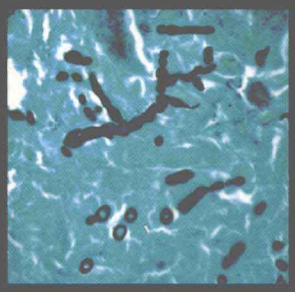
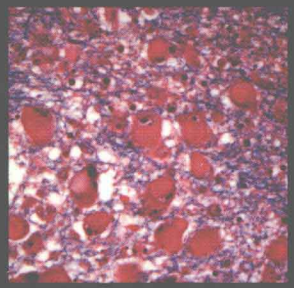
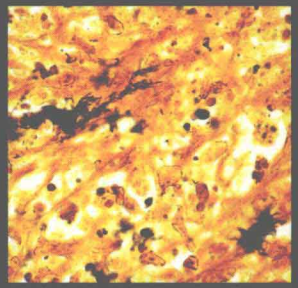
RICHARD L. KRADIN

Diagnostic Pathology of INFECTIOUS DISEASE







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Diagnostic Pathology of Infectious Disease

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Dedication

I dedicate this textbook to
my wife, Karen, and our six children: Rachel, Sarah, Ben,
Michael (×2), and Daniel, who have all been consistently
supportive of the many gyrations that
my career has taken.

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Preface

During my residency in internal medicine some 30 years ago, I was strongly drawn to the clinical practice of infectious disease. My teachers at the University of Pennsylvania were astute clinicians, formidably knowledgeable, and keen observers. During my subsequent training in anatomic pathology at the Massachusetts General Hospital, I considered a career in infectious disease pathology but was hard-pressed to identify it as a viable independent specialty. As a result, I compromised and devoted the next years of my training to specialty interests in both pulmonary medicine and lung pathology, in part because I was aware that many infections affect the lungs.

My subsequent training included research in cellular immunology, and I learned that the primary principles of host defense had largely evolved in response and in parallel to the challenges of infection. Relatively late in my career, I volunteered for the job of being a dedicated expert in infectious disease pathology, and as there was no competition, I got it. In many respects it has proved to be the most rewarding role of my career.

Most surgical pathology departments today are primarily focused on the field of neoplasia; infection has largely become the domain of the microbiology laboratory. Yet the amount of infectious disease pathology that is seen regularly in the practice of surgical pathology in most hospitals is substantial, varied, and diagnostically challenging. In a single week, I often see tens of cases of infection, some of them common, others extraordinary and exotic. It is my considered opinion that the challenges of expert infectious disease pathology diagnosis rival and frequently exceed those of diagnostic tumor pathology.

The nuances of the specialty are unique. They include a degree of clinical expertise, the knowledge of how diseases are geographically distributed, experience in identifying the varied morphological features of a host of pathogens, awareness of how in-host responses vary with levels of immunosuppression, and recognizing when one is not dealing with infection in responses that can mimic it. While most surgical pathologists manage to do a very reasonable job in diagnosing infection, most would admit that their level of sophistication in this area is too frequently limited.

Whereas most textbooks on the topic of infectious disease pathology emphasize details of microbial identification, it is evident that the practicing surgical pathologist primarily needs a firm grounding in recognizing the spectrum of histological responses by the host that can be seen in infection. In a hospital such as my own where pathologists are sub-specialized, surgical pathologists become well versed in how to diagnose the infections that frequently present in their organ of specialized interest. This is unfortunate for those of us who choose to practice infectious disease pathology as a primary subspecialty, as many interesting cases never reach my microscope. But this is easily remedied by maintaining a working relationship with the hospital clinical infectious disease specialists, who invariably make me aware of the cases of interest!

Frankly, few busy pathologists have the time or the inclination to specialize in infection, yet it is just this group that needs access to a single handy resource that will help them to establish an accurate diagnosis. That was the rationale for the present text. As I have noted elsewhere, this text may not invariably provide the level of detail that may be gleaned in the in-depth study of infectious disease morphology. For example, exhaustive detail has not been included with respect to the diagnosis of rare parasitic disorders, but there are already excellent textbooks available that can address these features. This is also not a source book on the molecular aspects of infection; this too can be found elsewhere. What the reader will find here, hopefully, is a practical, accessible, and well illustrated text of the surgical pathology of infection.

Acknowledgments

It is difficult to know where to begin with respect to acknowledgments. Perhaps the best place is with my fellow authors, with one exception, all colleagues in the Massachusetts General Hospital Surgical Pathology Unit of the Department of Pathology. If truth be told, some of them were initially reticent to sign up for the project. Their reasons varied: some were busy, few had a strong primary interest in infection, and so on. But what I knew after many years of working with them was that they are all expert diagnosticians and that once on board they would produce quality chapters, as they did. I hope that it was not too much of a hardship for them and that they have as a result become more comfortable with their own expertise in this area of surgical pathology.

The MGH Infectious Disease Unit is simply superb. I attend their weekly case conferences and am always impressed by the insight that they bring to the diagnosis and management of challenging cases. They have been consistently supportive of my activities, and I hope that I have added to their experience of the study and treatment of infectious disease. I must specifically thank Dr. Jay Fishman, a wise clinician with a good sense of humor who agreed, late in the process, to contribute an important chapter to this text.

