40) **(C)** Photomicrograph of mediastinal lymph node showing abundant granular antigen-staining and bacillary fragments using *B. anthracis* cell wall antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×63)
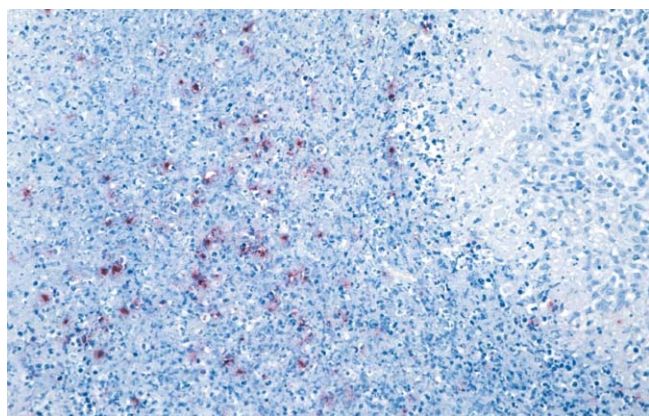
Fig. 2.24 Tularemia. Immunohistochemistry of lymph node showing a stellate abscess with *F. tularensis* antigen bearing macrophages in the central necrotic area using a mouse monoclonal antibody against the lipopolysaccharide of *F. tularensis*. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×10)

Fig. 2.25 Plague. Immunohistochemical stain of a lung demonstrating abundant bacterial and granular antigen staining in the alveolar spaces using a mouse monoclonal antibody against F1 of *Y. pestis*. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×20)

Fig. 2.26 Immunostaining of viral antigens in neurons and neuronal processes in CNS using a mouse anti-EEE antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, original magnification ×10)

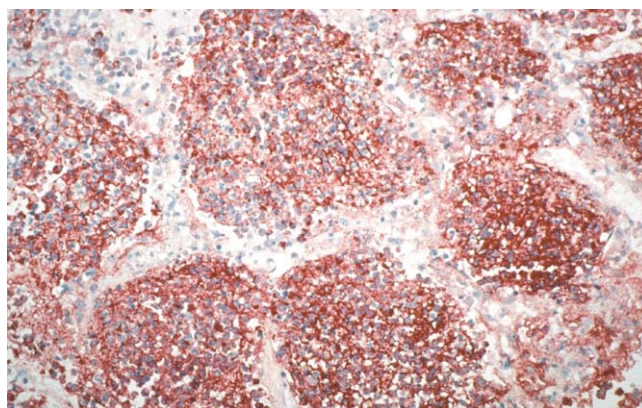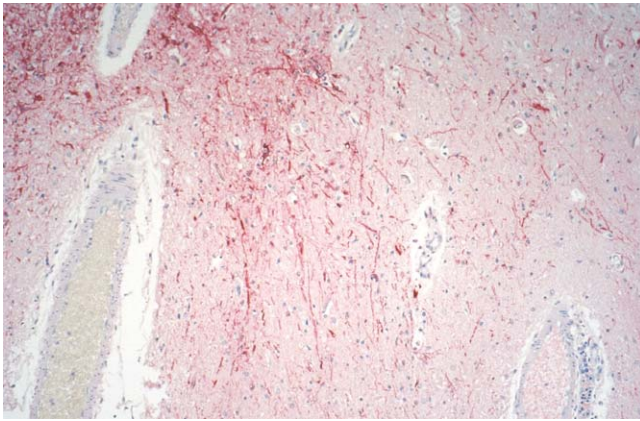identifying *Y. pestis* in different lung locations (i.e., alveolar versus interstitial).[258]

Immunohistochemical methods using polyclonal or monoclonal antibodies have been applied to the identification of several other potential biological terrorism agents, including antibodies to the causative agents of brucellosis,[5] Q fever,[5,125,263,264] viral encephalitides (Eastern equine encephalitis) (Fig. 2.26),[5,109–111] rickettsioses (typhus and Rocky Mountain spotted fever),[125–128,160] and viral hemorrhagic fevers (Ebola, Marburg).[5,77–83]

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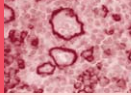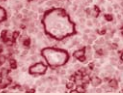
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