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Pneumocystis jiroveci Pneumonia

Abida K. Haque and Patrick A. Adegboyega

Pneumocystis pneumonia (PCP) is one of the most common pulmonary infections in persons with impaired cell-mediated immunity, and particularly those infected with human immunodeficiency virus (HIV).[1-7] Pneumocystis was first described in the lungs of guinea pigs, during experiments on American trypanosomiasis by Carlos Chagas[8] in 1909 and by Antonio Carinii[9] in 1910. Both considered the cysts of pneumocystis as part of the trypanosome's life cycle. Shortly afterward the Delanoes[10] found identical forms in the lungs of rats that had not been infected with trypanosomes and recognized the organism as a separate species. The name *Pneumocystis carinii*, was given to this organism as a generic name (Greek:*pneumon*, "lung"; *kystis*, "cyst"), honoring Carinii.[11]

The organism attained medical significance, when van der Meer and Brug[12] in 1942, and later Vanek, Jirovec, and Luke suggested it to be the cause of interstitial plasma cell pneumonia, a disease affecting premature and debilitated infants in central and eastern Europe.[12,13] In the 1960s *P. carinii* was recognized as an important cause of pneumonia in immunocompromised adults on corticosteroids and cancer chemotherapy, in organ transplant recipients, and in children with primary immunodeficiency syndromes.[6] The emergence of acquired immune deficiency syndrome (AIDS) in the 1980s thrust *P. carinii* to the forefront once again, as a leading cause of morbidity and mortality in immunocompromised individuals.[14] Pneumocystis organisms infecting human beings have recently been named *P. jiroveci*.[15]

The complete identification and classification of pneumocystis has taken many decades. Although initially considered to be a protozoan, it is now generally agreed that pneumocystis is a fungus. The ribosomal RNA is homologous to that found in fungi.[16,17] A study of the small subunits of ribosomal RNA (16S-like rRNA) of *P. carinii* and the fungus *Saccharomyces cerevisiae* shows close evolutionary linkage between the two.[16] The pneumocystis organisms also stain with methenamine silver stains, further supporting a closer link to fungi rather than pro-

tozoa. Recent molecular genetic studies that demonstrate the thymidylate synthase and dihydrofolate reductase genes to be similar to their counterparts in *S. cerevisiae* support the classification of pneumocystis as a fungus.[17] Furthermore, ultrastructural studies have failed to show the cytoskeletal elements and complex organelle systems characteristic of protozoa.[18,19]

Pneumocystis organisms are ubiquitous and globally distributed, having been identified in virtually every mammalian species including humans as well as rabbits, dogs, goats, cats, swine, chimpanzees, owl monkeys, and horses.[20-26] The organisms have a wide range of genetic characteristics that are host specific.[27] The pneumocystis that infects humans, *P. jiroveci*, is different from the one that infects rats, and there is no cross-species infection.[15,27] This observation was confirmed when polymerase chain reaction (PCR) applied to the human pneumocystis identified only *P. jiroveci*.[28,29]

Epidemiology

The epidemiologic features of *P. jiroveci* are poorly understood. Experimental studies have shown that the infection is acquired by inhalation.[30-33] There does not appear to be a natural transmission of *P. jiroveci* across species.[27,34-36] Immunosuppressed rats and nude mice acquire the infection by direct and distant contact with infected animals. In humans, the major predisposing factor is impaired cellular immunity as seen in AIDS, protein-calorie malnutrition, primary immunodeficiency diseases, immunosuppressive therapy with corticosteroids or other agents, and prematurity.[37] The organisms are present in practically every part of the world, including the temperate, tropical, and polar regions.[38] The use of molecular technology through the dihydropteroate synthase (DHPS) locus analysis has facilitated epidemiologic study of the prevalence of *P. jiroveci* in the human population. Identical genotypes of *P. jiroveci* were found in

the two groups of immunocompetent infants and adults with pneumocystis infection in a study from France, suggesting that the transmission cycles of infection in all individuals parasitized by *P. jiroveci* are linked with a common human reservoir.[39,40]

It is not clear whether there is an environmental reservoir for *P. jiroveci,* although mammalian lung appears to be a natural home.[35] The isolation of pneumocystis DNA from rural outdoor locations and a seasonal variation in infection in patients suggest that there may be an environmental reservoir.[41] Primary exposure to pneumocystis occurs early in life, so that most children have serum antibody by the age of 2 to 3 years.[42–45] This is presumed to be an asymptomatic infection. The organisms remain latent within the host, and propagate when the host immune system becomes compromised. Several studies using PCR have found no evidence of pneumocystis DNA in the lungs of immunocompetent individuals, and do not support the latent reactivation theory.[41,46–48] There are also experimental data in rat models that suggest that pneumocystis organisms do not persist in the lungs of immunocompetent individuals.[49] Transmission of pneumocystis has been shown to occur animal to animal when they had a common air supply, in immunosuppressed as well as immunocompetent models, although the latter had a subclinical transient infection.[41,50,51] Clinical studies have suggested the occurrence of human to human transmission of pneumocystis organisms.[52–56]

Acquired immune deficiency syndrome patients have been found to develop immunoglobulin M (IgM) antibodies with recurrent episodes of pneumocystis pneumonia.[57] It has also been shown by immunoblotting that *P. jiroveci (carinii)* antigen recognition patterns in bronchoalveolar lavage (BAL) fluid can change with recurrent episodes of pneumonia.[58] These findings may represent infections with different antigenic strains or antigenic changes in the existing strain of *P. jiroveci.* Recently, mutations in the *DHPS* gene of *P. jiroveci* were identified and used in epidemiologic studies of *P. jiroveci.* In one study of 139 HIV-infected patients with pneumocystis infection, 19% of patients with prior sulfa treatment had the gene mutation, compared to 4% of those without treatment.[59] Co-infection with multiple strains of *P. jiroveci* was found in 20% to 30% of cases, suggesting that recurrent infections may be related to reinfection with a new strain rather than reactivation.[60] The *DHPS* gene–type variation was related to the place of diagnosis and not the place of birth, suggesting the infection to be recently acquired. Additionally, 54% of *P. jiroveci* strains in newly diagnosed HIV-infected patients demonstrate *DHPS* gene mutations. Since these patients were not treated with sulfa drugs, such mutations suggest that the infection was acquired from patients who had received prophylactic sulfa. The authors therefore believed that these findings represent evidence for person-to-person

transmission of pneumocystis. Person-to-person spread of *P. jiroveci* is also suggested by outbreaks of infection in malnourished infants in orphanages and in hospitals caring for immunosuppressed patients.[37]

Life Cycle

The major obstacle to study the life cycle and biology of pneumocystis is the inability to sustain propagation of the organism outside the lung.[28] Our understanding of the life cycle of *P. jiroveci* derives mainly from detailed ultrastructural studies.[61–65] Four developmental forms are described: trophozoites, cysts, precysts, and sporozoites (also known as intracystic bodies).[66,67] All investigators have consistently identified the trophic (trophozoite) and the cyst stage, and also an intermediate precyst stage. Experiments using *P. carinii* from lungs of infected rats and human lung cell cultures have been partially successful in growing the organisms to study the life cycle.[68] Based on the cell culture studies, it was proposed that several developmental pathways may exist.[68] The environment may play a significant role in determining the predominant method of replication as it does in many yeast or other microorganisms.[69,70] In tissue culture, at least two methods of development were proposed: an asexual cycle and a sexual cycle. Figure 13.1 shows the two cycles of *P. jiroveci* pneumonia development. The asexual cycle involves mitotic replication of the trophic forms. The sexual cycle involves the continued development of precyst stages to mature cyst forms, and the development of elongated daughter forms within the cyst, followed by excystation and collapse of the cyst.

The vegetative forms of pneumocystis, the trophozoites, are 2 to 8 μm in diameter, and attach to type I alveolar epithelial cells. Although initially haploid, the trophozoites are believed to attain a diploid chromosomal number by gametic fusion.[37] The trophozoites enlarge and develop into diploid precysts through a process of cell wall thickening.[37] Sporozoites then develop within the precysts following meiosis and mitosis, a process referred to as asporogony.[67,69] Mature cysts contain eight haploid sporozoites that become trophozoites, following rupture of the cyst wall, and recapitulate the life cycle.

The cyst is the largest and most easily recognized developmental stage of *P. jiroveci.* As demonstrated with Gomori's methenamine silver (GMS) stain, the cysts are thick-walled spherules, 5 to 7 μm in diameter, that assume a cup or crescent shape when collapsed. The cyst wall is trilaminar, 70 to 160 nm in thickness, and shows a thick electron-dense outer layer, an electron-lucent middle layer, and a thin inner cell membrane.[37] The cyst wall stains well with methenamine silver stain, cresyl echt
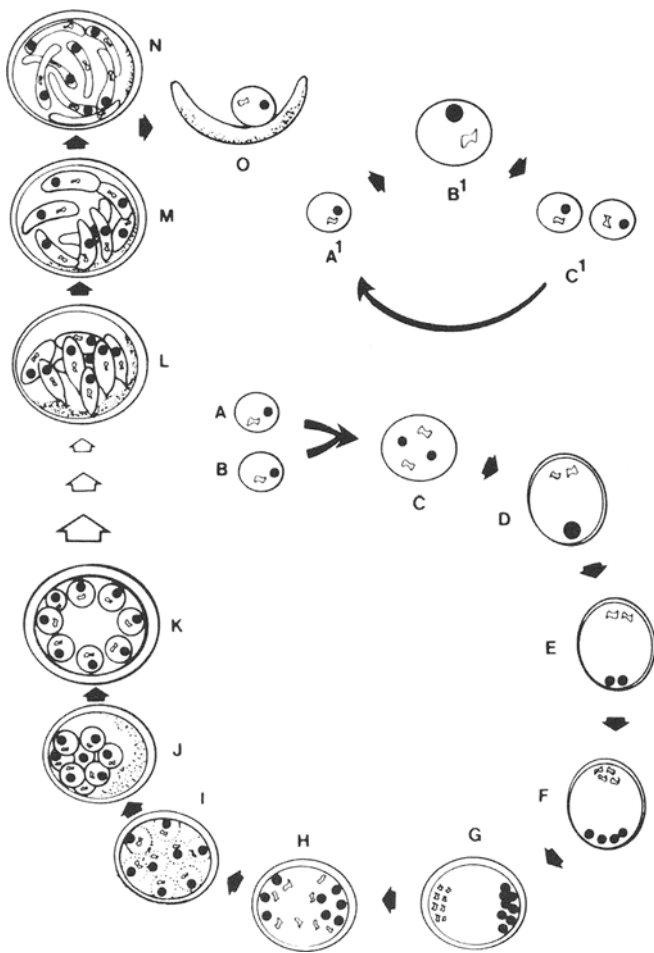
FIGURE 13.1. Diagrammatic representation of the developmental cycles of *P. carinii*, in vitro. The asexual cycle: A1, trophic form; B1, mitotic replication of trophic form; C1, trophic forms, products of binary fission. The sexual cycle: A,B, isogametic forms; C, karyogamy; D, early precyst, diploid zygote; E, intermediate precyst, beginning of mitotic replication of nuclei; F, intermediate precyst, four nuclei; G, intermediate precyst, completion of nuclear replication, eight nuclei; H, late precyst, migration of nuclei to periphery; I, late precyst, initiation of compartmentalization of daughter forms; J, early cyst, completed separation of daughter forms; K, mature cyst, eight rounded daughter forms within a thick wall; L, cyst containing ellipsoidal daughter forms; M, cyst containing elongated daughter forms; N, cyst containing thin, very elongated daughter forms; O, collapsed, excysted cyst with trophic form. The progressive elongation of the daughter forms within the cyst stages L to N may represent the process required for excystation. These forms have been seen repeatedly in culture and in infected rat lung homogenates, although the actual process of excystment was not observed. (From Cushion et al.[68] Copyright 1988, with permission from Macmillan Publishers Ltd)

violet, and toluidine blue. It has been shown that the silver particles are deposited only on the electron-lucent middle layer of the cell wall; this explains the lack of staining of the trophozoites, which lack this layer.[71] Many

studies have examined the composition of the cell wall. The cyst wall is rich in carbohydrates, lectins, β-1,3-glucan, and lipids, including sphingolipid fatty acids and cholesterol.[68,72–74] Treatment of *P. carinii* cysts with zymolyase, a β-1,3-glucanase, disrupts the cyst wall and liberates the surface antigens.[75] Ergosterol has not been detected in many studies, which explains the resistance of *P. jiroveci* to sterol synthesis inhibitors, such as amphotericin B and ketoconazole.[76]

Most cysts contain up to eight spherical daughter forms or intracystic bodies, also known as sporozoites that are 1 to 2 um in diameter and surrounded by a double unit membrane. The sporozoites have a single nucleus, a mitochondrion, abundant endoplasmic reticulum, ribosomes, as well as microtubules and vacuoles. They are reported to be attached to each other and to the cyst wall by a thread or stalk-like structure.[37] Many cysts contain single or paired, comma-shaped structures that are localized areas of thickening of the inner layer of the cyst wall. These distinctive argyrophilic structures help to distinguish the cysts of *P. jiroveci* from nonbudding yeast-like fungi and other pathogens. Although seen best with GMS, the thickened foci can also be stained with toluidine blue O, but not reliably with the Gram-Weigert stain. None of the cyst wall stains demonstrate the sporozoites or trophozoites, although the membranes of the latter may stain faintly in specimens heavily overstained with GMS.