My colleagues in the MGH Pulmonary/Critical Care Unit and most especially, Dr. Walter O'Donnell, who has shown a consistent interest in pulmonary infectious disease pathology and has been a source of erudition and support.

Dr. Eugene J. Mark, my colleague and co-author for many years in MGH Pulmonary and Autopsy Pathology, who encouraged me to develop this area of expertise.

The Infectious Disease Pathology Branch at the Armed Forces Institute of Pathology, which has made enormous contributions to the area of diagnostic surgical pathology of infection, and where I spent an enjoyable week some years back reviewing their extraordinary slide collection.

Drs. Sherif Zaki, Francis Chandler, and David Walker, whose lectures on infectious disease pathology at the MGH were both instructive and inspiring to those interested in this area.

To the late Dr. Walter Putschar, whose steadfast approach to discovering the truth was an inspiration, especially with respect to his knowledge of exotic parasitic infections.

To the staff of the London School of Hygiene and Tropical Medicine, who gave me the opportunity to study for a Diplomate in Tropical Infectious Diseases.

To the editorial staff at Elsevier, including Bill Schmitt, who recognized the merit in this project, and Katie DeFrancesco, who has patiently shepherded it along.

Contents

1 Introduction

Richard L. Kradin

CHAPTER

Infectious diseases account for the majority of human diseases. Indeed, in much of the world, infection is the leading cause of debilitating chronic disease and death. In recent decades, medicine has witnessed great strides in the diagnosis and treatment of infection, but it has also seen the emergence of new and deadly pathogens, including the human immunodeficiency virus, that have profoundly influenced how modern medicine is practiced. New treatment modalities including potent immunosuppressive regimens that weaken host defenses have contributed to this emergence of new pathogens and to the recrudescence of others that would normally not be considered pathogens.

In response to the challenge of infection, surgical pathologists are increasingly called on to render diagnoses from both cytology specimens and biopsy specimens. In an effort to decrease patient morbidity with respect to the biopsy procedure, both noninvasive and minimally invasive approaches have been developed that challenge the practicing pathologist to opine on the basis of smaller samples. Furthermore, in addition to establishing the cause of infection, the pathologist must consider a range of disorders in the differential diagnosis with regard to underlying factors that might predispose the host to infection and can mimic the histology of infection. Finally, the histologic response to infection may be the best indication of immunocompetence and indicate prognosis.

Biopsy samples from immunosuppressed hosts can be difficult to assess with accuracy, and they constitute a challenge that some might wish to avoid. Whereas the microbiology laboratory and medical specialists have become increasingly skilled in the diagnosis of infectious diseases, the same has not been uniformly true of surgical pathologists, who may prefer to defer to their clinical colleagues in this area. This is compounded by a trend among surgical pathologists to focus primarily in their practice on the diagnosis of neoplasia, where surgical pathologists maintain preeminent expertise. Their choice is fostered by the frustration of trying to identify small numbers of small pathogens, the delay in diagnosis that results from ancillary histochemical staining and other testing, and difficulties in diagnosing organisms with accuracy due to the morphologic distortions that can ensue after antimicrobial therapies. Taken together, the time, effort, and expense of diagnosing infection can at times seem nongratifying for a busy surgical pathologist.

Nevertheless, the ubiquity of infectious diseases makes it highly unlikely that surgical pathologists can avoid being confronted with their diagnosis in practice, so it behooves them to be aware of the intricacies of how infection manifests in situ. The primary aim of this text is to rekindle the interest that most surgical pathologists once held for the pathologic diagnosis of infectious diseases. Although this may seem like a tall order, it is certainly a worthy one.

The text is organized unlike most other textbooks of infectious disease pathology. The editor has long recognized that most subspecialists in surgical pathology establish expertise in diagnosing the infections that primarily affect the organ system of their specialty, although they may confess to limited interest in the details of infectious diagnosis in other tissues. For this reason, this text has been primarily organized based on organ systems rather than a litany of specific infectious organisms. As a consequence, the reader will be exposed to these disorders as they are actually encountered in a subspecialty practice of pathology. The nuances of infectious diagnoses are presented together with their differential diagnosis, so that the reader can better glean from the text how to narrow the differential diagnosis in practice.

The text includes a preliminary discussion of the types of inflammatory responses that can be elicited by various microorganisms and how host defenses modify these responses. There is a detailed explanation of how to apply histochemical stains differentially in order to narrow the differential diagnosis with respect to microbial morphology. The roles of immunohistochemical staining, in situ hybridization, and the polymerase chain reaction are discussed before the discussion of each of the major organ systems.

Because many microorganisms can affect a variety of human tissues, there is necessarily some redundancy in their description. However, on balance, the superimposed constraints of tissue microanatomy lead to diversity with respect to the morphologic appearances of infection at different sites, so that repetitiveness in this regard has a didactic purpose. In addition, for the busy practitioner, this text may be used as a single resource concerning infection in an organ system of specific interest, in a case-dependent fashion, without having to consult a series of subspecialty texts.

This text is meant to be functionally complete but not encyclopedic. There is much information regarding the clinical, epidemiologic, and mechanistic bases of infection that will not be found here. In addition, some exotic parasitic disorders have not been included. Other texts that include these data are available, and a pathologist may wish to refer to them at times. However, for the most part, all that is required to diagnose the vast majority of infections can be found in the pages of this text.

