10

Pneumocystis carinii and Parasitic Infections in the Immunocompromised Host

JAY ALAN FISHMAN

1. Introduction

International travel and shifting patterns of immigration have increased the importance of awareness of the major clinical syndromes associated with infections due to parasites. In the immunocompromised individual, life-threatening infection may emerge decades after a forgotten exposure in an endemic area. Most clinicians have some familiarity with the major clinical syndromes associated with malaria, Chagas' disease, giardiasis, amebiasis, or the helminthic diseases. The spectrum of immune deficits is almost as broad as the tens of thousands of species of parasites to which humans are exposed. However, prior to the recognition of the acquired immunodeficiency syndrome (AIDS), important parasites in the immunocompromised host were largely limited to infections with *Toxoplasma gondii, Pneumocystis carinii, Strongyloides stercoralis,* and occasionally babesiosis or malaria related to transfusions in splenectomized patients. Many new human parasites, both pathogens and nonpathogens of the normal host, must now be added to this list [see Table 1 (Section 1.2)]. The presence, progression, and manifestations of some parasitic diseases are altered by immune compromise. These organisms provide the focus of this discussion.

Successful parasitism is defined by the adaptation of an organism to the host environment. In the absence of an immunologic niche for the organism, the parasite will either fail to establish infection or overwhelm the host. The effects of immune compromise on the manifestations of infections due to parasites are defined by the "natural" mode of evasion/interaction with the host's immune system and by the nature of the immune lesion(s) (see Table 1). While *P. carinii* can no longer be considered a protozoan parasite, the great morbidity due to this organism in patients with AIDS illustrates the effects of immune compromise on the development of disease due to an organism of low native virulence. In the past, sophisticated diagnostic and therapeutic technology has not been available in the areas of greatest prevalence of parasitic disease. However, increasing numbers of patients with immune compromise due to AIDS, cancer chemotherapy, immunosuppressive therapy, and malnutrition, coupled with increased travel and interest in parasitic diseases, have increased the frequency and the recognition of infections due to these organisms.

1.1. Parasite Factors: Development and Distribution

Each year, parasites cause over 2 billion infections worldwide. It is predictable that some of these infections occur in individuals immunocompromised by malnutrition or underlying disease (notably cancer or AIDS) or by immunosuppressive therapy. A history of travel to endemic areas (recent or distant) or of exposures to food, water, animals, blood products, or other vectors of parasitic disease may provide a source of acute or reactivated infection during immune suppression. Some infections are prevalent in subgroups of immunologically normal hosts in developed regions. Homosexual males have an increased incidence of infections with intestinal parasites (including *Trichuris* and pathogenic *Entamoeba histolytica, Giardia, Strongyloides*) and nonpathogenic protozoa. Infection with other pathogens (cytomegalovirus,

JAY ALAN FISHMAN • Infectious Disease Unit, Medical Service and Transplantation Unit, Surgical Service, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114.

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R. H. Rubin et al. (eds.), *Clinical Approach to Infection in the Compromised Host*

Salmonella, Shigella) in this population may contribute to the pathogenesis or severity of concomitant infections. Day care and chronic care centers are common sources for infections with *Giardia,* amebae, and *Cryptosporidium.*

The parasite's life cycle determines the nature and duration of the exposure of the organism to the host's immune system (see Section 2.1) and the clinical manifestations of infection. Because the immune system interferes with the completion of the parasite life cycle, immune suppression has the greatest effect on the growth of organisms that replicate *within* the human host. The protozoa as a group have the capability of completing their life cycle within the human host. Depression of immune barriers may predispose to invasive disease by gastrointestinal parasites, as in amebiasis, or to dissemination of intracellular organisms that overwhelm the reticuloendothelial system in the absence of cytokines (interleuken-2 and interferon-γ), as can be seen in some cases of leishmaniasis in AIDS. Similar predisposition to infection due to latent protozoans accounts for most infections due to *P. carinii* and *T. gondii. Strongyloides stercoralis* is unusual as a nematode because of its ability to complete its life cycle within the human host. Thus, it is predictable that the manifestations of infection due to this organism would be exacerbated by immune suppression as it is not for other worms.

By contrast, the intensity of infection by organisms that require both development *outside* the host and subsequent penetration (often ingestion) into the host is limited by the size of the initial inoculum. The burden of this latter group of organisms cannot increase during the course of disease. As a result, helminths (worms) tend to cause mechanical obstruction due to size and location and are generally limited to the gastrointestinal (GI) tract. Accelerated growth of protozoans derepressed by immune dysfunction produces systemic disease that reflects the initial sites (organs) of infection and the ultimate location of reemergent disease. With other parasites, the pattern of disease (e.g., invasiveness) changes with immune suppression.

1.2. Host–Parasite Interactions and Mechanisms of Immune Evasion

Significant infections due to parasites occur when the balance between host protective mechanisms and parasite growth is disrupted. Specific immune lesions (e.g., hypogammaglobulinemia) may not predispose to parasites normally controlled by other mechanisms (e.g., T lymphocytes). Some infections (e.g., malaria, amebae) are not appreciably exacerbated by immune suppression. A second group causes little or no disease (subclinical or mild, commensal, or latent infection) until activated in the setting of immune compromise. Some of the most "successful" parasites have the ability to avoid detection or killing or both by the immune system. Some of the common mechanisms of immune evasion are listed in Table 1. A number of parasites are resistant to antibody- and complement-mediated lysis (e.g., *Schis-*

TABLE 1. Mechanisms for the Evasion of the Host Immune Response in Parasites

Host response	Mediator	Mechanism	Examples
Nonspecific inflammation	Anti-inflammatory molecules	—	Amebae, *T. taeniaeformis*
Humoral	Complement	Surface resistance	*T. cruzi,* schistosomes, *Leishmania*
Humoral	Antibody	Shedding antigen	*Trichinella,* schistosomes
		Antigenic variation	Trypanosomes, *Giardia*
		Antigenic mimicry	Schistosomes
		Antibody destruction	Filaria, *T. cruzi*
		Host antigen coat	*P. carinii, T. vivax*
		Polyclonal stimulation	Trypanosomes
Cellular	Macrophage	Block fusion/acidification	*T. gondii*
Phagocytosis	—	Escape phagolysosome	*T. cruzi*
		Evade oxidative burst	*T. gondii, L. donovani*
		Alter macrophage function[a] (decreased IL-1, MHC)	*Leishmania, T. brucei*
	Eosinophil	Inhibit attachment	Schistosomes
None	Privileged site	Escape into gut	*Ascarius,* hookworms
		Eye	*Ochocercus*
		Lymphoblast	*Theileria*
		Liver	Malaria
		Muscle	*Sarcocystis*
		Intestinal epithelia	Coccidia

[a](IL-1) Interleukin 1; (MHC) gene products of the major histocompatibility locus.

TABLE 2. Parasitic Infections of Importance in the Immunocompromised Host[a]

Mechanism	Organisms
Neutrophil inflammation	*P. carinii*[b]
Humoral immunity	*G. lamblia, Cryptosporidium*
Cellular immunity	*P. carinii,*[b] *T. gondii, Cryptosporidium, Strongyloides, Leishmania,* Microsporidia, *Isospora belli, G. lamblia, E. histolytica*

[a]This table does not list common parasitic infections that occur in immunocompromised patients from endemic regions or in "at risk" populations but are not increased in severity or frequency.
[b]*Pneumocystis carinii* is included for purposes of this discussion, but is not now considered to be a protozoan parasite.

tosoma, Trypanosoma cruzi).[1–3] Others vary or shed surface antigens to avoid detection. Still others become coated with host proteins to diminish immune detection. Filaria, *Leishmania,* and trypanosomes are capable of inducing defects in cell-mediated immunity.[4] Patients with defects in cell-mediated immunity are particularly susceptible to infections due to *Pneumocystis, T. gondii, Cryptosporidium* species, *Leishmania* species, and *S. stercoralis* (see Table 2). Subgroups of organisms of particular importance to the compromised host are the intracellular parasites (*T. gondii, Leishmania,* and *T. cruzi*) that evade killing by host macrophages. All share the need to evade the humoral (complement and antibody) immune response and intracellular oxidative killing mechanisms prior to establishing intracellular residence.

Perhaps the best example of the interaction of a parasite with the immune system is that of *Leishmania* species. The manifestations of cutaneous leishmaniasis (*L. mexicana* complex, *L. braziliensis* complex, *L. tropica, L. major*) range from localized cutaneous disease to diffuse cutaneous or mucocutaneous ("espundia") involvement. Disseminated disease (visceral leishmaniasis or kala-azar) involving the liver, spleen, bone marrow, and reticuloendothelial system also occurs (*L. donovani, L. chagasi, L. infantum*). In the presence of a normal cell-mediated immune response and in the absence of specific antibody, cutaneous lesions often heal spontaneously. Patients with diffuse cutaneous leishmaniasis generally have high levels of specific antibody without antigen-specific delayed-type hypersensitivity (DTH). Relapsing (recidivans) and mucocutaneous disease occurs in the presence of DTH, but macrophage dysfunction is suggested by the paucity of granulomata in affected tissues. Visceral disease occurs in the absence of cell-mediated immunity and in the presence of specific antibody. Fatal disease has been reported in AIDS patients in the setting of marked T-lymphocyte deficiency.

1.3. Missing Infections in Acquired Immunodeficiency Syndrome

A subgroup of common parasitic infections have not increased substantially in frequency or severity in individuals infected with the human immunodeficiency virus (HIV).[5] These infections include *S. stercoralis,* malaria, *E. histolytica,* and trypanosomiasis. Unrecognized or unreported infections (especially *Strongyloides*) may account for some of the reduction in expected infection frequencies. However, the immune lesion(s) seen in AIDS may not include some relevant immune defenses (e.g., of the intestinal mucosa). These organisms cause significant disease in other immunocompromised hosts. Multiple potential pathogens are often found in diarrheal stools from individuals infected with HIV.[6,7] The organism(s) causing disease (e.g., cytomegalovirus microsporidia, or Cryptosporidia) may be demonstrated microscopically in the small intestine but remain undetected in stool samples. Because of the importance of diarrheal illness in AIDS patients, particularly in Africa (i.e., "slim disease") and in homosexual males, it is important clinically to separate pathogenic organisms from the common commensal organisms, which include *G. lamblia, E. histolytica,* and *Blastocystis hominis,* as well as viral, bacterial, and fungal pathogens.

2. *Pneumocystis carinii*

Despite the description of *Pneumocystis carinii* in 1909 by Chagas and again in 1910 by Carini, *P. carinii* was not recognized as a pathogen of humans until 1942. The first clear association of *P. carinii* with human disease was in 1951, when Vanek and Jirovec[8] found the organism in the lungs of malnourished infants and neonates with an "interstitial plasma cell pneumonitis."[9–16] This unusual disease had been associated with epidemics of pneumonia in malnourished children in the aftermath of each of the major wars.[13,17,18] *Pneumocystis carinii* was first recognized in patients receiving corticosteroids and chemotherapeutic drugs in the 1950s with clusters of cases in clinical oncology centers in the 1970s.[9,10,18–25] The emergence of *P. carinii* as a major pathogen of individuals with AIDS has revolutionized the approach to diagnosis and management of patients with *Pneumocystis* pneumonia.[26–30] Molecular studies of the organisms have also suggested that *Pneumocystis* may be more closely related to the fungi than to the protozoan parasites.[31–34] Such molecular studies, new animal models, and the increasing number of patients with *Pneumocystis* pneumonia in AIDS have added impetus to improvements in the care of patients with this disease.

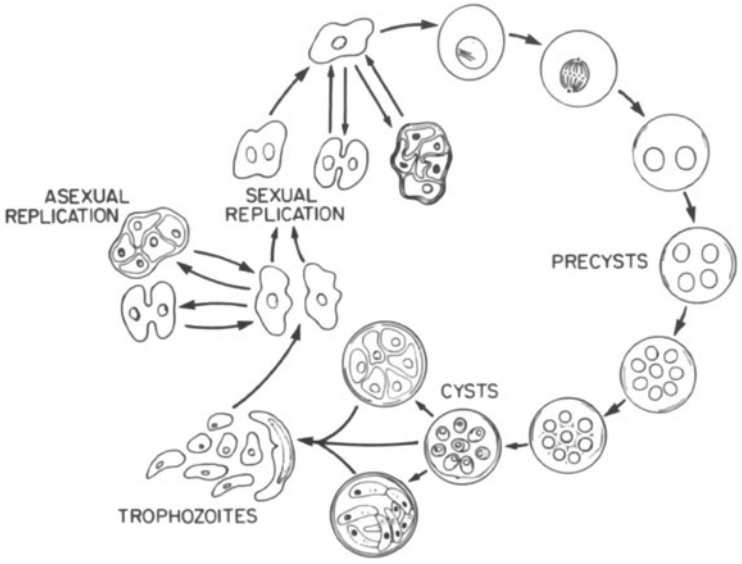

FIGURE 1. Life cycle of *Pneumocystis carinii*. Both sexual and asexual reproduction have been postulated. Glucan synthesis is necessary for cyst wall formation. Only the cyst form stains with methenamine–silver stains.

2.1. The Organism: Taxonomy and Life Cycle

The taxonomic position of *P. carinii* remains uncertain. The organism bears resemblance to both the fungi and the protozoan parasites.[26,31–34] The appearance of the organism *in vivo* is most similar to that of the protozoa, including the thick-walled cyst form with multiple internal sporozoites and the small, thin-walled trophozoites (Fig. 1). Antibiotics used to treat protozoan infections including *T. gondii* and malaria have been successful in the treatment of *Pneumocystis* pneumonia. By contrast, the cyst wall contains β-1,3-glucans and stains with both methenamine–silver and the periodic acid-Schiff (PAS) stains typically used for fungi. Two important enzymes of folate metabolism (dihydrofolate reductase and thymidylate synthase) are encoded on separate genes encoding distinct proteins. This contrasts with one gene encoding a bifunctional protein (both enzymatic activities) in the protozoa.[35,36] The airborne spread of infection supports identification with the dimorphic fungi. Phylogenetic mapping based on ribosomal messenger RNA sequences also places the organism more closely with the yeasts than with the protozoa.[31,34] On the basis of the common derivation of both the fungi and the protozoa from the classic Protista, it may well be that *Pneumocystis* represents a unique phylogenetic niche and will bear relationships with multiple groups of organisms including the fungi, protozoa, algae, and slime molds (*Dictyostelium*). Further genetic data may clarify these questions.

Pneumocystis carinii is an extracellular organism that appears in three forms in the pulmonary alveolus (Fig. 1). Up to 95% of the organisms are trophozoites: motile, pleomorphic, thin-walled (20 nm) nucleated organisms 2–6 μm in diameter with pseudopodia and a dense covering of small filopodia ("tubular expansions") of uncertain functions.[37–39] The trophozoite contains a nucleolus, nuclear pores, primitive mitochondria, endoplasmic reticulum (ER), and ribosomes, but apparently lacks Golgi, flagellae, and cilia. The "cyst" form is a thick-walled (100–200 nm) sphere 4–7 μm in diameter that contains up to eight internal daughter cells called "intracystic bodies" or "sporozoites." The cyst wall has an electron-lucent middle layer that is stained by the methenamine–silver technique and is absent in trophozoites (Fig. 2). Thus, the silver stains commonly used to detect *P. carinii* in tissue samples or in sputum samples will detect only 3–10% of the organism burden. The sporozoites each have a nucleus, mitochondrion, and large numbers of ribosomes and ER. Intermediate forms between the trophozoite and the cysts have been termed "precysts." These have an oval shape, intermediate cell wall thickness, loss of tubular expansions, and occasionally a nuclear "synaptonemal complex" consistent with meiotic division. It is postulated that the eight daughter nuclei are the product of two meiotic divisions and one mitosis (see Fig. 1). While some reports exist of intracytoplasmic location for *P. carinii,* this observation has not been common. In general, the organisms are embedded in a layer of alveolar material along the epithelial surface. Some organisms are seen surrounded by cytoplasmic protrusions from type I epithelial cells and within vesicles of alveolar macrophages. The trophozoites are often closely adherent to the epithelial surface with interdigitation of the cell membranes.[38,40] In areas of epithelial cell loss, organisms adhere to the basement membrane. The mechanisms of cell injury and the nature of the interaction between the lung cells and *P.*