The sporozoites and trophozoites can be visualized with the appropriate special stains in smears and imprints, mainly because of their nuclear staining. The sporozoites are seen as clusters of small nuclei surrounded by an unstained cyst wall, whereas the trophozoites appear as a meshwork of honeycombed spaces, many of which contain a dot-like nucleus.

The trophozoites may be difficult to distinguish from background fibrin or tissue elements. In lung sections they appear ameboid, with broad pseudopodia and polar aggregates of tubular cytomembranous processes, termed "filopodia." The filopodia increase the surface area of the trophozoites and may help in nutrition and transport of enzymes to the environment; they are not organelles of attachment.[37] In unfixed preparations the trophozoites appear as oval bodies resembling a cluster of grapes.[37] The trophozoites have a cell wall with three layers similar to the cyst; however, the middle electron-lucent layer is thin and poorly developed. Each trophozoite contains a single nucleus, cytoplasmic organelles, and glycogen, although not all of these elements are seen in every trophozoite. Electron micrographs of the trophozoites demonstrate the filopodia and the relationship between the filopodia and the pneumocytes (Figs. 13.2 and 13.3). Occasionally, trophozoites assume shapes that suggest reproduction by either binary fission or endogeny. The trophozoites tend to cluster in large aggregates, where

FIGURE 13.2. Electron micrograph of alveolar exudates in AIDS patient with pneumocystis pneumonia. Cyst containing six merozoites is seen between arrows. Next to the cyst is a maturing trophozoite (T) without filopodia. Two collapsed cysts (C) are also present. Note that the upper cyst has discrete areas of capsular thickening corresponding to the darkly staining bodies seen with Gomori's methenamine silver stain. Uranyl acetate, lead citrate. (Courtesy of Dr. Richard Sobonya, University of Arizona.)

they mold against each other in sheet-like pieces of a jigsaw puzzle. Each large cluster of trophozoites contains only a few admixed cysts. It is the clustering of the trophozoites that produces the appearance of alveolar "honeycomb exudate" seen on light microscopy. The

intermediate stage between the trophozoite and the cyst forms the precyst stage, which demonstrates considerable morphologic variability. The precysts are oval in shape and 4 to 6 μm in diameter. The cell wall is up to 100 nm in thickness, with an electron-lucent layer that is thicker than that of trophozoites. The cytoplasm contains one or more nuclei, mitochondria, free ribosomes, microtubules, vacuoles, and glycogen granules. Ultrastructural studies have identified three subtypes of the precyst stage, characterized by progressive thickening of the cell wall and an increasing number of nuclei from one nucleus in the early precyst to four or more in the late precyst.[38,66,77,78]

The developmental forms of P. jiroveci are not seen clearly in routine histologic sections. A variety of special stains may be used to make the diagnosis. These stains are grouped into two categories: those that stain the cyst wall, and those that stain the nuclei of the trophozoites and sporozoites.[79] The latter group, which can be applied only to imprint smears and cytology specimens, includes a variety of Romanowsky stains (Giemsa, Wright, Diff-Quik), polychrome methylene blue, and Gram's stain. The cyst wall stains can be applied to tissue sections as well, and are preferred for routine diagnostic work. These stains include Gomori's (Grocott) methenamine silver (GMS) and its rapid variants, toluidine blue O, and Gram-Weigert methods; GMS is preferred by most pathologists.[80]

Stains for sporozoites/trophozoites have been advocated for diagnosis because of their simplicity and rapidity. However, a well-prepared GMS stain is by far the most reliable and sensitive nonimmunologic stain for the diagnosis of P. jiroveci. Recently described rapid GMS variants have shortened the time required for specimen staining.[80–84] A microwave method, described originally by Brinn,[83] can provide high-contrast slides of optimal
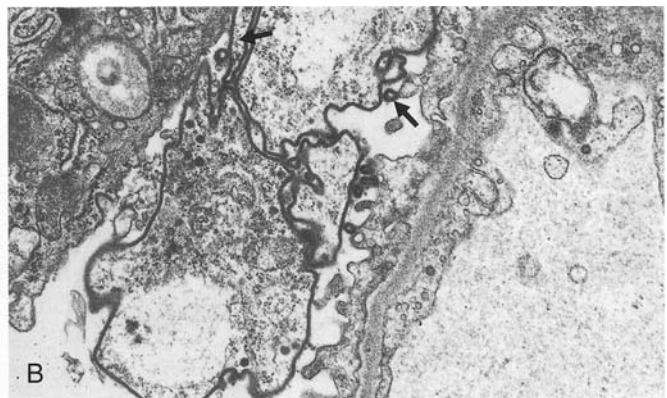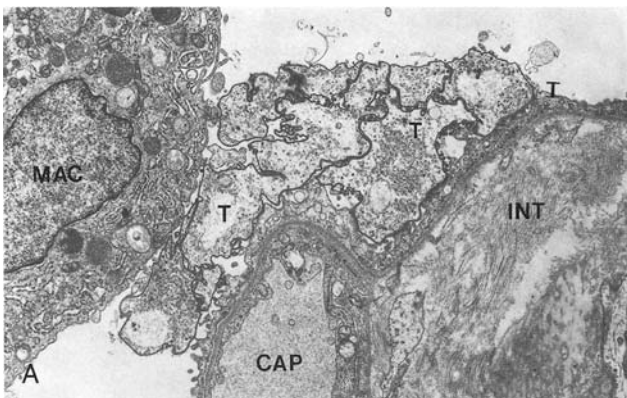

FIGURE 13.3. A. Electron micrograph of several trophozoites (T) attached to type I pneumocyte epithelium of the alveolar wall (I). MAC, macrophage; INT, alveolar interstitium; CAP, alveolar capillary. Uranyl acetate, lead citrate. B. Details of electron micrograph in A, taken from the area to left of CAP. Filopodia (arrows) are apparent especially in conjunction with type I pneumocyte epithelium. Uranyl acetate, lead citrate. (Courtesy of Dr. Richard Sobonya.)

quality in about 20 minutes and can be applied to any standard specimen. Combined methods that demonstrate both cyst walls and the nuclei of sporozoites and trophozoites, although aesthetically pleasing, offer no advantage over a good methenamine-silver stain.

Molecular Biology

Molecular studies of rat-derived *P. carinii* show the genome to be between 7×10^6 and 1×10^7 based on summation of *P. carinii* chromosomes in pulse-field gel electrophoresis.[85,86] This genetic material is arranged as 13 to 19 chromosomes and is between 295 and 710 kilobase (kb) in size. The karyotype of human-derived *P. jiroveci* appears different from that of rat-derived organisms, but has not been well characterized.[86] Three genes have been cloned from rat-derived *P. carinii*, including 18S RNA, dihydrofolate reductase, and thymidylate synthase gene.[87–89] Since then, the *DHPS* gene and the internal transcribed spacer (ITS) region of *P. jiroveci* have been studied extensively in human pneumocystis infections. *DHPS* gene mutations are reported to be associated with sulfa/sulfone resistance in patients who had previously received prophylaxis.[90,91] The prevalence of *DHPS* gene mutations was reported to be markedly increased in the United States since the use of prophylactic sulfa drugs.[92] However, another study from Portugal reports no association between *DHPS* types or therapy and response to anti-*Pneumocystis* therapy.[93] This same study found a subtype of the ITS region of nuclear rRNA, type Ne, to be associated with treatment failure, childhood infection, and early death.

Immunobiology of Pneumocystis

Antigenic studies of pneumocystis using Western blot technique have revealed several major antigens.[75,94–96] The most prominent surface antigen of pneumocystis is seen as a band of 116kDa on polyacrylamide gel electrophoresis.[37] There is cross-reactivity between this antigen from humans and other mammals. Another major class of antigens in rodent-derived *P. carinii* migrates in the 45- to 50-kDa range. The relationship of this antigen to the 116-kDa antigen is not clear. A third antigen that is the most prominent feature of human-derived pneumocystis is a broad intensely staining band between 35 and 45kDa.[75,94–96] This is the most common band found in the lungs and bronchoalveolar fluid of patients with *P. jiroveci* infection, while the 116-kDa band is less prominent in these specimens.[96]

Exposure to pneumocystis organisms evokes an immune response in the host.[97] Serologic studies using indirect *immunofluorescence* (IF) and enzyme-linked

immunoabsorbent assay (ELISA) have demonstrated serum antibodies to *P. jiroveci* in childhood under conditions of natural exposure.[43,98] These antibodies are primarily directed against the 35- to 45-kDa band antigen, although antibodies to other bands were also recognized.[57] This study also found that >90% of AIDS patients developed IgM or IgG antibodies to the 35- to 45-kDa band and other antigens with recurrent episodes of pneumocystis infection. The role of these antibodies is unclear; however, there is some evidence that they may function as opsonins.[99]

The development of PCP is related to impairment of cell-mediated immunity. The most prominent disease in this category is AIDS, although there has been an increase in non-AIDS patients also. In AIDS and HIV infection, there is a decline in the number and function of circulating $CD4^+$ T-helper cells, the increased incidence of PCP being directly proportional to the fall in $CD4^+$ T lymphocytes.[100]

Pneumocystis organisms are host-specific, but the reason for this stringent specificity is not clear.[27] *P. jiroveci*, the only pneumocystis identified in humans, has a unique tropism for the lung, where it exists primarily as an alveolar pathogen. A few cases of invasive infection with dissemination are reported with severe immunosuppression or overwhelming infection of the host.[101] The availability of molecular techniques has identified key molecules in the cell cycle, signal transduction, and metabolic pathways of pneumocystis. The first specific molecule identified was glycoprotein A, a major surface glycoprotein with an integral role in the attachment of pneumocystis to host cells.[45,102–104] This surface glycoprotein is immunogenic and antigenically distinct in each form of pneumocystis infecting various mammalian hosts.[102,104] The major component of the cyst wall is β-1,3-glucan, and the pneumocystis β-1,3-glucan synthetase gene, *GSC1*, which mediates the polymerization of uridine 5'-diphosphoglucose into β-1,3-glucan.[105] Inhibitors of β-1,3-glucan synthetase are effective in clearing the cyst forms of pneumocystis from lungs of infected mammals.[106,107] The cyst wall also contains chitins and other complex polymers, including melanins that provide stability to the cell wall and invoke an inflammatory response in the lungs.[108,109]

Several signal-transduction molecules have been recently identified in pneumocystis. These include cdc2 cyclin-dependent kinase, cdc13 B-type cyclin, cdc25 mitotic phosphatase, and pneumocystis mitogen (PCM)-activated protein kinase.[110–115] Furthermore, the finding of enhanced activity of PCM in the trophozoites as compared with the cyst forms suggests that the trophozoites may use this pathway for transitions in the life cycle.[113]

During infection, the trophozoites adhere tightly to the alveolar epithelium, activating specific signaling pathways in the organism, including the gene encoding PCSTE20

kinase, which signals responses for mating and proliferation in fungal organisms.[114] Other signaling molecules identified in pneumocystis include the pheromone receptors, heterotrimeric G-protein subunits, and transcription factors.[115,116] Currently, some of these specific molecules are under intense research studies as potential drug targets. These include dihydrofolate reductase (target of trimethoprim), thymidylate synthase, inosine monophosphate dehydrogenase (target of mycophenolic acid), S-adenosyl-L-methionine:sterol C-24 methyl transferase (involved in biosynthesis of sterol), and lanosterol 14(α)-demethylase (the target enzyme of azole antifungal drugs).[59,117,118]

