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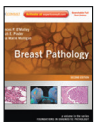

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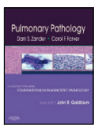

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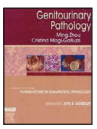

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PATHOLOGY OF INFECTIOUS DISEASES

A Volume in the Series
FOUNDATIONS IN DIAGNOSTIC PATHOLOGY

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To John Leon Procop, my father, who taught me what to do when the going gets tough and at the same time was always a fun dad.

–Gary Procop

To my parents, John and Sue Pritt, for their love and inspiration.

–Bobbi Pritt

1

Principles of Infectious Disease Pathology: An Introduction

■ Michael L. Wilson ■ Gary W. Procop
■ Bobbi S. Pritt

■ DIAGNOSIS OF INFECTIOUS DISEASES: AN INTEGRATED APPROACH

The diagnosis of infectious diseases ranges from straightforward clinical diagnoses to those that are possible only with the use of advanced molecular methods. Between these two extremes are the many infectious diseases for which an accurate and timely diagnosis requires the combined use of microbiologic cultures, histopathology/cytopathology, and molecular methods. It is this broad group of infectious diseases where the histopathologist and medical microbiologist, either alone or together, play a central role in diagnosis.

As with all clinical or pathologic evaluations, pathologists need all available clinical, radiographic, and laboratory findings in order to make accurate and timely diagnoses. This is particularly important in infectious disease pathology: many infectious diseases are restricted geographically, present with distinctive (or at least highly suggestive) clinical signs and symptoms, can present as localized or disseminated disease, may be associated with environmental or zoonotic exposure, and, because many are contagious, can present as part of an outbreak or be linked to transmission from another host. The immune status of the host also is important, as some infectious manifestations are seen primarily in the setting of immune compromise. Therefore, the amount of information necessary for the diagnosis and treatment of infectious diseases can be substantially greater than that needed for many noninfectious diseases. It is imperative that clinicians provide this information to pathologists, and that pathologists make an active attempt to obtain it when it is not initially received.

The histopathologic and cytopathologic diagnoses of infectious diseases are progressive and sequential processes that move from the general to the specific based on results from a combination of diagnostic methods. The traditional approach is shown graphically in Figure 1-1, where the starting point is gross examination of specimens, frozen sections, aspirate smears, or hematoxylin and eosin (H&E)–stained permanent sections. In this approach, the evaluation is limited to the sequential use of histologic methods with or without the accompanying use of microbiologic cultures. This is a cost-effective and adequate approach for many common infectious diseases. For more challenging cases, an integrated approach as shown in Figure 1-2 is more appropriate. In such an approach, several methods are used simultaneously and in parallel to obtain the most accurate and timely diagnosis, including correlating histopathologic findings with the results of clinical signs and symptoms, other laboratory test results, radiographic findings, and, when appropriate, consultation with infectious disease pathology specialists. Ideally, the result of this process is an integrated, composite report that incorporates all of these findings into a summary and interpretation.

■ THE INFLAMMATORY RESPONSE IN THE DIAGNOSIS OF INFECTIOUS DISEASES

The inflammatory response to pathogenic microorganisms is sufficiently consistent and predictable to usually allow pathologists to identify that an infection is present. The type of inflammatory response—as well as its distribution in tissues, fluids, and organs—also may allow the pathologist to categorize the infection as one likely caused by bacteria, fungi, mycobacteria, or parasites (Table 1-1). Some histopathologic clues, when combined with other findings in tissue, help narrow the differential diagnosis. One example is the Splendor-Hoeppli phenomenon, which is a radial aggregate of eosinophilic material surrounding a nidus of infection. The protein was once believed to be composed of aggregates of antigen-antibody complexes, but it is now known to be composed of major basic protein. When it is identified

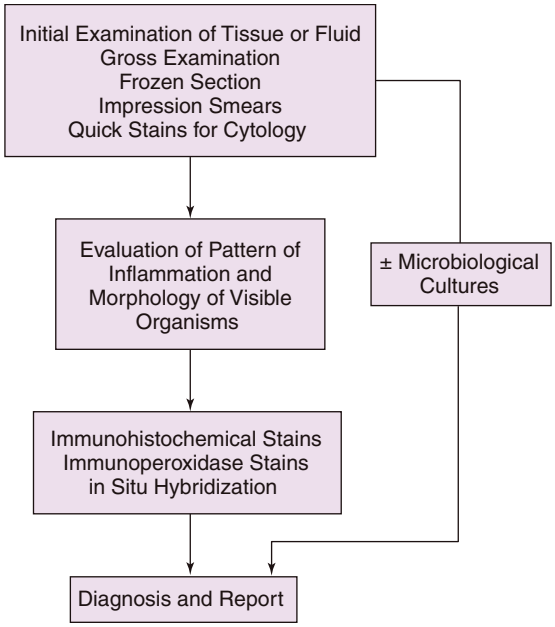

FIGURE 1-1

Conceptual approaches to diagnosis: traditional approach.

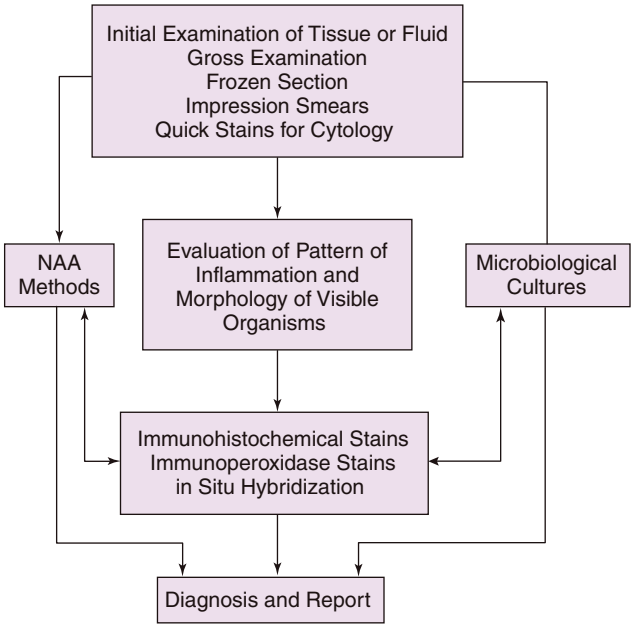

FIGURE 1-2

Conceptual approaches to diagnosis: integrated approach.

in tissue sections it suggests that an infection is caused by *Sporothrix,* some other fungi, *Schistosoma* eggs, and others. Although it is not a specific finding, it does help the pathologist narrow the differential diagnosis. Thus, although infections in tissues may show considerable overlap in their histopathologic characteristics, the type and distribution of inflammation—and often a combination of otherwise nonspecific findings—provides sufficient information for pathologists to order additional studies in a logical and sequential manner.

TABLE 1-1

Patterns of inflammation associated with infection

Pattern of Inflammation	Likely Pathogens
Acute Inflammation	Bacterial
	Early stages of mycobacterial, fungal, and parasitic infections
Granulomatous inflammation	Mycobacterial, fungal, rare bacterial, parasitic
Endothelial damage, focal acute hemorrhage	Viral (e.g., viral hemorrhagic fevers)
	Rickettsial
	Pseudomonas aeruginosa
Focal necrosis	Viral
	Bacterial
Ulceration	Bacterial
	Mycobacterial
	Fungal
	Viral
	Parasitic

Bacteria

Most common bacterial pathogens elicit the acute inflammatory immune response, a typical example being acute bronchopneumonia caused by *Streptococcus pneumoniae*. The acute inflammatory response consists primarily of an infiltrate of segmented neutrophils, although in more severe or prolonged cases the infiltrate can also contain neutrophil precursors. The surrounding tissue is often edematous, shows vascular congestion with margination of neutrophils along the vascular endothelium, and may show focal necrosis with severe inflammation. As the segmented neutrophils lyse, they release cellular debris that can mimic the appearance of extracellular bacterial cocci, but in general the cellular debris varies more in size and shape than do cocci. If a tissue Gram stain is performed, histopathologists should remember to look for both free bacteria and intracellular bacteria. The location of bacteria is determined in part by the nature of the infection (e.g., intracellular gram-positive cocci in pneumococcal pneumonia) and also by the degree of cellular lysis, severity of infection, and stage of infection.

A small number of pathogenic bacteria are associated with inflammatory responses other than typical acute inflammation. Rickettsia and other pathogens associated with endothelial infections typically do not cause acute inflammation but rather cause vascular leakage with edema of affected tissues. Vascular thromboses and vascular necrosis may also occur, depending on which rickettsial pathogen is the cause of the infection. Affected

vessels may show a surrounding cuff of mononuclear inflammatory cells, but in general these bacterial infections are not associated with an infiltrate of neutrophils. Similarly, *Pseudomonas aeruginosa* pneumonia is characterized by damage to the walls of blood vessels with the subsequent development of acute hemorrhage with or without associated acute inflammation. Bacterial infections that are caused by toxin-producing bacteria, such as *Clostridium* species, may show only extensive tissue necrosis with minimal or no inflammation. Rapidly progressive infections caused by *Streptococcus pyogenes* in skin and subcutaneous soft tissues may progress so rapidly that extensive tissue necrosis occurs before acute inflammation can develop. One of the less intuitive inflammatory responses is that elicited by *Brucella* spp., *Yersinia pestis, Francisella tularensis, Bartonella henselae* (the causative agent of cat-scratch disease), and the *Chlamydia trachomatis* strains that cause lymphogranuloma venereum. Unlike most other bacterial pathogens, these bacteria are associated with the formation of stellate necrotizing granulomas in infected tissues.

Some bacterial infections are associated with patterns of infection that are distinctive but may not be recognized due to their relative infrequency in routine clinical practice. The foamy macrophages observed with Whipple disease in gastrointestinal biopsies is one example, as is the similar tissue reaction observed in the spleen with infections caused by *Mycobacterium avium* complex in patients with severe immunosuppression caused by HIV infection. Infections caused by *Rhodococcus* are typically associated with malakoplakia. The finding of xanthogranulomatous inflammation, while nonspecific, strongly suggests a chronic bacterial infection rather than an ongoing fungal or mycobacterial infection.