One final point: The diagnosis of infection is in many respects comparable to that of neoplasia—it requires experience. The morphologic appearances of infection are at least as diverse

as those of malignancy. The variations encountered are virtually inexhaustible, and no textbook can suffice to illustrate all that may be encountered in practice. At times textbooks tend to focus on one aspect of an infection, and the inexperienced pathologist in this area may be misled, expecting to encounter examples that are comparable to those within selected illustrations. Let the reader be forewarned that this text cannot replace experience. But, once the diverse appearances of infection are appreciated and accepted, the surgical pathologist may derive substantial pleasure from pondering its fine distinctions and take pride in the growing sense of competence that develops from experience in this area.

General Principles in the Diagnosis of Infection

Richard L. Kradin and A. John Iafrate

2 CHAPTER

Introduction

The identification of infection in biopsied tissues is the primary responsibility of the surgical pathologist. In an age when both noninvasive and minimally invasive approaches and techniques have increased, it is important to revisit the role of the biopsy in the diagnosis of infection (Table 2-1). Isolating microorganisms in the microbiology laboratory is a sensitive and accurate approach to their identification, but it has several important limitations. First, it cannot distinguish infection from colonization, nor can it ascertain the significance of the isolated organism. Only the presence of an organism in situ, together with an expected inflammatory response by the host, constitutes acceptable evidence of its role in infection.

For example, consider how to interpret the clinical significance of a fungus isolated from the airways of a patient with bronchiectasis who also has a new pulmonary infiltrate in the setting of immunosuppression. Is the fungal isolate the likely cause of the opportunistic infection, or might it be a benign commensal? Studies have attempted to address this question[1] with guidelines formulated for practice, but these are indeed merely "guidelines," because only identification of a potential pathogen within a site of infection can provide substantive evidence that the fungus is an invasive pathogen. For this and other reasons to be addressed in this text, the pathologic diagnosis of infection is a critical element in formulating optimal therapy.

Sampling

Tissue sampling is fundamentally important in the diagnosis of infection. All excised tissues should be considered as potentially infective. This approach fosters due diligence with respect to the possibility of contagion, as well as thoughtful concern as to how the tissues will be handled to optimize the chances of establishing an accurate diagnosis (Table 2-2). Samples of excised tissues should be harvested by sterile technique and sent to the microbiology laboratory with information concerning the types of organism that are being considered diagnostically. Directions to consider anaerobic and fastidious species should be clearly stated.

The surgical pathologist must ascertain that all diagnostic possibilities have been considered. Consultation with an infectious disease specialist can be invaluable in ensuring that specimens are properly handled *ab initio*. What must be avoided is thoughtlessly placing a biopsy specimen directly into formalin fixative without first considering a diagnosis of infection.

Touch imprints should be routinely prepared and can be stained in the frozen-section suite or in the microbiology laboratory. In general, 5 to 10 touch imprints will suffice, with sampling from the most suspicious portions of the biopsy specimen (e.g., areas of necrosis or suppuration).

Harvesting a portion of the biopsy specimen for ultrastructural analysis can foster the accurate diagnosis of many organisms (e.g., viruses, *Tropheryma whippelii*, microsporidia).[2] Specimens may be harvested for polymerase chain reaction (PCR) testing to establish the diagnosis of others (e.g., *Coxiella*, mycobacteria, rickettsia).[3]

The rapid diagnosis of a frozen section can help to focus the diagnostic workup. All of the pertinent histochemical and ancillary studies can ideally be ordered before the permanent sections are processed, to avoid undue delay in diagnosis.

Diagnosing Infection In Situ

Because host immune mechanisms can greatly amplify the host response, the actual numbers of pathogens present in tissues is frequently surprisingly small. This means that many sections may need to be examined before a pathogen is identified. Although

Table 2-1 Role of the Surgical Pathologist in the Diagnosis of Infection

Establish morphologic diagnosis of infection
Assess immunocompetence of the host
Narrow the differential diagnosis of possible pathogens
Confirm results of microbiologic cultures
Refute the relevance of microbiologic cultures
Establish diagnosis unrelated to infection
Identify concomitant infection in a primary inflammatory or neoplastic disorder
Identify new pathogens

Table 2-2 Optimal Handling of Tissue Biopsies: Always Consider Infection!

Make touch imprints for histochemical staining
Handle samples for microbiologic culture with sterile technique
Harvest samples for ultrastructural examination in glutaraldehyde fixative
Harvest fresh samples for appropriate polymerase chain reaction assays
Freeze portion of biopsy specimen for research
After all of this is done, place biopsy specimen in formalin

few surgical pathologists would balk at the idea of ordering additional sections to exclude malignancy in a biopsy they deemed suspicious, it is not uncommon for a pathologist to examine only a single histochemically stained tissue section in the diagnostic process of infection.[4] More egregious is the fantasy that the causative infectious agent will eventually be diagnosed by the microbiology laboratory, so there is no need for the surgical pathologist to belabor the process.