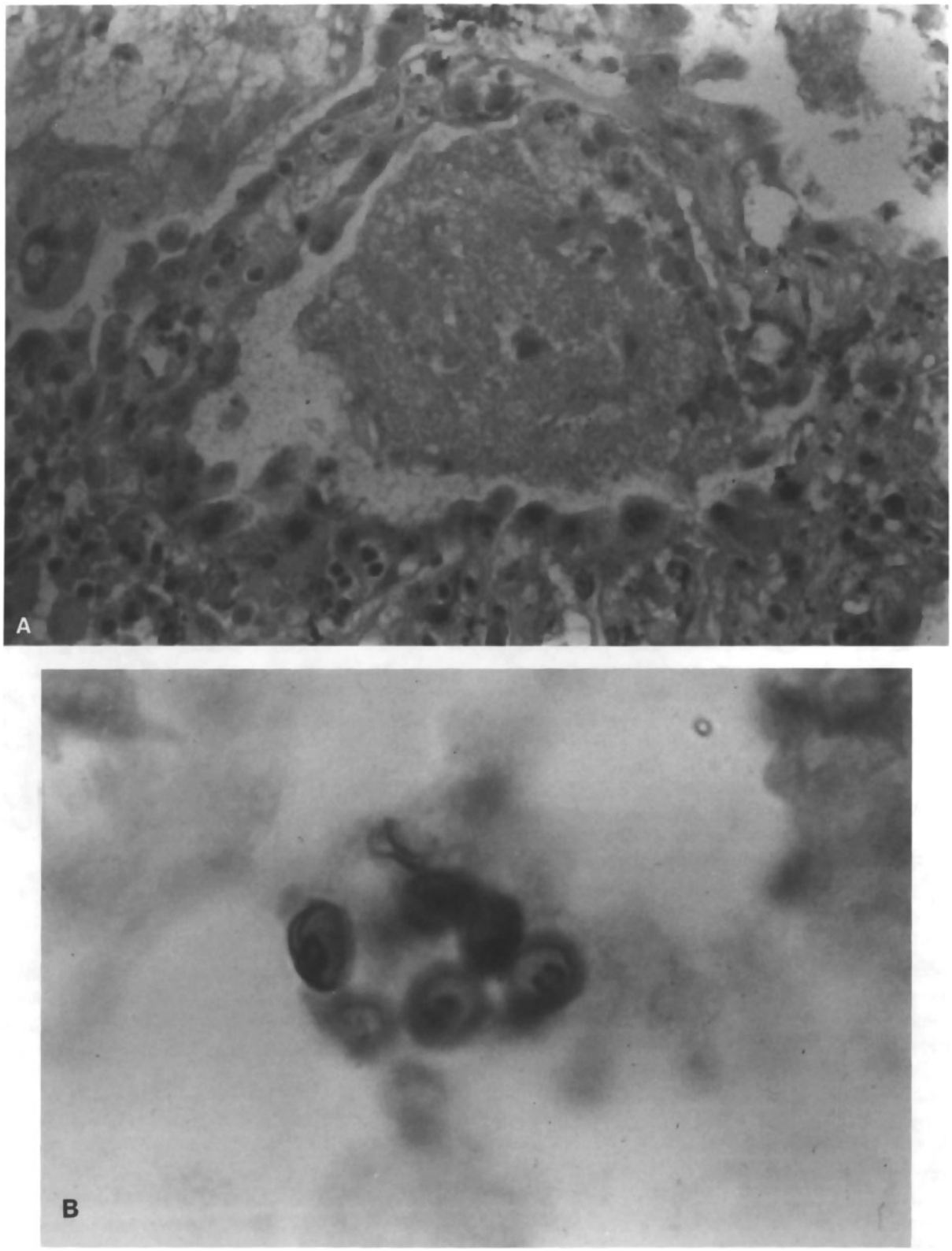

FIGURE 2. (A) Hematoxylin–eosin stain of *Pneumocystis*-infected lung demonstrating pathognomonic intra-alveolar "frothy" material, interstitial widening, and minimal inflammatory cell infiltration. The biopsy was obtained from a patient with AIDS. (B) Induced sputum examination reveals multiple cyst forms stained with a rapid methenamine–silver stain. Immunofluorescent staining is preferred for rapidity and because both cyst and trophozoite forms of *P. carinii* are detected.

carinii are unknown. Productive culture *in vitro* has not been achieved without cell contact.

The absence of a continuous *in vitro* cultivation system for *P. carinii* has made studies of the life cycle difficult.[38,41,42] Observation of the organism in tissue sections or with a feeder layer of mammalian tissue culture cells *in vitro* suggest the scheme diagrammed in Fig. 1. Both sexual and asexual replication have been postulated. Trophozoites mature into the aforementioned early cyst forms ("precysts") with up to eight visible nuclei and a thick outer cell wall. It is the cell wall maturation step that appears to be blocked by inhibitors of glucan synthesis. This group of glucan synthase inhibitors (echinocandins and others) blocks the production of cysts *in vivo* and inhibits increases in organism burden during exposure to these agents. Cell membranes form around each of the internal nuclei of cysts, forming the aforementioned internal "sporozoites." The cysts rupture to release immature trophozoites of a variety of shapes and sizes to restart the cycle. *In vitro* cultivation for periods up to 10 days has been achieved using rat-lung-derived organisms cultured on a variety of mammalian cell lines. Despite extensive efforts, the system has been improved very little since the first *in vitro* cultivation by Pifer et al.[41,46] and Latorre et al.[45] in 1977.[43,44] Reports of successful axenic culture of organisms using fungal media have not provided growth beyond 48–72 hr after inoculation.[42] The culture systems have assisted in "cleaning up" preparations of organisms that are contaminated by host lung cells and proteins. Viability tests for *P. carinii* are in their infancy. Reviews of the biology of *P. carinii* have been published.[25,26,40,43]

2.2. Epidemiology

2.2.1. Animal Studies and Serology

The natural reservoir for *P. carinii* is unknown. The association of protein–calorie malnutrition and of immune suppression with the development of *P. carinii* pneumonitis has been documented in the rat and mouse models of this disease.[40,44,47,48] The rat model has changed little since the description of the induction of *P. carinii* pneumonia in animals treated with cortisone acetate by Weller in 1955 and by Frenkel et al.[50] in 1966.[49,51] The animal model has been modified to utilize "virus-free" rats using transtracheal inoculation of *P. carinii;* this modification results in fewer infections in these immunosuppressed animals due to other pathogens and a more consistent level of infection.[52] These studies suggested that pneumonitis was the result of the emergence of latent infection during immune suppression. Aerosol transmission of the organism has also been demonstrated in the animal model by Hughes and others. Clusters of infection in clinical oncology centers and serologic studies support the aerosol transmission of infection from environmental or human sources or both. Recent studies using the corticosteroid-treated animal model of infection suggest that few organisms (<100 cysts) are needed to cause infection in the immunocompromised host.

The role of T lymphocytes in protection against infection is best illustrated by the use of cyclosporin A in rats and the use of antibodies to T-helper lymphocytes (CD4+) in mice to deplete the host immune response.[53,54] The protective effect of the passive transfer of T lymphocytes in the mouse model also supports the primacy of the T-cell response in prevention of *P. carinii* infection.[55,56] Augmentation of the macrophage response using interferon-γ (IFN-γ) appears to reduce the amount of antibiotic needed to clear infection.[57,58] The roles of colony-stimulating factors [granulocyte- and granulocyte–macrophage-stimulating factors (G-, GM-, and M-CSF)] in the clearance of infection are not yet known. Passive immunization with monoclonal antibodies is partially protective against *P. carinii* infection, suggesting a role for both cellular and humoral immune mechanisms.

A major role for animal models has been the evaluation of therapies for the treatment of *Pneumocystis* pneumonia. The efficacy of antibiotics in the rat model has been shown to correlate with successful outcome in clinical applications. Most of the new agents available for treatment of *P. carinii* (aerosolized pentamidine, clindamycin–primaquine, BW566c80, dapsone–trimethoprim, glucan synthase inhibitors, azithromycin, trimetrexate, erythromycin–sulfa) have been developed and tested using the rat and mouse models.

A limited number of antigens have been detected on *P. carinii.*[59–63] Monoclonal antisera raised to these moieties have been useful in the development of immunofluorescent staining of clinical specimens for the diagnosis of *P. carinii* pneumonia. The major antigens detected in human organisms by Western immunoblotting are of molecular weights 110–116, 50–55, 60–65, 35–45, and 22–25 kDa. These molecules are poorly soluble and very "sticky," accounting for the low level of antigenemia seen in *Pneumocystis* pneumonia and the clumping of organisms. A number of other antigens are variably detected. There is variation in the pattern of glycosylation of *P. carinii* antigens isolated from different species. The role of these antigens in immunity to *P. carinii* is not known.[60,63,65] As was noted, passive transfer of T cells, but not serum, from immune animals is protective against *Pneumocystis* infection.[55] Up to 87% of adults have lymphocyte proliferation in response to stimulation with *P. carinii* antigens.[64–66] Solubilized (and particulate) glycoproteins of the 55–60 kDa and

100–116 kDa ranges stimulate T-lymphocyte proliferation from sensitized hosts[60,64] In the lungs, antigenic processing by accessory cells (dendritic cells and macrophages) is needed for the generation of *Pneumocystis*-specific T-cell proliferation. IFN-γ and Dapsone appear to enhance intracellular killing of *P. carinii* by macrophages. Opsonization by immune serum is not essential, but improves phagocytosis by nonimmune macrophages.[67] The organisms are subsequently degraded without evidence of intracellular replication. Infection by HIV decreases internalization but not adhesion of *P. carinii* to alveolar macrophages. The production of cytokines (TNF-α and IL-1β) by macrophages in response to *P. carinii* is also blocked by infection with HIV.

2.2.2. The Susceptible Host

Pneumocystis has been documented as a cause of pneumonia in a broad range of immunocompromised patients (Table 3). In the non-AIDS patient, the propensity for the development of *Pneumocystis* pneumonia is related to three factors: (1) the duration of immune suppression, (2) the specific drugs to which a patient has been exposed, and (3) the nature of the underlying disease.[68] The presentation of disease will vary based on the underlying predisposing condition. As a general rule, more severe disease is seen in T-lymphocyte deficiencies or hematopoietic malignancies.[69] Individuals who are malnourished or treated with corticosteroids or both tend to have greater susceptibility than do patients with other induced immune deficiencies.[70–72] The manifestations of disease are frequently most muted in patients receiving a range of immunosuppressive agents or in AIDS patients with advanced disease.

Serologic studies suggest that seroconversion to *P. carinii* usually occurs some time after the 3rd year of life. The earliest studies of *Pneumocystis* occurring in the epidemic form as "interstitial plasma cell pneumonitis" in malnourished children in orphanages demonstrate that low serum immunoglobulin and low serum albumin levels are associated with both the occurrence and the poor outcome of this form of the disease.[10,11,40]

TABLE 3. Conditions Associated with *Pneumocystis carinii* Pneumonia

Acquired immunodeficiency syndrome (AIDS)	Malignancies (especially hematopoietic)
Chemotherapy (especially corticosteroids)	Congenital immune deficiency diseases (cellular, humoral, combined)
Radiation therapy	Collagen vascular disease
Organ transplantation	Hematologic disorders
Prematurity	Cushing's syndrome
Malnutrition (protein and calorie)	Nephrotic syndrome

The absence of immunity to *P. carinii* in babies is illustrated in children with congenital HIV infection. These patients can be expected to survive for less than 1 year. Patients developing AIDS or HIV infection after 1 year of age do somewhat better. Both the malnourished infants and congenitally infected AIDS patients develop *Pneumocystis* pneumonia on average by 6 months of age.

Pneumocystis carinii is an organism of low native virulence. Apparent enhanced virulence of *P. carinii* in some individuals may be a function of coinfection alone, immune suppression due to coexistent viral (CMV, HIV) infection, or the possibility that coinfection with certain agents may enhance the virulence of infection due to *P. carinii*. The association of cytomegalovirus (CMV) with *Pneumocystis* is commonly observed.[73,74] This association is due largely to the frequency of CMV infection in the population of immunocompromised individuals. CMV is well known as a systemic immunosuppressive agent, but its effects on the pathogenesis of *Pneumocystis* remain unclear. CMV does not appear to increase the morbidity or mortality due to *P. carinii* pneumonia in AIDS, but may increase the severity of the pneumonia and may require additional therapy.[75]

Recent reports of *Pneumocystis* pneumonia in immunologically normal hosts have raised suspicions about an increased environmental exposure, possibly due to *P. carinii* in AIDS.[21,76] However, these case reports lack clear documentation of *P. carinii* infection and of normal immune function. Autopsy studies do not support the existence of the organism as a commensal. However, serologic studies that subclinical exposure occurs in most individuals before the age of 5.[62,77–81] Various tests of immunoglobulin G (IgG) serum antibodies [immunofluorescence, enzyme-linked immunosorbent assay (ELISA)] have detected infection in 1–100% of normal adults and in 30–100% of infected adults.[77,80] Significant titers of antibody to *P. carinii* are detected in most patients at the time of diagnosis of *Pneumocystis* pneumonia.[78,79] Detection of circulating antigen would be preferred for establishing the presence of *P. carinii*. Antigen detection systems developed to date have had low specificity due to impure antigen preparations used to generate the detector antibodies. Improvements in antigen isolation (gel electrophoresis, molecular cloning) and in antibody development (i.e., monoclonal antibodies) may make clinical antigen detection feasible.

2.3. Acquired Immunodeficiency Syndrome

Prior to the use of prophylactic antibiotics, *P. carinii* pneumonia was the major complication and diagnostic manifestation of AIDS. Without prophylaxis, over 80% of individuals infected with HIV would be expected to develop significant *Pneumocystis* pneumonia. The

severity and the incidence of *Pneumocystis* pneumonia have been reduced by the use of antiviral therapy in AIDS patients [azidothymidine (AZT), 2′,3′-dideoxyinosine (DDI)] and by the ability to treat simultaneous viral infection due to CMV (ganciclovir, foscarnet). The frequency and the manifestations of *Pneumocystis* have also been altered by the use of prophylactic therapy. The beneficial effect of AZT does not seem to persist indefinitely. The incidence of pneumocystosis in AIDS patients is greatest with fewer than 200 CD4+ lymphocytes/ml or in whom fewer than 20% of circulating lymphocytes are CD4+ [54,69] It is likely that alveolar macrophage activity against the organism is decreased by HIV infection.

2.4. Clinical Pneumocystosis

The clinical manifestations of *Pneumocystis* pneumonia depend on the patient's condition: preexisting lung injury, immune function, concomitant infections, or drug therapies (Table 4).[61] In the adult without AIDS, *P. carinii* pneumonia is usually subacute to acute in onset, developing over a few days to weeks (Fig. 3). The patient develops progressive dyspnea, tachypnea, cyanosis, and a nonproductive cough. Auscultatory findings at the onset are minimal, generally no more than scattered rales and somewhat diminished breath sounds. In the adult with AIDS, the manifestations of the initial episode of *P. carinii* pneumonia, usually dyspnea and fever, evolve more slowly, often over 2–5 weeks.[29,82] Subsequent relapses may evolve more rapidly, especially in the setting of other infections (e.g., CMV) or fibrosis

TABLE 4. Clinical Presentation of *Pneumocystis carinii* Pneumonia[a]

Clinical sign	Non-AIDS	AIDS
Dyspnea	Common	Common
Cough	Common	Common
Fever	Common	Common
Progression[a]	Rapid (7–21 days)	Gradual (2–5 weeks)
Hypoxemia	Severe	Moderate to severe
Leukocytosis	Often absent (neutropenic)	Often absent (lymphopenic)
Chest radiograph	Diffuse bilateral interstitial infiltrate (much variability)	Asymmetric or bilateral interstitial infiltrate (often normal)
Response Initial therapy	Rapid (3–5 days)	Slow (5–9 days)
Recurrence	Unusual	Common[b]
Response Repeat therapy	Good	Decremental; residual lung injury?
Side effects of therapy	Usually mild	Common; some severe

[a]Altered by type and duration of immunodeficiency.
[b]Patients taking zidovudine (AZT) or DDI may have less severe infections with delayed recurrences.

or emphysematous changes from previous infections (Fig. 3).

By the time of hospitalization, arterial hypoxemia is generally moderate to severe and the alveolar–arterial O_2 gradient is considerably widened: The degree of arterial hypoxemia is out of proportion to the physical and radiologic findings. Dyspnea and arterial hypoxemia often occur in the face of a normal chest radiograph. Fever is common, usually low-grade, and precedes the development of respiratory symptoms. In the patient undergoing chemotherapy, clinical manifestations of pulmonary disease often intensify after the immunosuppressive agents are discontinued, and pulmonary infiltrates appear on the chest radiograph as the host's inflammatory response reemerges (Fig. 4). Conversely, the use of corticosteroids or cyclosporine therapy may entirely mask the signs and symptoms of *Pneumocystis* pneumonia until late in the course of disease. Manifestations of extrapulmonary disease due to *P. carinii* depend on the location of infection.[83] Mass lesions of the liver or spleen may be silent. Colonic and omental lesions have caused obstructions, and embolid phenomena have been seen in virtually every organ system.