The histopathologic changes associated with bacterial infections are discussed in detail in Chapters 12, 13, 15, 16, 17, 18, and 19.

Fungi

Fungal infections are typically associated with granulomatous inflammation, which may be necrotizing or non-necrotizing depending on the stage of the infection. Early stages of fungal infections are typically associated with acute inflammation, with the evolution of granulomatous inflammation occurring as cell-mediated immunity develops. As occurs with mycobacterial infections, the early stages of fungal infections are rarely seen: by the time the patient is symptomatic, or a biopsy is necessary, the infection almost always has progressed to the stage at which granulomatous inflammation has developed. One notable exception occurs with cutaneous fungal infections, where mixed acute and granulomatous inflammation occurs. In addition, many granulomas in cutaneous fungal infections have a necrotic center, but rather than be filled with necrotic debris the centers of the granulomas are filled with neutrophils. These granulomas do not have a specific name, being referred to as pyogenic granulomas or mixed inflammation, among other terms. When identified in skin and subcutaneous tissues, they are highly suggestive of a cutaneous fungal infection such as phaeohyphomycosis or chromoblastomycosis. As with mycobacteria and other causes of granulomas, necrosis of the center of the granulomas is more typical of infections that have persisted for some time. It is important for the diagnostician to remember that granulomatous inflammation varies considerably in its histopathologic appearance.

One distinctive tissue reaction to fungal infection occurs with the diverse group of fungi that cause what is variably termed *zygomycosis, phycomycosis,* or *mucormycosis*. Because the causative fungi all belong to the class Zygomycetes, perhaps the best term is *zygomycosis*. Infection with any of these agents, which are indistinguishable in tissue sections, results in invasion of arterial walls with subsequent vascular occlusion and thrombosis. Similar findings occur when members of the hyaline hyphomycetes (such as *Aspergillus*) invade pulmonary arteries. Necrosis of infected tissues is the result of these fungal infections, with minimal acute inflammation in the early stages of infection.

More comprehensive descriptions of the histopathologic changes associated with fungal infections are presented in Chapters 23 to 26.

Mycobacteria

As with fungi, mycobacterial infections are classically associated with granulomatous inflammation. As with bacteria and fungi, however, mycobacteria elicit a spectrum of inflammatory reactions depending on the type of mycobacterium, the site of infection, and the stage of infection. Although the immediate tissue response to mycobacterial infection is that of acute inflammation, this is rarely (if ever) seen in diagnostic specimens: by the time clinical signs and symptoms develop, the inflammatory response has evolved to the stage of granulomatous inflammation. Early infections show non-necrotizing granulomas; if infections persist, the granulomas will begin to show necrosis of the central portions, which eventually become fully necrotic, eventually assuming the classic finding of caseation. At this stage of the infection, a rim of viable tissue, consisting mostly of epithelioid histiocytes, surrounds the central area of necrosis. From a practical standpoint, the most important feature for the histopathologist is that residual mycobacteria are limited to the interface between the necrotic and viable tissue; mycobacteria are almost never found in either the necrotic or the viable granulomatous tissue.

A number of unique patterns of inflammation with mycobacterial infections have also been described. Buruli ulcer, a tropical infection caused by *Mycobacterium ulcerans,* is manifested by the development of chronic skin ulcers that show acute and chronic inflammation at the leading edge of ulcers, with minimal granulomatous inflammation along the edge of ulcers. Tuberculoid leprosy shows a granulomatous inflammation, particularly in a perineural pattern, whereas lepromatous leprosy shows aggregates of lipid-laden macrophages that may be filled with *Mycobacterium leprae* bacilli. The absence of granulomas in lepromatous leprosy reflects a lack of an effective T-cell immune response. *Mycobacterium avium complex* infections in patients with profound immunosuppression also are characterized by foamy macrophages distended by innumerable bacilli, and again the absence of granulomas reflects a lack of an effective T-cell immune response. *Mycobacterium marinum* causes small subcutaneous granulomas in the skin (usually of the extremities) where the body temperature is sufficiently low to support the growth of the bacterium.

The histopathologic changes associated with mycobacterial infections are discussed in detail in Chapters 20, 21, and 22.

Viruses

Excluding viral cytopathic effects, most viral infections are not associated with characteristic inflammatory or other tissue reactions in tissues or organs, although the distribution of changes within tissue or organs may suggest certain viral infections. For example, changes limited to the liver would suggest certain viral infections but not others due to the organ- and cell-specific tropism for many viruses. Other examples include the pattern(s) of acute hemorrhage associated with viral hemorrhagic fevers, changes associated with progressive multifocal leukoencephalopathy caused by JC virus infection, and viral myocarditis. That is not to say that a histopathologic diagnosis of viral infections is not possible, rather that the pattern of inflammation per se is not as characteristic as that of many bacterial, fungal, or mycobacterial infections.

It is important for pathologists to remember that many viral infections can be accompanied by secondary bacterial infections. Classic examples include bacterial superinfection during and following viral pneumonias and bacterial infections following ulcers caused by viruses (e.g., esophageal ulcers caused by the *Herpes simplex virus* or *Cytomegalovirus*). Depending on the timing of any biopsy or collection of fluid, the acute inflammatory infiltrate associated with the secondary bacterial infection can easily obscure the underlying viral infection.

The histopathologic changes associated with viral infections are discussed in detail in Chapters 2 to 9.

Parasites

The classic description of the histopathologic changes associated with invasive parasitic infection is that of a chronic inflammatory infiltrate with a marked eosinophilic component. This description, however, is inadequate in many ways. For example, some parasitic infections are associated with minimal or no tissue reaction at all, such as the cysts of *Toxoplasma gondii* observed in brain or skeletal muscle during the dormant state of infection. Other parasitic infections are associated with granulomatous inflammation, such as cutaneous or mucocutaneous forms of leishmaniasis or the granulomatous pulmonary arteritis seen when schistosome eggs circulate to the lungs. With many parasitic infections, longstanding infection may result in marked fibrosis of affected tissues, the best example being the so-called pipe-stem fibrosis seen in livers infected with *Schistosoma japonicum*. Last, cutaneous or mucocutaneous forms of parasitic infections may be associated with secondary bacterial infections, which can result in acute inflammation of the affected site, thereby partially obscuring the nature of the underlying infection. It is important to remember that the various tissue reactions to parasitic infections are for the most part nonspecific: unless parasites are directly visualized, it is not possible to make a definitive diagnosis in most cases.

As with other types of infections, for many parasitic infections the only clues as to the causative agent are the distribution of affected tissues or organs, epidemiologic information, and a past medical history suggestive of a given parasite. A careful clinical history is a mandatory part of the evaluation of these patients: it is necessary to obtain detailed information as to the patient's birthplace, residence or travel to endemic regions, past clinical signs and symptoms, and previous evaluation and treatment. Serologic tests may be useful for documenting past exposure to parasites, but pathologists should remember that serologic tests may only document exposure, not disease, and they suffer from a number of pitfalls including lack of sensitivity or specificity as well as cross-reactions with similar parasites. Moreover, accurate serologic tests are not widely available for many parasitic infections and are typically limited to large reference laboratories and public health agencies (e.g., the U.S. Centers for Disease Control and Prevention).

The histopathologic changes associated with parasitic infections are discussed in detail in Chapters 27 and 28.

CLASSICAL HISTOPATHOLOGY AND CYTOPATHOLOGY

The first step in histopathologic or cytopathologic diagnosis is the recognition of findings that are consistent with an infection. Although in many cases this

will be the presence of an acute inflammatory infiltrate, it is important to remember that many infections are not associated with acute inflammation or any visible inflammation at all. Some of the clues that an infection is present are shown in Table 1-1. The second step in histopathologic diagnosis is the identification of patterns of histopathologic changes that suggest the nature of the infecting microorganisms. For example, granulomatous inflammation may be nonspecific, but in many cases there are a number of patterns associated with different pathogens. Necrotizing granulomas suggest mycobacterial or fungal infections, whereas bacteria such as *Brucella* spp., *Francisella tularensis,* or the *Chlamydia trachomatis* infection lymphogranuloma venereum more often cause stellate necrotizing granulomas. The third step in histopathologic diagnosis is the recognition of specific infections. The characteristic cytopathic effects of common viruses such as cytomegalovirus, herpes simplex virus, varicella zoster virus, or molluscum contagiosum virus can, in many cases, be identified definitively on both cytologic and histopathologic sections without the need for any special stains or use of other diagnostic methods (Table 1-2). In the same way, identification of yeasts, yeast-like microorganisms, pseudohyphae, or hyphae may be sufficient to establish the identity of the infecting pathogen. At the very least, the histologic appearance provides a strong basis for selecting subsequent tests to evaluate specimens (Tables 1-3 and 1-4).