This approach is wrong-minded for several reasons. First, the microbiology laboratory may fail to identify a causative organism.[5] Second, the organism isolated by the laboratory may not represent the actual infective agent in vivo. The analogy is the role for Gram staining of secretions in chronically intubated patients to determine whether there is a neutrophilic exudate consistent with infection and whether there is a predominating organism—steps that can promote the choice of appropriate antibiotic therapy.[6] In this setting, undue emphasis on culture results can lead to a seemingly endless process of adding or eliminating antibiotics in patients who are merely colonized by bacteria and not actually infected. Treatment decisions that do not take into account the host response and dominating organisms will tend to favor the production of increasingly antibiotic-resistant isolates and may potentially compromise public health. This is only one of several compelling reasons to consider diagnostic biopsies in patients with infections in situations that do not readily yield to noninvasive approaches.

Potential Limits of Biopsy Interpretation

Despite the merits of examining biopsy specimens in the diagnosis of infection, one must be aware of those situations in which

Table 2-3 Tissue Responses to Infection

Type of Inflammation	Example
Exudative inflammation	Pyogenic bacteria
Necrotizing inflammation	Gram-negative bacteria, amebiasis
Granulomatous inflammation	Mycobacteria, fungi
Histiocytic inflammation	*Rhodococcus, Legionella,* Whipple's disease
Eosinophilic inflammation	Fungi, parasites
Cytopathic changes	Viruses
No response	Host anergy

the sensitivity and specificity of histochemically stained sections is limited. An example is tuberculosis, in which biopsies can fail to demonstrate mycobacteria in almost half of cases.[7] But even in this setting, the appearance of the inflammatory response in situ should foster a working diagnosis that is often sufficiently reliable to institute empirical treatment.

Classification of Patterns of Infection

There is currently no uniformly accepted classification schema for the histologic patterns of response yielded by microorganisms. The inflammatory response in infection is a function of the host response, which is in turn a function of (1) the anatomy of the affected organ, (2) the virulence factors produced by the infective agent, and (3) host immunocompetence. The surgical pathologist must be aware that a single species of microorganism may be capable of evoking a variety of different patterns of inflammation. An example is the broad spectrum of disorders produced in response to infection with *Aspergillus* spp., which ranges from benign colonization, to hypersensitivity responses, to malignant angioinvasive infection.[8]

The characteristic types of inflammation elicited by infection (Table 2-3) can be broadly categorized as follows.

1. **Pyogenic responses.** In these responses, neutrophils predominate, leading to pus formation. They are evoked primarily by bacteria, although viruses and fungi can also elicit them (Fig. 2-1).
2. **Necrotizing inflammation.** Tissue necrosis can occur in several forms. In certain infections, such as those caused by amebas or gram-negative bacteria, liquefactive necrosis is frequently seen (Fig. 2-2). Other forms, such as ischemic, mummefactive, and caseous necrosis, are often seen in mycobacterial and fungal infections.
3. **Granulomatous inflammation.** This response is characterized by the presence of epithelioid macrophages with multikaryon (giant cell) formation. It appears to reflect cell-mediated immunity to poorly catabolized antigens and is evoked by mycobacteria, fungi, and parasites (Fig. 2-3).
4. **Histiocytic inflammation.** These responses are characterized primarily by the presence of foamy macrophages and are a prominent component of infections caused by *Legionella,*

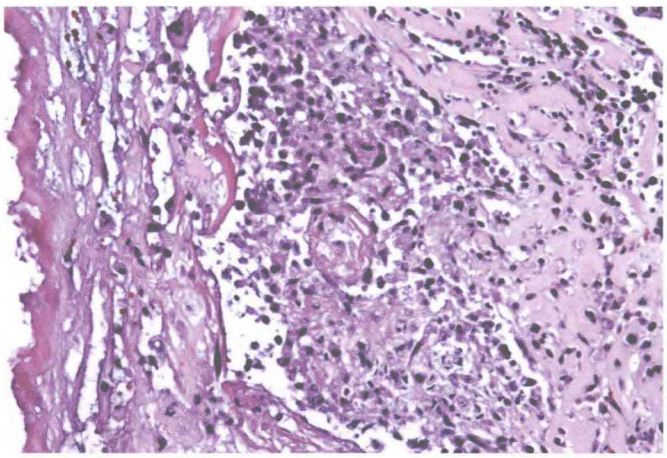

Figure 2-1. Pyogenic response in acute infective endocarditis due to *Streptococcus* spp. with neutrophilic exudate. (×400)

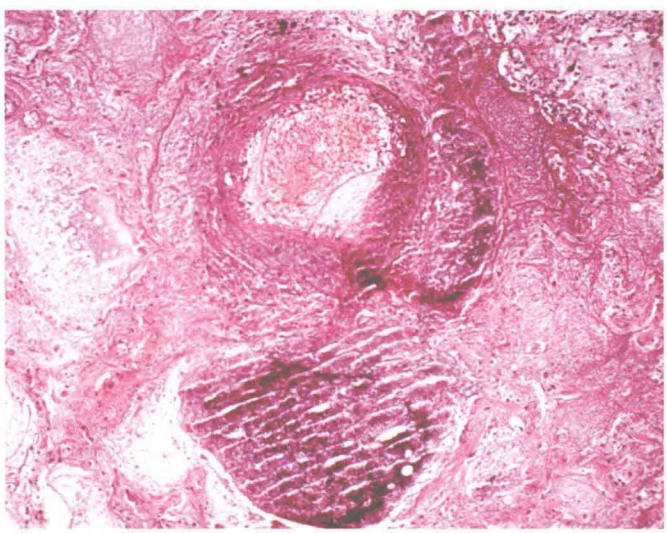

Figure 2-2. Necrotizing response to *Pseudomonas aeruginosa*, showing liquefactive destruction of lung tissue. (×250)