In the organ transplant recipient, *P. carinii* pneumonia will occur approximately 8 weeks after the initiation of immunosuppressive therapy or during periods of increased immunosuppression for treatment of episodes of organ rejection. The incidence of *Pneumocystis* pneumonia depends on the center where transplantation is performed and the immunosuppression regimens and prophylactic regimens employed there. In patients receiving heart–lung and single-lung transplants, the incidence of asymptomatic *Pneumocystis* isolation from these organs approaches two thirds of the total number of patients in some centers. Of these, approximately half will be expected to develop symptomatic disease in the absence of treatment or prophylaxis. By contrast, among other organ transplant recipients, including heart transplants, only 5–10% will be expected to carry or develop *Pneumocystis* infections. These patients are very instructive in terms of the pulmonary inflammatory response to *Pneumocystis* infection. They tend to have a lymphocyte-predominant response to the acute infection, with the recruitment of macrophages during and after therapy. Despite therapy with cyclosporine, lymphocytes are found in the infected transplanted lung in large numbers. These are primarily T lymphocytes with normal helper/suppressor ratios. Over half of this group of patients with *Pneumocystis* pneumonia will also have a secondary bacterial or viral infection. The heart and heart–lung transplant recipients are particularly susceptible to co-infection with CMV. The cytotoxic T-lymphocyte-mediated response to pulmonary CMV may be difficult to separate from organ rejection. The incidence of

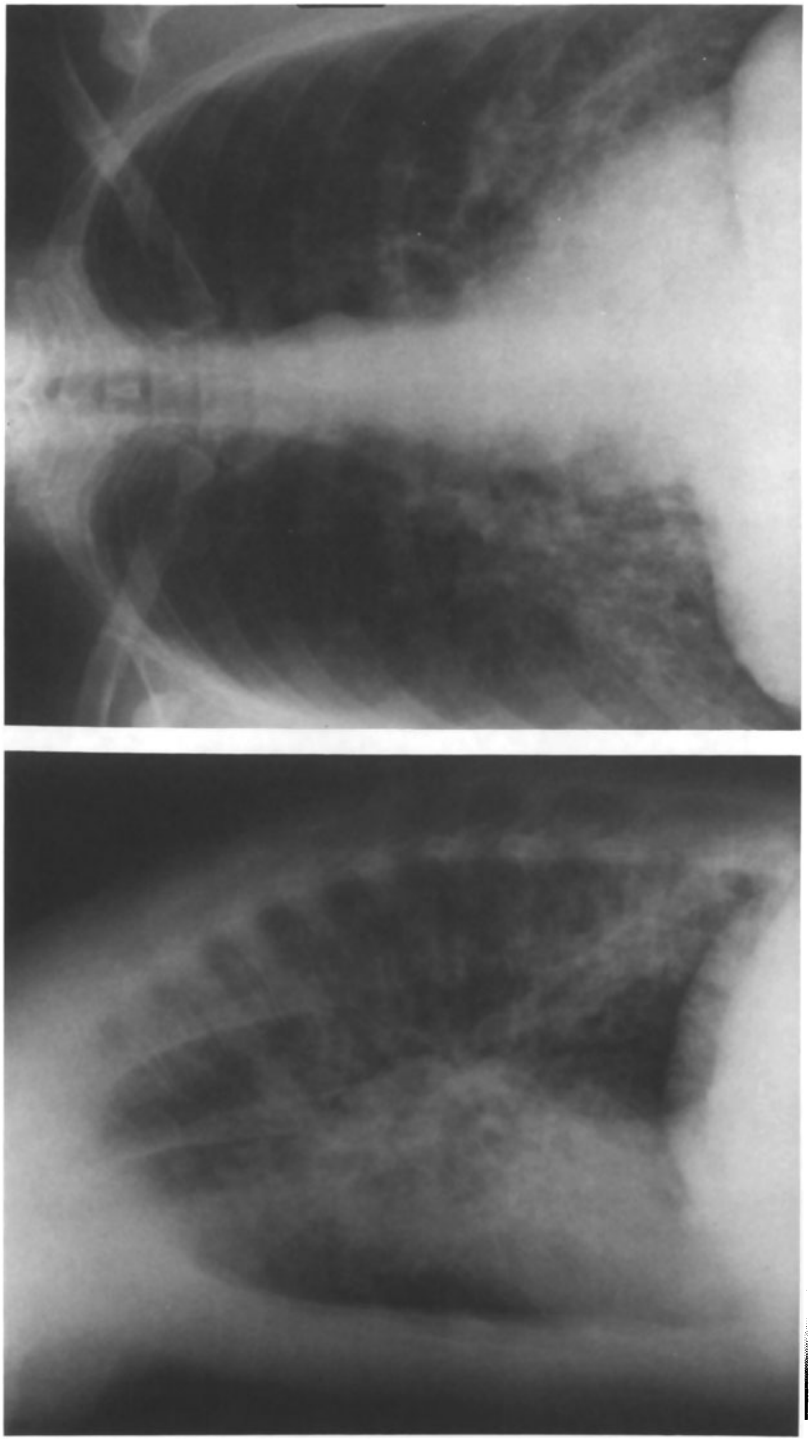

FIGURE 3. Chest radiograph of a 36-year-old man with *P. carinii* pneumonia following chemotherapy for a non-Hodgkin's lymphoma. A complete clinical history appears in Illustrative Case 1 (Section 2.9). Typical diffuse and bilateral, fine, interstitial infiltrates are observed.

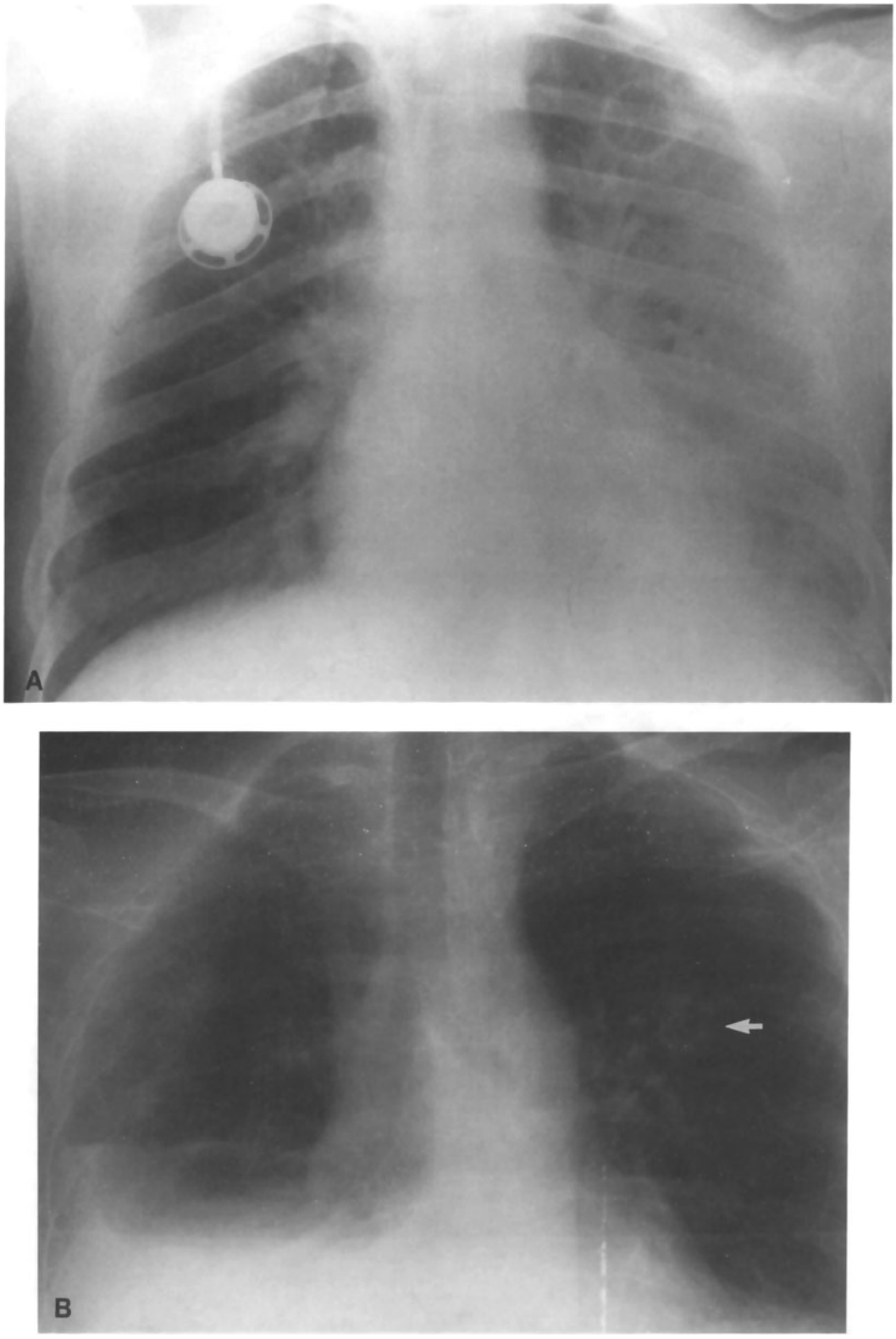

FIGURE 4. (A) Chest radiograph of a 38-year-old man with AIDS who presented with fever, cough, and malaise of 3 weeks' duration. His CD4+-lymphocyte count was 87 at the time of admission. An abscess cavity was seen in the left upper lobe. Bronchoscopic biopsy revealed only *P. carinii*. (B) Chest radiograph of a 43-year-old woman who became febrile and dyspneic 6 weeks following liver transplantation. A small abscess cavity was seen (⇨) in addition to a benign right-sided pleural effusion. Percutaneous needle aspiration of the abscess cavity revealed *P. carinii*. (C) A CT scan of the chest demonstrates progression of the abscess despite therapy with intravenous pentamidine. The patient recovered completely after 4 weeks of therapy.

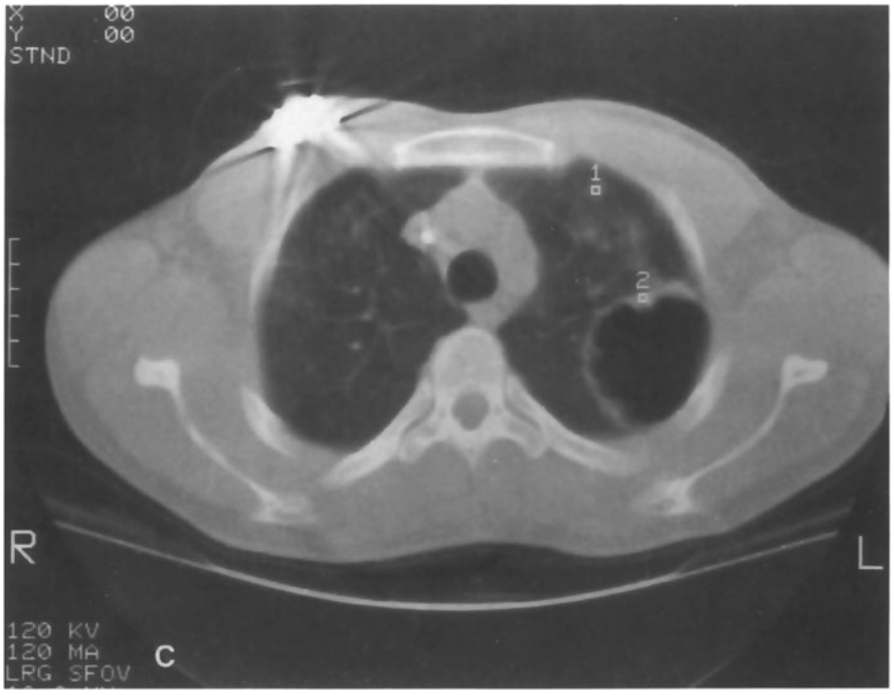

FIGURE 4. (*Continued*)

fungal infection has increased with the use of cyclosporine therapy, while bacterial infection has been reduced. A number of centers have noted that patients with *Pneumocystis* pneumonia while on cyclosporine have an increased mortality over other immunocompromised patients with *Pneumocystis*.

Bacterial infection of the lung remains more common than *Pneumocystis* pneumonia in the pediatric immunocompromised population.[84] Early signs of pneumocystosis include diarrhea, poor feeding, and coryza. The respiratory manifestations progress to nasal flaring, intercostal retraction, and cyanosis. Fever may be absent. As in the adult, arterial hypoxemia is generally present along with respiratory alkalosis (pH 7.45–7.6; P_{CO_2} 20–40 mm Hg). *Pneumocystis* infection in HIV-infected children less than 1 year of age is a predictor of very poor short-term survival.[84]

2.5. Radiology of *Pneumocystis carinii* Pneumonia

The variability of the clinical presentation of *P. carinii* is matched by the radiographic picture. Like many of the "atypical" pneumonias (pulmonary infection without sputum production), no diagnostic pattern exists for *Pneumocystis* pneumonia on routine chest X ray. The chest radiograph may be entirely normal despite significant hypoxemia and diffuse parenchymal involvement.[85,86] Diffuse, fine, "ground-glass" interstitial infiltrates with a perihilar predominance are common (Fig. 3).[87] These infiltrates may progress to involve the entire lung with progressive consolidation. "Atypical features" are often seen: small effusions, asymmetry or focal consolidation, small nodules or cavities, linear opacities, lymphadenopathy.[88–90] Distortions of the radiographic pattern may occur in the presence of preexisting pulmonary disease (e.g., radiation or cytotoxic injury). Accentuation may be noted in the presence of superimposed viral (CMV) infection or after weaning of immunosuppressive agents (Fig. 3). Abscess formation may be due to *Pneumocystis* alone, when *P. carinii* develops in a preexisting cavity, or with bacterial or fungal superinfection (Fig. 4).

In the AIDS patient, radiographic disease will commonly progress despite appropriate therapy. While this progression may reflect superinfection, it is more often an indication of the greater organism load seen in this patient subpopulation. The intravenous drug abuser will often have small cysts and bulli in the peripheral lung fields; these changes are more often perihilar with pneumocystosis.[90] The use of aerosolized pentamidine for prophylaxis against *P. carinii* in AIDS patients (and in non-AIDS patients) has resulted in a series of otherwise unusual radiologic presentations of *Pneumocystis* pneumonia (Fig. 5). Maldistribution of drug may account for the development of *Pneumocystis* only in the upper lobes. This distribution of disease, coupled with the apparent tendency of these patients to develop cystic changes in the parenchyma, also explains a predilection for spontaneous pneumothoraces. Pneumothorax may

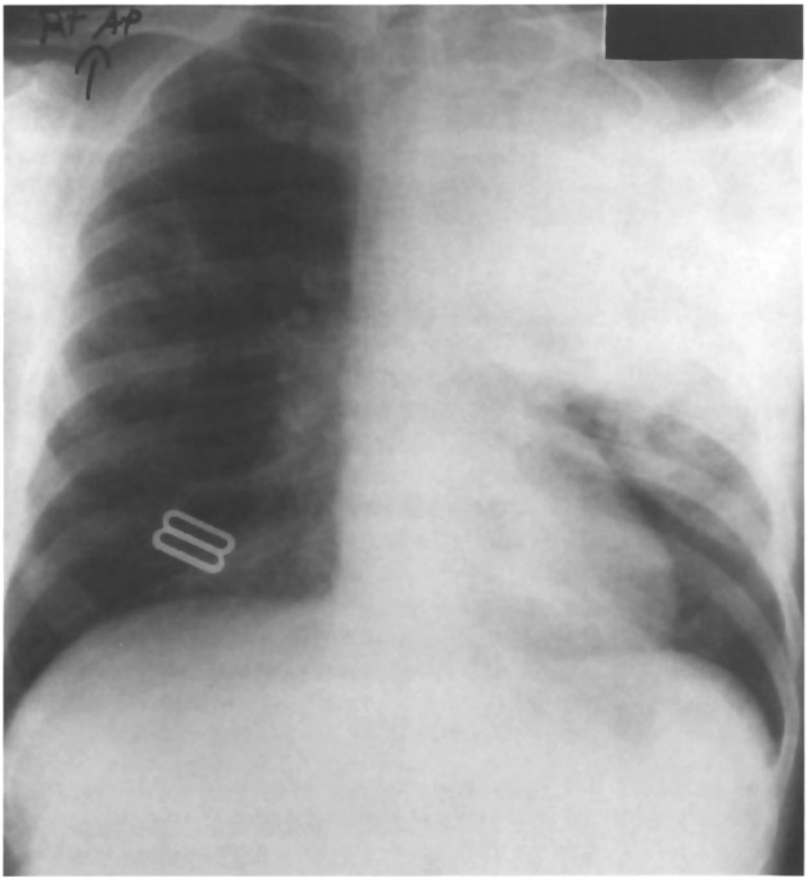

FIGURE 5. Simultaneous upper lobe infection with *P. carinii* and *Legionella pneumophila* in a 46-year-old patient with AIDS while on prophylaxis with aerosolized pentamidine.

also complicate the therapy of intubated patients with infection or residual fibrosis from previous *Pneumocystis* infection.[90] The development of extrapulmonary pneumocystosis, while rarely seen in non-AIDS patients, is probably due to the reduced systemic absorption of pentamidine during aerosol administration. The clinical presentation is generally a mass lesion of the liver or spleen.

In transplanted lungs, infection may be separated from rejection of the transplanted organ. Rejection may cause nodular and interstitial infiltrates indistinguishable from *P. carinii* pneumonia. These changes are more common in the period up to 6 weeks after transplantation. Infection in these hosts is due to CMV more often than to any other single pathogen.

Children with epidemic "interstitial plasma cell pneumonitis" on the basis of malnutrition and crowded or institutional living quarters have a slowed progression of the chest radiograph. Vascular markings and atelectasis are more commonly seen, with hyperinflation and intercostal widening preceding consolidation. In AIDS, lymphocytic interstitial pneumonitis (LIP) may mimic the radiologic appearance of *Pneumocystis* infection. This complication involves a diffuse lymphoid hyperplasia and infiltration of the interstitial space with lymphocytes.