Pathogenesis of Infection

Pneumocystis infection is acquired by inhalation. When the organism is deposited in the alveoli, the first critical step in the establishment of infection is attachment to the type I pneumocyte, through interdigitation of their cell membrane to the alveolar cell membrane.[119] This binding is facilitated by the interaction of host proteins, fibronectin and vitronectin, which bind to the surface of the trophozoites and mediate the attachment to integrin receptors of the alveolar cells.[120] The subsequent events depend on the immune status of the host; the organism can remain quiescent for a long period of time, and then proliferate when the host becomes immunocompromised.[119] Other investigators suggest that the organisms remain in the lungs for only a short time. In an immunocompromised host, the organisms start to proliferate, and the alveoli become progressively filled with masses of the pneumocystis organisms. In animal models, P. carinii organisms increase from <10^5 to 10^9 or 10^{10} in 8 to 10 weeks. It is presumed that P. jiroveci replicates in a similar manner in humans. The proliferation of P. jiroveci results in the accumulation of the typical foamy, honeycomb-like alveolar exudates. Although the type I alveolar cells appear vacuolated and eroded in infected tissues, there is no evident disruption of structure or barrier function in lung epithelial cell cultures.[121,122] Ultrastructural studies have shown the alveolar exudate to contain abundant fibronectin, vitronectin, and increased surfactant proteins A and D.[123] Surfactant protein B, in contrast, is reduced during pneumocystis infection.[124] The reasons for the increased surfactant protein A (SP-A) levels are poorly understood; however, both surfactant proteins A and D interact with the surface glycoprotein A component of pneumocystis, and modulate its interaction with macrophages.[125–127] Changes in the type I pneumocytes are followed by type II pneumocyte hyperplasia, suggesting a reparative response. Long-term survival of patients with pneumocystis infection is often associated with alveolar damage and interstitial alveolar fibrosis.[128]

Host Immune Responses

Pneumocystis infection elicits both humoral and cellular immune responses in the host. Epidemiologic studies have shown that antibodies to the organism are acquired in early childhood.[43] There is increasing evidence to support the role of humoral immunity in host defense against pneumocystis. Passive immunization with specific monoclonal antibody is shown to confer partial protection against P. carinii in animal models.[129]

Pneumocystis infection invokes a cellular response with production of inflammatory cytokines and chemokines. These inflammatory responses are required to control the infection, but an exuberant inflammatory response may result in pulmonary injury. Complex interactions between the inflammatory cells and the soluble mediators produced by these cells facilitate the clearing of infection, but may result also in lung injury. The key inflammatory cells include CD4$^+$ T lymphocytes, alveolar macrophages, and neutrophils. The complex interaction among the inflammatory cells, the soluble mediators, and the immune responses is presented in Figure 13.4.

Lymphocytes

The activity of CD4$^+$ T lymphocytes is pivotal in host defense against pneumocystis, in both humans and animals. A reduction in CD4$^+$ T-lymphocyte count to less than 200/mm^3 increases the risk of pneumocystis infection.[101,130] CD4$^+$ T cells proliferate in response to the
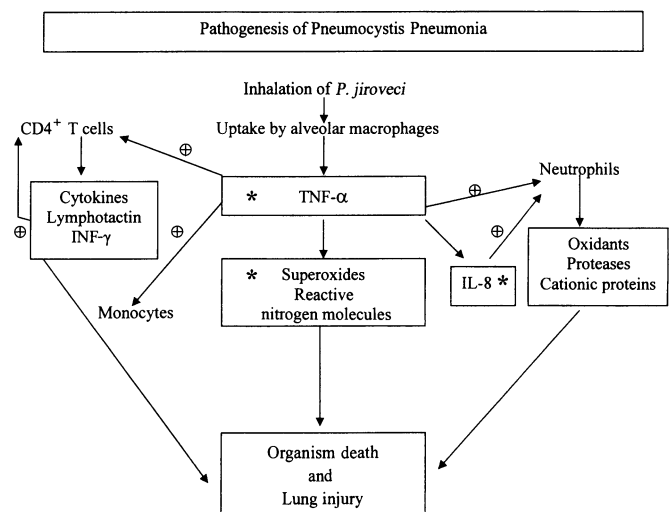

FIGURE 13.4. The host immune response to infection with *Pneumocystis jeroveci* is complex and includes both humoral and cellular reactions. CD4$^+$ lymphocytes and macrophages interact to produce cytokines and chemokines that are protective, but also induce lung injury. IFN-γ, (interferon-γ); IL-8, (interleukin-8); TNF-α, (tumor necrosis factor-α). $\oplus$, cell recruitment and/or activation; *, produced by alveolar macrophages.

pneumocystis antigens and generate cytokine mediators, including lymphotactin and interferon-γ.[131] Lymphotactin is a chemokine that serves as a potent chemoattractant for lymphocyte recruitment.[132] Interferon-γ activates macrophages to produce tumor necrosis factor-α (TNF-α), superoxides, and reactive nitrogen radicals.[133,134] Aerosolized interferon-γ has been shown to reduce pneumocystis infection in rats, regardless of CD4$^+$ cell depletion.[133] Macrophage-derived TNF-α and interleukin-1 (IL-1) are necessary for initiating pulmonary responses that are mediated by CD4$^+$ T cells. An undesirable effect of T-lymphocyte activation, however, is pulmonary injury and functional impairment.[132] Pneumocystis infection also results in a marked accumulation of CD8$^+$ T lymphocytes in the lung.[133]

Macrophages

Alveolar macrophages are the principal cells mediating the uptake and degradation of pneumocystis in the lung, mediated either through the opsonins in the epithelial-lining fluid, or through the macrophage mannose receptors that interact with the surface mannoprotein, glycoprotein A, of the pneumocystis.[135] Once phagocytosed, the organisms are incorporated into phagolysosomes and degraded.[136] In response to the phagocytosed pneumocystis organisms, a variety of inflammatory cytokines, chemokines, and eicosanoid metabolites are produced.[136] These mediators, however, in addition to eradicating pneumocystis, also produce lung injury. The macrophage function is impaired in patients with AIDS and malignancies, resulting in impaired clearance of pneumocystis.[137]

Cytokines and Chemokines

The production of TNF-α by alveolar macrophages is mediated by recognition of the β-glucan component of the pneumocystis cell wall.[138] Macrophages possess several potential receptors for glucans, including CD11b/CD18 integrin (CR3), dectin-1, and toll-like receptor 2.[139] The activation of macrophages is facilitated by host vitronectin and fibronectin that bind glucan components on pneumocystis cell wall.[138] Tumor necrosis factor-α plays a significant role in the clearance of pneumocystis. It promotes the recruitment of neutrophils, lymphocytes, and monocytes, and induces the production of other cytokines and chemokines, including IL-8 and interferon-γ, which stimulate further recruitment and activation of inflammatory cells.[140,141]

Chemokines such as IL-8, macrophage-inflammatory protein-2, and the interferon-inducible protein of 10 kDa are chemoattractants for neutrophils and important inflammatory mediators during pneumocystis infection. Interleukin-8 production is correlated with neutrophil infiltration with release of reactive oxygen radicals, proteases, and cationic proteins, resulting in capillary endothelial and alveolar epithelial cell injury, in turn resulting in impaired gas exchange. Increased levels of IL-8 in BAL fluid are correlated with poor prognosis.[142]

Clinical Features of Pneumocystis Pneumonia

There are two major clinical forms of *P. jiroveci* pneumonia, each with a different epidemiologic pattern: (1) the infantile form, designated plasma cell interstitial pneumonia; and (2) the adult form, or pneumonia in immunocompromised host.

Plasma cell interstitial pneumonia, also called the "epidemic form," historically occurred in institutional settings in underdeveloped countries, mainly affecting premature, malnourished children.[11,143,144] The infection is characterized clinically by progressive respiratory distress in infants, and histologically by a prominent pulmonary interstitial plasma cell infiltrate.[143,144] This disease has practically disappeared from the Western world, but is still found in developing countries. Clinically, the infants gradually develop respiratory difficulty characterized by tachypnea, cyanosis, cough, and progressive respiratory failure. The chest roentgenogram shows diffuse pulmonary infiltrates and focal or diffuse consolidation.

The adult infection, also called the "sporadic form," is one of the leading causes of fatal opportunistic infection in AIDS patients and other immunocompromised hosts. This infection is more frequent in the United States and other developed countries, and indicates an underlying cellular immune deficiency with impairment of CD4$^+$ (T-helper) lymphocyte function.[145] Diseases that predispose to pneumocystis pneumonia include congenital immunodeficiency diseases, immunodeficiency induced by cytotoxic agents and corticosteroids, and other acquired immunodeficiency.[6,146,147] Before the AIDS epidemic, most adult patients with pneumocystis either had acute leukemia or were leukopenic from chemotherapy.[6,147] Now these patients are vastly outnumbered by AIDS-associated pneumocystis. The incidence of PCP has undergone a significant reduction with the institution of chemoprophylaxis.[148] At the beginning of the AIDS epidemic, PCP represented the most common AIDS index diagnosis, and the most life-threatening opportunistic infection, with an incidence of 44% to 74%.[149] Approximately 80% of AIDS patients developed PCP during the course of their disease.[150] The incidence of PCP in HIV-seropositive patients started to decrease in 1987, with only 16% of new index AIDS cases reported in 1993, almost exclusively due to the institution of chemoprophylaxis and antiretroviral therapy.[149,151–157] In those individuals

not receiving chemoprophylaxis, it still represents a serious pulmonary infection.[151] Rarely, PCP may develop in patients without a recognized predisposing illness.[158]

The clinical and radiographic findings in sporadic *P. jiroveci* infection are not specific, and mimic those of other opportunistic infections. In AIDS patients, the onset of symptoms is more insidious. The fall in the circulating CD4^{+} T cell count usually correlates with the development of the pneumonia, with the vast majority of infections occurring when the CD4^{+} lymphocyte count is <200 cells.[100,153] The typical clinical presentation in HIV-seropositive patients is an insidious onset, including fever, dyspnea, tachypnea, and nonproductive cough.[6,7,159] Less frequently, weight loss, chest pain, night sweats, chills, fatigue, and malaise have been reported.[160–165] This is in contrast to the more acute presentation of HIV-seronegative immunocompromised patients.[166] Physical findings are often mild compared to the degree of symptomatic respiratory impairment. There is, however, a subset of HIV-seropositive patients that presents with an acute, fulminant onset, progressing to respiratory failure within 1 to 2 weeks.[160,162,167] The disease in non-AIDS patients may vary from an acute illness with an abrupt onset to a more indolent disease. Symptoms often start after corticosteroids are tapered or discontinued, and are present for a short time, 1 to 2 weeks before a diagnosis of pneumocystis pneumonia is established.

In general, patients with AIDS-associated PCP have significantly more pneumocystis organisms in their lungs, with fewer neutrophils, than those patients without AIDS.[166] The smaller number of inflammatory cells in AIDS-related pneumocystosis correlates with better oxygenation and survival compared with non–AIDS-related infection. The mortality rate in patients with AIDS is 10% to 20% during the initial infection, but the rate increases substantially with the need for mechanical ventilation.[168] Patients with non–AIDS-related pneumocystosis present with an abrupt onset of respiratory insufficiency that may correlate with a tapering or increased dose of immunosuppressive therapy. These patients have more neutrophils and fewer pneumocystis organisms in the lungs than do patients with AIDS-associated pneumocystis pneumonia.[166] The mortality rate in patients with non–AIDS-related pneumocystis pneumonia is 30% to 60%, with a greater risk of death among patients with cancer than among patients with transplants or other diseases such as connective tissue disorders.[4,169]

Extrapulmonary Infection

Extrapulmonary infection is being recognized with increasing frequency, and appears to correlate with the use of inhaled prophylactic pentamidine therapy.[170,171] A review of autopsy cases with AIDS shows an incidence of extrapulmonary pneumocystosis of 2.5% to 2.8%

between 1980 and 1994.[172–175] Concomitant pneumocystis pneumonia was present in 45% of patients. The most frequent spread of infection is to the hilar lymph node, followed by bone marrow, liver, spleen, and adrenal glands, followed by gastrointestinal tract, thyroid, genitourinary tract, heart, pancreas, eyes, and ears.[174] Less frequently, skin, cerebral cortex, pituitary gland, and parathyroid glands are involved.[172,175] Most patients who have disseminated extrapulmonary pneumocystosis have no specific symptoms. Some patients, however, may experience local pain or a mass; rarely, there is clinical and laboratory evidence of organ (e.g., renal, otic, or bone marrow) failure.[172,175] A case of digital vasospasm and necrosis secondary to microemboli containing pneumocystis organisms has been reported.[176] *P. jiroveci* probably spreads from the lungs by hematogenous or lymphatic routes to these sites.