In most cases, however, gross examination of tissues, or microscopic examination of frozen sections, H&E-stained slides, or fine-needle aspirate preparations, does not provide sufficient information as to the specific nature of the infecting agent. For example, although some common viral infections can be identified with certainty in histologic or cytologic preparations, in many cases viral cytopathic effects in and of themselves are not diagnostic. This may be a result of a low number of infected cells, sampling artifact (only part of an infected cell is on a tissue section), or the presence of viral cytopathic effects that are not specific to any given virus. One of the best examples is the eosinophilic Cowdry A intranuclear inclusions caused by herpes simplex virus, cytomegalovirus, varicella zoster virus, poxviruses, measles virus, and adenovirus. A number of texts suggest that the Cowdry

TABLE 1-2
Differentiating characteristics of viruses

Organism	Cell Size	Intranuclear	Cytoplasmic Inclusions	Other
Adenovirus	Normal	Basophilic "smudge" cells without peripheral margination of chromatin; occasional basophilic Cowdry A	—	May closely resemble herpetic inclusions
Cytomegalovirus	Enlarged	Basophilic Cowdry A	Basophilic	Nucleolus preserved
Herpes simplex virus	Normal to enlarged (multinucleated)	Eosinophilic Cowdry A; eosinophilic ground glass with margination of chromatin	—	Multinucleated cells common, with molding of adjacent nuclei
JC virus	Normal	Eosinophilic to amphophilic ground glass with margination	—	Oligodendrocytes contain inclusions
Varicella zoster virus	Normal to enlarged (multinucleated)	Eosinophilic Cowdry A; eosinophilic ground glass with margination of chromatin	—	Multinucleated cells common, with molding of adjacent nuclei; found only in stratified squamous epithelium
Measles virus	Normal to syncytial	Eosinophilic Cowdry A; eosinophilic ground glass with margination of chromatin	Eosinophilic	Multinucleated cells called Warthin-Finkeldey cells
Pox viruses	Normal	Eosinophilic Cowdry A; small	Eosinophilic	Inclusions (Guarnieri's bodies) can undergo ballooning degeneration
Respiratory syncytial virus	Normal to syncytial	None	Eosinophilic	Infrequent in clinical specimens
Rabies virus	Normal	None	Amphophilic	Inclusions (Negri bodies) have sharp borders

TABLE 1-3

Differentiating characteristics of common yeasts and yeast-like microorganisms (ordered by size)

				Staining Pattern	
Organism	**Typical Size**	**Morphology**	**Budding Pattern**	*GMS*	*PAS*
Trypanosoma cruzi (amastigotes)	1.5-5 μm	Within a pseudocyst; kinetoplast present	N/A	+	±
Candida (Torulopsis) glabrata	2-5 μm	Arranged in tight clusters, no pseudohyphae formed; usually extracellular	Single, narrow-based	+	+
Coccidioides immitis/posadasii (endospores)	2-5 μm	More spherical; immature and mature spherules may be present; septate hyphae may be found in cavitary lesions	N/A (endospores within spherule are formed by division planes)	+	+
Leishmania spp. (amastigotes)	2-5 μm	Intracellular (within histiocytes); kinetoplast present	N/A	–	–
Histoplasma capsulatum	2-5 μm	Intracellular or extracellular; appear encapsulated when intracellular due to poorly staining cell wall ("pseudocapsule")	Single, narrow-based	+	±
Penicillium marneffei	2-5 μm	Round, oval, and curved "sausage" forms	N/A (divide by forming transverse septa)	+	+
Cryptococcus neoformans/gattii	2-20 μm	Extreme variation in size; round, oval, and collapsed forms (size not including the capsule); rarely pseudohyphae seen; capsule diagnostic	Single or multiple, narrow-based	+	+
Sporothrix schenckii	2-10 μm	Round, oval, or cigar-shaped	Single or double at poles	+	+
Toxoplasma gondii	2-8 μm	Cysts or free tachyzoites	N/A	–	±
Pneumocystis jiroveci	3-7 μm	Helmet or watermelon seed appearance; intra-cystic bodies; characteristic frothy exudate; extracellular	N/A	+	–
Candida albicans	5-8 μm	Yeast forms, pseudohyphae, and true septate hyphae may be present	Single, narrow-based	+	+
Chromoblastomycosis group	6-12 μm	Muriform or sclerotic bodies (also known as "copper pennies"); pigmented forms often with internal septations	N/A	+	+
Histoplasma duboisii	8-15 μm	Thick cell wall	Single, narrow-based, forming "figure-eight" forms	+	+
Blastomyces dermatitidis	8-20 μm	Thick, doubly refractile wall; marked variation in size	Single, broad-based	+	+
Paracoccidioides brasiliensis	8-20 μm	Thick cell wall	Multiple with "Mariner's wheel" pattern, narrow-based	+	+
Coccidioides immitis/posadasii (spherules)	10-80 μm	May or may not contain endospores; septate hyphae may be present in cavitary lesions	N/A	+	+

N/A, not applicable.

A nuclear inclusions of cytomegalovirus are basophilic rather than eosinophilic, but the tinctorial characteristics of inclusions can be influenced substantially by the quality of the H&E stain. Another example is the ground-glass intranuclear inclusions that occur with adenovirus, JC virus, and *Herpes simplex* virus infections. Whereas in infections such as JC virus infection of the central nervous system the cell type that is infected as well as the clinical history and presentation enable the histopathologist to make a definitive

TABLE 1-4
Differentiating characteristics of fungal hyphae and hyphal-like forms

Organism	Width	Morphology	Septations	Branching Pattern
Actinomyces spp.	≤ 1 μm	Filamentous, branching; may appear beaded; gram positive, non-acid-fast; form granules with prominent Splendore-Hoeppli material in tissues; individual organisms not seen in inflammatory infiltrate	+	Branch at right angles
Aspergillus spp. and other members of the hyalohyphomycosis group (hyaline molds)	3-10 μm	Parallel cell walls; dilated forms may be present; *Aspergillus* sporulation may occur in cavitary infections (presence of fruiting bodies); when fruiting bodies are seen, a definitive diagnosis of *Aspergillus* may be made; otherwise, *Aspergillus* spp. cannot be reliably differentiated from other hyaline molds	+	45 degrees to right angles; may be constricted where arise from parent hyphae; *Aspergillus* classically has 45 degrees dichotomous branching, but this can be seen with other hyaline molds as well
Candida albicans	3-6 μm	Constrictions at septae give a bulging appearance to pseudohyphae (resembling sausage links); true septate hyphae may also be present; yeast forms are typically present in addition to pseudohyphae/hyphae.	+	Irregular; true hyphae may resemble hyphae of *Aspergillus* spp.
Geotrichum spp.	3-6 μm	Parallel cell walls; hyphae break into arthroconidia; arthroconidia may also be seen with *Coccidioides* and *Trichosporon* spp.	+	Infrequent branching
Nocardia spp.	≤ 1 μm	Filamentous, branching; not easily visible on H&E, gram positive, often beaded appearance, weakly acid-fast; do not form granules in tissue (except in mycetomas); individual organisms scattered throughout inflammatory infiltrate	+	Branch at right angles
Phaeohyphomycosis group (pigmented molds)	3-10 μm	Dark yellow to brown pigmented hyphae; nonpigmented hyphae may also be seen; irregular morphology; short hyphal segments; chlamydoconidia-like structures occasionally seen	+	Irregular
Zygomycetes	3-30 μm	Thin-walled, broad and irregular hyphae; cell walls not parallel	+/–	Irregular

diagnosis, when only ground-glass intranuclear inclusions are identified in other tissues, it is not possible to render a definitive diagnosis, and the use of additional methods becomes necessary.

There are few published data regarding the diagnostic sensitivity of histopathologic methods for detecting microbial pathogens in infected tissues. This is due to a number of factors, including (1) the multiplicity of stains used to detect and characterize pathogens, (2) the rigor of the study (i.e., how many sections are cut, how much time is spent examining each slide), (3) varying skills of the examining pathologists, (4) conceptual difficulties in designing meaningful clinical trials, and (5) the difficulty in standardizing the infected tissues (i.e., defining severity of infection so that the microbial burden is comparable between cases). As a general rule, the sensitivity of detecting pathogens in tissue is considered to be less than 50%. This is particularly true in the later stages of infection, where the sensitivity is likely to be substantially less than 50%. As a result, practitioners should not rely on histopathology for a definitive diagnosis of infection.

The advantages to histopathology—the relative speed of the diagnosis and the ability to see the background inflammatory response—are useful but often need to be supplemented by more definitive means for identifying the causative pathogen.

Histochemical Stains

Histochemical stains remain an inexpensive and useful tool in the histopathology of infectious diseases. The most widespread use of histochemical stains is to highlight the presence of bacteria, fungi, and mycobacteria. A large number of tissue Gram stains are available, with fewer stains for fungi and mycobacteria. A number of references and textbooks advocate the use of a Giemsa stain to highlight the presence of a number of parasites, but in fact the tissue Giemsa stain provides little additional benefit over the H&E stain.

Of the tissue Gram stains, the Brown-Brenn (B&B) and Brown-Hopps (B&H) are among the most widely used. The B&B is said to stain gram-positive cocci better than gram-negative bacteria, and the B&H is said to have the opposite pattern. There are no published data to support this claim, however, and because the tissue Gram stain is compromised in many ways (due to fixation and processing of tissues), any differences between the two stains are likely to be minimal. Other common tissue Gram stains included the Gridley, Taylor, and McCallum-Goodpasture. No one of these has any substantial advantage over another, and all are about equally difficult to perform and interpret.

A number of other stains have been used to help both locate and identify bacteria in tissue sections. The silver stains Warthin-Starry and Steiner are used for identifying spirochetes, as well as some bacteria that do not stain well with the Gram stains, but both of these stains are technically difficult to perform and have fallen out of common use in most pathology departments. The periodic acid Schiff (PAS) with diastase (PAS-D) stain is useful for identifying *Tropheryma whipplei* in tissue sections. A variety of acid-fast stains have been developed for identifying mycobacteria and for differentiating among filamentous bacteria. The Ziehl-Neelsen and Kinyoun stains were developed for identifying *Mycobacterium tuberculosis* in sputum specimens, and they were subsequently modified to enable them to identify mycobacteria in tissue sections. The Fite stain is useful for staining *Mycobacterium leprae* and may also be useful for staining *Nocardia* (see Chapter 13).