Alternatives to plain radiographic imaging include the computerized tomography (CT) and nuclear magnetic resonance imaging (MRI) scans, ultrasound, and nuclear medicine imaging including gallium, radiolabeled immunoglobulin, and white blood cell scans. The tissue–air interface is poorly imaged by MRI and makes this modality less useful. The CT scan often reveals diffuse and nodular parenchymal involvement of the *Pneumocystis*-infected lungs in the setting of normal or nearly normal routine chest radiographs (Fig. 6). The CT scan is sensitive to emerging or atypical patterns of lung injury, including cysts and microabscesses (Figs. 4 and 6). The correlation of CT scans with histopathology is quite good; imaging demonstrates the patchy distribution of lung involvement and the apposition of normal parenchyma with consolidated tissue. Ultrasound and CT scanning are both useful in the evaluation of extrapulmonary masses due to *P. carinii*. This presentation needs to be separated from other infections (e.g., fungi, mycobacteria) and from lymphoma or metastatic tumor. Multiple small lesions may be seen in the liver or spleen with punctate or rim calcifications. These foci are often better identified by ultrasound than by CT scan. They are clumped hypoechoic masses that develop an echogenic

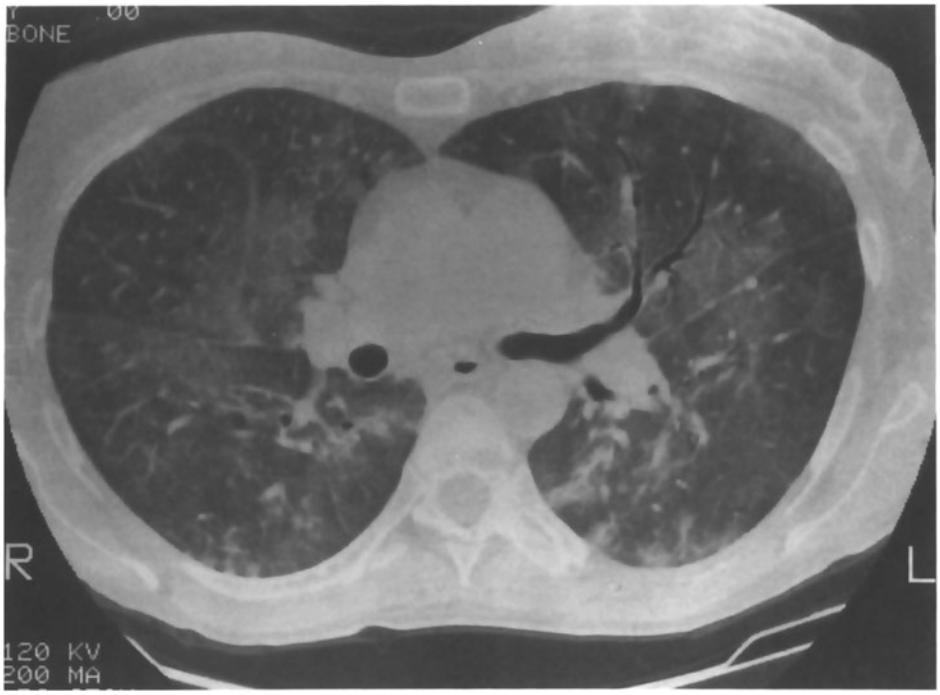

FIGURE 6. CT scan of the chest of an AIDS patient with *P. carinii* pneumonia and a normal chest radiograph. Multiple small interstitial densities and areas of parenchymal consolidation are seen.

rim during therapy. Biopsy can be performed using ultrasound or fluoroscopic guidance.

Each of the nuclear medicine imaging techniques is limited by the need for tissue inflammation to accumulate the imaging agent and to produce a localized image. In marked neutropenia or uremia or in infections that do not induce much local inflammation, images may not develop. Conversely, the diffuse inflammation that is often observed in the lungs of patients with AIDS (possibly due to cytotoxic lymphocytes for HIV or CMV) may produce false-positive images. However, nuclear medicine imaging may detect inflammation earlier than other techniques. Furthermore, the ability to scan the entire body reveals unexpected findings in up to 15% of scans.

Gallium citrate (^{67}Ga scintography) scanning, radiolabeled human serum immunoglobulin ([^{111}In]-IgG) imaging, ^{99m}Tc and diethylenetriamine pentacetic acid (DTPA) scans are abnormal in *Pneumocystis* pneumonia.[86,91–93] The diffuse uptake of ^{67}Ga in the lungs coupled with hypoxemia and a decreased diffusion capacity (D_LCO) to carbon monoxide have been used in many centers to make a presumptive diagnosis of *Pneumocystis* pneumonia in AIDS patients. These tests are also abnormal in non-AIDS patients with *P. carinii* infection. The main deficiency of this method, as with all noninvasive imaging techniques, is a lack of specificity. Half the positive images seen in pulmonary gallium scans of AIDS patients will be due to *P. carinii*. Lymph node uptake (AIDS-related complex) is common. Drug reactions, adult respiratory distress syndrome (ARDS), CMV, mycobacteria, radiation injury, and other insults may provoke a positive image. However, the image will precede demonstrable infection in many patients by as much as 4–6 weeks. A negative gallium scan is rarely seen (<7%) in pneumocystosis. Normal images should be seen by 3–5 weeks after the start of therapy in the absence of other processes.

Immunoglobulin scans utilize the localization of human IgG to areas of capillary permeability and white cell accumulation. The IgG scan provides a more intense image than the gallium scan with a much lower total dose of radioactivity. This sensitivity to inflammation allows the detection of focal processes (i.e., abscesses) as well as the more common diffuse picture seen with *P. carinii* infection. This capability also allows earlier detection of infection and the ability to follow the resolution of inflammation. DTPA scans are a function of fluid movement and label clearance out of the alveolar space. While abnormal in *Pneumocystis* pneumonia, DTPA scans are nondiagnostic. The positron emission tomographic (PET) scan may provide useful information about the course of infection as metabolic labels for the growth of *P. carinii* are developed.

2.6. Laboratory Evaluation

Laboratory evaluation of the immunocompromised patient with pulmonary symptoms provides information

TABLE 5. Diagnostic Techniques for *Pneumocystis carinii*[a]

Technique	Yield	Complications	Comments
Routine sputum	Poor	Rare	Cultures needed
Induced sputum	30–55%	Rare	First choice; excellent in AIDS
Transtracheal aspiration	Fair (in AIDS only)	Common: bleeding; subcutaneous air	Rarely worthwhile
Gallium scan, D_LCO	Nonspecific	Injection site	Positive in >95% of infected patients
Bronchoalveolar lavage (BAL)	>50% (>95% in AIDS)	Bleeding, aspiration fever, bronchospasm	Wedged terminal BAL with immunofluorescence
BAL/brushing	As for BAL alone	As for BAL	Not useful for *P. carinii*
BAL/transbronchial biopsy	Over 90% (all patients)	See BAL; pneumothorax	Impression smears; cultures; pathology
Open lung biopsy	Over 95% (all patients)	Anesthesia, air leakage, altered respiration, wound infection	"Gold standard" for noninfectious and infectious processes; adequate sample needed
Needle aspirate	Up to 60%	Pneumothorax, bleeding	Best in localized disease

[a]All samples should be cultured and stained for bacteria (including mycobacteria), fungi, viruses, and protozoa and handled with caution. Optimal procedures will depend on the locally available expertise.

about the susceptibility and the prognosis of the patient with *Pneumocystis* pneumonia. The level of serum lactic dehydrogenase (LDH) is elevated in most patients with *Pneumocystis* pneumonia [>300 international units (IU)/ml]). Very high levels indicate that large amounts of lung tissue are involved, and levels over 600 or 700 IU/ml carry a poor prognosis. Other diffuse pulmonary processes, including pulmonary emboli with infarction, lymphoma, other pneumonias, and LIP, also raise serum LDH levels. The characteristic hypoxemia of *Pneumocystis* pneumonia produces a broad alveolar–arterial Po_2 gradient; gradients in excess of 30 mm Hg tend to have a higher mortality. Another indicator of diffuse lung injury is an elevation in the level of angiotensin-converting enzyme. This level is also increased by smoking and by sarcoidosis. Pulmonary function testing is not useful diagnostically, but may indicate abnormalities in oxygen exchange. However, arterial blood gas measurements are very useful in the management of patients in making decisions in regard to intubation and the use of corticosteroids as adjunctive therapy to antibiotics. Corticosteroids have been shown to be of benefit in hastening improvement in oxygenation in nonintubated patients with a Pao_2 between 35 and 75 mm Hg while breathing room air or a "hypoxemia ratio" (Pao_2/Fio_2) between 75 and 350. In the markedly neutropenic or lymphopenic patient, consideration may also be given to the use of CSFs to augment the host response.

2.7. Histopathologic Diagnosis

Identification of *P. carinii* as a specific etiologic agent of pneumonia in an immunocompromised patient should lead to successful treatment (Table 5). Given the toxicity of the agents currently available for the treatment of *Pneumocystis* pneumonia in both AIDS- and non-AIDS-infected patients, it is advantageous to have histopathologic confirmation of the diagnosis prior to initiating therapy.[37,94] In the absence of data suggesting that antibiotic-resistant organisms exist, the failure of therapy in the setting of known *Pneumocystis* infection should suggest the presence of another simultaneous process. Further, in the non-AIDS patients, no more than 15–25% of pulmonary infiltrates are caused by *Pneumocystis*. The broad antibacterial spectrum of trimethoprim–sulfamethoxazole may delay or obscure the ability to make an alternative diagnosis. Practically, in many patients, it may not be possible to make a diagnosis invasively due to the patient's clinical condition. In AIDS patients, the frequency of *Pneumocystis* pneumonia in patients not receiving prophylaxis may make a therapeutic trial more appropriate than invasive diagnostic tests. The optimal approach must therefore be based on the patient's clinical condition. For the patient treated empirically, the physician must have a low threshold to adopt a more invasive posture should the clinical situation deteriorate.

A distinction should be made between the diagnosis of *Pneumocystis* infection in AIDS and in non-AIDS patients (see Table 4).[82,95,96] The burden of organisms in infected AIDS patients appears greater than that of the otherwise immunocompromised host. Thus, the identification of organisms by noninvasive techniques is more often achieved in the AIDS patient (see Fig. 2). In general, noninvasive testing should be employed to make the initial diagnosis of *Pneumocystis* pneumonia, but invasive techniques should be used when necessary and clinically feasible to identify problems such as carcinoma

impinging on the airway, viral or fungal coinfection, pulmonary embolism, or congestive heart failure. The most commonly used techniques in order of increasing invasiveness are outlined in Table 5. It is important to adapt these recommendations for the techniques available at a given institution and for the relative skill of the practitioners involved in providing these diagnostic techniques. That is, in a given institution, it may be preferable to perform an open lung biopsy or needle aspiration, rather than pulmonary bronchoscopy. Similarly, the yield of diagnostically useful material may be greater from pathology specimens than from the clinical laboratory that examines sputum or bronchoscopy specimens. This judgment must be made by the physician.

2.7.1. Histology of Infection

The diagnosis of *P. carinii* infection has been improved by the use of induced sputum samples and of immunofluorescent monoclonal antibodies to detect the organism in clinical specimens.[61] The recognition of small numbers of organisms in an individual without symptoms of pulmonary disease or without a history of prior PCP is of uncertain diagnostic value. This is to say that given the presence of both cellular and serologic exposure to *Pneumocystis* in the general population, it might be expected that *Pneumocystis* could be isolated in a non-immunocompromised host as either a nonpathogen or during a minor infection in an immunologically normal host. However, therapy should be initiated with the isolation of this organism in an individual with altered immune function, especially T-lymphocyte function.[69] Conversely, the identification of this organism in a normal host should initiate a search for immune deficiency.

In the lungs, *P. carinii* produces a characteristic interstitial and alveolar infiltrate (see Fig. 2).[38] This infiltrate is diagnostic even in the absence of clearly identifiable organisms. In the malnourished infant or neonate, the reaction to *Pneumocystis* pneumonia ("epidemic pneumocystosis") is primarily a disease of the interstitium. The pathology of "interstitial plasma cell pneumonia" of the neonate includes interstitial edema with infiltration of plasma cells and lymphocytes with a characteristic frothy exudate in the alveolar space. In the immunosuppressed adult and child, the disease tends to be more alveolar. The alveolar space is filled with a frothy eosinophilic material that contains organisms and debris of macrophages and alveolar epithelial cells as well as edema fluid and protein.[67] The distribution of disease is often patchy, with normal lung adjacent to areas of dense consolidation. Identification of organisms requires special stains. The most commonly used tissue stain is the methenamine–silver, which stains only the cyst forms of the organism (Fig. 2). Because cysts represent only 5–10% of the total infectious burden of *Pneumocystis* in the lungs, the silver stain greatly underestimates the organism load. To identify the presence of trophozoites, a polychrome stain needs to be done, usually on impression smears made from the cut surface of a lung biopsy specimen or from sputum smears. These are discussed in some greater detail below.

The primacy of the interstitial injury probably accounts for the marked hypoxemia seen in *Pneumocystis* pneumonia. While early disease is characterized by clumps of organisms at the alveolar epithelial surface, progressive infection causes epithelial injury and sloughing with interstitial cellular infiltration. In normal animals, *P. carinii* elicits primarily a polymorphonuclear leukocyte response in addition to alveolar macrophages early in disease. In the T-cell-deficient host, the inflammatory response is muted. The nature of the infiltrate depends on the nature of either the underlying immune defect or the immune suppressive regimen that is being used. The pathognomonic frothy alveolar infiltrate should be distinguished from "hyaline membranes" that may line alveoli in oxygen toxicity, alveolar proteinosis, or the adult respiratory distress syndrome (ARDS). All these conditions can coexist with *Pneumocystis*. In pediatric AIDS patients, LIP (see Section 2.5) may occur in the absence of clear infectious etiology. This is a systemic proliferation of lymphocytes and of lymphoid tissue, but may produce the same radiologic picture as *Pneumocystis* pneumonia in this patient population. In the pediatric AIDS population, bacterial infection is more common than is *P. carinii* pneumonia.

In tissue sections or on smears, *P. carinii* may be demonstrated by a variety of staining methods. Direct immunofluorescent staining of organisms using monoclonal antibodies is very useful for screening induced sputum specimens. These antibodies generally bind both cysts and trophozoites. The cyst wall can be displayed by a variety of staining techniques; of these, the Gomori methenamine–silver nitrate method (which stains organisms brown or black) is most reliable, even though it is susceptible to artifacts. Sporozoites and trophozoites are stained by polychrome stains, particularly the Giemsa stain. The Giemsa, Wright's, toluidine blue O, or Grocott's rapid silver stain technique is most useful in dealing with the lung imprints, bronchial lavage fluid, or pulmonary aspirates. Rapid polychrome staining (Diff-Quick, American Scientific Products, Inc.) and a rapid silver staining technique are useful in screening smears. When a silver stain is used, a counterstain such as Gram's, Wright's, Giemsa, hematoxylin, or trichrome may be required to identify intracystic bodies and to distinguish cysts from red blood cells and yeasts.

Following the resolution of acute infection, interstitial fibrosis and small areas of emphysema are often

seen. The relative roles of *P. carinii*, drug therapy, and concomitant infection (e.g., HIV, CMV) in this pulmonary picture are unclear. In AIDS patients, residual organisms are commonly detected months after the completion of successful therapy. These organisms do not correlate with the incidence of recurrent disease and are not thought to represent "resistant" organisms.

Extrapulmonary disease has been reported in both AIDS and non-AIDS patients. Extrapulmonary organisms have occasionally been identified in lymphoid tissue, blood, bone marrow, liver, spleen, heart, kidney, pancreas, adrenal, thyroid, thymus, mesentery, ear, and eye tissue.[97–100] In extrapulmonary sites, care must be taken to avoid confusing yeast forms with *Pneumocystis*. In the AIDS patient population, dissemination is most often associated with prophylactic therapy with aerosolized pentamidine or with the absence of prophylaxis against *Pneumocystis* pneumonia. The patients present with mass lesions in the liver or spleen and may develop ischemic injury when clumps of organisms embolize to small blood vessels. These lesions must be biopsied to distinguish them from metastatic tumor, lymphoma, or focal fungal infections.

2.7.2. Sputum Examination

Sputum collected for routine bacterial and fungal stains and cultures is rarely usable for the diagnosis of *Pneumocystis* pneumonia.[37,101] The technique of sputum induction has been very useful in the diagnosis of *Pneumocystis* infection in all immunocompromised individuals when coupled with the use of immunofluorescent antibodies for the detection of *Pneumocystis* in these specimens.[95,102–106] Sputum induction has become the diagnostic technique of choice for *P. carinii*. It should be noted that many bacteria will not grow after exposure to hypertonic saline, so it is important that routine sputum collection be utilized for bacterial and fungal diagnosis. Patients are exposed to aerosolized hypertonic saline or water for up to 30 min, and smears are prepared from the mucoid portion of the collected specimens. Smears can be prepared in a number of ways, including after treatment of the specimen with a mucolytic agent (acetylcysteine, Mucomyst) or dithiothreitol just prior to making the smear. The cytocentrifuge has also been very useful for this purpose. Smears should be stained with Giemsa or Diff-Quik stains for the intercystic bodies or with toluidine blue O or rapid silver stain, which stain the cyst wall (Fig. 2). Because cyst stains detect only 5–10% of the total organisms, the Giemsa stain is preferred over the more complex silver stain. However, the Giemsa stains are difficult to read. This problem has been overcome by the use of monoclonal antibodies directed against surface epitopes from *P. carinii*.[101,104] With some experience, these commercially available kits are easy to use with a relatively low level of background. The use of immunofluorescent microscopy should increase the detection of *Pneumocystis* by up to 10–20% over conventional staining. The same techniques are used to process bronchoalveolar lavage specimens. It is advantageous to concentrate these specimens using a cytocentrifuge or a microcentrifuge prior to preparing smears due to the effect of large fluid volumes associated with bronchoalveolar lavage.

2.7.3. Fiberoptic Bronchoscopy

In experienced hands, pulmonary bronchoscopy with multiple biopsies will provide the diagnosis of *Pneumocystis* pneumonia in over 90% of all patients.[94,96,107–112] Wedged terminal lavage in aliquots of at least 50 cc in at least three aliquots should be sufficient to detect *Pneumocystis* infection without biopsy in over 80% of all patients and in up to 95% of patients with AIDS. The presence of other pathogens in lavage specimens is often difficult to interpret. For example, the frequent colonization of the upper airway with *Candida* and the frequent isolation of CMV from such samples is of uncertain importance without histopathologic confirmation. Further, the ability to use bronchoscopic lavage for diagnosis is completely dependent on the skill of the laboratories handling the specimens. Biopsies are not generally needed to make the diagnosis of *P. carinii* pneumonia in AIDS, but will often provide useful information about the patient's status in regard to interstitial injury after chemo- or radiotherapy, viral infection, ARDS, or response to therapy. The complication rate is institution-dependent, but generally low. Biopsies (open or bronchoscopic) may be preferred if the clinical laboratories lack experience with *P. carinii*.