Clinical manifestations of extrapulmonary pneumocystosis are variable, and may range from asymptomatic infection to end-organ failure.[172,174] Computed tomograms may show low-attenuation lesions that progressively calcify in a rim-like or punctuate manner.[177,178] Although chemoprophylaxis is considered a risk factor in extrapulmonary infection, only 50% of the patients had received the therapy in one series.[174] The detection, using PCR amplification, of *P. jiroveci (carinii)* DNA in the blood of patients with PCP provides supporting information in cases with extrapulmonary infection.[179,180] Pathologic findings in the extrapulmonary sites consist of the typical eosinophilic foamy material with a variable inflammatory infiltrate. The organisms can be demonstrated with the usual silver stains or immunoperoxidase stain.[37]

Radiographic Features

Typical radiographic findings in PCP include bilateral diffuse interstitial and alveolar infiltrates, initially predominating in parahilar regions and lower lung fields, which may progress to extensive areas of air-space consolidation involving much of both lungs. The radiographic picture presents a combination of air-space consolidation with variable interstitial infiltrate. Atypical manifestations include unilateral and upper lobe infiltrates, nodular lesions, lobar consolidation, cavitation within a mass, localized nodule or consolidation, pneumatocele, pneumothorax, pneumomediastinum, subsegmental atelectasis, bronchiectasis, hilar and mediastinal lymphadenopathy, and pleural effusion.[181,182] Classically, ground-glass perihilar interstitial infiltrates are seen in the early stages, which progress to involve all lung fields in untreated patients. The infiltrates may develop a coarse pattern with homogeneous consolidation and air bronchograms.[183,184] The lung apices are relatively spared, but the use of prophylactic inhaled pentamidine has resulted in involvement of

the apices, presumably due to the relatively poor distribution of aerosol pentamidine to these regions. The radiographic features of PCP in those with and without HIV infection and with and without chemoprophylaxis are summarized elsewhere.[185,186] As many as 10% to 20% of AIDS patients with PCP present initially with no detectable radiographic abnormalities.[186] In these cases, high-resolution computed tomography may reveal extensive ground-glass attenuation or cystic lesions.

Immune Reconstitution Syndrome

Recently, the phenomenon of immune reconstitution syndrome was described in a small group of patients who developed a pneumonic illness after treatment for pneumocystis pneumonia and subsequent highly active anti-retroviral therapy (HAART) initiation.[187] During the second illness, none of these patients had an identifiable pathogen on bronchoscopy. The mean BAL CD4/CD8 ratio was 0.54, which was much higher than the ratio of 0.07 seen in a cohort of HIV-infected patients undergoing bronchoscopy for a variety of respiratory complaints. The authors suggested that this influx of CD4 cells might represent immune reconstitution. Another similar case was reported in which the BAL and tissue samples taken at the time of recurrent respiratory illness were negative on routine stains, but PCR confirmed the presence of pneumocystis.[188] These reports suggest that subclinical infection with *P. jiroveci* can be present after clinical response to therapy and may trigger an inflammatory response upon reconstitution of the immune system.

Pathology of *Pneumocystis jiroveci* Pneumonia

The pathologic features of PCP have been described in patients with the epidemic or infantile infection, and more recently in the sporadic or adult infection.[189–193]

Epidemic Pneumocystis Pneumonia (Infantile PCP)

Interstitial plasma cell pneumonia, also known as the epidemic or infantile form of non–AIDS-related PCP, is rarely encountered in the United States. It has an intense plasmacytic and lymphocytic interstitial inflammation.[143,144] This morphologically nonspecific interstitial pneumonia resembles pneumonia associated with other opportunistic (especially viral) infections that are also prone to occur in premature, debilitated, or malnourished infants. Often, the correct diagnosis is suggested by the "honeycomb exudate" in alveolar spaces, but definitive

diagnosis requires demonstration of the pathogen with an appropriate special stain or immunohistochemical reagent (see Fig. 7.42 in Chapter 7).[143]

Sporadic Pneumocystis Pneumonia (Adult PCP)

Pathologic features of the sporadic *P. jiroveci* pneumonia, almost exclusively seen in immunocompromised adults, can be categorized in two major histopathologic groups: typical and atypical (Table 13.1).

Typical Pathologic Features

The typical or classical features are seen in the lungs of patients who are not on prophylactic treatment (HAART). Grossly, the lungs are heavy with a pale gray or tan, granular, firm, consolidated cut surface as seen in Figure 13.5. Nodules and cavities are occasionally encountered. Microscopically, a variety of patterns have been described in patients with and without AIDS.

The typical histopathologic pattern in AIDS-associated PCP is characterized by a mild interstitial chronic inflammation and type II pneumocyte hyperplasia, associated with eosinophilic, foamy intraalveolar exudates that expand the alveolar spaces (Fig. 13.6). At higher magnification, the exudate is punctuated with round basophilic dots that correspond to the nuclei of the sporozoites and trophozoites, and are best seen in tissue preserved with a good nuclear fixative, such as B5 solution.[189] Recognition of the basophilic nuclear "dots" is helpful in the differential diagnosis of pneumocystis from alveolar edema and alveolar proteinosis, both with eosinophilic alveolar exudates, without any demonstrable nuclei. The alveolar exudate is composed predominantly of abundant *P. jiroveci* trophozoites admixed with cysts, membranotubular

TABLE 13.1. Histologic patterns of *Pneumocystis* infection

Classical (typical) pattern
Plasma cell interstitial pneumonia (epidemic PCP)
Mild interstitial chronic inflammation, type II pneumocyte hyperplasia (sporadic PCP)
Atypical patterns
Diffuse alveolar damage
Diffuse interstitial fibrosis
Necrotizing PCP
Vasculitis
Calcification
Thin-walled cavities
Granulomatous PCP
Nonnecrotizing, poorly formed
Necrotizing
Fibrocaseous nodules (solitary or multiple)
Miliary granulomas

PCP, *Pneumocystis* pneumonia.

FIGURE 13.5. Gross photograph of lung from a patient who died within 5 days of onset of respiratory failure. The lung weighed 1750 g and showed a tan solid cut surface, consistent with diffuse alveolar damage (DAD).

hyaline membranes, and reactive epithelial cell proliferation may be seen.[189,196] Because *P. jiroveci* cannot be cultivated on cell-free media in the clinical laboratory, the diagnosis of PCP depends on demonstration of the organisms in biopsy or cytology specimens.

Atypical Pathologic Features

Several studies have suggested that the histologic spectrum of pneumocystis pneumonia is much broader than the typical pattern described above. The first study that documented a broad spectrum of patterns was conducted before the onset of the AIDS epidemic; only about one third of the lung biopsy specimens showed the typical pattern of PCP.[197] Notably, the foamy alveolar material was not found in approximately 50% of the specimens. Atypical findings included dense interstitial lymphocytic infiltrates, interstitial fibrosis, epithelioid granulomas, multinucleated giant cells, and focal calcifications (Table 13.1). This spectrum of changes in PCP has been confirmed in subsequent studies.[192,197]

In a study from the National Institutes of Health (NIH), the atypical manifestations reported were interstitial chronic inflammation (57%), fibrosis (50%), numerous alveolar macrophages with desquamative interstitial pneumonia-like pattern (9%), granulomatous inflammation (5%), hyaline membranes (4%), interstitial pneumonitis (3%), cystic or cavitary lesions (2%), microcalcifications (2%), and vascular infiltration and vasculitis (1%). In 19% of the patients, alveolar exudates were absent, and in 2% there was a minimal histologic reaction.[194,195]

extensions, surfactant, and cellular debris enmeshed in fibrin. The alveolar exudate may be focal or diffuse, depending on the severity of infection. In formalin-fixed tissue, the exudate is often separated from the adjacent alveolar septa by an artifactual clear space due to retraction. The histologic appearance is so characteristic that a presumptive diagnosis of pneumocystis pneumonia can be made on hematoxylin and eosin (H&E)-stained slides, in the appropriate clinical setting.[190–193] The eosinophilic alveolar exudate stains with periodic acid-Schiff (PAS) stain.[194] Cysts are round to oval and focally curved disk-shaped or boat-shaped, and stain well with GMS or toluidine blue O (Fig. 13.7).[194,195] Trophozoites are not seen on H&E stain, but easily can be seen in smears with Giemsa, Wright-Giemsa, or Diff-Quick stains (Table 13.2).[196]

In a progressive and chronic infection, interstitial and intraluminal fibrosis may be seen. One study reported interstitial and intraluminal fibrosis in 63% and 36% of lung biopsies, respectively.[194,195] In acute progressive infection with respiratory failure, diffuse alveolar damage,

Interstitial Chronic Inflammation

The presence of interstitial inflammation is the most common finding in PCP. The lymphocytic and plasma cell infiltrates within the alveolar septa are associated with mild expansion of the septa with type II pneumocyte hyperplasia. In one large series of biopsies of HIV-positive patients, an interstitial infiltrate was graded only as mild or moderate in nearly 90% of the patients.[195] Figure 13.8 shows the classical features of interstitial chronic inflammation. If severe, the chronic infiltrate may be misinterpreted as lymphocytic interstitial pneumonia (LIP) or nonspecific interstitial pneumonia (NSIP), particularly if the typical foamy exudate is minimal. Intraalveolar cellular infiltrates are not a usual feature of active PCP, and when present, consist of macrophages that may contain intracytoplasmic pneumocystis cysts by silver stains.[189] The presence of numerous intraalveolar macrophages may simulate desquamative interstitial pneumonia (DIP).[197] Cysts have been described in the alveolar pneumocyte cytoplasm in the early phase of PCP.
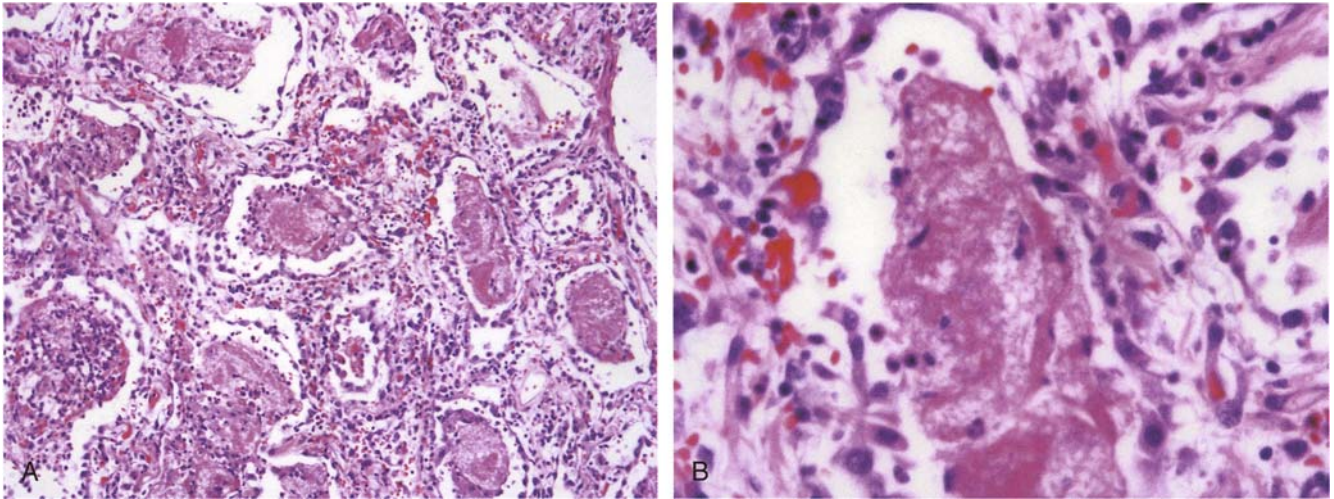
FIGURE 13.6. **A.** Low-magnification photomicrograph shows thickened alveolar septa with mild interstitial inflammation and fibrosis, lined by hyperplastic type II pneumocytes. The alveoli are filled with the typical foamy, eosinophilic exudate of *Pneumocystis*. **B.** Higher magnification shows an alveolus with the foamy/bubbly eosinophilic exudates and a few mononuclear cells. Basophilic dots are visible within the exudate. The alveolar septa have a few inflammatory cells and collagen, and are lined by hyperplastic type II pneumocytes. **C.** Immunostain demonstrates the cyst forms of *Pneumocystis* organisms, surrounded by inflammatory cells. The trophozoites also stain with the immunostain; however, these are not as clearly visualized as the cyst forms.
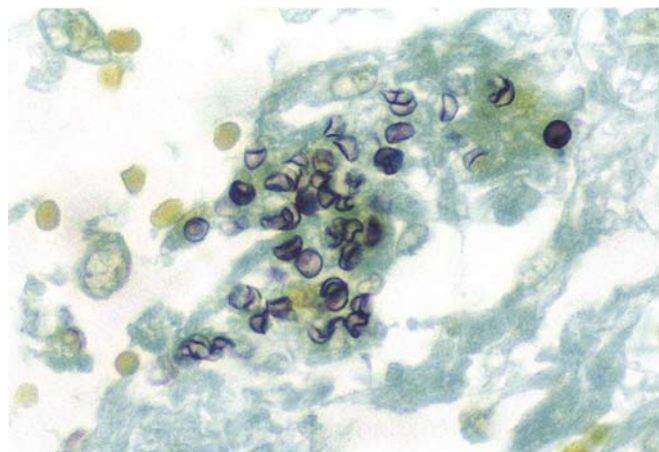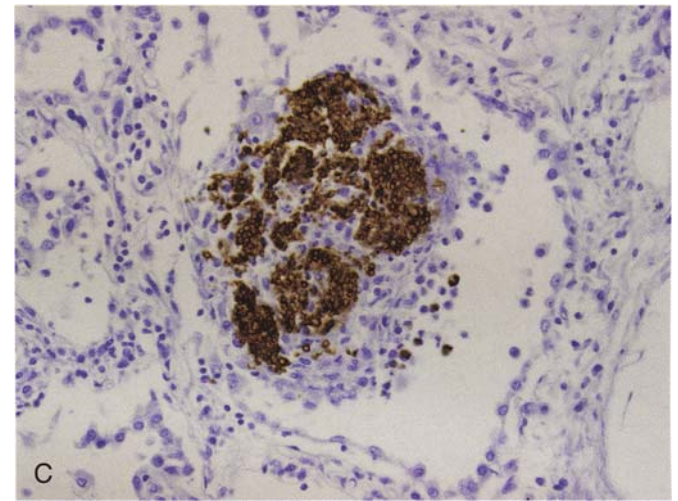

FIGURE 13.7. Gomori's methenamine silver (GMS) stain shows the typical round and collapsed crescent or boat-shaped cyst forms of *Pneumocystis*.