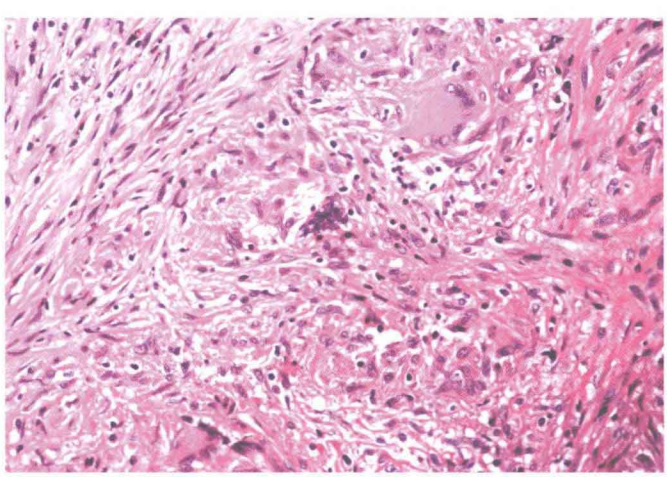

Figure 2-3. Granulomatous response to *Mycobacterium tuberculosis*. (×25)

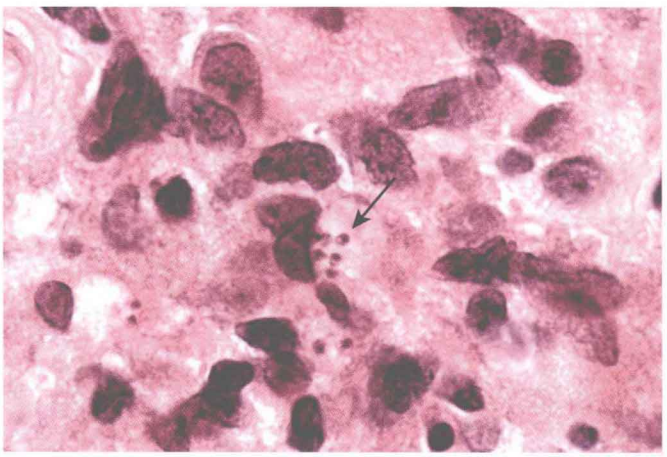

Figure 2-4. Histiocytic response shows "foamy" macrophages containing *Leishmania donovani* (*arrow*). (×600)

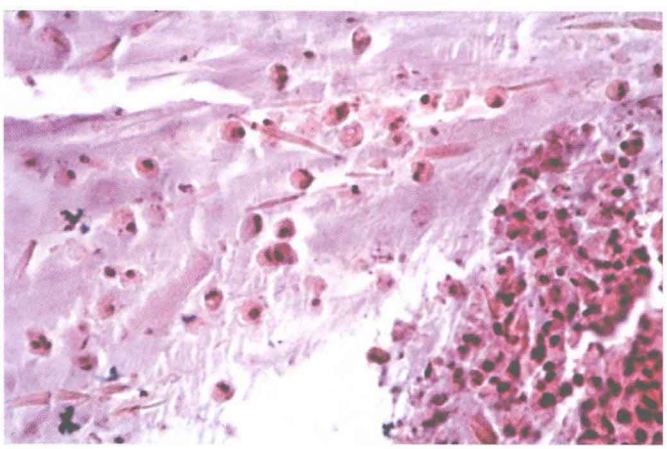

Figure 2-5. Eosinophilic response to *Aspergillus fumigatus*. (×400)

Rhodococcus, *Calymmatobacterium*, *Leishmania*, and *T. whippelii* (Fig. 2-4). In patients who are severely immunocompromised, organisms that normally elicit granulomatous inflammation may instead evoke histiocytic infiltrates.

5. **Eosinophilic inflammation.** This is seen in response to multicellular parasites and certain fungi (Fig. 2-5).
6. **Cytopathic changes.** Although this is not properly a type of inflammation, cytopathic changes do reflect a response to viral infection. Nuclear inclusions are part of the response to DNA viruses, whereas cytologic inclusions are seen with some RNA and DNA viral infections, such as cytomegalovirus (Fig. 2-6).
7. **Null responses.** In the setting of profound immunosuppression, one may not see inflammation; only the uninhibited growth of microorganisms is apparent (Fig. 2-7).