Identification and characterization of structures such as spores, septations, budding and branching patterns, and the presence or absence of parallel cell walls form the morphologic basis of identifying fungi by histopathologic examination. These structures cannot always be identified using the H&E stain, which means that histopathologists need alternative special stains to locate and identify fungi in tissue sections. The two most common types of histochemical stains for fungi are the methenamine silver stains (Grocott and Gomori) and the periodic acid Schiff (PAS) stain. Methenamine silver stains highlight fungi in a gray-to-black color, making fungi easier to find as well as highlighting fungal morphology. Methenamine silver stains have a secondary advantage of staining bacterial cell walls, a phenomenon that is particularly useful for identifying infections caused by filamentous bacteria such as *Actinomyces* or *Nocardia,* as well as in partially treated bacterial infections where the Gram stains may not be useful. The PAS stain provides some of the same benefits, but as a broad generalization it does not provide the same level of cellular detail. Moreover, although there have been claims that an added benefit to the PAS stain is that it stains only viable fungi, there are no data to support this claim. There is abundant anecdotal evidence to refute it. The greatest disadvantage to the GMS stains is that they are technically difficult to perform well and too often show nonspecific staining of the cellular background, particularly staining of elastin, neutrophil granules, and mucin.

For both types of fungal stain, and many other histochemical stains, the type of background is an important consideration. With the methenamine silver stains, a green background (counter-) stain or an H&E counterstain can be used, as either provides good contrast for silver stains. Some green counterstains do not show background tissues very well, making it difficult to find small numbers of fungal elements. The type of counterstain used with the PAS stain is probably less important, although an H&E counterstain that has too much eosin can make it difficult to see the red PAS-stained fungal structures against an eosinophilic background.

The most common histologic stains for mycobacteria are Kinyoun, Ziehl-Neesen, Fite, and Fite-Faraco stains. All are based on the same histochemical principles of binding of an acid-fast red dye to the cell wall of mycobacteria, imparting a red color to the bacteria against either a blue or green counterstain. The Ziehl-Neesen is a hot stain, in that heat must be applied to the slides during part of the staining. Conversely, the Kinyoun is a cold stain; slides are not heated. The two stains give equivalent results. The Fite and Fite-Faraco acid-fast stains are variants of the Kinyoun stain and are used primarily to stain either *Mycobacterium leprae* or *Nocardia* spp. As with fungal stains, a number of background stains can be used. Only the H&E counterstain cannot be used, as the low numbers of mycobacteria makes it necessary to have a background with a high degree of contrast such as a bright blue or green.

Ancillary Studies

Immunohistochemistry

Immunohistochemical stains have the same benefits as histochemical stains: they increase the analytic and diagnostic sensitivity of the test by highlighting microorganisms, as well as providing specific information as to the identity of the microorganisms. In the case of immunohistochemical stains, however, the information regarding the identity of the microorganisms is an inherent part of the test, as opposed to histochemical stains where more structures are highlighted but the identity of microorganisms requires interpretation by the histopathologist. A large number of antibodies are available for use in immunohistochemistry, including antibodies that have specificity for epitopes on bacteria, fungi, mycobacteria, and viruses. Few of these have undergone any type of controlled comparison, many are user developed and may not be available commercially, and many were developed for purposes other than diagnostic immunohistochemistry (such as research purposes). As a result, histopathologists and histotechnologists should exercise caution in selecting antibodies for use in immunohistochemistry.

As with all immunohistochemical stains, rigorous quality control and the use of appropriate controls are crucial. Moreover, nonspecific reactions can occur with background inflammatory cells—particularly with plasma cells—and the necrotic cellular debris that is often associated with infections may show nonspecific staining. There are also different types of staining associated with various immunohistochemical stains. Some of the antibodies with specificity for human cytomegalovirus, for example, stain nuclear antigens, whereas others stain both nuclear and cytoplasmic antigens. Last, as with any type of immunohistochemical stain, decalcification of bone or other calcified tissue can result in a loss of immunoreactivity and false-negative test results.

The most common use of immunohistochemical stains is for the identification of viral infections. Not only do immunohistochemical stains increase the sensitivity of histopathologic examination of tissues, and when positive provide a definitive diagnosis, they are also invaluable for the identification of viral pathogens more quickly than is possible with viral cultures (and sometimes even more quickly than nucleic-acid amplification [NAA] tests). They have the added benefit over culture and NAA tests in that the histopathologist can observe the cell types that are infected and correlate the result within the context of other findings.

In Situ Hybridization

In situ hybridization plays much the same role as immunohistochemistry. It also has the same advantages: increased analytic and diagnostic sensitivity, the ability to observe staining within the context of other findings, and greater specificity. Because in situ hybridization of nucleic acid sequences allows for greater specificity compared with binding of antibodies to antigens, it provides somewhat greater specificity than immunohistochemistry. The primary disadvantages of in situ hybridization include (compared to immunohistochemistry) the availability of only a relatively small number of probes and a more technically challenging type of assay. In situ hybridization is likely to replace a number of immunohistochemical tests, as the greater specificity compared with immunohistochemistry is a significant advantage.

In situ hybridization has been used primarily to look for viral infections, often Epstein-Barr virus infections. To date it has had only limited utility in the diagnosis of most other pathogenic microorganisms, mostly because of the limited availability of reagents. Whether probes (or immunoperoxidase reagents) for the diagnosis of other pathogens will be developed or research and development efforts will focus on other molecular methods is not known. One of the drawbacks of molecular methods that do not require visual examination of tissue or fluid specimens is the inability to interpret test results in the context of the infection and the changes that it causes.

Nucleic Acid Amplification and Other Advanced Molecular Methods

Several nucleic acid amplification (NAA) methods have been developed, the most common of which is the polymerase chain reaction (PCR). The basis for all NAA methods is similar, in that specific nucleic acid sequences are identified by one of several means, subjected to repeated copying (amplification), and the copied material detected by one of several methods. Other approaches to detecting nucleic acid sequences are used in clinical diagnostic tests, such as signal amplification, but are not used widely in histopathology.

Nucleic acid amplification is both similar and dissimilar to immunoperoxidase stains and in situ hybridization. It is similar in that it both increases the diagnostic sensitivity of histopathologic diagnosis and provides specific information as to the identity of the infecting pathogen. Of the three approaches, it is the most sensitive. It is not without drawbacks, however, and should not be viewed as a method that is useful in every situation. Not all nucleic acid sequences are good targets for amplification, specific primers and probes do not exist for all sequences of interest, a number of inhibitors to amplification may be present in tissue sections, and degradation of nucleic acid sequences during tissue processing and prolonged storage in formalin can affect

amplification. For these reasons, NAA methods are, in many cases, performed on a portion of tissue or fluid that is not processed for histopathology or cytopathology, and in such cases NAA methods are used as an adjunct to histopathology or cytopathology rather than as an integral part of the process of examining tissues or fluids microscopically. In that sense, NAA methods are more similar to microbiologic cultures than they are to other histopathologic methods.

The specific role of NAA methods is determined by a number of factors. For common bacterial and fungal infections, the combined use of histopathologic or cytopathologic examination of tissues along with microbiologic cultures is sufficient in almost all cases. It is of particular importance in fungal infections, where it is of paramount importance that histopathologic examination be used to document the presence of an invasive fungal infection, as opposed to merely isolating a fungal pathogen in culture. For bacterial or fungal infections that grow poorly, slowly, or not at all in microbiologic cultures (e.g., *Pneumocystis jiroveci, Mycobacterium leprae, Tropheryma whipplei*), the use of NAA methods is important. When a viral infection is suspected, it may be best to start with NAA methods, as a definitive diagnosis of many viral infections is not possible with histopathologic examination, and viral cultures (if a virus can be cultured at all) take 2 to 3 days, even with use of rapid methods such as shell-vial cultures. Conventional viral tube cultures can take 14 days or more to yield the etiologic agent. Nucleic acid amplification methods may also be useful in cases where patients have received antimicrobial therapy, thereby delaying or preventing isolation of pathogenic microorganisms by culture.

In the past, NAA methods were used less often because they were not as widely available (even today, some NAA methods are available from only a small number of laboratories), took a prolonged period of time to obtain results, and were expensive. Today, use of the technology has become more widespread, results are typically available in a few days, and the cost has decreased substantially. It is, however, important for histopathologists to remember that these improvements have occurred primarily in the testing of tissues that have not been formalin fixed and paraffin embedded, moving NAA methods further into an adjunct role in the histopathologic or cytopathologic diagnosis of infectious diseases.

With a few notable exceptions, nucleic acid sequencing is not widely used as a diagnostic method for detecting pathogens in tissue specimens. It is used more widely to test fluid specimens. It is particularly useful for identifying viral infections such as influenza, viral hemorrhagic fevers, or viral infections of the central nervous system in tissue sections, where the histopathologic findings may be nonspecific and the differential diagnosis of a viral infection was made only after the tissue was obtained. Analysis of tissue sections by this method is done in only a limited number of reference laboratories, limiting the availability of the method. Importantly, the results usually are not available in time to influence clinical decisions or treatment. Whether this method will become more widespread is not apparent: the method is relatively expensive and requires equipment and expertise not available in most clinical laboratories.

COMPOSITE REPORTS: LINKING RESULTS OF MICROBIOLOGY, PATHOLOGY, AND MOLECULAR DIAGNOSTICS

As stated previously, the accurate diagnosis of infectious diseases often requires the combined use of microbiologic cultures, histopathology, cytopathology, and molecular methods. One of the new roles of pathologists, in addition to using this information to make a diagnosis, is to combine various test results, observations, and other data into a composite report that effectively synthesizes and communicates the information to providers. As health care becomes increasingly specialized, communicating interpretations and diagnoses across specialties is of paramount importance.

A composite report should provide the context in which the different components can be interpreted, list each of these components, and present a final interpretation. As with other types of histopathology or cytopathology reports, the individual components will be available at different times. As a result, the pathologist must decide whether to issue a final report early in the process, followed by amendments as individual components become available, or to withhold issuing a final report until all of the individual components are available. The benefits and drawbacks to either approach are obvious: issuing a final report before all of the information is available can result in misdiagnosis or a partial diagnosis, whereas delaying the final report until all of the information is available can result in delayed diagnosis. The usual approach—issuing preliminary reports—does not address either concern satisfactorily. Preliminary reports are still based on inadequate or incomplete information. Moreover, preliminary reports have the additional disadvantage of requiring notification or follow-up by providers, which in busy settings all too often does not occur. It is too easy for providers to (1) assume that preliminary reports will not change, (2) place too much faith in preliminary diagnoses, or (3) forget or fail to review final reports.