2.7.4. Transtracheal Aspiration and Percutaneous Needle Aspiration

Transtracheal aspiration for the diagnosis of *Pneumocystis* infection is to be avoided. The incidence of complications outweighs the potential benefit of the rapid production of a diagnostic specimen. Given the advantages of immunofluorescent staining coupled to induced sputum or bronchoscopy, the inexperienced practitioner should avoid transtracheal aspiration. In experienced hands, the diagnostic yield of tracheal aspiration is lower than that achieved by induced-sputum examination when both are coupled to immunofluorescent microscopy.

Radiologically guided percutaneous needle aspiration of the lung produces diagnostic specimens in up to 60% of patients with *P. carinii* pneumonia.[113] This technique is useful both in diffuse lung disease and in the

TABLE 6. Treatment of *Pneumocystis carinii* infections[a]

Agent(s) (route)[b]	Dose	Options[b]
Trimethoprim And sulfamethoxazole (IV/PO)	15 mg/kg/day TMP (to 20 mg) 75 mg/kg/day SMZ (to 100 mg)	Treat through rash: Reduce TMP or SMZ by one half; desensitize
Pentamidine isethionate (IV)	4 mg/kg/day; 300 mg/day maximum	Lower dose (2–3 mg/kg); IM route not advised
Dapsone (PO) With TMP (PO/IV)	100 mg/day 15–20 mg/kg/day (900 mg)	Methemoglobinemia; G6PD; may be tolerated in sulfadiazine allergy
Clindamycin (IV/PO) And primaquine	600–900 mg q6h 15–30 mg base qd	Methemoglobinemia; diarrhea
Trimetrexate (IV) With folinic acid (leucovorin)	30–45 mg/m^2/day 80–100 mg/m^2/day	Leukopenia, anemia; thrombocytopenia; relapse common
Pyrimethamine	Load 50 mg bid × 2 days, then 25–50 mg qd	Not studied fully
With sulfadiazine	Load 75 mg/kg, then 100 mg/kg/day	Maximum 4 g in two doses; up to 8 g
Piritrexim With folinic acid	Under study	Like trimetrexate
566C80 (investigational)	Under study (750 mg bid–qid, PO)	Variable absorbance, better with food; rash

Prophylaxis (after *P. carinii* pneumonia or with <20% CD4+ lymphocytes/ml or <20% CD4+ lymphocytes or with rapid disease progression): AZT or DDI; aerosolized or intravenous pentamidine (up to 300 mg q3–4 weeks); TMP-SMZ (160 mg TMP, PO bid/qd or 3 days/week); dapsone (100 mg/day; rule out G6PD, methemoglobinemia); Fansidar (1 tab qwk; rule out hepatitis, erythema multiforme).

[a]*Adjunctive therapies* (see Section 2.8.4): corticosteroids (high-dose with rapid taper); interferon-γ; macrophage colony-stimulating factor; aerosolized pentamidine.
[b]Based on the physician's clinical judgment; some agents are not FDA-approved for this indication.

evaluation of focal and peripheral processes seen on chest radiograph or CT scan. Pneumothorax is common (up to a third of cases in some series); 20% of pneumothoraces require chest-tube insertion. Inadequate specimens and bleeding are the other main complications of this technique.

2.7.5. Open Lung Biopsy

Surgical open lung biopsy remains the "gold standard" for the evaluation of pulmonary processes in the immunocompromised host.[114] When the procedure is performed by skilled surgeons, the complication rate of thoracotomy and biopsy should be low. This approach provides the best specimen for cultures and histopathology. The sampling of a larger segment of the lung may assist in clinical decision-making. The presence of CMV in alveolar cells is useful data; culturing CMV from lavage fluid is nondiagnostic. Impression smears taken from the cut surface of the lung biopsy are often adequate for the diagnosis and treatment of *P. carinii* pneumonia.

2.8. Therapy of *Pneumocystis carinii* Infections

Due to the frequency of *P. carinii* pneumonia in patients with AIDS, the number of therapeutic options available for the treatment of *Pneumocystis* pneumonia has increased.[25,115] For most of the available antibiotics, the potential for side effects such as rash, hepatitis or pancreatitis, or GI intolerance must be balanced against the potential for bone marrow suppression, which is common to almost all the agents discussed in this section and in Table 6.[116] A few general points may be made about therapy:

1. The most effective systemic therapy for the treatment of *P. carinii* pneumonia in all patients remains trimethoprim–sulfamethoxazole (cotrimoxazole, TMP-SMZ).[117–120] This consideration includes such factors as the onset of action and ease of administration (availability in all forms).

2. The use of adjunctive therapies (colony-stimulating factors, immune modulators, aerosolized pentamidine, corticosteroids, antibodies) must be tailored to the individual patient.

3. Experience in AIDS patients suggests that treatment can be continued through the occurrence of mild side effects including rash, mild elevation of serum liver function tests, and slight bone marrow depression. Such treatment may require adjustments in dosage, the interval of administration, or the form of the antibiotic given. These guidelines are not universally applicable to non-AIDS patients, in part because the incidence of adverse reaction in this population is much lower.

4. *Pneumocystis carinii* that is resistant to antibiot-

ics has not yet been demonstrated. The apparent failure of an individual patient to respond to therapy may reflect either inadequate serum or tissue levels of antibiotic or greater lung injury. Switching agents for reasons other than toxicity is not recommended.[116,120,121] Of interest is the observation of a reduction in the incidence of antibiotic side effects in groups treated with corticosteroids as an adjunct to antibiotic therapy.

5. Coinfection with pathogens in addition to *P. carinii* is common.

6. The duration of therapy in the immunocompromised patient with *Pneumocystis* has not been studied carefully. The choice of 14-day courses of therapy in non-AIDS patients and of 21-day courses of therapy in AIDS patients is largely arbitrary. Shorter courses may well be effective, especially in the setting of antibiotics with long serum half-lives. In patients with persistent immune deficiency, prophylaxis should be considered. Residual organisms present in bronchoalveolar lavage specimens at the completion of therapy are of uncertain importance, but are largely nonviable. These organisms do not correlate with the incidence of recurrent disease. Non-AIDS patients respond to therapy and prophylaxis as well as or better than patients with AIDS, and with fewer adverse reactions.

2.8.1. Pentamidine

Pentamidine isethionate was the first agent employed for the successful therapy of *P. carinii* pneumonia.[10,40,117,122,123] Pentamidine was first administered intramuscularly in an epidemic of infantile *Pneumocystis* pneumonia. In this population, it reduced mortality from 50% to 3.5%. The success of pentamidine therapy in subsequent trials varied widely, with survival rates of 25–85% of affected individuals. While pentamidine therapy is generally successful in up to 75% of individuals, over half will have adverse effects when receiving this drug via the intramuscular route, the major complications being sterile abscesses at the site of injection. Currently, pentamidine isethionate is given intravenously by infusion over 1–2 hr in a 5% glucose solution at a dose between 2 and 4 mg/kg per day. Because of the prolonged half-life of this drug and the high levels of tissue binding, it may be advantageous to begin therapy at the 4 mg/kg per day level and to use lower doses subsequently. Therapeutic efficacy is achieved more slowly than with other agents, often requiring 5–7 days before clinical improvement is observed. Therapeutic levels persist in the lungs long after treatment is completed. Pentamidine may play a role in the reduction of symptoms due to reduced secretion of tumor necrosis factor by macrophages involved in the phagocytosis of *P. carinii*. In Europe, pentamidine methanesulfonate is also available and requires different dosing than the isethionate form available in the United States. Pentamidine is a very useful drug in the treatment of AIDS patients allergic to cotrimoxazole, but is probably a drug of second choice in the non-AIDS patients who tolerate TMP-SMZ.

The incidence of side effects to intravenously administered pentamidine is roughly equivalent to that seen with cotrimoxazole in AIDS patients.[120] Adverse reactions are both idiosyncratic and dose-related. Administration of pentamidine in the presence of renal dysfunction is associated with an increased incidence of most of the side effects of pentamidine therapy. These adverse reactions include hypoglycemia, hyperglycemia, neutropenia, thrombocytopenia, azotemia, pancreatitis, nausea, and altered taste sensation. Pancreatic dysfunction is more common after a total dose exceeding 3 g pentamidine. This injury may occur after the cessation of therapy because of the prolonged half-life of the drug, which is frequently over 2 months. Hypoglycemia or hyperglycemia may precede permanent insulin dependence. Despite initial enthusiasm, aerosolized pentamidine has not proven useful for the initial therapy of *Pneumocystis* pneumonia, but may be useful as an adjunct to therapy. A hepatic metabolite of pentamidine may be responsible for most of the toxicities of this agent. New diamidines with fewer side effects and greater efficacies are under development.

2.8.2. Trimethoprim–Sulfamethoxazole (TMP-SMZ, Cotrimoxazole)

Trimethoprim–sulfamethoxazole (Bactrim, Septra) is the drug of first choice for the treatment of *P. carinii* pneumonia in patients who tolerate this agent.[24,26,118–120,124–127] This preference is based on (1) the availability of both intravenous and oral formulations of the drug, which enhances the ease of administration; (2) the ability to follow serum levels of the sulfa component; and (3) the efficacy of this drug for both therapy and prophylaxis. The onset of action for cotrimoxazole is rapid; clinical responses are seen as early as 3 days into therapy. Serum levels with orally administered drug are equivalent to intravenous levels, given normal GI function. Therapy is generally initiated with a total dose of 20 mg/kg per day of TMP coupled with 100 mg/kg per day of SMZ divided into 4 daily doses. Peak serum levels are reached within 2 hr after oral administration and probably in the range of 5–15 μg/ml of TMP and 100–150 μg/ml of SMZ. Serum SMZ levels of over 200 μg/ml are associated with a somewhat higher incidence of drug toxicity, in particular bone marrow suppression. The rapid onset of action of this agent may provide the margin necessary to avoid intubation of the critically ill patient.

In the non-AIDS patient, TMP-SMZ evokes many fewer adverse reactions than does pentamidine. The rates of adverse reactions in the AIDS patient population are roughly equivalent and amount to 50% of all patients who take either agent. A course of 14 days of therapy is adequate if immune suppression can be reduced or reversed; 21 days of therapy is preferred in AIDS patients or patients on chronic immune suppression (organ transplant recipients) in whom immune suppression cannot be varied. Chronic immune suppression requires prophylactic antibiotic therapy in patients who have had an episode of *Pneumocystis* pneumonia.

The proper dosing of TMP-SMZ in adults has not been studied.[121,125] The dosing regimens in common use were developed in children with leukemia and have not been reevaluated in adults with any form of underlying immune deficiency. While successful, these levels may be excessive, and it is worth monitoring serum levels at some point during the course of hospitalization. In the immunocompromised host, TMP-SMZ covers a broad spectrum of organisms, including *Listeria, Nocardia,* and many of the common bacterial pathogens including both encapsulated and unencapsulated gram-negative and gram-positive organisms. Thus, there may be unexpected beneficial effects when treating a patient for *Pneumocystis* with this agent.

The toxic side effects of TMP-SMZ are generally those of sulfa allergy. In the AIDS patient population, the adverse reactions to this drug include reactions to TMP and to the carriers and dyes present in various formulations. Some of the allergies in AIDS patients can be quite severe, including Stevens–Johnson's syndrome, hepatotoxicity with eosinophilia and cell necrosis, erythema multiforme exudativum, and nephrotoxicity. Both components in the combination can produce bone marrow suppression, including thrombocytopenia and neutropenia; these side effects are frequently reversible by reducing the total dose of the drug or supplementing with folinic acid. The bone marrow suppressive effects are greater in patients with underlying hematologic disorders or those receiving cytotoxic chemotherapy. Folinic acid should probably not be used in patients with acute leukemias.

2.8.3. Alternative Regimens

Multiple new drugs and drug combinations have been used in the treatment of *Pneumocystis* since the onset of the AIDS epidemic. Because of the frequency of adverse reactions in AIDS patients, most of these regimens have been used almost exclusively in the subpopulation of AIDS patients with allergies both to TMP-SMZ and to pentamidine. The combination of TMP (at 20 mg/kg per day in 4 doses) and dapsone (4′4′-diaminodiphenyl sulfone, Jacobus, 100 mg orally/day) has been effective.[128–131] Dapsone has been tolerated in many patients with proven sulfa-drug allergy. Particular caution must be exercised when using dapsone in this patient group, however, because hypersensitivity may cross between sulfa- and sulfone-containing drugs, and dapsone has a prolonged half-life, which makes side effects not rapidly reversible. The absorption of dapsone from the GI tract may be reduced by antiviral therapy with DDI (2′,3′-dideoxyinosine, Videx).[132] Preliminary data supporting a number of other combinations have also been accumulated. Clindamycin–primaquine and pyrimethamine–sulfadiazine have been useful both in the animal model and in human trials.[133–136] Clindamycin given intravenously (600–900 mg IV q8h for 10 days) is slightly more effective than oral administration (600 mg PO q6–8h) for mild to moderately severe *Pneumocystis* pneumonia. Clindamycin is given with oral primaquine (15–30 mg EPO base per day), with the major toxicities being gastrointestinal. The macrolides (erythromycin, clarithromycin, or azithromycin) coupled with SMZ have also proven useful in animal models and in small groups of patients. The erythromycin and sulfa combination is of particular interest because of the broad spectrum of coverage available in the immunocompromised host and because of the long history of use of this combination in pediatric infectious disease patients. Of interest, few of these agents have much activity on their own against *P. carinii*. Synergism between these agents can be demonstrated in animal models. The optimal dosing of these combinations has not been established for the treatment of *Pneumocystis* pneumonia. Some recommendations are presented in Table 6.

Further alternative regimens include trimetrexate, a dihydrofolate reductase (DHFR) inhibitor originally developed as a chemotherapeutic agent for cancer patients.[137–139] This drug has high affinity for isolated DHFR from *Pneumocystis* as compared to human DHFR, but is consistently toxic to the bone marrow, requiring folinic acid (leucovorin) to allow administration. This combination is expensive and remains investigational. Trimetrexate has the unique advantage of being lipid-soluble with a long serum half-life of up to 34 hr.[140] Side effects (fever, rash, leukopenia, liver function test abnormalities) occur in over a third of patients, and recurrence of *Pneumocystis* is very common. Another lipid-soluble DHFR inhibitor, piritrexim, coupled with sulfonamide also appears useful, and a variety of newer inhibitors (BW 566C80) are under study.[141] Atovaquone (Mepron, Burroughs-Wellcome, 566C80) has been approved for the treatment of mild-to-moderate infections. DFMO (α-difluoromethylornithine, Merrell Dow) has been used to treat *Pneumocystis* pneumonia in some patients with AIDS who do not respond or who are intolerant of other regimens.[142] Most of the patients successfully

treated with this regimen had completed some course of therapy with pentamidine. The prolonged half-life of this latter agent may count for those early successes. Subsequent experience has been more disappointing.

2.8.4. Adjunctive Therapies

Many patients with *P. carinii* pneumonia will suffer disease progression despite appropriate antibiotic therapy. Especially prone to do so are debilitated patients or those with marginal oxygenation. In this setting, the initial delay of 4–7 days before responding to therapy may necessitate intubation with mechanical ventilation. It may well be that the successful killing of intra-alveolar organisms may contribute to the local inflammatory process and further diminish oxygenation. These patients will often require supplemental oxygen and are at risk for bacterial and fungal superinfection after intubation. One approach to this problem has been the judicious use of corticosteroids in selected patients with *Pneumocystis* pneumonia with hypoxemia and prior to intubation early in the patient's course. Recent clinical trials have demonstrated that corticosteroids administered in the first 72 hr of therapy for *Pneumocystis* pneumonia are of significant benefit in AIDS patients in terms of morbidity, mortality, and the avoidance of intubation in patients with an arterial Po_2 on room air between 35 and 72 mm Hg or with a hypoxemia ratio (Pa_2/FIO_2) between 75 and 350.[143–146] Experience with both neutropenic and organ transplant patients with *Pneumocystis* pneumonia has been equally gratifying. In AIDS patients, the benefits of early steroid therapy were as follows: up to a 50% reduction in patients requiring intubation, a marked reduction in the number of patients experiencing deterioration in oxygenation during the first 7 days of therapy, a reduction in the number of side effects due to antibiotics observed, a significant decrease in patient mortality in the first 84 days after hospitalization (to 50%), and a persistent improvement in exercise tolerance after the completion of therapy. In addition, many of these patients were found to eat better. Patients with undiagnosed CMV adrenalitis may also benefit. The incidence of side effects was surprisingly low when the patient was given a maximum of 14 days of tapering steroid therapy. Patients in whom steroid therapy is not tapered are prone to recrudescence of hypoxemia and of acute pulmonary symptoms. The optimal dose of steroids has not been established. One useful regimen is a dose of 40–60 mg prednisone or prednisolone given orally or intravenously twice a day. After 5–7 days, the steroids are tapered over a period of 7 days to 2 weeks. Predictable side effects of steroid excess are rarely seen with a short course of modest steroid doses. An excess incidence of opportunistic infection, gastric irritation, or acceleration of the underlying disease due to HIV was not observed. Patients did observe an increased incidence of oral herpes simplex with oral thrush, both of which are improved with careful attention to oral care.