TABLE 13.2. Staining features of *Pneumocystis*

| Stain	Structure identified	Tissue section	Fluid smears/ imprints
Histochemical			
Gomori's methenamine silver (GMS)	Cyst	+	+
Rapid GMS	Cyst	Frozen section	+
Romanowsky stains			
Wright	Trophozoites	–	+
Giemsa	Sporozoites		
Diff-Quik			
Gram			
Methylene blue			
Chemofluorescence			
Papanicolaou stain	Cyst	–	+
Calcofluor white	Cyst	+	+
Immunofluorescence	Cyst	Frozen section	+
	Trophozoites		
	Sporozoites		
Immunohistochemistry	Cyst	+	+
	Trophozoites		
	Sporozoites		
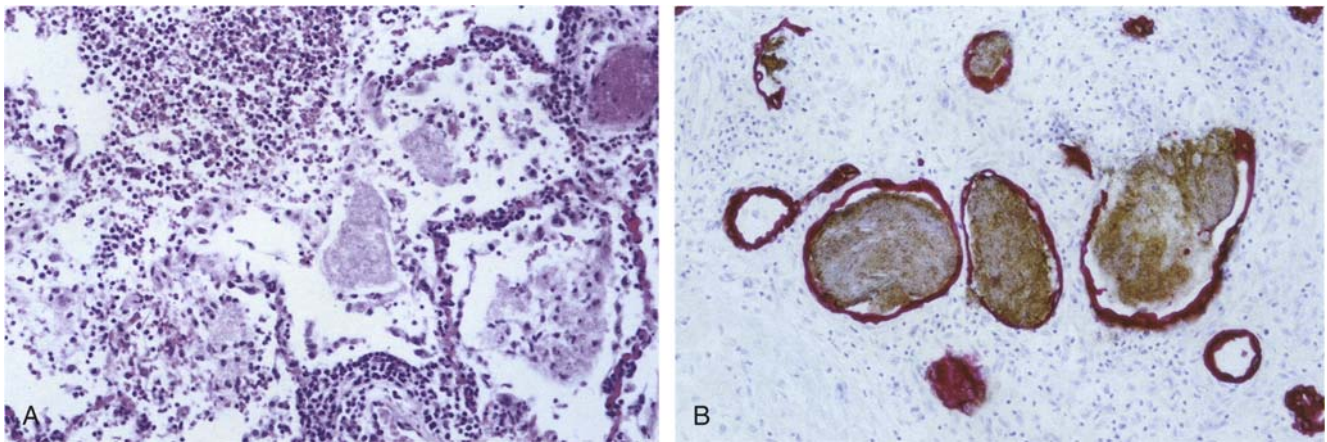
FIGURE 13.8. **A.** The widened alveolar septa and alveolar spaces contain chronic inflammatory cells. The foamy exudates can be seen in the alveolar spaces. **B.** Double immunostain using red counterstain for pneumocytes and brown stain for *Pneumocystis* highlights the chronic interstitial inflammation. One of the alveolar walls is focally devoid of pneumocytes.

Interstitial Fibrosis

This pattern of *P. jiroveci* pneumonia may complicate long-standing, treated, or recurrent PCP.[190,192] Interstitial fibrosis may be seen in >50% of patients with chronic PCP.[195] This usually presents as a mild thickening and fibrosis of the alveolar septa, with deposition of loose, edematous fibrous tissue, and without architectural distortion of the lung parenchyma (Fig. 13.9A). In some cases, alveolar intraluminal fibrosis consistent with organizing pneumonia may be seen (Fig. 13.10). Many, if not most, of these cases are consistent with the organizing or proliferative phase of diffuse alveolar damage (DAD), which may also be the result of oxygen toxicity, concurrent viral infection, shock, or other recognized causes of DAD in HIV-positive patients, rather than injury by *P. jiroveci* (Fig. 13.9).

Diffuse Alveolar Damage

Diffuse alveolar damage may be seen both in AIDS patients and in other immunodeficient patients.[198–200] Respiratory failure as a terminal event is one of the leading causes of death in AIDS patients. In a study of 196 autopsied AIDS patients, 34% died of acute respiratory distress syndrome (ARDS), expressed histologically as DAD. *P. jiroveci* was the most common organism, identified in 55% of these patients.[201] Often, there is very little foamy material in the alveolar spaces, and the changes, therefore, may be misinterpreted as due to viral infection, oxygen toxicity, or a drug reaction unless an appropriate stain is used to demonstrate the cysts. The cysts may be quite sparse, and are usually found within the hyaline membranes lining the walls of alveolar ducts and alveoli (Fig. 13.11A–C). Figure 13.5 shows gross findings in the lung from a patient with DAD. Figure 13.11 demonstrates the features of DAD in the lung shown in Figure 13.5. The pneumocystis organisms are plastered along the alveolar walls within the hyaline membranes, and also present in the alveolar exudate (Fig. 13.11B). Dual immunostain for pneumocystis and alveolar pneumocytes (cytokeratin AE1/3) shows invasion of the alveolar interstitium and destruction of type II pneumocytes by the pneumocystis organisms (Fig. 13.8B). This finding may not be appreciated by the routine H&E or the usual single immunostain for pneumocystis.

Necrotizing PCP

Necrotizing PCP, seen mainly in patients with AIDS, is characterized by confluent parenchymal necrosis with lysis of alveolar septa. Grossly, parenchymal nodules and cavities ranging from 1 to 6 cm in diameter may be seen (Fig. 13.12).[189,198] Histologically parenchyma is replaced by confluent eosinophilic foamy material in which there are abundant cysts of *P. jiroveci* (Fig. 13.13). Pneumocystis exudate infiltrates alveolar septa, a pattern referred to as "septal lysis," resulting in lung necrosis and cavity formation (Fig. 13.13).[189] Rarely, parenchymal necrosis is associated with pneumocystis vasculitis, characterized by massive infiltration of blood vessel walls by characteristic foamy material accompanied by mononuclear inflammatory cells and mural necrosis (Fig. 13.14).[202] The histogenesis of the cavitary lesions is not clear; however, pulmonary infarction due to vascular invasion by pneumocystis trophozoites is considered a possible mechanism.[202] Interestingly, about half of the patients with necrotizing pneumocystis pneumonia also have extrapulmonary pneumocystosis; vascular invasion is a possible mechanism of spread of infection.
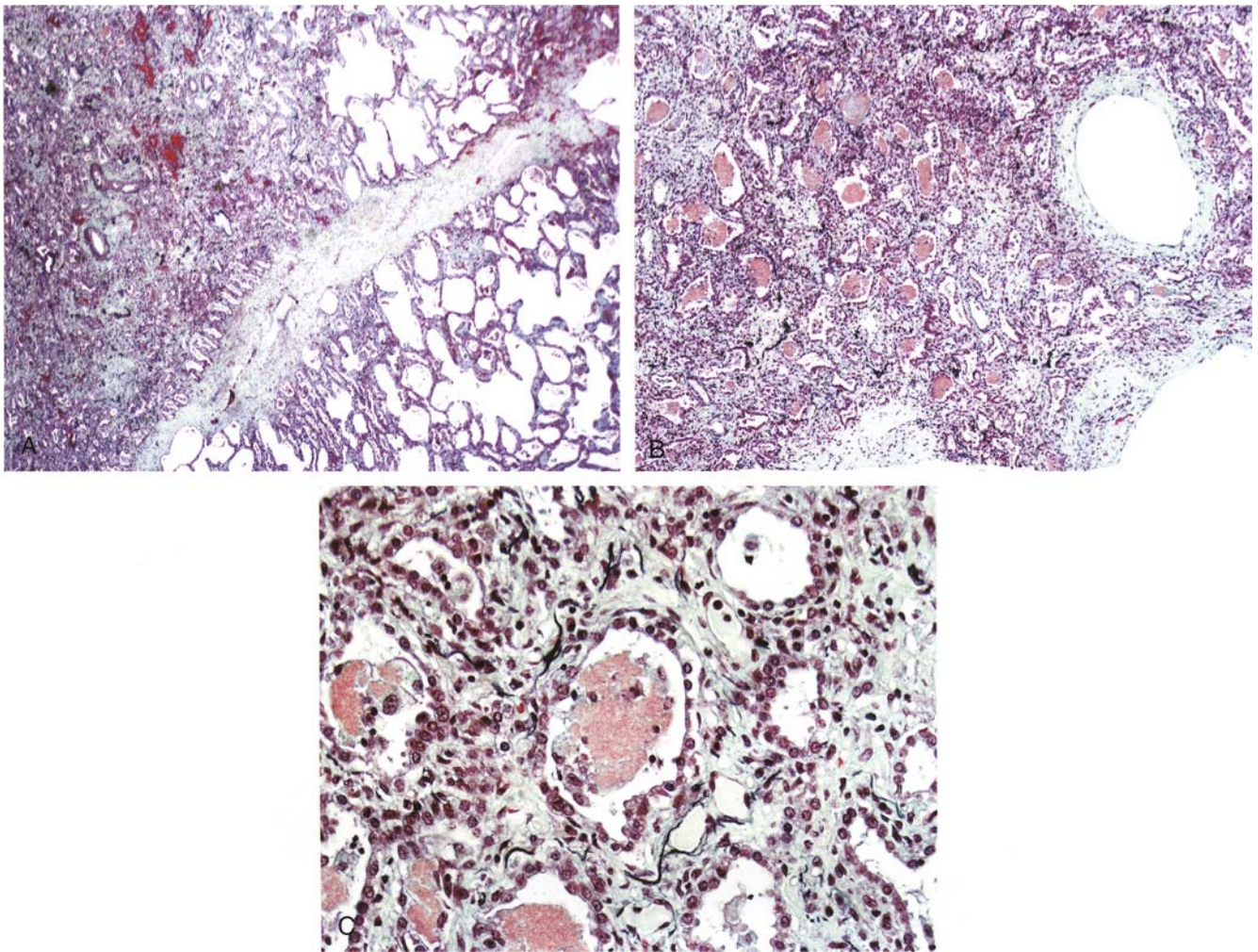
FIGURE 13.9. **A.** Lung section from a patient with pulmonary fibrosis shows mildly fibrotic alveolar septa to the right of the thickened interlobular septum. There is diffuse fibrosis to the left of the septum. (Movat.) **B.** Another area of lung from the same patient shows alveoli filled with *Pneumocystis* exudate surrounded by fibrosis. Note dilated alveolar ducts with peripheral, ring-like fibrosis, consistent with fibrotic phase of DAD. (Movat.) **C.** Higher magnification of **B** shows the *Pneumocystis* exudate surrounded by hyperplastic type II pneumocytes. (Movat.)
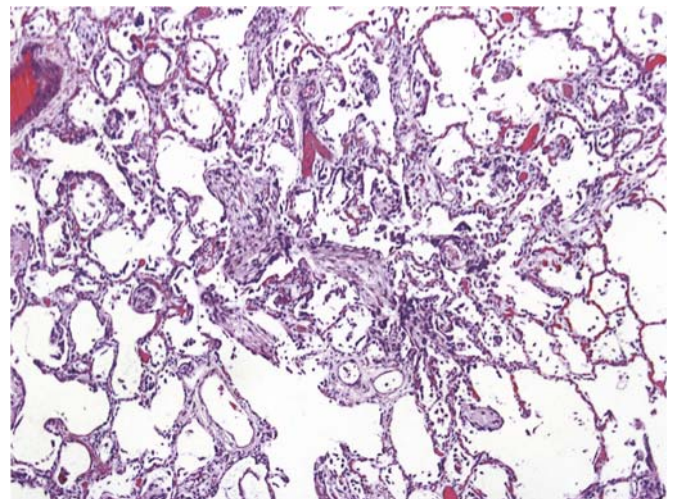