As with any histopathologic or cytopathologic report, composite reports should include a complete record of the specimen. Information that should be contained within the report includes patient demographic information;

other pertinent patient information (e.g., clinical history); a description of the specimen; a record of what was done with and to the specimen; the results of any special stains, microbiologic cultures, or molecular tests; and final diagnoses with or without interpretive comments. Pathologists should make every effort—as should their clinical counterparts—to avoid the needless use of jargon. Modern pathology reports can run many pages in length and contain information (such as flow cytometry or cytogenetic information) about which many providers have little familiarity. As a result, any or all jargon should be eliminated from final reports so as to provide the clearest and most concise information to providers. Composite reports do exist and are used as part of routine practice for a few infections. Examples would include the cytopathology report for cervical cancer screening, which includes information about the cytopathologic findings, correlations with cervical biopsy results, and the results of testing for human papillomavirus (HPV) infection. Another example would be lymph node biopsy specimens, which often contain information regarding histopathologic findings, cytopathologic findings (touch preparations), flow cytometric findings, cytogenetic test results, and the results of microbiologic cultures.

One of the challenges of creating composite reports is the growing complexity of information technology (IT) in many hospitals and clinics. The many components of a composite report typically are derived from different IT systems such as the anatomic pathology computer, laboratory information system (LIS), interfaces from reference laboratories, and the hospital IT system. Moreover, even within the LIS the different modules (e.g., microbiology) often do not lend themselves to integration of data due to the way the modules are built. As a result, generating a composite report is, in most facilities, a largely manual effort that is time consuming. As IT systems evolve toward what is termed *enterprise systems,* which are systems designed to better share information and data across test platforms and IT modules, it is hoped that the generation of composite reports will someday become automatic.

Another challenge of creating composite reports is the issue of collecting information among the different departments involved in testing specimens. This is not due to a deliberate or intentional lack of collaboration but is a symptom of how increasingly specialized diagnostic laboratories work in isolation from one another, and in particular from anatomic pathology departments. Not only are these various laboratories physically separate from one another, but the results of testing are available at widely varying times, reports are often generated from platforms that do not communicate electronically with one another, and even interpretation of results often requires expertise that is unique to the testing laboratory personnel.

These challenges, while daunting, present important opportunities for pathologists. First, because no other medical specialty has the expertise to interpret histopathologic and cytopathologic findings, only pathologists are in a position to create composite reports. Second, in most hospitals and clinics, the diagnostic laboratories are part of pathology departments, which gives pathologists the most direct access to data. Third, LISs and anatomic pathology computer systems are specialized IT systems that pathologists and laboratory scientists are uniquely qualified to help customize, implement, and use. Finally, most American pathologists receive training in both anatomic and clinical pathology, so they are often the only physicians in hospitals or clinics with formal training in laboratory medicine. For pathologists trained in other countries, where pathologists train in either anatomic (i.e., histopathology) or clinical pathology, it will be imperative for pathologists to develop the skills needed to interpret and integrate the many parts of a composite report.

ARTIFACTS AND PITFALLS

Many artifacts are associated with the histopathologic and cytopathologic diagnoses of infectious diseases. These artifacts fall into two broad categories: artifacts within tissues that mimic microorganisms and artifacts of staining that may cause false-positive test results. These artifacts can occur with any of the diagnostic methods mentioned in this chapter.

Artifacts That Mimic Microorganisms

A number of common artifacts have been reported that mimic pathogenic microorganisms. With H&E-stained tissue sections, some of the more common artifacts include the presence of calcific bodies that mimic yeasts, nuclear clearing that mimics viral cytopathic effects, large eosinophilic nucleoli that mimic the changes of Cowdry A intranuclear viral cytopathic effect, and degenerating strands of connective tissue that can mimic fungal hyphae. A number of similar artifacts also have been described in standard cytologic preparations.

Artifacts of Staining Causing False-Positive Results

As the level of specificity increases from H&E-stained section to histochemical stains, immunohistochemistry, in situ hybridization, and advanced molecular methods, the number of artifacts and other types of false-positive test results should decrease. Histochemical stains help increase the sensitivity and specificity of the histopathologic

examination of tissues, but a number of artifacts do occur with histochemical stains. Notable examples include staining of mammalian nuclei with methenamine silver stains, mimicking fungal hyphae; staining of cellular debris (particularly debris from segmented neutrophils) by methenamine silver stains, mimicking bacteria; and staining of erythrocytes by silver stains, mimicking yeasts. Silver stains in particular are associated with nonspecific staining of cells and tissues. Immunohistochemical stains show fewer artifacts but do show nonspecific staining of tissue sections that can lead to confusion or misdiagnosis. Endogenous peroxidase activity and inadequate rinsing of slides during preparation can both result in nonspecific staining. Staining of plasma cells is a common type of nonspecific staining with immunohistochemical stains, particularly in bone marrow and lymph nodes. One of the more common causes of false-negative test results is the overdecalcification of bone marrow biopsy (and other bone) specimens. In situ hybridization shows less nonspecific staining when compared with immunohistochemical stains, but incomplete washes can result in areas of staining that mimic hybridization. As a general rule, this artifact is easy to identify with larger pieces of tissue compared with small pieces of tissue such as biopsy specimens. By its nature, in situ hybridization shows less nonspecific staining compared with immunoperoxidase stains, yet it is more technically difficult to perform. As a result, false-negative results are of concern (discussed later). For a further discussion on infectious mimics, see Chapter 29.

Pitfalls

One of the main drawbacks to all of the methods described in this chapter is a lack of data regarding the performance characteristics of each diagnostic approach. Calculations of sensitivity, specificity, and predictive values require comparisons against a gold standard diagnostic method, but comparing the results of histopathologic or cytopathologic tests against methods such as microbiologic culture or NAA usually yield invalid outcomes. This is because the methods provide fundamentally different types of information (e.g., detection of DNA or RNA sequences as opposed to the histologic identification of patterns of inflammation with special methods to identify pathogens); specimens are divided for different assays, which introduces sampling bias; specimens are handled differently based on the type of method used (e.g., tissue submitted for culture or NAA testing is preferably not formalin fixed and paraffin embedded); and the method of interpretation is fundamentally different: visual and (by its very nature) somewhat subjective interpretation of histopathologic or cytopathologic findings cannot be compared directly to detection of nucleic acid sequences or melt curves. As a result, there is a marked paucity of information regarding the performance characteristics of histopathologic and cytopathologic methods. Histopathologic methods can be compared with each other, but as none of these methods is likely to be a gold standard, the only conclusions that should be drawn are *relative* performance characteristics.

Many of the benefits and drawbacks of the approaches to the diagnosis of infectious diseases by histopathology and cytopathology are described in this chapter. Of the drawbacks, the most important are the relative lack of diagnostic sensitivity, the relative lack of specificity of most methods, and the many artifacts that can be seen in tissue sections or on cytology slides. Even the addition of molecular methods does not resolve these issues, because too often an infection is not suspected at the time a specimen is collected and neither microbiologic cultures nor many molecular methods can be reliably performed on the specimen. Despite their potential for improving the performance characteristics of histopathology and the ability to provide rapid results, at the present time molecular methods have only limited utility in diagnostic histopathology and cytopathology for the detection and identification of pathogenic microorganisms.

SUMMARY

The histopathologic and cytopathologic diagnoses of infection diseases are sequential and progressive processes that in many cases require the traditional approach of applying basic diagnostic methods such as routine histology and cytology stains, with or without the need for microbiologic cultures. More challenging cases require the application of an integrated process that combines histopathologic and cytopathologic techniques with the routine use of microbiologic cultures and molecular methods. The information obtained from all of these methods must be integrated into a composite report that contains all of the information necessary for the treating provider and for other pathologists who might review the case at a later time.

Suggested Readings available on Expert Consult.

2

Herpes Virus Infections

Tess Karre

Herpesviridae are characterized by an ability to establish latency within specific tissues and reactivate at a later time. The latent viral genetic material may exist extrachromosomally or it may become integrated into the host cell DNA. Eight herpes viruses are currently recognized and are classified into α, β, and γ groups (Box 2-1).

HERPES SIMPLEX VIRUS TYPES 1 AND 2

Herpes simplex virus (HSV) consists of two closely related viruses termed HSV types 1 and 2. HSV has a worldwide distribution and causes a wide range of clinical disease from mild stomatitis to life-threatening disseminated infection. The clinical manifestations and severity of disease depend on several factors including the site of infection and host immune status.

Transmission of both HSV types 1 and 2 generally requires intimate contact between a person with active infection and a susceptible host. HSV initially infects the epithelial cells of mucous membranes or (broken) skin. Following an incubation period of 4 to 6 days, the virus undergoes replication within the epithelial cells, resulting in cell lysis and inflammation. The inflammation and cell lysis are manifested clinically as painful fluid-filled vesicles that rupture to form shallow ulcers. The virus establishes latency by spreading in an ascending fashion from the peripheral sensory nerves to the dorsal root ganglia. Reactivation involves retrograde axonal spread of the replicating virus along the peripheral sensory nerves to the mucosal or skin surface.

Clinical Features

Based on seropositivity studies, greater than 50% of adults in the United States have been infected with HSV-1, whereas 20% or more have been infected with HSV-2. Primary infection with HSV most commonly occurs in early childhood. As many as one out of five cases will present with acute HSV gingivomastitis (herpes labialis), characterized by the sudden appearance of a multitude of ulcers on the cheeks and gums. Primary infection is often preceded by a prodrome of fever, malaise, anorexia, and lymphadenopathy. The virus will then undergo latency and may subsequently recur when triggered by factors such as stress, illness, and hormonal changes. Genital herpes is a less common manifestation of HSV infection and may involve the genital mucosa, cervix, and surrounding skin. Like gingivomastitis, primary herpes genitalis is typically more severe than recurrent infections. Herpes simplex infections involving the oral mucosa are most frequently associated with HSV-1, although a smaller proportion of these infections may be caused by HSV-2. In contrast, most genital herpes simplex infections were traditionally thought to be caused by HSV-2, but an increasing number of these infections have been found to be caused by HSV-1, particularly in young adults.