An alternative approach to the suppression of the acute inflammatory response is the augmentation of the immune response to *Pneumocystis* using immune modulators.[58] Animal studies and some clinical anecdotes have suggested that interferon-γ (IFN-γ) has the effect of reducing the amount of *Pneumocystis* found in infected lungs, probably by enhancement of the macrophage response. IFN-γ administered either intravenously or by aerosol may accelerate the clearance of organisms without greatly enhancing the local inflammatory response.[57,58] In leukopenic patients or in AIDS, an alternative would be the utilization of macrophage or granulocyte–macrophage colony-stimulating factors (M-CSF or GM-CSF). Unlike the effect of IFN-γ, the effects of CSFs on local immunity may require T-lymphocyte function. There is controversy regarding the use of CSFs in patients infected with HIV due to increased viral replication in the presence of M-CSF and GM-CSF. It is not yet clear whether or not these effects are of physiologic significance.

The role of aerosolized pentamidine remains unclear in the acute management of patients with *Pneumocystis* pneumonia. The distinct advantage of having an agent that does not disseminate systemically and obtains high local concentrations appears obvious. It may be that aerosolization of pentamidine is a useful supplement to intravenous pentamidine therapy in the first few days prior to obtaining good lung tissue levels of this agent. Other immune modulators are being evaluated for the treatment of *Pneumocystis*. Preliminary data suggest that some of the newer antibiotic agents (e.g., the echinocandins or quinolones) will also be useful.

2.8.5. Response to Therapy

The manifestations of *Pneumocystis* pneumonia have changed both because of new prophylactic antibiotic therapies and because of new immunosuppressive regimens, including those with cyclosporine. The response to therapy has also changed with these new regimens. AIDS patients are presenting with advanced disease and with atypical manifestations (upper lobe disease, multiple infections, extrapulmonary disease) or with a history of adverse reactions to one or another of the primary therapeutic agents. In general, the non-AIDS immunosuppressed patient does better with initial therapy for *P. carinii* pneumonia than does the AIDS patient. The response is more rapid, and recurrence is relatively uncommon. Failure of a patient to respond to cotrimoxazole therapy in 5–7 days is unusual. However, little

benefit is likely to be seen if a switch to pentamidine is made before 7 days into the course of therapy. The chest radiograph may progress while oxygenation and nonspecific indicators of lung injury gradually improve. Failure to improve is more often due to other factors than it is to failure of a given antibiotic. Adding pentamidine to TMP-SMZ offers no advantage over simply switching agents, and there may be antagonism between these agents when used in combination. Patients switched from cotrimoxazole to pentamidine for reasons of therapeutic failure generally do less well than patients who are able to be treated for 2–3 weeks on either agent. Of the newer agents, there appears to be antagonism in the animal model between erythromycin and 566C80. Antibiotic resistance has not been demonstrated in *P. carinii*. However, this may reflect the limitations of the *in vitro* culture system. The clinical assessment of the patient is usually the best guide to subsequent therapy. Patients who fail to respond to antibiotics within 7 days or so are good candidates for bronchoscopy with biopsy or lavage or both to clarify the nature of their progressive pulmonary disease.

The survival of patients on anti-*Pneumocystis* therapy has improved to between 80% and 90% at most medical centers. AIDS patients receiving zidovudine (AZT) have fewer and less severe episodes of *Pneumocystis* pneumonia. However, of patients developing opportunistic infections, the fraction with *Pneumocystis* remains about the same, at 50–60%. The benefits of AZT therapy appear to diminish somewhat over time. Patients on prophylactic antibiotics may avoid recurrence of *P. carinii* pneumonia. Complications of therapy are more common in the AIDS population, including a 10-fold increase in the incidence of significant skin rash and fever. Hepatic toxicity occurs in up to 20% of these patients. Minor adverse reactions (skin rash or transient liver function test abnormalities) may be due to either component, and these are often reversed by continuing the drug at the initial or reduced levels. It is worth checking serum SMZ levels if side effects occur. Serious toxicity requires switching to an alternative regimen.

2.9. Prevention of *Pneumocystis carinii* Pneumonia in the Susceptible Host

Pioneering studies in children with hematopoietic malignancies, especially those on corticosteroid therapy, led to the development of TMP-SMZ for the prevention of *Pneumocystis* pneumonia.[71,72,119,124,147,148] The successful experience with prophylaxis at the Saint Jude's Children's Research Hospital was broadened to include immunocompromised adults and in patients with severe combined immunodeficiency syndrome (SCID) at risk for *Pneumocystis* infection.[147] The advantages of cotrimoxazole were the prevention of infection due to *Pneumocystis* as well as toxoplasmosis, *Listeria, Nocardia,* and other bacterial pathogens. The incidence of side effects was fairly modest with relatively short periods of prophylaxis. In the non-AIDS patient, prophylactic therapy should be reserved for patient populations known to have a predictably high incidence of *Pneumocystis* infection or those who have had recurrent opportunistic infections or *Pneumocystis* infections in the past. Oral administration of 5 mg/kg per day of TMP as cotrimoxazole divided into two daily doses or 150 mg/m^2 per day successfully prevents the development of *Pneumocystis* infection. Even on this modest dosage, bone marrow suppression is relatively common, but usually mild. It is equally effective to administer cotrimoxazole 3 days each week (either consecutive or alternative) with one double-strength tablet at bedtime. Lower doses of antibiotic have also been effective.[147] These doses of drug are usually well tolerated, with minimal incidence of side effects and relatively modest bone marrow suppression. Alternative regimens are occasionally necessary in non-AIDS patients requiring prophylaxis. These patients will tolerate pentamidine 300 mg by aerosol or intravenously every 3–4 weeks. In these patients, dapsone (100 mg orally a day) is also effective, as is weekly Fansidar (pyrimethamine–sulfadoxine).[149] The use of these latter two agents should be guided by knowledge of their side effects (see below).

In AIDS patients, prophylaxis against *P. carinii* is critical. The incidence of *Pneumocystis* is greatest in patients with less than 200 CD4+ lymphocytes/ml blood or less than 20% CD4+ lymphocytes total. While these guidelines are useful, the incidence of *Pneumocystis* pneumonia is also very high in patients with rapidly progressive immune deterioration, or a prior history of *Pneumocystis* infection, or both. Recent evidence suggests that while aerosolized pentamidine is still very popular, it has been less successful in preventing *Pneumocystis* infection than has TMP-SMZ. TMP-SMZ is now the agent of choice in patients who tolerate it for the prevention of *Pneumocystis* infection.[150,151] Lower-dose regimens of TMP-SMZ are generally well tolerated (in up to 75–80% of AIDS patients); potential side effects are those seen in therapy. In patients completing therapy with cotrimoxazole, prophylactic therapy should be initiated immediately to prevent sensitization to this agent.

In patients who do not tolerate cotrimoxazole, pentamidine aerosol has been very useful. This mode of drug administration has been associated with atypical presentations of *Pneumocystis* pneumonia, including extrapulmonary *Pneumocystis* infection and pneumothoraces in some patients. Successful administration of aerosolized pentamidine depends not only on the dosage and schedule of administration, but also on the nebulizer

used to create the aerosol. It has been found that health care personnel may be exposed to both pentamidine and infectious agents in the lungs of AIDS patients during the course of nebulization. The nebulizer must produce a mist of 1–3 μm droplets to be successful. Careful positioning of the patient assists in the proper distribution of this drug. A tightly closed nebulizer system and dedicated room must be considered for the safe administration of this agent to AIDS patients. Patients who are intolerant of aerosolized pentamidine may benefit from the use of bronchodilators prior to administration or may be treated intravenously. The side effects of pentamidine will develop more slowly in the aerosolized treatment group, but can still be expected to occur in patients after a total dose of 3 g. Aerosolized pentamidine also has the effect of reducing the number of organisms such that diagnosis of recurrent or of upper lobe disease is occasionally delayed based on induced sputum examination. However, this agent has proven very useful in large population studies.

Some of the longer-acting sulfa-derived agents including Fansidar and dapsone have been useful in preventing infection at relatively low cost.[149–152] Breakthrough infections have been seen with dapsone at 50 mg/day doses, and side effects are more common at the recommended 100 mg/day dose. This drug is occasionally associated with nausea, asymptomatic methemoglobinemia, and hemolytic anemia, especially in glucose-6-phosphate dehydrogenase (G6PD)-deficient patients. There is some suggestion that DDI use in the treatment of HIV infection may interfere with the absorption of dapsone from the GI tract. Fansidar administered weekly has also been effective in clinical trials. Concerns about this agent are derived from its long half-life and the occasional episode of severe hepatitis in some individuals taking Fansidar for prophylaxis against malaria. Atovaquone may also be useful for prevention of *Pneumocystis* infection, especially in patients intolerant of sulfa drugs.

Concerns about the prevention of *Pneumocystis* have also led to considerations about the possible person-to-person spread of this organism. While serologic studies have suggested that such transmission is possible, the strongest suggestion of person-to-person transmission is the increased incidence of *Pneumocystis* pneumonia in non-AIDS patients in institutions caring for AIDS patients with *Pneumocystis* infection. Patients infected with *Pneumocystis* should not share rooms with other immunocompromised patients. This problem merits study and may benefit from the ability to do epidemiological chromosomal mapping of *P. carinii* isolates.

Illustrative Case 1

The patient is a 36-year-old man with a non-Hodgkin's lymphoma treated with high-dose corticosteroids and cytotoxic chemotherapy who presented with fevers, a diffuse pulmonary process, and hypoxemia. His chest X ray [Fig. 3 (Section 2.4)] had both alveolar and interstitial infiltrates at the time of transfer from an outside hospital. The patient has been followed for a stage 1 high-grade B-cell lymphoma diagnosed 2 years prior to admission. The pathology on this tumor included cells varying from small noncleaved, non-Burkitt's cells to immunoblasts. He was initially irradiated in the left groin area, but had a recurrence in the right groin 1 year later with the same histology. At the time, his abdominal CT and bone marrow biopsy did not reveal tumor, and he started treatment with ProMACE-cyta-BOM: multiple courses of cytoxan, adriamycin, and VP 16, followed by bleomycin, vincristine, methotrexate, and cytosine arabinoside, followed by high-dose prednisone. He had completed four cycles of therapy and was on high-dose prednisone at the time of his admission to the Massachusetts General Hospital. His presentation followed the development of diffuse bilateral pulmonary infiltrates over a period of 4 days. He had been treated with oral amoxicillin–clavulanate. He developed a rapid deterioration of pulmonary function. He was markedly hypoxemic, with diffuse rales and rhonchi, fever to 102°F, with a total white blood count of 2000 (42% polymorphonuclear leukocytes).

After transfer, initial antibiotic coverage was broad; his antibiotics included cotrimoxazole (TMP-SMZ) for the presumed diagnosis of *Pneumocystis carinii* pneumonia as well as erythromycin, ticarcillin, gentamicin, and vancomycin. Induced sputum was noted to contain *P. carinii* by direct immunofluorescence. Initial blood, urine, and sputum cultures were negative for other pathogens, including CMV.

When first seen in consultation, he had completed approximately 14 days of pentamidine isethionate after 7 days of TMP-SMZ and was on a gradual steroid taper. His course had been complicated by a skin rash and fever, probably due to TMP-SMZ, and thrombocytopenia due to pentamidine. Notable in his course was that despite therapy for *Pneumocystis* for 21 days, his chest radiograph had failed to improve, with diffuse infiltrates consistent with adult respiratory distress syndrome. The effects of past chemotherapy were also considered.

It was recommended that the patient be taken for open lung biopsy due to the failure of his chest X ray and pulmonary functions to improve, and the inability to isolate further pathogens. At the same time, his anti-*Pneumocystis* therapy was stopped (other than prophylactic pentamidine, 300 mg intravenously every 3 weeks). The lung specimen grew CMV overnight via Shell vial. Pathology demonstrated an interstitial process consistent with lung toxicity due to cytoxan in addition to the CMV infection and rare *P. carinii* cysts. On the basis of these results, he was treated with ganciclovir (DHPG), at an initial dose of 450 mg intravenously every 12 hr. His steroid taper was continued. Despite some early radiologic improvements with lysis of his fevers, the chest X ray failed to improve greatly; however, his oxygenation improved markedly during therapy. He completed 21 days of antiviral therapy and was discharged home. He has received ganciclovir and dapsone prophylaxis for subsequent periods of chemotherapy. Bacterial and fungal cultures remain negative.

Comments. This case illustrates the complexity of the management of immunocompromised patients with the "febrile pneumonitis syndrome." The likelihood of significant infection rises with the amount and the duration of immune suppression. The rapid progression of *P. carinii* is typical for this infection in a non-AIDS patient. The frequency of side effects of therapy for *P. carinii* is illustrated by the leukopenia (due to cotrimoxazole) and thrombocytopenia (due to pentamidine) seen in this host. Initial management with oral amoxicillin-clavulanate in a sick, compromised patient and in the absence of microbiologic (Gram's stain) evidence for bacterial infection is worrisome. Delays in the recognition and treatment of *Pneumocystis* pneumonia contributed to this patient's progressive deterioration. The use of corticosteroids in chemotherapy proved to be a microbiologic double-edged sword. Steroid therapy is probably the main factor in the develop-

ment of infection in this host, but may decrease pulmonary inflammation and transiently improve oxygenation in the acute setting. While well studied in AIDS patients, steroid therapy for *P. carinii* pneumonia has not been examined in the heterogeneous non-AIDS population.

Failure to improve pulmonary function by day 7–10 of therapy for *P. carinii* infection should suggest additional evaluations. Congestive heart failure, pulmonary embolus, and bacterial superinfection [Fig. 5 (Section 2.5)] are common cofactors. Because antibiotic resistance has not yet been demonstrated in *P. carinii*, switching therapy for reasons other than toxicity is *not* recommended. An aggressive approach to histopathologic diagnosis is *often* required in the absence of a reasonable microbiologic diagnosis. The ability of the patient to tolerate invasive procedures is best earlier in the course of illness. In this patient, the successful treatment of *P. carinii* pneumonia was masked by CMV pneumonitis and by pulmonary toxicity due to chemotherapy. Treatment of CMV pneumonia with ganciclovir (not an FDA-approved indication) produced a rapid clinical improvement. The likelihood of recrudescence of infection due to *P. carinii* or CMV or both necessitates prophylaxis for subsequent periods of neutropenia and corticosteroid therapy.

3. *Toxoplasma gondii*

Serologic evidence suggests that up to 70% of all individuals are exposed to *Toxoplasma gondii* at some point during their lives. Thus, it should be considered surprising that *Toxoplasma* causes significant infection only infrequently outside the immunocompromised host. In immunocompromised adults and in fetuses, this relatively benign organism causes significant morbidity. Toxoplasmosis represents a spectrum of diseases caused by infection with *T. gondii*. The presence or absence of the organism in tissues ("infection") is not indicative of clinical disease ("toxoplasmosis") in the absence of the appropriate clinical presentation. Acute infection in the immunocompromised individual requires prompt diagnosis and therapy to avoid significant injury. The important role of infection of the central nervous system (CNS) by this organism complicates both the diagnosis and the treatment of toxoplasmosis. This problem is further compounded by the lack of reliable and reproducible serologic tests for use in immunocompromised individuals.

Since its description in the gondii, a rodent from North Africa, and in rabbits, it has become apparent that there are strain differences among *T. gondii* isolates from various regions of the world.[153,154]

3.1. The Organism

3.1.1. Life Cycle

Toxoplasma gondii is an obligate intracellular protozoan of the order Coccidia. The multiple developmental stages of *T. gondii* are relevant to the clinical manifestations of toxoplasmosis (Fig. 7). Oocysts initiate the life cycle of *T. gondii* and are produced only in the intestines of members of the cat family. This form of organism is oval in shape, measuring 10–15 μm in diameter, and is produced in the small intestine following both asexual (schizogeny) and sexual (gametogeny) reproduction.

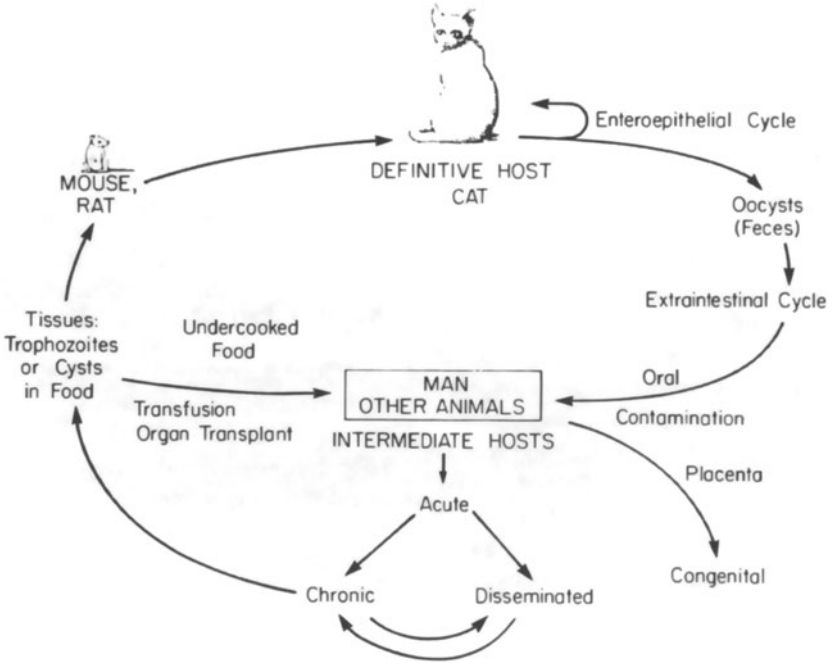

FIGURE 7. Life cycle of *Toxoplasma gondii*.