FIGURE 13.10. Organizing pneumonia pattern. Lung section taken from a patient with an early stage of *Pneumocystis* infection shows mildly fibrotic alveolar septa. Two small alveolar ducts in the center of the field contain fibrous plugs.
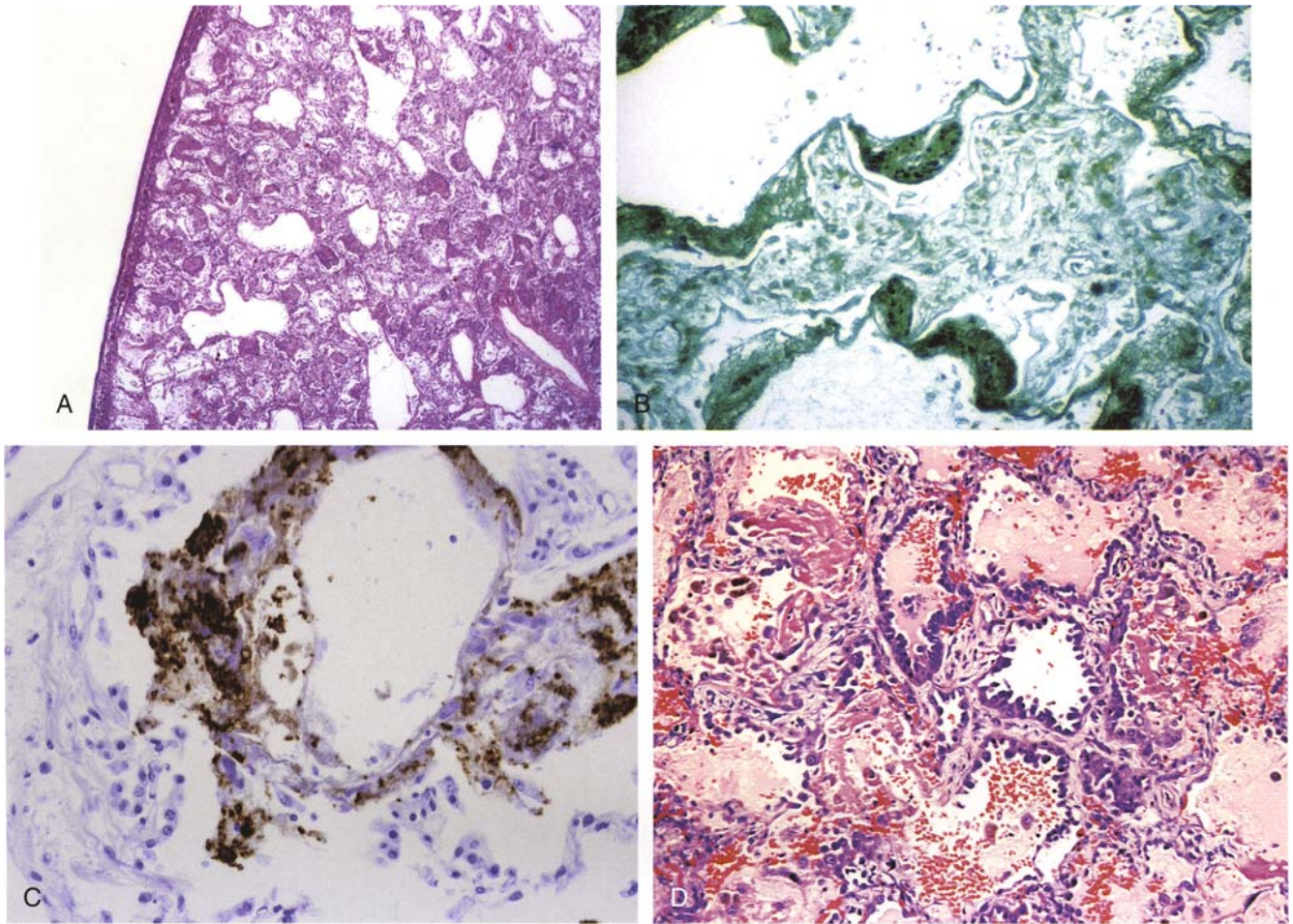
FIGURE 13.11. **A.** Photomicrograph of lung seen in Figure 13.5 shows the typical histologic pattern of exudative stage of DAD. Alveolar ducts are dilated and lined by hyaline membranes, and surrounded by collapsed alveoli. Intraalveolar foamy exudates of pneumocystis are also visible. **B.** Higher magnification of the hyaline membranes show embedded pneumocystis cysts. (GMS.) **C.** Immunostain for pneumocystis may also be used to demonstrate the cysts and trophozoites in the hyaline membranes. **D.** Photomicrograph of lung from a patient with long-standing pneumocystis infection shows enlarged and atypical type II pneumocytes with high nuclear cytoplasmic ratio, as seen in atypical alveolar hyperplasia.

FIGURE 13.12. Necrotizing *Pneumocystis jiroveci* pneumonia. Apical subpleural cavity due to necrotizing PCP in a patient with AIDS.
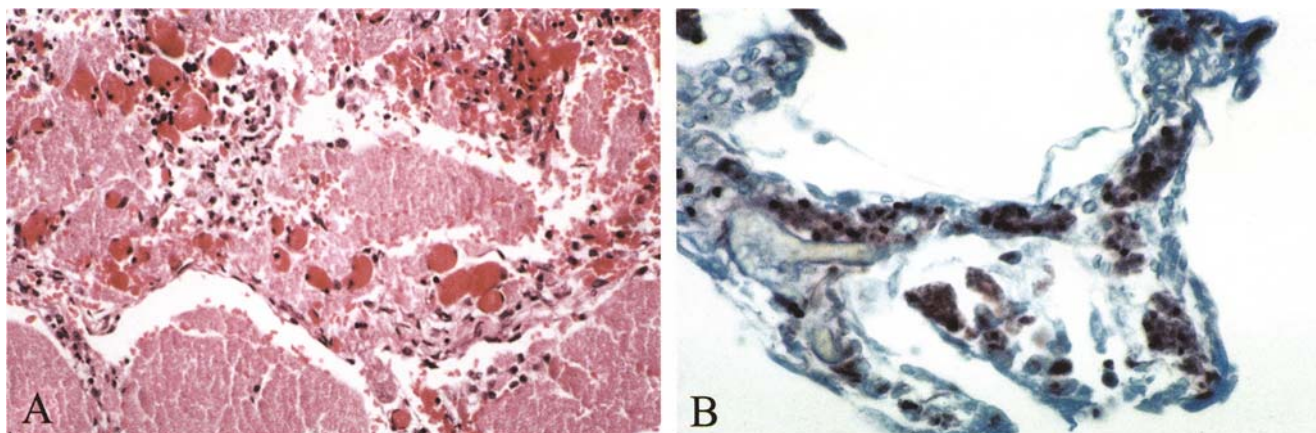
FIGURE 13.13. Necrotizing PCP. **A.** Dense infiltration of alveolar septa and alveolar spaces by pneumocystis exudate. There is early septal necrosis and dissolution. **B.** Pneumocystis cysts within a necrotic alveolar wall. (GMS.)

Other lesions associated with necrotizing PCP include poorly formed granulomas (Fig. 13.15) and microcalcifications (Fig. 13.16), described in more detail below.

Cysts

Thin-walled, pneumatocele-like cysts may be seen in PCP, either in the parenchyma or in the subpleural areas (Fig. 13.17). Initially the walls of the cysts may be formed by alveoli filled with pneumocystis exudates in necrotizing PCP. Chronic or healed cavities have a thin fibrous wall with focal chronic inflammation (Fig. 13.17B,C). Focal microcalcifications may be present in the cyst wall. The large subpleural cysts presumably develop from either rupture or confluence of smaller parenchymal cavities.[195] The cysts are usually empty, or contain a few macrophages and lymphocytes. Occasionally cysts may be colonized by organisms other than pneumocystis such as aspergillus or mycobacteria. Rupture of cavities leads to spontaneous pneumothorax.[203]

Microcalcifications

Microcalcifications are an important atypical feature of PCP, and may be seen with or without the foamy exudates.[204] Several patterns of calcification may be seen, including a "bubbly" pattern with vacuolations, a plate-like pattern, an elongated pattern, and a conchoidal pattern; these patterns were described in a study of 13 patients with microcalcifications.[204] The authors postulate that the initial lesion is manifested by a form of long, thin calcifications that are always associated with active PCP. The "bubbly" plate-like calcifications are usually
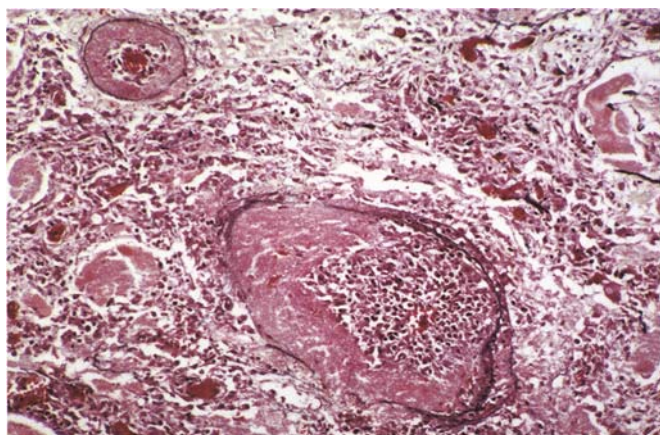

FIGURE 13.14. Vascular infiltration of pneumocystis exudate associated with chronic inflammation in patient with AIDS. (Movat.)
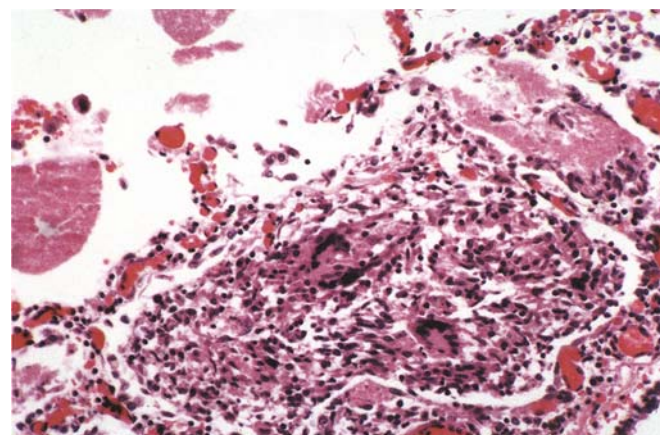

FIGURE 13.15. Granulomatous features. Poorly formed granuloma fills alveolus in patient with AIDS and PCP.
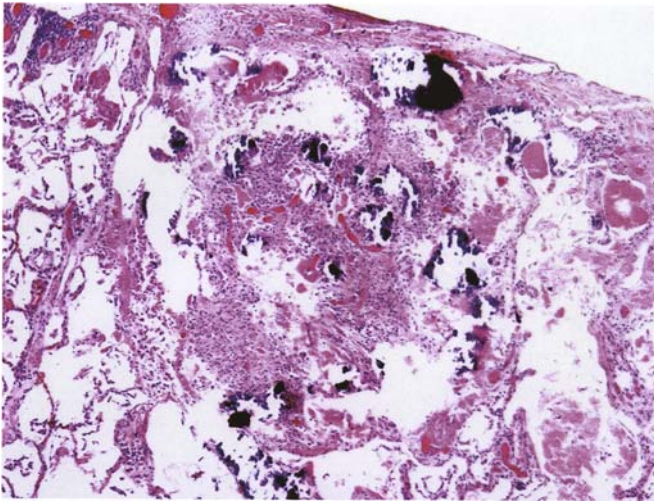
FIGURE 13.16. Necrotizing PCP with dystrophic calcifications.

associated with focal interstitial fibrosis and are a later phase of calcification. All calcifications contained *P. jiroveci* cysts on GMS stain, and thus represent dystrophic calcification of degenerated *P. jiroveci* organisms.[204] The margins of cavitary lesions, as well as subpleural areas, may also develop microcalcifications. Figure 13.16 shows plate-like and conchoidal calcifications in the lung of a patient with AIDS.