Superficial lesions may rarely occur in other anatomic sites such as the eyes and nonmucosal surfaces such as herpetic whitlow (typically fingers) or superinfection of traumatized skin (e.g., burn injury). Herpetic whitlows are most commonly seen in health care workers following contact with infected oral secretions.

In adults, HSV-1 central nervous system (CNS) infection is most commonly manifested as encephalitis, whereas HSV-2 tends to cause meningitis. In contrast, nearly 50% of infants who acquire HSV-2 during the birth process develop encephalitis. Herpes encephalitis frequently presents clinically in adults as altered mood, memory, and behavior. The course of HSV-1 encephalitis may be subacute, developing over a 4- to 6-week period. Concurrent infection with the human immunodeficiency virus (HIV) may result in a more acute presentation.

Neonatal herpes simplex infection is a rare condition that results from vertical transmission of HSV. The risk of transmission to the infant is highest during primary maternal infections. Neonatal herpes simplex virus is most often caused by HSV-2 (70%) and typically occurs when the neonate contacts infected genital secretions in the birth canal. Rarely, transmission may result from in utero or postnatal exposure. Three clinical categories of neonatal

Box 2-1

α-Group Viruses

Herpes simplex virus (HSV) types 1 and 2
Varicella zoster virus (VZV)

β-Group Viruses

Cytomegalovirus (CMV)
Human herpes virus 6 (HHV-6)*
Human herpes virus 7 (HHV-7)*

γ-Group Viruses

Epstein-Barr virus (EBV)
Human herpes virus 8 (HHV-8)/Kaposi's sarcoma virus (KSV)

*Some sources classify HHV-6 and HHV-7 as γ-herpes viruses rather than as β-herpes viruses; however they are genetically most closely related to CMV and have similar host-range properties.

herpes simplex infection may be observed: ocular/mucocutaneous, encephalitis, and disseminated infection. These categories do demonstrate some overlap with extension of ocular/mucocutaneous or disseminated infection to the CNS. The ocular/mucocutaneous form involves the eyes, skin, and mucous membranes and may be localized without involvement of other organ systems. Neonatal HSV encephalitis most commonly involves the cerebral cortex, although occasionally the brainstem may be affected. It is not necessarily accompanied by fever or systemic symptoms and may not become clinically evident for several months after birth. When CNS disease is present, the cerebrospinal fluid indices usually show elevated protein and a mononuclear pleocytosis. The untreated mortality rate for neonatal HSV encephalitis is 15%. Disseminated HSV infection is the most serious category and is fatal in 60% to 85% of neonates if left untreated. Symptoms are generally observed during the first week of life and may include respiratory distress, seizures, irritability, jaundice, disseminated intravascular coagulation, and hemorrhagic pneumonitis. These may be accompanied by a vesicular rash.

Diagnosis

The most rapid method for identifying HSV in mucocutaneous lesions is through observation of classic intranuclear inclusions using a Tzanck preparation. This technique is performed by unroofing a vesicle, scraping the base and sides of the underlying lesion, and then placing the material on a glass slide. The slide is typically air dried and stained with Diff-Quick or Giemsa and examined for the presence of intranuclear inclusions or giant cells. It should be noted that this is a relatively insensitive method and that lesions of varicella zoster virus (VZV) cannot be differentiated from those of HSV. Some laboratories instead perform direct fluorescent monoclonal antibody staining on the lesional material, which allows for more specific diagnosis and differentiation of HSV types 1 and 2 and VZV.

The gold standard method for diagnosis of HSV has traditionally been viral culture, as the virus grows readily in multiple cell lines in a relatively short period of time (often in 24 hours). It is a sensitive method for detecting virus from mucocutaneous lesions, and typing is easily performed using fluorescent antibodies to HSV-1 and HSV-2. However, the sensitivity when testing sources such as cerebrospinal fluid and blood is unacceptably low, and more sensitive methods such as polymerase chain reaction (PCR) are recommended for these sources. PCR of cerebrospinal fluid has now supplanted brain biopsy with culture as the test of choice for diagnosis of disseminated disease and involvement of the central nervous system.

In general, serologic testing is not helpful in the diagnosis of acute HSV infection, but it may be useful for determining if a patient has been previously exposed to HSV. Type-specific serology may be useful for evaluating the risk of reactivation, as HSV-2 genital infection is more likely to cause recurrent lesions than HSV-1 genital infection. Finally, serologic studies may be useful in pregnant

HERPES SIMPLEX VIRUS (HSV)—TYPES 1 AND 2 FACT SHEET

Definition

- Herpes simplex virus types 1 and 2 causes a wide spectrum of disease.
- They include orolabial and genital infections and dermal lesions (herpetic whitlow).
- Severe or disseminated infections may occur in immunocompromised or neonatal patients.

Epidemiology

- The majority of people have been exposed to HSV by early adulthood.
- Most orolabial infections are caused by HSV-1 and most genital infections are caused by HSV-2, although either virus can infect either site.

Clinical Features

- Primary and recurrent infection may be asymptomatic.
- Symptomatic mucocutaneous disease is characterized by itching, followed by the appearance of tiny vesicles that rupture and form painful ulcers.
- Complications of mucocutaneous HSV may include difficulty eating (oropharyngeal ulcers) and bacterial superinfection.
- Recurrences are common and may be triggered by stress, illness, and hormonal changes.
- Systemic disease may involve the lungs, liver, brain, and other organs.

Prognosis and Therapy

- Treatment for uncomplicated mucocutaneous infections is generally supportive.
- Suppressive antiviral therapy may be used for recurrent infections.
- Severe or disseminated infections may require intravenous antiviral therapy.

women to determine if previous exposure to either HSV-1 or HSV-2 has occurred, because a negative serostatus indicates a potential risk for primary infection during pregnancy.

Pathologic Features

Gross Findings

The initial lesions of HSV-1 and HSV-2 on the skin or mucosal surfaces appear as clusters of closely grouped, fluid-filled vesicles on an erythematous base. Later, lesions often appear as larger crusted superficial ulcerations. Involvement of other organs is typically associated with edema, necrosis, and hemorrhage. In children and adults, encephalitis is usually localized to the temporal lobes, whereas disease in neonates is typically generalized.

Microscopic Findings

Early cutaneous or mucosal lesions demonstrate intraepidermal or intramucosal acantholysis with edema and accumulation of proteinaceous fluid. This later develops into vesicles or "blisters" containing fluid with an overlying thin layer of epidermis (Figure 2-1A). The base of the vesicles may demonstrate marked ballooning degeneration in which the keratinocytes are enlarged and often contain viral inclusions (Figure 2-1B). Two types of viral inclusions may be observed: Cowdry type A inclusions and "ground-glass" nuclear inclusions. Cowdry Type A inclusions are characterized by a condensed eosinophilic central structure surrounded by a cleared area, which may impart a targetoid appearance, whereas "ground-glass" inclusions are indistinct eosinophilic inclusions that obscure the entire nucleus. Infected cells may demonstrate the classic 3 Ms of HSV infection, with *m*ultinucleation, nuclear *m*olding, and *m*argination of the nuclear chromatin (Figure 2-2). Acute inflammatory cells are frequently seen within the upper dermis as well as within the vesicle fluid. Inflammation and ulceration may be extensive and obscure the correct diagnosis when classic inclusions are not readily visible.

HERPES SIMPLEX VIRUS TYPES 1 AND 2—PATHOLOGIC FEATURES

- Closely grouped fluid-filled vesicles exist on an erythematous base.
- Two types of intranuclear inclusions are involved: Cowdry type A and "ground glass."
- Infected cells may demonstrate the "3 Ms" of HSV cytopathic changes: *m*ultinucleation, nuclear *m*olding, *m*argination of chromatin.
- Ulceration or acute inflammation may obscure the diagnosis.

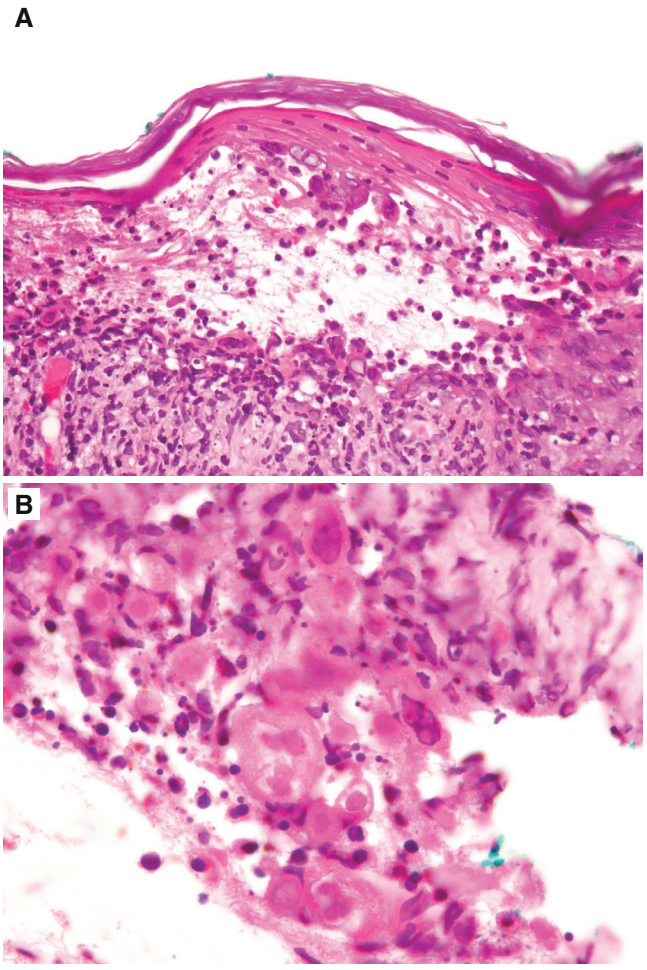

FIGURE 2-1

Herpes simplex virus. **A,** Low-power view of a blister demonstrating intraepidermal acantholysis with ballooning degeneration and viral inclusions in the epidermis at the base of the vesicle. **B,** High-power view showing both Cowdry type A and ground-glass inclusions. The cells with ground-glass inclusions also demonstrate the "3 Ms": multinucleation, molding, and margination of the nuclear chromatin.