Self-propagating infection (the enteroepithelial cycle) within the intestinal epithelium generates millions of oocysts per day in cat feces. Fecal oocysts will survive for over a year in moist soil. These oocysts undergo sporulation over 2–3 days after excretion and become infective. This form can be ingested directly by humans (fecal–oral route) to initiate infection in the human (intermediate) host. Infected oocysts are killed by boiling or adequately cooking (over 65°C) meats or vegetables.

Proteolytic disruption of oocysts or of tissue cysts occurs after ingestion. The released sporozoites are motile and penetrate nucleated cells throughout the body via direct invasion of local intestinal epithelial cells or to distant sites via the bloodstream and lymphatics. Sporozoites mature into trophozoites within vacuoles of reticuloendothelial cells and of other tissues.[155] Trophozoites are 4–8 μm crescentic nucleated organisms that move by body flexion without a flagellum. These trophozoites multiply within a cell by internal division (endodyogeny) until the cell ruptures, initiating a new cycle of invasion.[155] In the face of an immune response and for unclear reasons, infectious spread may be interrupted by the development of tissue cysts. These cysts are large (up to 200 μm) and contain thousands of slowly dividing, relatively inactive trophozoites (sometimes called "bradyzoites"). These cysts act as a reservoir of infection throughout the life of the host. Rupture of cysts due to ingestion of undercooked meats or due to immunosuppression of the host initiates another cycle of infection. Tissue cysts can be disrupted by freezing and thawing or by cooking. Bradyzoites are killed by heat and by normal gastric secretions.

3.1.2. Epidemiology

The infected cat produces millions of oocysts each day for a period of up to 3 weeks. The organism has been found in most animal species that consume either plants or meat. Up to 25% of lamb and pork have been shown to contain tissue cysts. Uncommonly, *Toxoplasma* has been isolated from beef, goat's milk, and eggs, as well as from vegetables consumed by seropositive vegetarians. *Toxoplasma gondii* is found worldwide. Seropositivity increases with age.[153] Infectious oocysts may be carried by coprophagous organisms including cockroaches, flies, worms, and snails. Significant infection has occurred in laboratory workers who have become inoculated with organisms. *Toxoplasma* has prolonged survival in refrigerated blood samples and may be transmitted by transfusion of either whole blood or white blood cells.[156] Transmission of toxoplasmosis has occurred in the setting of organ transplantation into a seronegative recipient as well as by reactivation of latent disease by immune suppression.[157] There are multiple subpopulations of *Toxoplasma* that vary in virulence and may have differing developmental characteristics, including a predilection for different organ systems. The determinants of specific organ involvement are unclear. Parasitization is most common in the brain, heart, lungs, pericardium, and lymphoid tissues.[158] Trophozoites and cysts coexist during active infection, and tissue cysts persist after the clearance of an acute infection.

The incidence and virulence of *Toxoplasma* infections vary by region. In Europe, seroprevalence approaches 75%, while estimates in the United States vary from 5% to 40%. Clinically apparent infection occurs in up to 30% of *T. gondii*–seropositive, HIV-infected individuals. While patients on maintenance therapy for toxoplasmosis do not develop *Pneumocystis* pneumonia, cotrimoxazole prophylaxis is incompletely protective against activation of *T. gondii*.

T lymphocytes mediate much immunity to *T. gondii*, largely via the mechanism of macrophage activation.[159,160] Without specific activation, *Toxoplasma* blocks the fusion and acidification of phagolysomes. Agents that interfere with either T-cell or monocyte function (steroids, HIV, antithymocyte globulin, lymphoma) predispose to toxoplasmosis.[159,161–174] Stimulation of monocytes (IFN, interleukin-2, CSFs) by cytokines may enhance killing of *T. gondii*.[159,173] In the non-AIDS patient, the majority of cases of toxoplasmosis occur in patients with hematopoietic malignancy (lymphoma, leukemia) under chemotherapy (especially regimens including corticosteroids) and in organ transplantation recipients, notably of heart and bone marrow.[167,168,175–179] The predilection for CNS involvement suggests that immune clearance of *T. gondii* is less effective in the CNS. The roles of antibody and complement in the killing clearance of *T. gondii* remain uncertain, although the combination can kill extracellular trophozoites. The development of T- and B-cell immunity to *T. gondii* coincides with the clearance of extraneural organisms, the regression of tissue invasion, and the development of tissue cysts.

3.2. The Patient

Toxoplasmosis occurs with four distinct clinical presentations: (1) congenital, (2) acquired in immunocompetent individuals, (3) disseminated in immunocompromised individuals, and (4) as reactivation of latent infection within the eye (ocular toxoplasmosis).

3.2.1. Congenital Toxoplasmosis

Congenital infections result from acute infections during pregnancy. They are usually asymptomatic infec-

tions occurring in immunocompetent individuals. However, infected women who become immunocompromised may reactivate infection and transmit the organism to the fetus. Women who are infected and seroconvert prior to conception have a low risk of transmission to the fetus. The incidence of fetal infection resulting in abortion (stillbirth) or in significant congenital disease rises during the course of pregnancy: from 25% in the first trimester to two thirds of third-trimester fetuses. While the incidence of infection is quite high, the majority of infants infected during the latter trimesters do not show signs of infection, and treatment of the mother with specific antibiotics significantly reduces the incidence of congenital infection (by over 50%).

The primary manifestations of congenital disease are of CNS disease, particularly chorioretinitis or hydrocephalus. Sequelae of infection may not be immediately evident at birth. Children may have blindness, psychomotor or mental retardation, jaundice, thrombocytopenia, anemia, encephalitis, microcephaly, hypothermia, or pneumonitis. Most of these congenital malformations are not specific to *T. gondii,* but are related to inflammation and scarring in the sites of greatest infection. Mild disease is seen restricted to hepatosplenomegaly or lymphadenopathy. These individuals may ultimately develop CNS disease. It is currently thought that most children infected *in utero* will develop *some* disease related to congenital toxoplasmosis.

3.2.2. Acquired Toxoplasmosis in Immunocompetent Individuals

Over 80% of individuals with *Toxoplasma* infection will be asymptomatic. Alternatively, toxoplasma will present with nontender lymphadenopathy affecting cervical lymph nodes or systemic lymph glands.[180–184] In this setting, toxoplasmosis may be confused with a "flu-like" syndrome or infectious mononucleosis. Atypical features such as those seen in lymphoma may include fever, hepatosplenomegaly, atypical lymphocytosis, sweats, muscle aches, sore throat, and maculopapular rashes. The lymph nodes may be tender, but rarely become fluctuant without superinfection. Rarely, chorioretinitis may occur in acute infection. While the clinical course is relatively benign, symptoms may persist for up to a year. Fluctuating adenopathy and persistence of lymphadenopathy is occasionally seen. More severe disease involving the heart, lungs, and CNS is rarely observed.[185] Because of the broad differential diagnosis of lymphadenopathy syndromes, the diagnosis of toxoplasmosis is dependent on serologic testing or the identification of organisms. Occasionally, lymphadenopathy is not significant in the setting of multisystem involvement including hepatitis, myositis, or fever of unknown etiology.

3.2.3. Ocular Toxoplasmosis

Toxoplasma gondii is a common cause of chorioretinitis throughout the world, usually as a result of reactivation of latent congenital infection.[169,170,186] This form of infection occurs in immunologically normal young adults, affecting the retina and underlying choroid and presenting clinically as unilateral or bilateral chorioretinitis. Congenital ocular toxoplasmosis may cause developmental abnormalities of the optic neuraxis, including such common manifestations as strabismus, cataracts, nystagmus, or optic neuritis. The characteristic lesion on funduscopic exam is a cluster of areas of focal necrotizing retinitis, giving a white-to-yellow raised cotton patch with blurred margins. Healing lesions are initially pale and gradually take on black pigment. Occasionally, associated acute panuveitis, papillitis, or optic nerve atrophy may occur. Recurrent disease is common (up to 30% after specific chemotherapy), often in areas of scars from previous infection. Ocular toxoplasmosis usually occurs in the absence of systemic symptoms. Bilateral infection is characteristic of congenital disease, while acute disease is usually unilateral. Distinctive characteristics of ocular toxoplasmosis include the clarity of vitreous and aqueous humors, the presence of bilateral macular involvement, and the appearance of a normal retinal exam in the presence of focal areas of retinal degeneration.

3.2.4. Toxoplasmosis in Immunocompromised Hosts

The true incidence of toxoplasmosis in immunocompromised hosts is unclear. Toxoplasmosis of the immunocompromised individual is usually due to the reactivation of latent infection in the absence of a limiting immune response. Acute infection in this population generally results from organ transplantation or the direct infusion of contaminated blood or blood products.[171,175,177,187–192] The occurrence of toxoplasmosis in a seronegative individual in the absence of organ or blood transfusion is uncommon. Blood products from patients with chronic myelogenous leukemia (CML) tend to maintain high levels of parasitemia despite high antibody titers. Because of the survival of organisms in stored citrated blood for up to 2 months at 4°C, patients with CML should not be used for blood or organ donation. The serologic status of organ donors should be assessed prior to transplantation.

Because of the large number of protean manifestations of toxoplasmosis, this infection must be considered in the differential diagnosis of many systemic illnesses in the immunocompromised host. The non-AIDS immunosuppressed patient is more likely to have some of the

systemic manifestations of toxoplasmosis than are seen in the immunocompetent host. A "mono"-like prodrome with fever and lymphadenopathy may precede other manifestations. In this setting, disseminated infection will involve the brain, liver, bone marrow, heart, omentum, spleen, and other organs. Multiple brain lesions are common. The presentation of toxoplasmosis in the immunodeficient host is often that of encephalitis, with diffuse CNS impairment or multiple focal neurologic deficits.[186] Some 10% (United States) to 25% (Europe) of acutely infected immunocompromised individuals will have a neurologic presentation (see below). Pulmonary infection may also be significant.[193,194] The clinical onset of pneumonia is associated with fever, dyspnea, cough, and occasionally hemoptysis. Chest X rays reveal a diffuse bilateral pulmonary infiltrate with atypical features. In this setting, it should be possible to isolate organisms from lung washings.[195] In the organ transplant recipient, a similar pattern emerges, with the exception that the disease may be due to dissemination from the transplanted organ.[157,168] Recipients who develop infections are generally seronegative. This form of infection is seen most often in cardiac transplantation, given the predilection of the organism for the myocardium. Identification of organisms, including tissue cysts, in a seronegative individual should raise the specter of acute disseminated disease.

In AIDS patients, as in most immunocompromised individuals, toxoplasmosis is usually disseminated at the time of presentation.[196,197]

In the AIDS population, lymphadenopathy is commonly due to HIV; lymph node biopsies generally are not diagnostic. The occurrence of disseminated *Toxoplasma* infection is usually correlated with low circulating CD4+ lymphocyte levels (<50). *Toxoplasma* encephalitis is a common presentation for patients with AIDS. Between 5% and 50% of seropositive HIV-infected individuals may develop CNS toxoplasmosis, based on autopsy studies. AIDS patients from endemic areas due to consumption of undercooked pork or lamb or contaminated vegetables are at particular risk. In patients from such areas (e.g., Haiti, much of Africa), *T. gondii* infection is a commonly diagnosed opportunistic infection in individuals with AIDS. Signs of meningitis are uncommon, while seizures, confusion, depression, visual changes, and hydrocephalus are more common.

The CNS lesions of toxoplasmosis must be distinguished from other infections or tumor (Fig. 8).[175,197–205] The infection can be either focal abscesses or diffuse meningoencephalitis, based on the distribution of the preexisting latent lesions. Acute, primary toxoplasmosis has also been reported. The presentation can be as mild as slightly altered mental status, minor neurologic signs, or fever with headache. Seizures and gross motor deficits may occur in up to 10% of individuals. The progression of *Toxoplasma*-induced mental status changes is subacute when compared to that due to HIV alone. HIV leukoencephalopathy or progressive multifocal leukoencephalopathy (PML) forms part of the "AIDS dementia complex," which can also present with focal or

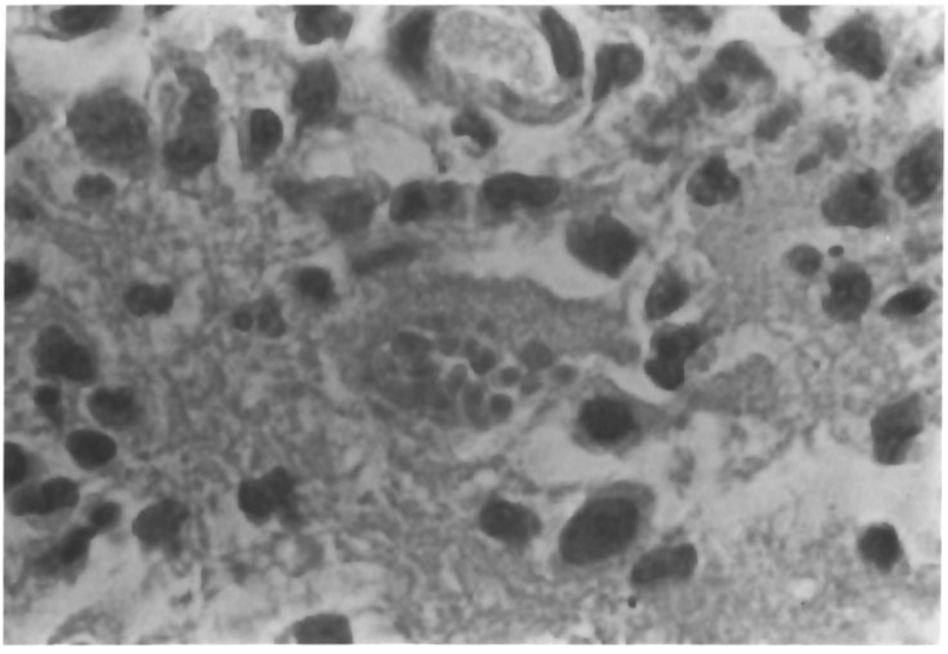

FIGURE 8. Hematoxylin–eosin stain of a brain biopsy specimen from the edge of a large anterior brain abscess from a patient with AIDS. A tissue cyst contains multiple intracystic bodies or bradyzoites consistent with a diagnosis of toxoplasmosis.

global CNS deterioration and may coexist with acute infections of the brain. Some of our patients have presented with multiple organisms in a single small brain abscess, including *T. gondii* with anaerobic bacteria, *Aspergillus* species, and mycobacteria, including tuberculosis and *M. avium* complex. Because serologic testing in immunodeficient patients often is not helpful in establishing a diagnosis of toxoplasmosis, biopsies of infected areas may be required to confirm a diagnosis.

3.3. Diagnosis

3.3.1. Laboratory Evaluation for *Toxoplasma gondii*

The diagnosis of infection due to *Toxoplasma* is difficult, most notably in the immunocompromised individual, in whom early diagnosis is most important and serologic tests are least helpful.[206] Demonstration of the tissue cyst form of the organism or of a positive serum IgG level suggests the possibility of infection due to *T. gondii,* but does not prove toxoplasmosis. Tissue cysts persist in the brain, lung, liver, lymph node, heart, and spleen for years after acute infection. The presence of trophozoites in lymph node, blood, brain, cerebrospinal fluid (CSF), or other tissues during acute infection, or the demonstration of an acute (IgM) immune response to the organism, is needed for confirmation.

Brain disease features prominently in the presentation of immunocompromised patients with toxoplasmosis (Figs. 8 and 9).[202] Decisions about the management of suspected toxoplasmosis involving the brain are complicated by the risk of performing definitive diagnostic procedures, including lesion aspirates or open brain biopsies, and the potential for toxicity of antibiotics (notably pyrimethamine or sulfa), which approaches 50% with the antibiotics commonly used in AIDS patients. The lumbar puncture is nondiagnostic in most cases. The CSF will have slightly elevated protein levels and few white blood cells. These patients will have a normal to slightly decreased glucose concentration in the CSF; hypoglycorrachia is usually associated with the rupture of organisms directly into the CSF. Elevations in antibodies to *T. gondii* in the CSF are highly suggestive of acute infection.

Empiric therapeutic trials are frequently the best approach in stable patients. In the presence of rapidly progressive brain lesions, clinical deterioration, lesions unresponsive to empiric therapy, or serologic testing not consistent with the clinical picture, a brain biopsy may allow the definitive separation of toxoplasmosis from other processes. Biopsy of brain lesions has the added advantage of identifying other potentially treatable processes. In addition to meningoencephalitis, encephalomyelitis, or mass effect due to brain abscess, the patient may present with pneumonitis, myocarditis, or signs of hepatitis. *Toxoplasma gondii* trophozoites are occasionally found in lung lavage or lung biopsy sample from patients with pneumonitis.

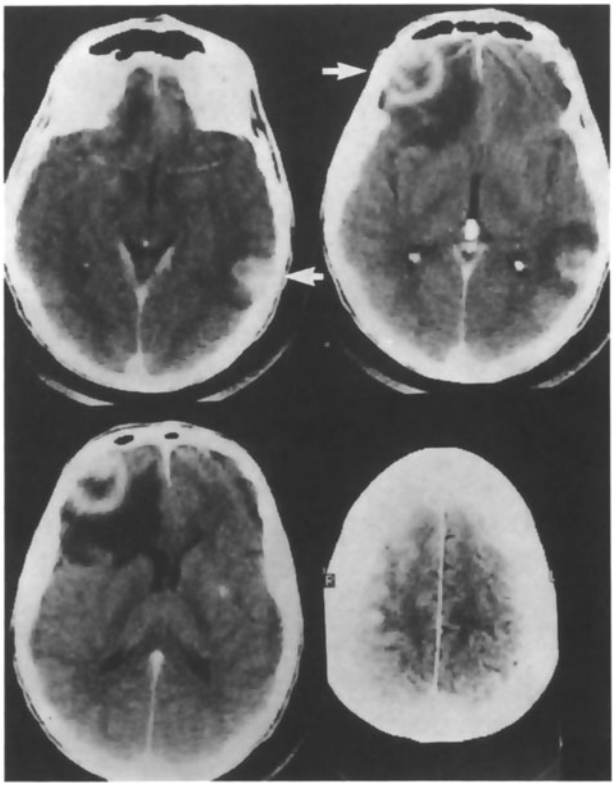

FIGURE 9. CT scan from the patient in Fig. 8 reveals multiple and bilateral contrast-enhancing brain abscesses surrounded by edema. This presentation is seen in *T. gondii* infection more often than the large solitary lesions often associated with CNS lymphoma.