This classification schema is only a crude approximation, because overlap patterns of inflammation are common, as with necrotizing granulomatous inflammation, granulohistiocytic inflammation (Fig. 2-8), and granulomatous inflammation with tissue eosinophilia (Fig. 2-9). The primary didactic element is

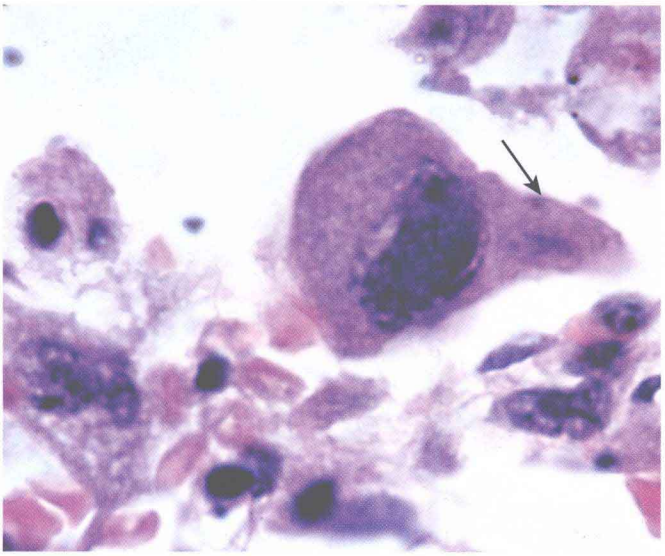

Figure 2-6. Cytopathic response to *Cytomegalovirus* with both nuclear and cytoplasmic (*arrow*) inclusions. (×600)

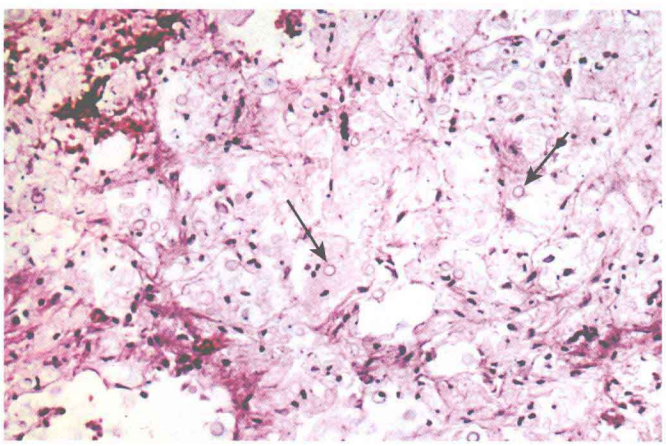

Figure 2-7. Null response to *Cryptococcus neoformans* (*arrows*). (×400)

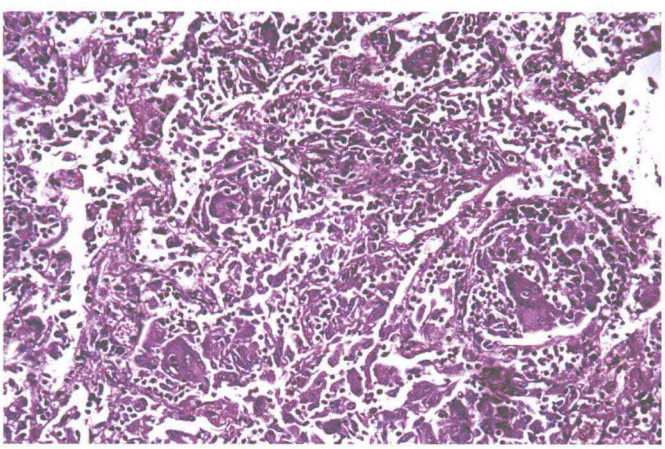

Figure 2-8. Granulohistiocytic response to *Blastomyces dermatitidis*. (×250)

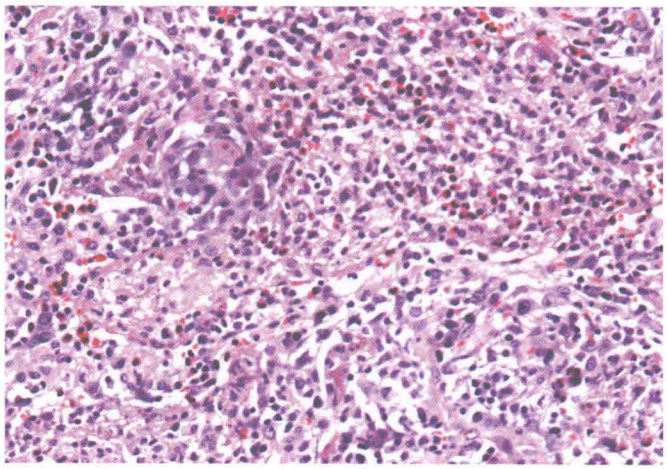

Figure 2-9. Granulomatous response with tissue eosinophilia due to *Coccidioides immitis*. (×250)

that careful consideration of the histological response in situ can help to narrow what would otherwise be a very broad differential diagnosis and can also provide invaluable information concerning host immunocompetence. For this reason, surgical pathologists must develop expertise concerning the inflammatory patterns that can accompany reduced immunocompetence resulting from genetic factors, age, toxins, and drugs, because they can skew the expected pattern of inflammation and at times confound the diagnosis.

Histochemical Stains

The identification of microorganisms in biopsy samples is enhanced by the selective application of widely available histochemical stains (Table 2-4). Pathologists should be aware of the spectrum of histochemical staining by microorganisms and knowledgeable with respect to how to formulate combinations of stains to enhance diagnostic specificity.[9]

Hematoxylin and Eosin

The majority of pathogens can be identified with the standard hematoxylin and eosin (H&E) stain. These include cytopathic viruses, some bacteria, most fungi, and virtually all parasites (Table 2-5).