Differential Diagnosis

The differential diagnosis of mucocutaneous herpes simplex virus may include varicella zoster virus, as this also produces vesicular skin eruptions with similar appearing cytopathic changes. In addition, other infectious and noninfectious blistering diseases such as bullous impetigo, bullous pemphigoid, and pemphigus vulgaris may be considered, depending on the clinical presentation.

Ancillary Studies

Immunohistochemistry or in situ hybridization (ISH) performed on tissue sections may aid in the diagnosis of HSV when the characteristic viral inclusions are not

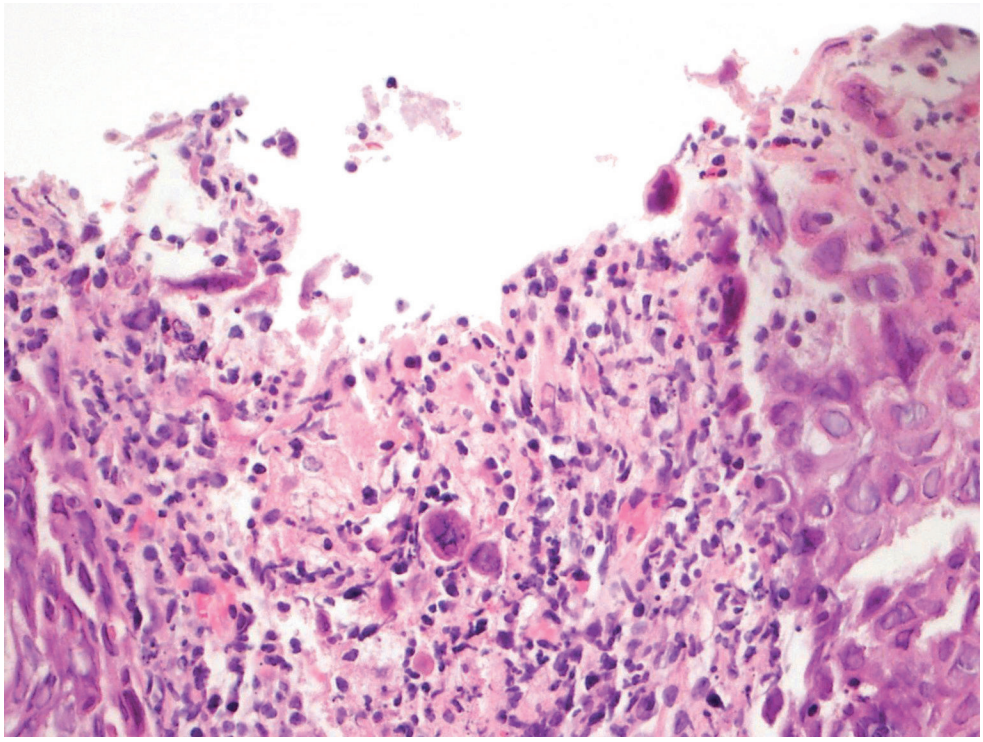

FIGURE 2-2

Herpes simplex esophagitis. Cells with ground-glass inclusions demonstrating multinucleation, molding, and margination of the nuclear chromatin.

observed on hematoxylin and eosin (H&E) staining or when it is necessary to distinguish from varicella zoster virus.

Resistance to acyclovir is uncommon, and if suspected it is detected by viral culture with subsequent susceptibility testing at specialized laboratories.

Prognosis and Therapy

Primary infections with HSV are generally not treated with antiviral medications, although recurrent reactivations of mucocutaneous or genital HSV may be treated using suppressive therapy with acyclovir.

Early diagnosis and treatment is critical in CNS and disseminated infections, as these are frequently fatal if left untreated. Treatment with antiviral agents including acyclovir, valacyclovir, or famciclovir may significantly alter the course of infections with HSV. Severe or disseminated HSV infections may require intravenous therapy.

VARICELLA ZOSTER VIRUS

Varicella zoster virus is responsible for two distinct syndromes in immunocompetent patients: varicella (also known as chickenpox) and herpes zoster (also known as shingles).

Clinical Features

Varicella zoster virus is distributed worldwide. Most primary infections occur in early childhood, although nonimmune adults may also become infected. Transmission may occur through inhalation of infectious respiratory secretions or direct contact with lesions on the skin or mucous membranes. The peak season for primary infection with VZV occurs in the winter and spring.

Primary VZV infection presents as an exanthematous syndrome referred to as chickenpox. Following an incubation period of approximately 14 days, patients develop a prodrome consisting of fever, chills, headache, and anorexia. After 2 to 3 days, a rash composed of crops of closely grouped maculopapular lesions appears on the trunk. The rash spreads to the extremities and head, and the lesions begin to develop into fluid-filled vesicles on an erythematous base (so-called "dewdrop on a rose petal"). These are frequently associated with intense pruritus. The lesions generally become crusted over within 8 to 12 hours; however, new crops of vesicles continually appear within a 2- to 5-day period, so lesions at multiple stages of development may be present together. Disseminated infection, with or without neurologic involvement, is unusual in immunocompetent patients. Following primary infection, the virus establishes latency within the dorsal root or trigeminal ganglia.

Serious complications of primary infection are uncommon and are more likely to occur in patients with impaired cell-mediated immunity. These may include

varicella pneumonia, disseminated infection, hepatitis, and central nervous system involvement.

Reactivation of VZV is characterized by a painful vesicular skin eruption in a dermatomal distribution, referred to as *shingles* or *herpes zoster*. Triggers may include stress, localized trauma, or a decrease in immune function. Frequently, patients will experience a prodrome of pain, itching, numbness, or burning in the specific dermatome. Fever or other systemic symptoms may also be present. The most common dermatomes involved are the thoracic, cranial, lumbar, cervical, and sacral. Herpes zoster is generally a self-limited process, although some patients, particularly adults over age 60, experience persistent pain for months or even years in the area where the shingles occurred, a syndrome known as postherpetic neuralgia. Ocular involvement may occur when the ophthalmic branch of the trigeminal nerve is involved (known as herpes zoster ophthalmicus) and can result in conjunctival ulcers, iritis, keratitis, and chorioretinitis. Similarly, Ramsay-Hunt syndrome is a peripheral facial nerve palsy that occurs as a complication of herpes zoster. The features typically include unilateral facial weakness associated with vesicles in the ipsilateral ear, hard palate, or tongue. Other symptoms may include tinnitus, hearing loss, nausea, vomiting, vertigo, or nystagmus.

Congenital VZV may be associated with congenital abnormalities, particularly if maternal infection occurs prior to 20 weeks' gestation. Congenital varicella syndrome is associated with a high mortality rate (up to 30%) and is characterized by low birth weight and congenital skin lesions in a dermatomal distribution. Other features that may be present include microcephaly, neurologic signs, eye defects, limb deformities, and neonatal seizures.

VARICELLA ZOSTER VIRUS (VZV)—FACT SHEET

Definition

- VZV causes two distinct clinical entities: varicella (chicken pox) and herpes zoster (shingles).

Epidemiology

- Varicella generally occurs in children < 10 years.
- Herpes zoster generally occurs in adults > 60 years.
- Congenital infections are rare but may occur if maternal exposure occurs (especially at < 20 weeks gestation).

Clinical Features

- Varicella is characterized by a fever and a generalized vesicular rash that progresses to scabbed lesions.
- The lesions of varicella typically occur in multiple stages (new vesicles and older crusted lesions), which differentiate them from the lesions of smallpox.
- Herpes zoster is characterized by a prodrome of pain, itching, or numbness followed by a vesicular rash in a dermatomal distribution.
- Congenital VZV infection has a high mortality rate and is characterized by low birth weight, dermatomal rash, microcephaly, neurologic signs, eye and limb defects, or seizures.

Prognosis And Therapy

- Treatment for primary VZV infections in immunocompetent patients is mostly symptomatic.
- Antiviral medication (acyclovir) may be recommended in exposed/infected immunocompromised patients or in patients with herpes zoster.
- Vaccination has reduced the number of cases of VZV.

Diagnosis

Primary infection with VZV is frequently diagnosed using clinical features alone.

In ambiguous cases, a Tzanck smear (discussed previously in the section on HSV) may be used to identify epithelial cell changes seen in VZV, although it is not specific for VZV. Like HSV, direct fluorescent antibody staining may also be performed on lesional material, which adds sensitivity and enables distinction from HSV.

Serology is not frequently used in the diagnosis of primary varicella infection, but a high titer IgM or a significant rise in IgG titer may suggest acute disease. Serology is more often used in the confirmation of immunity following vaccination or previous exposure, especially in pregnant women.

Culture may be used for isolation of VZV, but the virus grows poorly in culture and turnaround time is typically 2 weeks. Instead, PCR is recommended as the most sensitive and specific test for the detection of VZV in dermal lesions and other sources. PCR may be very useful in cases of atypical skin rashes where VZV and HSV are not easily distinguished clinically. Unfortunately, PCR is generally limited to the reference lab setting and may not be available at smaller laboratories.