Vascular Permeation and Vasculitis

Vascular permeation is not a common feature of PCP; however, when present, it may be a marker of extrapulmonary dissemination of *P. jiroveci*. The vascular wall shows an eccentric expansion of the intima by the characteristic eosinophilic foamy exudate, which may also infiltrate the muscular layer of the vessel wall. Chronic inflammation may be associated with the mural exudate (Fig. 13.14).
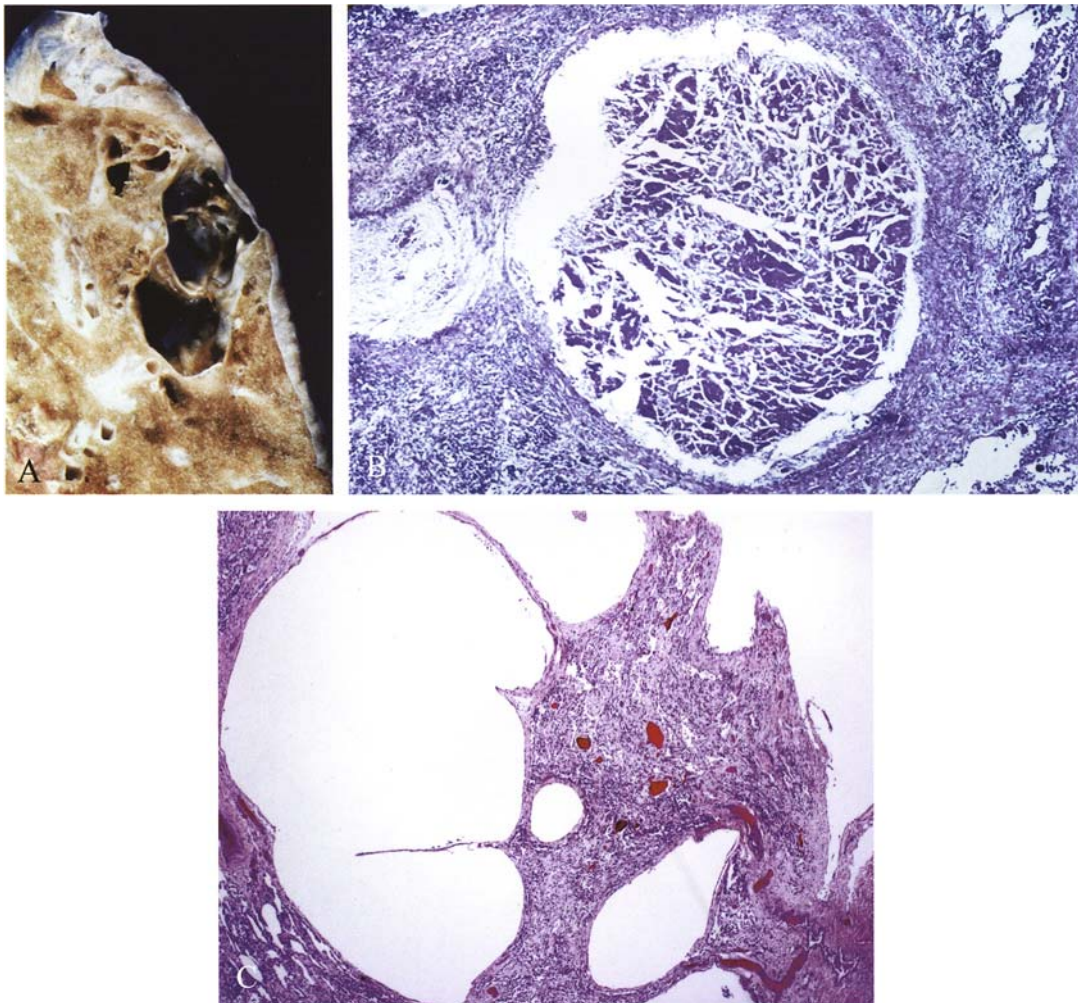

FIGURE 13.17. Cystic lesions. **A.** Subpleural thin-walled cysts, the residual of healed cavitary PCP in a patient with AIDS. **B.** Intermediate stage of cyst development showing fibrous wall and necrotic exudate. **C.** Photomicrograph of cystic lesion shows no discernible lining. The cyst wall is composed of collapsed and fibrotic lung parenchyma.
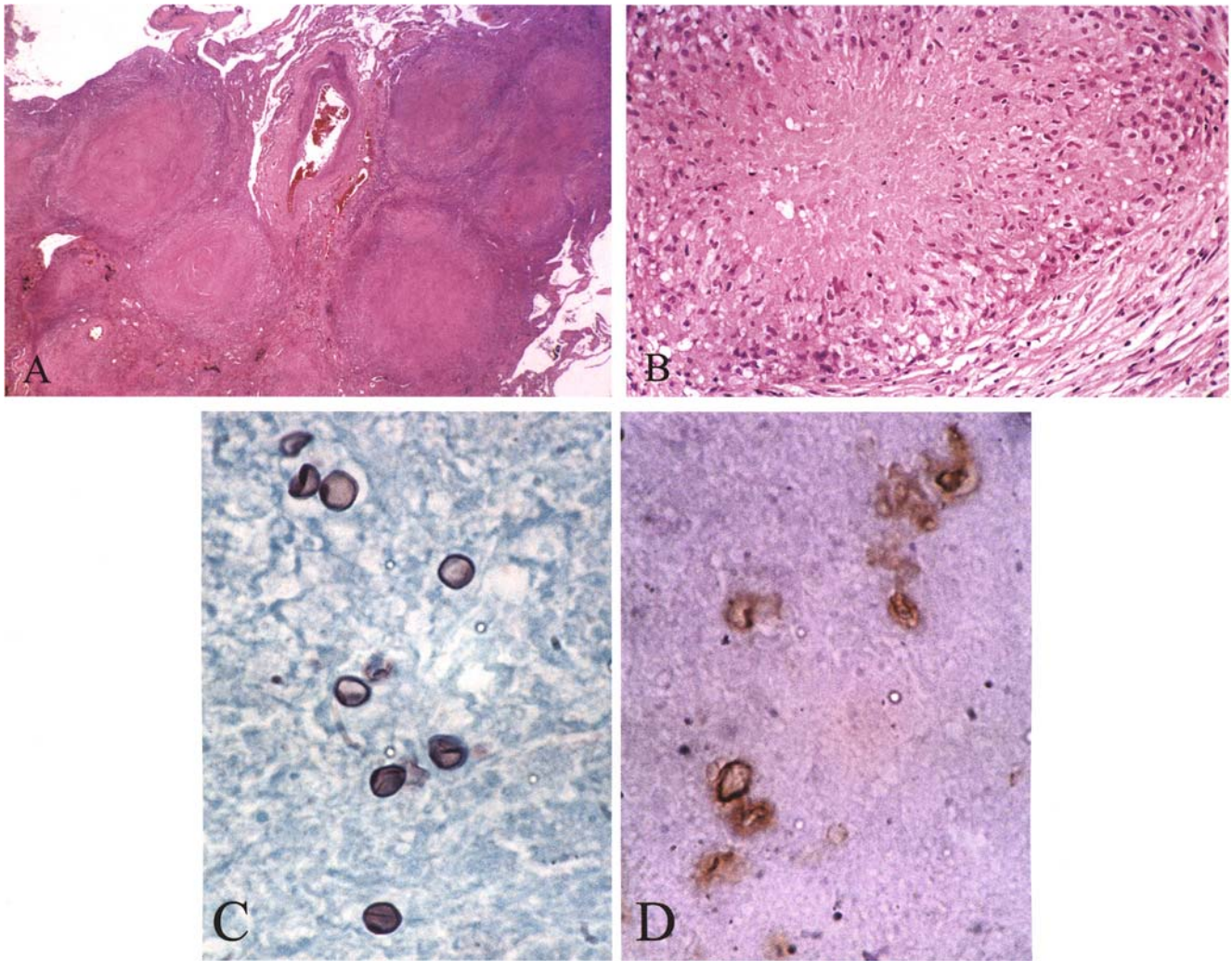
FIGURE 13.18. Necrotizing granulomas. **A.** Confluent necrotizing granulomas, resembling a mycobacterial or fungal infection, surround a pulmonary artery. **B.** Granuloma with central necrosis. **C.** Pneumocystis organisms stained with GMS in the central necrotic area. **D.** Immunostain for pneumocystis distinguishes the lesion from histoplasmosis.

Granulomatous Pattern

Granulomatous inflammation in response to pneumocystis infection has been described both in AIDS patients and in immunodeficient patients without AIDS. In some patients, it has been associated with a nodular or cavitary pattern on chest radiograph.[190,196,205,206] Frequently seen in necrotizing PCP, the granulomas are predominantly in alveolar spaces, and are usually loose and poorly formed, with or without necrosis (Fig. 13.15); see also Fig. 23.26 in Chapter 23).[207–209] The granulomatous reaction surrounds, or is adjacent to, eosinophilic, foamy material composed of trophozoites and cysts of *P. jiroveci*.

Rarely, well-formed granulomas with central necrosis may dominate the histologic picture in a manner reminiscent of miliary mycobacterial or fungal infection

(Fig. 13.18).[205,206,207] An even less frequent pattern of granulomatous PCP is that of a solitary nodule or a few discrete fibrocaseous lesions, similar radiographically and histologically, to tuberculomas or histoplasmomas.[210,211] Cyst forms may be sparse in the center of these well-formed necrotizing granulomas. An immunohistochemical stain for pneumocystis is helpful in locating sparse cyst forms and distinguishing them from histoplasma organisms (Fig. 13.18D).

Granulomatous PCP has been particularly associated with dissemination of pneumocystis to extrathoracic viscera.[210,211] It is speculated that granulomatous PCP occurs in patients who are marginally, rather than severely, immunosuppressed.[192,198] Because granulomatous reactions are infrequent in pneumocystis infection, every effort should be made to exclude other pathogens such as fungi and mycobacteria using special stains.
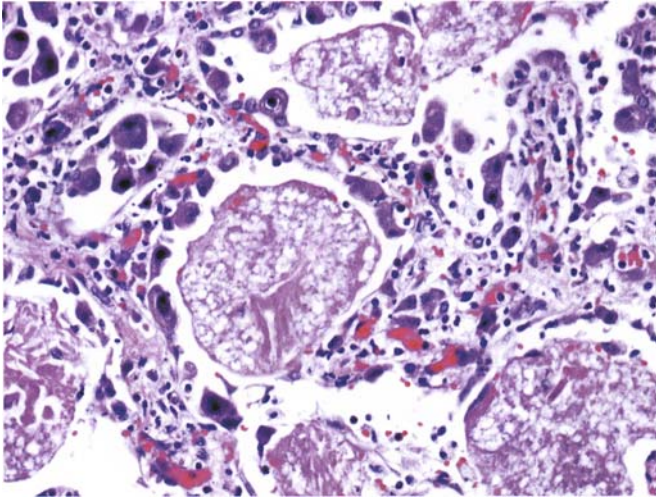
FIGURE 13.19. Photomicrograph shows *Pneumocystis* in the alveolar spaces and cytomegalovirus inclusions in the surrounding type II pneumocytes.

Hilar Lymphadenopathy

This is an infrequent manifestation of PCP. In some patients, lymphadenopathy is caused by underlying disease (e.g., lymphoma or leukemia) or reactive hyperplasia. In one patient, an intravenous drug abuser, enlarged lymph nodes contained typical foamy masses of *P. jiroveci* similar to those found in the alveolar spaces.[212] This patient also had disseminated pneumocystosis.

Other Findings

A PCP infection in patients with AIDS is often associated with other infections, cytomegalovirus infection being one of the commonest. Figure 13.19 shows both infections in the same alveolus in a patient who died with AIDS.

Pathology of Treated Pneumocystosis

The effects of therapy on pathologic features of pneumocystis pneumonia were studied in patients who died of fulminant infection following therapy.[213] Patients who died within the first week of therapy showed compact alveolar exudates that lacked the foamy appearance of typical infection, with fewer stainable organisms as compared to pretreatment biopsy. A second group of patients who died from 8 days to 8 weeks following therapy, had features of organizing DAD; however, all patients were treated with high concentration of oxygen. A third group of patients who responded to therapy and were considered to be cured of infection showed no residual pneumocystis organisms when autopsied 6 months to 2 years later. This study suggests that chronic interstitial lung disease does not develop as a result of pneumocystis infection in long-term survivors.