Gram Stain

The tissue Gram stain is a congener of the Gram stain used routinely to identify organisms in body secretions and fluids. The Brown-Hopps stain is currently the preparation of choice, because it enhances gram-negative bacteria and rickettsia to a greater degree than the Brown-Brenn. In addition, the latter can be hazardous to technical personnel and has largely fallen into disfavor. The tissue Gram stain colors the cell walls of gram-positive bacteria a deep violaceous blue (Fig. 2-10A) and gram-

Table 2-4 Histochemical Staining Characteristics of Microbes

Organism	Staining Characteristics
Viruses	
Influenza	No cytopathic change
Coronavirus (SARS)	No cytopathic change
Adenovirus	H&E (smudge cells); IHC
Cytomegalovirus	H&E (intranuclear and cytoplasmic inclusions); IHC; PAS and GMS (intracytoplasmic inclusions)
Herpesvirus	H&E (intranuclear inclusions); IHC
Measles	H&E (intranuclear inclusions, polykaryons)
Respiratory syncytial virus	H&E (polykaryons); IHC
Parainfluenza	H&E (intracytoplasmic inclusions)
Bacteria	
Gram-positive	Tissue Gram, GMS (all)
Gram-negative	Tissue Gram, GMS (some)
Legionella	Silver impregnation
Nocardia	Tissue Gram, GMS, modified ZN
Actinomyces	Tissue Gram, GMS
Mycobacteria tuberculosis	ZN and modified ZN; PCR
Atypical mycobacteria	Modified ZN, ± ZN, PCR
Fungi	
Histoplasma	GMS, PAS
Cryptococcus	H&E, GMS, PAS, mucicarmine; Fontana, IHC
Blastomyces	H&E, GMS, PAS, mucicarmine (weak)
Coccidiomyces	H&E, GMS, PAS
Candida	H&E, GMS, PAS, Gram stain; IHC
Aspergillus	H&E, GMS, PAS, IHC
Zygomyces	H&E, GMS, PAS
Pseudeallescheria	H&E, GMS, PAS
Alternaria and dematiaceous fungi	H&E, GMS, PAS, Fontana
Parasites	
Protozoa	H&E, PAS, Gram stain (microsporidia); IHC (*Toxoplasma*),
Metazoans	H&E, trichrome stain
Echinococcus	GMS in chitinous wall, modified ZN (hooklets)
Paragonimiasis	Ova birefringent
Schistosomiasis	Lateral and terminal spines stain with modified ZN

GMS, Gomori methenamine silver stain; H&E, hematoxylin and eosin stain; IHC, immunohistochemical methods; PAS, periodic acid–Schiff stain. PCR, polymerase chain reaction; SARS, severe acute respiratory syndrome; ZN, Ziehl-Neelsen stain.

Table 2-5 Microbes That Can Be Identified with Hematoxylin and Eosin Stain

Cytopathic viruses
Bacteria in colonies or in "granules"
Most fungi
Parasites

negative bacteria a pale salmon pink (see Fig. 2-10B). Consequently, it is far easier to detect gram-positive species, and one must be careful not to overlook the presence of faintly stained gram-negative species. Gram variability is a potential pitfall in interpretation, because it can raise the specter of polymicrobial infection. Attention to the uniform morphologic characteristics of stained organisms is the best way to avoid being misled by this phenomenon.

Nonbacterial pathogens can also be identified with the Gram stain. The blastoconidia (yeast) of *Candida* spp. (Fig. 2-11A) and the microconidia of *Aspergillus* spp. (see Fig. 2-11B) are gram-positive, and this feature can help in distinguishing these species from other fungi. Microsporidia can be well demonstrated as gram-positive intracellular inclusions within cells (Fig. 2-12).

Silver Impregnation

The impregnation of tissue sections with silver constitutes the basis of the Warthin-Starry, Dieterle, and Steiner stains. There is some controversy among experts as to whether these stains are equally efficacious in the identification of certain organisms, such as *Bartonella* spp., but in general they yield comparable results. In theory, all eubacteria, including mycobacteria, will stain positively with silver impregnation. However, in our experience, they do not do so reliably, and this approach cannot be recommended as a screening tool. In general, bacteria are enhanced both colorimetrically and in size by the deposition of silver salts on their cell walls, making them easier to identify but at times causing confusion in interpretation. Background staining presents a problem in interpretation, but the morphologic regularity of eubacteria usually allows for accurate identification, once experience has been established with the technique.

Certain weakly gram-reactive or non–gram-reactive bacteria cannot be demonstrated reliably by any other histochemical method. These include *Treponema* spp. (Fig. 2-13), *Borrelia* spp., *Bartonella* spp., *Leptospira* spp., and *Calymmatobacterium*. Weakly staining gram-negative bacteria, including *Legionella* spp., *Burkholderia* spp., *Francisella* spp., and *Helicobacter*, are also best demonstrated by silver impregnation.