Pathologic Features

Gross Findings

Cutaneous lesions appear as crops of small maculopapular lesions, which eventually develop into fluid-filled blisters on an erythematous base ("dewdrop on a rose petal"). These eventually become hard, dry, crusted lesions. Over time, new crops of maculopapular lesions and vesicles appear alongside older crusted lesions; this variation in lesion stage allows for differentiation of varicella from smallpox infection (variola). Systemic involvement may occur in the absence of cutaneous lesions (zoster sine herpete).

Microscopic Findings

The cytologic and histopathologic features of mucocutaneous VZV infections may be difficult to distinguish

from those of herpes simplex virus. Biopsy of an intact vesicle may demonstrate intraepidermal acantholysis and splitting at the suprabasal level (Figure 2-3A). Involvement of hair follicles and other adnexal structures is not uncommon. Viral inclusions similar to those seen in HSV are frequently present, with Cowdry type A or "ground-glass" inclusions, multinucleated cells, margination of chromatin, and nuclear molding (Figure 2-3B).

In contrast to HSV, VZV frequently demonstrates a leukocytoclastic vasculitis, characterized by fibrinoid degeneration of small vessels in the dermis with acute inflammation, accumulation of neutrophilic nuclear debris, and extravasation of red blood cells.

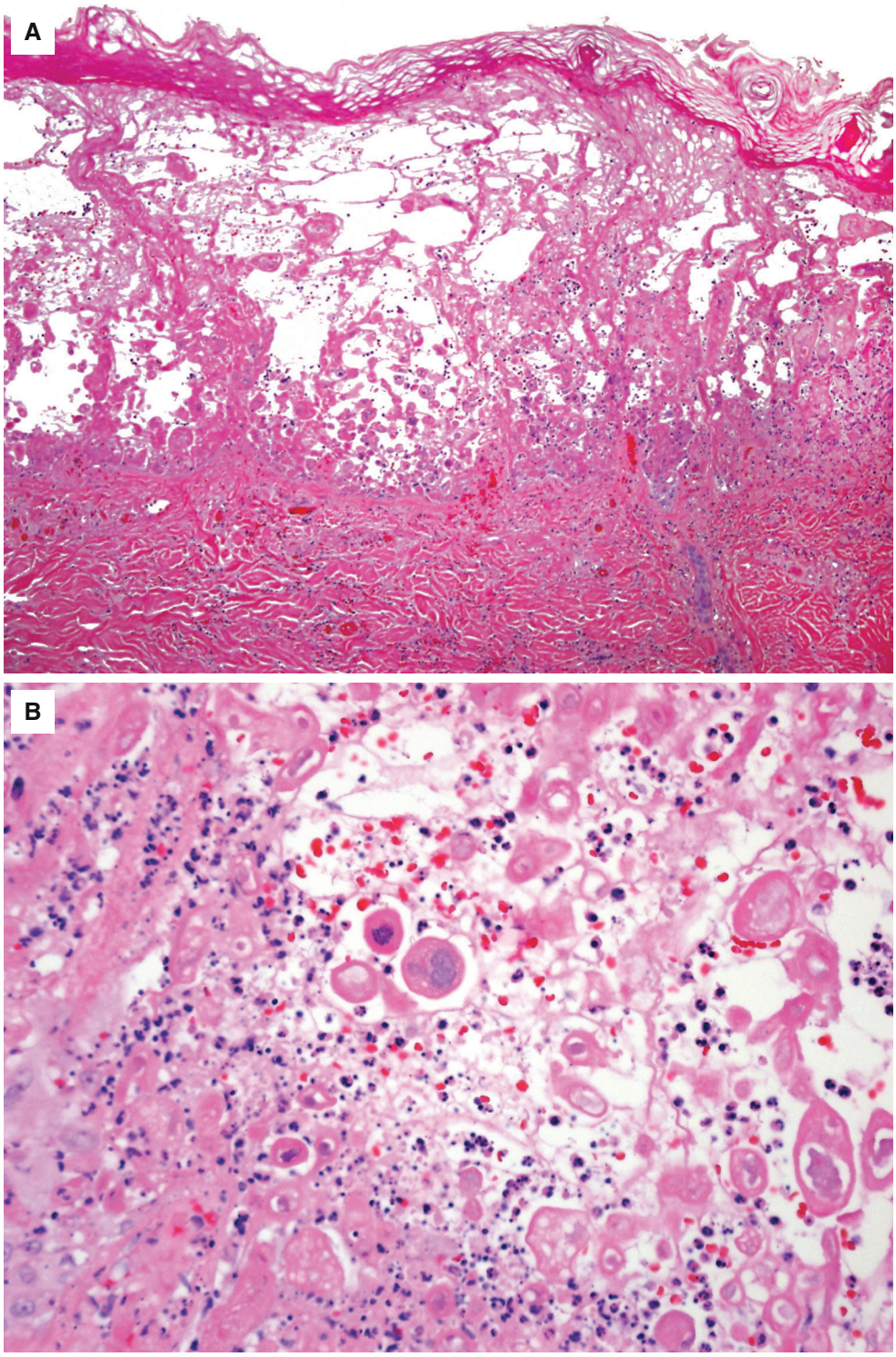

FIGURE 2-3

Varicella zoster virus. **A,** This low-power H&E demonstrates blister formation with intraepidermal acantholysis and inflammation. **B,** A higher-power view demonstrates multinucleation and glassy intranuclear inclusions. *(Courtesy of Lori A. Erickson, MD.)*

VARICELLA ZOSTER VIRUS (VZV)—PATHOLOGIC FEATURES

- Primary VZV lesions appear as crops of closely grouped fluid-filled vesicles on an erythematous base (so-called "dewdrops on a rose petal").
- Viral inclusions are similar to those seen in HSV, with Cowdry A or "ground-glass" type inclusions, multinucleated cells, marginated chromatin, and nuclear molding.
- May see leukocytoclastic vasculitis with fibrinoid degeneration of small vessels in the dermis, acute inflammation, and accumulation of nuclear debris (not seen in HSV infections).

Ancillary Studies

Immunohistochemical and in situ hybridization studies can identify VZV in histologic tissue sections, and this is especially helpful in distinguishing VZV from HSV (see Figure 2-4). It is also useful in cases where viral inclusions are not classic or are obscured by inflammation and ulceration.

Differential Diagnosis

Because both VZV and HSV present with blistering lesions, HSV may be considered in the differential where the diagnosis is unclear. Smallpox or monkeypox should also be considered, although the likelihood of disease with these agents is extremely low. Other noninfectious blistering disorders should also be considered.

Prognosis and Therapy

Treatment of primary VZV infections in immunocompetent patients is aimed primarily at reduction of symptoms (i.e., antipyretics for fever, antihistamines for itching). Antivirals are generally not recommended for this group. Patients at risk for severe complications (including immunocompromised patients) may be treated with acyclovir. Alternative agents include valacyclovir and famciclovir. For herpes zoster, antivirals started early have been shown to decrease pain and duration of symptoms. Childhood vaccination for VZV has been shown to successfully prevent VZV infection. Since the introduction of the vaccine, the number of deaths and hospitalizations due to VZV infection has significantly decreased. Vaccination is now available to prevent zoster and is recommended for individuals 60 and older.

EPSTEIN-BARR VIRUS (EBV)

Epstein-Barr virus (EBV) causes a wide spectrum of diseases ranging from benign infectious mononucleosis to malignant B-cell lymphomas. Prevalence serologic studies indicate that greater than 95 % of adults worldwide have been exposed to EBV. In regions of the world with poor hygiene and sanitation systems, infection is commonly acquired in early childhood. In resource-rich countries, on the other hand, infection occurs in later childhood and the early teen years. By the age of 20 years, most individuals (> 90 %) have been infected.

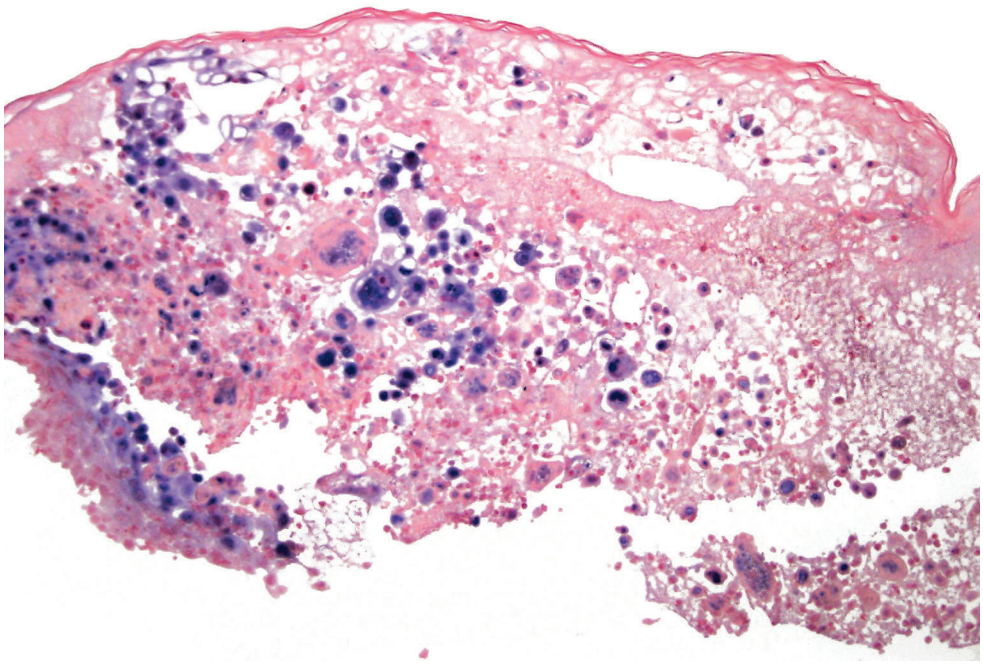

FIGURE 2-4
Varicella zoster virus. In situ hybridization for VZV. *(Courtesy of Lori A. Erickson, MD.)*