P. jiroveci can persist in the lungs of AIDS patients following therapy, even when there is a good clinical response. Pneumocystis cysts can persist in the lungs for up to 5 weeks or more following treatment with sulfa drugs. The viability of the trophozoites is not known, but if viable they may cause recurrent infection. Since cyst forms of *P. jiroveci* are known to persist for several weeks, the interpretation of repeat biopsy or cytology during this period should be cautious. There is also a case report that indicates preferential elimination of the cysts and persistence of trophozoites on treatment. In such cases, silver stain can be falsely negative, and immunohistochemical stains or electron microscopy should be used for diagnosis, if clinical suspicion of recurrence is high.[214]

Pathology of Extrapulmonary Pneumocystosis

Extrapulmonary disease is seen primarily in patients with overwhelming pulmonary infection, those with severe underlying immunodeficiency, and those (particularly HIV-infected patients) who received aerosolized pentamidine for prophylaxis against PCP.[189,215] Since 1982 there have been increasing reports of extrapulmonary dissemination of pneumocystis in AIDS patients receiving aerosol pentamidine.[176,215] Sites of extrapulmonary dissemination include lymph nodes, skin, ears, eyes, bone marrow, spleen, liver, kidneys, thyroid gland, gastrointestinal tract, and a variety of other organs and tissues.[189] The lesions of extrapulmonary pneumocystosis are gray-white or yellow nodules 0.5 to 4.9 mm or larger, and they resemble microabscesses. Histologically, the lesions are often angiocentric with eosinophilic foamy exudates similar to the ones seen in the lungs, and show abundant cyst forms on GMS stain. An inflammatory response is minimal or none, and when present it consists of histiocytes, lymphocytes, plasma cells, neutrophils, giant cells, and fibroblasts. In most cases the lesions gradually enlarge and contain all the developmental forms of pneumocystis, suggesting replication in anaerobic extrapulmonary sites. The characteristic morphology of the cysts on GMS stain and immunohistochemical stain is sufficient for making a diagnosis. Microscopically, the lesions consist of sheets of eosinophilic, foamy material identical to that described previously, composed of cysts and trophozoites, with little or no inflammatory response.[196] Smaller lesions and satellite nodules may have an angiocentric distribution.

The ultrastructural pathology of PCP is mainly of pathogenetic interest.[65,189] Ultrastructural studies have shown that the single or paired argyrophilic "intracystic bodies" are plaquelike thickenings of the cyst wall rather than sporozoites or other organelles.[189] Such studies also have confirmed that *P. jiroveci* is strictly an extracellular pathogen. Trophozoites attach to the surface of type 1 pneumocytes by specialized interdigitations, without membrane fusion or communication between

cytoplasmic compartments.[216,217] Trophozoite attachment may eventually cause epithelial and endothelial damage, resulting in acute diffuse alveolar damage.[218]

Cytopathology of Pneumocystis Infection

The cytologic features of PCP are well described and illustrated in the literature.[219,220] Cytologic examination of induced sputum and BAL specimens has become the primary diagnostic modality for PCP, particularly in an HIV-infected patient population in whom the sensitivity of cytodiagnosis has been shown to be reasonably high.[220] In Papanicolaou-stained smears, *P. jiroveci* is seen as aggregates of cysts and trophozoites with a granular, foamy, or honeycomb appearance (Fig. 13.20). These

aggregates are usually spherical, with considerable depth of focus, and approximately the size of a normal alveolar sac.[219] Sometimes referred to as "alveolar casts," these aggregates can be recognized at screening magnification if present in sufficient quantity. However, the diagnosis should be confirmed with GMS stain. The honeycomb aggregates in Papanicolaou-stained smears are fluorescent when viewed with ultraviolet light and a fluorescence microscope[221]; however, this method is no more sensitive or specific than GMS stain.[221,222] Also, the fluorescent method is not readily available in all laboratories, especially in those in the developing world.

Histologic Diagnosis of *Pneumocystis jiroveci*

Pneumocystis jiroveci can be demonstrated by special histochemical, fluorescent, and immunohistochemical stains. The staining features of *P. jiroveci* are presented in Table 13.2. The easiest and most reliable stain is GMS, which stains the cysts in tissue sections, smears, and cytologic preparations. The cysts are round to oval, 4 to 7 μm in maximum dimension, and often have collapsed crescent and helmet forms, as seen in Figures 13.6 and 13.7. An intracystic density "central dot" or "paired comma structures" due to a plaque-like thickening of the cyst wall is an important diagnostic feature of pneumocystis.[223] The alveolar foamy exudate is composed of trophozoites and sporozoites, which appear as basophilic dots on H&E-stained tissue sections, and can be seen in smears (but not tissue sections) by Romanowsky stains (Giemsa, Wright, Diff-Quick), and Gram and methylene blue stains.

Chemofluorescent techniques such as Calcofluor white highlight the cysts, but offer no advantage over GMS stain.[221,224] *Pneumocystis jiroveci* pneumonia is not auto-fluorescent, unlike other fungi.[221] Fluorescent antibody techniques, although specific, are time-consuming, require a fluorescent microscope, and do not allow visualization of morphologic details.[225] A variety of monoclonal antibodies are available commercially to detect and confirm the identity of *P. jiroveci* in routinely processed biopsy and cytology specimens.[226] These antibodies, which are specific and sensitive, identify both the cyst and trophozoite forms of *P. jiroveci*. For this reason, immunohistochemistry is considered to be more sensitive than GMS for the detection of *P. jiroveci* in clinical specimens.[227,228] The immunostain is particularly useful for the examination of induced sputum and BAL specimens, which may contain abundant argyrophilic mucus and debris that can obscure the organisms, and for specimens from treated patients.[226,229] An immunohistochemical stain provides a definitive diagnosis, and is more sensitive than GMS, especially in treated and extrapulmonary pneumocystis infection.[230,231]
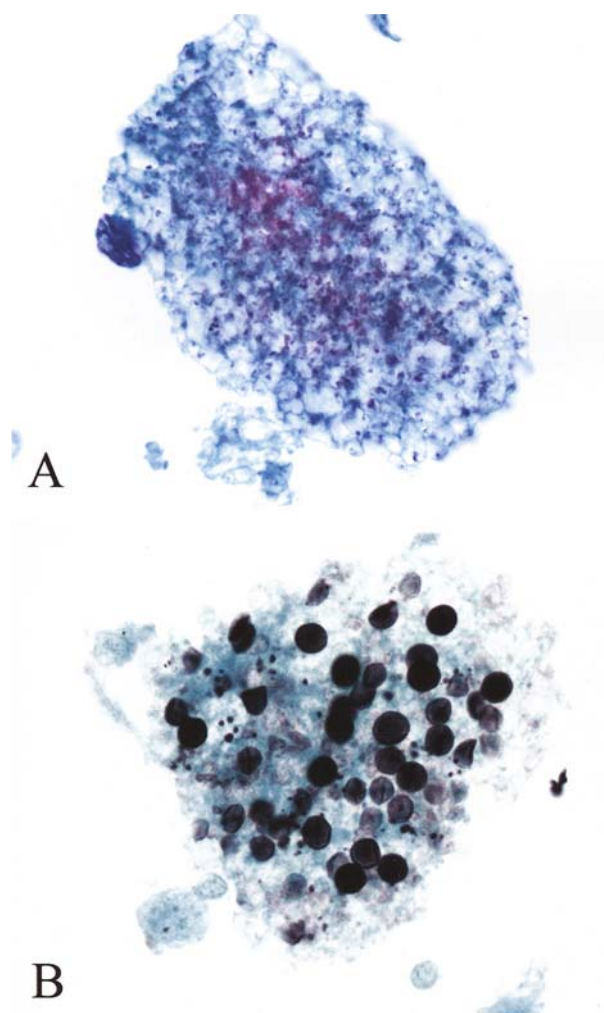

FIGURE 13.20. **A.** Bronchoalveolar lavage cytology specimen. Alveolar "cast" of pneumocystis exudate. Note central dot-like structures. (Papanicolaou stain). **B.** GMS stain showing PCP organisms in cytology smear.

TABLE 13.3. Comparative features of *Pneumocystis* and other fungi

	Pneumocystis	*Histoplasma*	*Cryptococcus*	*Candida glabrata*
Size	4–7 μm cysts	2–4 μm yeast	4–10 μm yeast	2–5 μm yeast
Morphology	Round, crescents, helmets	Ovoid	Pleomorphic with mucoid capsule (occasionally capsule-deficient forms)	Spherical, oval
Budding	–	+, single	+, single	+, single
Pseudohyphae	–	–	– (very rare)	– (+ in other *Candida* species)
H&E	Frothy exudate dot-like trophozoites	– Extracellular organisms + Weakly intracellular organisms	+ Faint, refractile with halo	+, amphophilic
Mucicarmine	–	–	+	–
Fontana-Masson	–	–	+, including capsule deficient forms	–
Gram	– Cyst + Trophozoite	–	–	+, usually (gram positive)

Differential Diagnosis

Although a large number of pathogenic microbes are stained with GMS, it is the yeast-like fungi, particularly *Histoplasma capsulatum*, *Candida glabrata*, and *Cryptococcus neoformans*, that are most difficult to distinguish from the cysts of *P. jiroveci*.[189] The comparative features of these organisms are presented in Table 13.3. The recognition of the single or paired, comma-shaped argyrophilic foci in the walls of *P. jiroveci* cysts is most important, since structures with this configuration are never seen in the walls of yeast. Production of blastoconidia by the process of external budding is a characteristic feature of yeast, but the cysts of *P. jiroveci* do not reproduce by budding. *Cryptococcus neoformans* has a mucopolysaccharide capsule that is mucicarmine positive and Fontana-Masson (melanin) stain positive (even in the capsule deficient organisms), which distinguishes it from cysts of *P. jiroveci*. The yeast forms of *Candida* spp. are usually accompanied by mycelial elements (pseudohyphae and true hyphae), which are not a feature of pneumocystis.

The intracytoplasmic inclusions of cytomegalovirus (CMV) are argyrophilic in GMS-stained sections.[232] However, they can be distinguished easily from pneumocystis cysts, because the viral inclusions are always located inside cells, and they "keep company" with typical Cowdry type A intranuclear inclusions, seen best in replicate sections stained with H&E. The cyst wall of *Toxoplasma gondii*, like that of *P. jiroveci*, is argyrophilic with GMS. The cysts of *T. gondii*, however, are many times larger than those of *P. jiroveci* and are clearly visible in H&E-stained sections, unlike the cysts of *P. jiroveci*. Finally, tissue elements such as leukocytes, erythrocytes, and mucous vacuoles are weakly argyrophilic and may complicate both screening and diagnosis in slides that are overstained with GMS.[189] For this reason, a positive control slide known to contain cyst forms of *P. jiroveci* must be stained in parallel with unknown histologic sections and cytology smears, to gauge the intensity of silver staining.

Molecular Diagnosis of *Pneumocystis jiroveci*

Amplification of *P. jiroveci* DNA by PCR recently has been used for the diagnosis of *P. jiroveci* in induced sputum and BAL specimens, and reported to have greater sensitivity over standard methods (special stains and immunohistochemistry) for the detection of *P. jiroveci*.[233–235] In a study of 29 patients with suspected PCP, PCR of *P. jiroveci* DNA in induced sputum was evaluated, and it was significantly more sensitive than cytology (54.5% positive versus 4.5% positive).[236] A quantitative real-time PCR assay for detection and quantitation of *P. jiroveci* in 53 BAL specimens based on the probe targeting the gene encoding β-tubulin compared to immunofluorescence microscopic examination and Giemsa stain showed that all PCR-negative samples were negative by microscopy. Among the PCR-positive BAL specimens, only 35% were positive by microscopy.[237] Polymerase chain reaction for detection of *P. jiroveci* appears to be a useful and noninvasive method that has high sensitivity and specificity for early diagnosis of PCP.[238]

Confirmation Testing and Diagnosis

Pneumocystis jiroveci can be sustained in cell culture on a limited number of cell lines.[239,240] To date, no artificial media support the growth of *P. jiroveci*. Serologic methods to detect antibodies to *P. jiroveci* are regarded as insensitive, mainly because many patients with PCP are unable to mount a detectable humoral response.[241,242]

Therefore, demonstration of cysts or trophozoites with an appropriate special stain or by immunohistochemistry remains the only dependable way to establish the diagnosis of pneumocystosis.

Treatment

The prevention and treatment of pneumocystosis are rapidly evolving controversial subjects that are currently the focus of considerable investigational interest. Two standard drugs used to treat most patients with PCP are trimethoprim-sulfamethoxazole (TMP-SMX) and pentamidine isothionate.[243] They appear to be equally effective and are associated with approximately the same incidence of adverse reactions, which may affect as many as 60% of patients with AIDS.[243] Early adjunctive therapy with corticosteroids can reduce the morbidity and mortality associated with moderate or severe PCP.[243–246] Patients at high risk for PCP, specifically HIV-infected patients with CD4$^+$ T-lymphocyte counts of less than 200 cells/mm^3, and all AIDS patients who have already had one or more episodes of PCP received prophylaxis with TMP-SMX and aerosolized pentamidine.[247,248] The latter regimen, however, may predispose to extrapulmonary pneumocystosis.[248]

The recent introduction of combination antiretroviral therapy, including HAART, have contributed to the reduced incidence of PCP. An investigational drug, 566CB0, used in mild to moderately severe episodes of PCP in AIDS patients has shown promising results.[249] Other drugs under development include echinocandins and pneumocandins, which inhibit β-glucan synthesis, or sordarins, which inhibit fungal protein synthesis.

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