Fungal Stains

The Gomori methenamine silver (GMS) and Gridley stains are the preferred methods for demonstrating fungi (Table 2-6). Because certain fungi demonstrated by GMS do not consistently stain well with periodic acid–Schiff (PAS), the latter should be

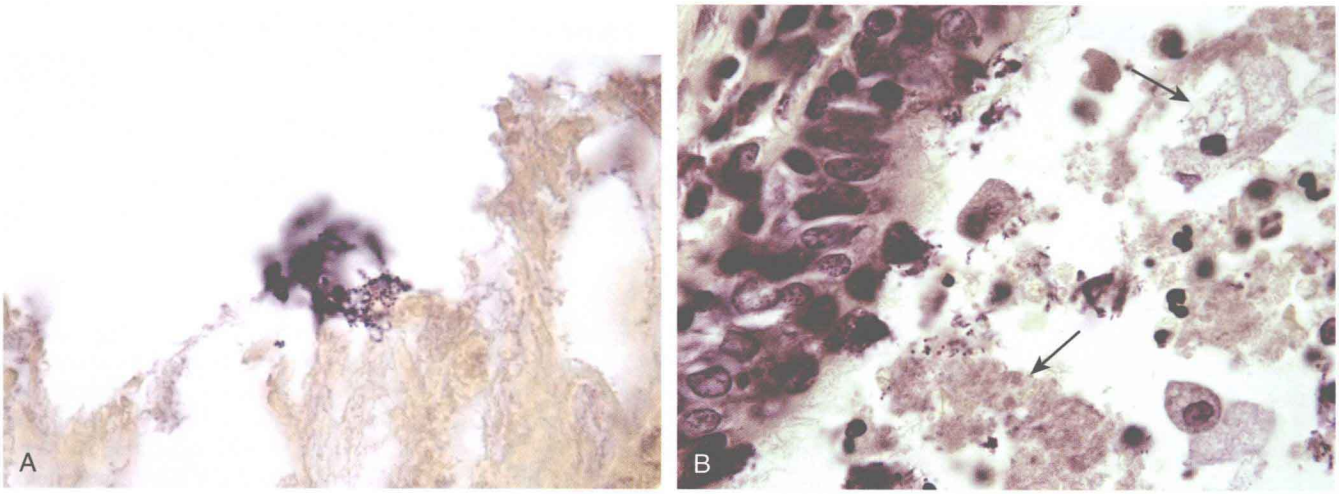

Figure 2-10. A, *Streptococcus* spp. stain deep blue-magenta. (×600) **B,** Gram-negative bacteria are pale salmon-pink (*arrows*). (×600)

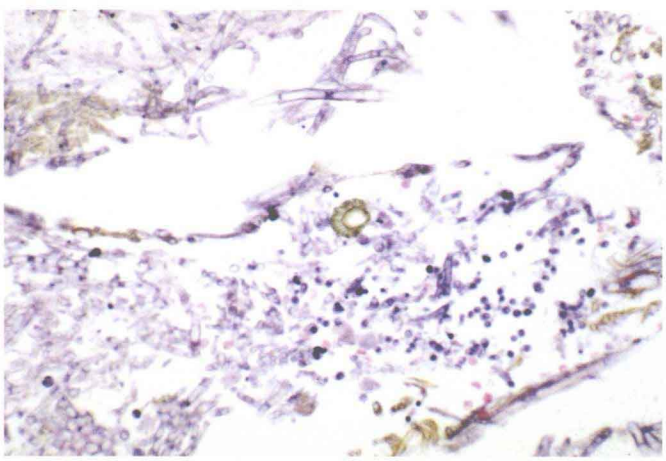

Figure 2-11. The microconidia of *Aspergillus fumigatus* stain intensely gram-positive. (×250)

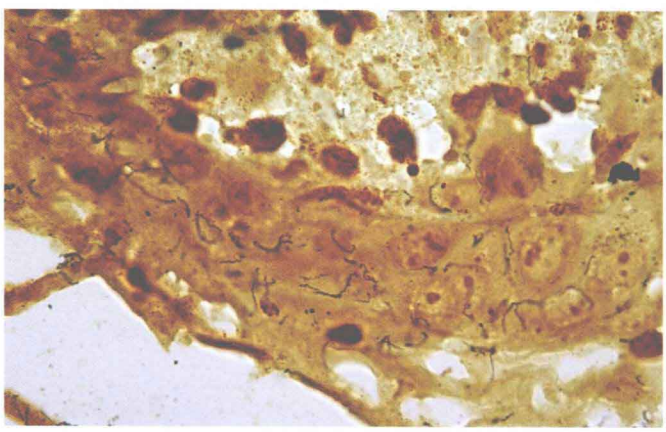

Figure 2-13. Spirochetes of *Treponema pallidum* stain with Warthin-Starry silver impregnation. (×400)

Figure 2-12. Gram-positive intracytoplasmic microsporidia. (×400)

reserved as a secondary approach, but it can at times enhance morphologic detail. Although the GMS is often counterstained with methyl green for contrast, other counterstains can be applied. It is possible, for example, to counterstain with H&E; this allows for a detailed assessment of the cellular immune response and promotes accurate identification of intravascular and perineural invasion by organisms.

All gram-positive bacteria, including the actinomycetes, stain with GMS (Fig. 2-14), as do some encapsulated gram-negative bacteria, such as *Klebsiella* spp. Bacteria that have been treated before tissue sampling (e.g., infective endocarditis), may not be well decorated by the Gram stain, but they often retain their GMS positivity. For this reason, both stains should be examined before excluding a gram-positive bacterial infection. The actinomycetes, including mycobacteria, are gram-positive eubacteria and consequently also stain with GMS. The GMS is the stain of choice for demonstrating *Pneumocystis jiroveci* (Fig. 2-15), and it highlights the trophozoites of *Entamoeba*