3.3.2. Radiology

Radiologic evaluation will generally begin with a head CT scan and chest radiograph. The chest radiograph is nonspecific, the picture being that of prominent hilar lymphadenopathy with diffuse interstitial infiltrates. Like other atypical pneumonias, atypical patterns including nodules and assymmetric patches are common. Cavitation or pleural effusions are uncommon. Chest radiographs do not tend to improve over a 14-day course of therapy unless corticosteroid therapy is employed.

In AIDS patients, the presence of multiple and bilateral intraparenchymal lesions that are contrast-enhancing on CT scans is often considered diagnostic of CNS toxoplasmosis (Fig. 9).[207,208] CT studies of AIDS patients with solitary CNS lesions show that about half will be due to *T. gondii,* with the balance being lymphoma, progressive multifocal leukoencephalopathy

(PML), and, less commonly, other infectious or malignant processes.[208] Non-AIDS patients will also have multiple CNS lesions, but the differential diagnosis must be broadened and invasive diagnosis considered earlier in the course. Up to 20% of patients will have single lesions without contrast enhancement. Occasionally, uptake of contrast dye may be delayed. Repeat scans after the administration of increased doses of contrast dye may demonstrate lesions not previously observed on routine scanning. CT scans without contrast are usually negative. The majority of lesions are nodular or ring-enhancing and have a predilection for the basal ganglia and the gray–white junction. Most lesions, especially those of the posterior fossa, are better seen on MRI scan, which may reveal multiple, bilateral, small lesions not seen by CT. Often seen are large focal abscesses that cannot be distinguished from necrotic tumor or abscesses due to other pathogens. Serologic testing, when positive, is most useful in this latter group. Radiologic evidence of encephalitis is seen less frequently.

3.3.3. Histopathology and Culture

The identification of *T. gondii* in clinical specimens is difficult. In general, organisms are not found in body fluids; they may be found in cytology specimens from lung washings or CSF or as "contaminants" of viral tissue cultures.[209,210] Impression smears of the tissues can be stained with Giemsa stain to demonstrate both the cyst and trophozoite forms. Tissue cyst walls are stained by PAS stain (see Fig. 8). Trophozoites are stained with either Wright or Giemsa stains and by fluorescent or peroxidase-tagged antibody to *T. gondii* on histologic sections. The presence of multiple tissue-cyst forms in areas of acute tissue inflammation in the absence of other pathogens may be used as presumptive evidence for the presence of acute infection. Tissues or fluids inoculated into mice have also been used for detection of *T. gondii* infection. This method is not practical for routine clinical diagnosis. Further, mice generally do not die from human *Toxoplasma* infection; serologic testing and pathology are generally necessary to confirm the diagnosis of toxoplasmosis using this system. Tissue culture methods have been improved with the identification of plaques in cell culture monoloayers and are more generally available. Growth of *T. gondii* from blood in tissue culture can be considered evidence of disseminated infection. Isolation of viable organisms from tissue does not assist in determining the acuteness of infection. *Toxoplasma gondii* organisms are uncommonly found in lymph nodes outside acute infection, but may be found in other tissues for months after therapy. The identification of organisms in fetal tissue or from the placenta is diagnostic of congenital infection. The detection of *Toxoplasma* in infected tissues may be improved by immunostaining amplification using peroxidase–antiperoxidase, fluorescent antibody, or ELISAs using unfixed tissues. All have proven useful clinically.

In the normal individual, the histopathologic changes seen in the lymph nodes are often diagnostic.[181,183,211] Epithelioid histiocytes appear in clusters, monocytes infiltrate the sinuses, and reactive hyperplasia is observed. Organisms and giant cells are rarely seen. In the eye, retinitis with necrosis, vascular proliferation, and granulomata are seen. Myositis may be seen in any muscle, but is most prominent in the heart. Diffuse patches of mononuclear infiltration may occur in the presence or absence of organisms. Diffuse meningoencephalitis will produce multiple areas of necrosis, particularly of the gray matter and microglial nodules. Organisms are found within blood vessel walls, surrounding areas of necrosis, and in normal tissues. Periventricular lesions are more often seen in infants, accounting for a high incidence of hydrocephalus. In the immunocompromised host, many small areas of coagulation necrosis may be seen; large abscesses occur in some patients.

In contrast to the lesions produced in other organs, *T. gondii* usually evokes diffuse disease in the lungs. This result may reflect the role played by the lungs as a filter for circulating organisms and the extensive resident phagocyte population.[193,194] The pneumonitis is predominantly interstitial and characterized by an infiltrate of macrophages, lymphocytes, plasma cells, and, occasionally, polymorphonuclear leukocytes. The local inflammatory response is usually mild. Areas of bronchopneumonia, endarteritis, and necrosis may develop. Discrete areas of interstitial infiltration may progress to areas of consolidation with necrosis or infarction. Proliferating stages of *T. gondii* are seen inside alveolar macrophages and in the alveolar space. With active myocarditis, organisms can also be found in the endocardium. Death may result from hepatic necrosis, cardiomyopathy, or secondary pulmonary edema. As in the brain, toxoplasmosis may occur in the setting of other opportunistic infections including *P. carinii* pneumonia, bacterial pneumonia, or fungal abscess.

3.3.4. Immunologic Diagnosis

Skin testing for delayed hypersensitivity is not useful in establishing the diagnosis of acute toxoplasmosis. Positive skin tests may not develop for months after acute infection and may never develop in immunocompromised patients, including those with AIDS. Skin testing may provide an alternative to serologic testing for population screening. Similarly, lymphocyte transformation using patients' cells stimulated with *Tox-*

TABLE 7. Serologic Assays for *Toxoplasma gondii* Infection[a]

Test	Titer		Comments
	Acute	Chronic	
Sabin–Feldman dye test (IgG)	≥1:1000	1:4–1:2000	Remains elevated; onset 1–2 weeks
Indirect flourescent antibody (IFA)-IgG[b]	≥1:1000	1:4–1:2000	Remains elevated; onset 2–3 weeks; some false-positives[b]
IFA-IgM[b]	≥1:64	0–1:20	Negative in months; first positive acutely (1 week)
Direct agglutination test (IgG)	≥1:1000 to ≥1:20	≥1:64,000	Remains elevated; BME[c] to block IgM agglutination
Indirect hemagglutination (IHA) (IgG)	≥1:1000	1:16–1:256	Remains elevated; delayed onset
Complement fixation (IgG)	≥1:32	0–1:8	Remains elevated; onset 2–3 weeks
ELISA[b]–double sandwich–IgM	≥1:256 (or ≥1.7 units)	0–1:256 (1.7–3.0)	Remains elevated; early onset; sensitive
Immunosorbent (IgM) (latex bead)	Positive	Positive	Simple, sensitive (vs. IFA); fewer false-positives

[a]A 2-tube or four-fold rise in titer to "acute" level is diagnostic for any test. Positive/diagnostic values for the various tests will vary among clinical laboratories, and some patients will fall outside these ranges. These represent adult values.
[b]May give a false-positive value in the presence of rheumatoid, antinuclear, or other autoantibodies; a single high titer is diagnostic of acute infection.
[c]BME, β-mercaptoethanol.

oplasma antigens is also an indicator of previous exposure to *T. gondii*. Inversion of the T-lymphocyte helper/suppressor ratio (an excess of suppressor T cells) is occasionally observed in the presence of acute toxoplasmosis. Such an inversion also occurs in the presence of viral infection, including HIV and CMV, and in a variety of other conditions. The demonstration of circulating antigen from *T. gondii* in sera is not routinely available, but may be helpful in establishing the acuteness of exposure.[212,213]

3.3.4a. Antibody Detection Tests. In the immunologically intact individual, serologic testing is diagnostic for *T. gondii* infection (Table 7).[184,214] The immunocompromised host will often fail to generate a specific antibody response to acute infection, or this response will be much delayed. In the patient receiving immunosuppressive therapy, false-positive serologic tests may occur in the setting of organ transplantation into seropositive organ recipients. These include both IgM and IgG antibodies. The absence of diagnostic serology may increase the need for a tissue diagnosis in the immunocompromised host. In the immunodeficient individual, the presence of a positive test is still of clinical importance. Because much of acute toxoplasmosis occurs in seropositive individuals, the value of serologic diagnosis in the absence of an elevation of the serum IgM titer is questionable. However, conversion from seronegative to seropositive or the presence of a fourfold rise in titer can be taken as indicative of acute toxoplasmosis for most of the tests currently available. The currently available tests are summarized in Table 7. The presence of a differential concentration of specific antibodies in CSF when compared with serum may be used to indicate the presence of primary infection of the CNS.[186,215,216]

Specific diagnostic tests merit comment. Positive values must be established for each laboratory. The standard serologic assay is the Sabin–Feldman dye test, which can be standardized to reference sera available from the World Health Organization.[214,217] Like most of the tests that measure primarily IgG antibodies, positive tests do not occur until after 2 weeks of infection, with peak titers occurring at times up to 2 months. While titers decline over 1–2 years, low titers persist for life. As with all serologic tests, the titer of antibody does not correlate with the severity of illness, but may provide information about the ability of the host to respond to new antigenic challenge. Because immunity to toxoplasmosis is largely T-cell-mediated, the presence or absence of antibody will not determine the ability of the host to respond to acute infection. The IgM titer correlates most closely with acute infection; a number of tests have been developed for this purpose.[218,219] The direct agglutination test using either fixed whole trophozoites or antigen-coated latex particles, the IgM immunofluorescent antibody test, and the conventional IgM ELISA all give false-positive results in the case of high endogenous levels of nonspecific IgM, rheumatoid factor, and antinuclear antibodies.[220–223] The double-sandwich IgM ELISA and IgM immunosorbent assay using solid phase do not suffer from such false-positive results and have much greater sensitivity than the alternatives.[218,222–224] In the immunocompetent individual, acute infection should be accompanied by seroconversion from negative to positive or by the demonstration of a fourfold (2-tube) rise from a low chronic titer to a high

acute titer in sera drawn at least 3 weeks apart and run simultaneously. If initial sera are drawn too late in the course of infection, such diagnostic increases may be missed.

3.3.4b. Diagnosis in the Immunodeficient Patient. In the immunodeficient host, rapid progression of disease or atypical presentations may necessitate tissue diagnosis.[225] Many patients will fail to demonstrate a rise in IgM serum titer. In AIDS patients, there may be some advantage to the solid-phase IgM immunosorbent assay that is available commercially. The failure of serologic testing points out the importance of obtaining baseline titers of *Toxoplasma* antibodies in AIDS or organ transplant patients. Demonstration of relatively enhanced antibody production in the CSF has been associated with *Toxoplasma* encephalitis. Local antibody production should be associated with a specific antibody level (as a fraction of total local IgG) greater than that fraction of specific antibody present in serum (as a fraction of total serum IgG). A similar concept has been applied to the diagnosis of ocular toxoplasmosis, in which serum titers are typically low. In some hosts, notably in newborns and in recent organ transplant (especially cardiac) recipients, elevated titers of anti-*Toxoplasma* antibody may be normal. IgG antibody titers in newborns may reflect maternal antibody titers. In congenital infection, the child may not produce specific antibodies before 2–9 months of age. Serial tests may be helpful in this situation. Cardiac and heart–lung transplant recipients may demonstrate significant titers even without acute infection. Elevated CSF IgM levels should always be taken as indication of possible brain involvement.

3.4. Therapy of *Toxoplasma gondii* Infection

Initial therapy for *T. gondii* infection should include a reduction in the immunosuppressive therapy, when possible. Cellular immunity is needed to eradicate intracellular organisms. Extracellular organisms are killed by antibody in conjunction with complement, while cyst forms are largely resistant to antibiotic therapy. Empiric therapy should be initiated in patients with significant clinical disease who are at risk for disseminated toxoplasmosis. Such conditions include CNS, pericardial, pulmonary, hepatic, splenic, or blood-borne disease in patients with AIDS, high-dose corticosteroid or cyclosporine therapy, bone marrow transplantation recipients, or acute hematopoietic malignancy.[206,209,225–229]

Antibiotic therapy is outlined in Table 8. A synergistic combination of antibiotics including pyrimethamine and sulfonamide or clindamycin is favored for *Toxoplasma* infection in all patients. Recent data in AIDS patients suggest that oral clindamycin (600 mg PO q6h) may be as effective as high-dose intravenous drug (i.e., 1200 mg IV q6h).[227–231] The most common sulfonamide used is sulfadiazine, but it is interchangeable with trisulfapyrimidine for this purpose.[228] Other sulfonamides are not equally effective.

Non-AIDS immunodeficient patients do better in general than AIDS patients suffering from disseminated toxoplasmosis. The main limitation of therapy in AIDS is drug toxicity, which occurs in at least half of individuals. Discontinuation of therapy is generally associated with a relapse of brain disease. Our own experience has supported lifelong therapy in AIDS patients. Relapse has been observed during prophylactic therapy with pyrimethamine, trimethoprim–sulfamethoxazole, and spi-

TABLE 8. Antibiotic Therapy for *Toxoplasma gondii* Infections[a]

Drug[b]	Dose	Duration	Comments
Pyrimethamine	100 mg PO × 2	Load	Bone marrow suppression: May give folinic acid 5 mg
and	Then: 25–50 mg PO qd or qod	3–6 weeks	PO/IM qod except in leukemia
Sulfonamide	Sulfadiazine 4 g PO		
	Then: 1–1.5 g PO qid	3–6 weeks	Decrease dose for neutropenia; sulfa allergy common
or	*or*		
	Trisulfapyrimidine		
	75–100 mg/kg/day		
Spiramycin	1 g PO tid or qid	3–6 weeks	In pregnancy or sulfa allergy with pyrimethamine; CNS data limited
or			
Clindamycin	600–1200 mg IV q6h	3–6 weeks	Slower resolution than with sulfa; *C. difficile* colitis
	or		
	600 mg PO q6h		

[a]Active infection: Twice-weekly blood counts are necessary to detect bone marrow suppression due to therapy. Lifelong prophylaxis after therapy for acute infection is recommended in AIDS patients. See Section 3.7 for preferred therapies.
[b]Investigational: trimetrexate, BW566c80 (atavaquone), macrolides, IFN-γ.

ramycin.[206,231,232] Higher-dose pyrimethamine (50–75 mg/day) has been used in some AIDS and transplant patients for therapy and prophylaxis to increase serum levels of antibiotic. In general, folinic acid supplementation is needed. Newer therapies that have promise and are under study include atovaquone, trimetrexate, and the new macrolides roxithromycin, azithromycin, and clarithromycin in combination with sulfonamide or pyrimethamine.[233,234] These latter agents have the advantage of excellent tissue penetration in excess of serum levels. Atovaquone may also have some activity against tissue cysts. In AIDS patients with toxoplasmosis, some early relapses have been seen with the macrolide regimens.[232] In combination with pyrimethamine, skin rash (38%), GI (38%), liver function test (77%), or hematologic (54%) toxicities are seen. Success is directly proportional to the penetration of the agent into the CNS and the patient's ability to tolerate drug side effects. Bone marrow suppression and skin rashes are common. In the setting of other immunosuppressive therapies or antiviral therapies, some patients will tolerate slightly reduced sulfonamide doses or use of another sulfonamide preparation in therapy or prophylaxis, rather than its being necessary to discontinue an agent that is causing minor side effects. While the use of corticosteroids may reduce mass effect and brain edema in the acute phase, it is not clear that there is a role for these agents in the long-term management of toxoplasmosis.[170] Immune modulators, including IFN-γ, may be useful as adjuncts to antibiotics in clearing intracellular organisms.

Therapy should be adjusted to the underlying immune disorder.[225] For most immunodeficient patients, pyrimethamine is given for up to 6 weeks at 25 or 50 mg/day. In AIDS patients, 50–100 mg/day is preferred. In most patients, this drug will induce bone marrow suppression, which may be relieved by calcium leucovorin. Some patients will experience an altered taste sensation, headaches, or GI upset while taking pyrimethamine. The sulfa drugs are given at a daily dose of up to 8 g after a loading dose of 4 g. The patient must be well hydrated to prevent crystalluria. Alternative therapies are less toxic and less active.[235] AIDS patients and some organ transplant recipients may require prolonged prophylactic therapy.[206] Most of the AIDS patients at risk for relapse of toxoplasmosis are also at risk for other infections, including *P. carinii* pneumonia. Seronegative recipients of heart transplants from seropositive donors should receive 6–8 weeks of pyrimethamine (50 mg/day) with sulfadiazine (2–4 g/day) or clindamycin (1200–1800 mg/day).[178,236] Like *Toxoplasma, Pneumocystis* can be prevented by the chronic use of a regimen containing sulfa drug.[228] The use of pyrimethamine (50 mg/day) and sulfadiazine (2 g/day) successfully prevents both diseases. Given the long serum half-life of pyrimethamine, a regimen of 3 days a week is generally adequate for the prevention of disease. Alternatively, Fansidar can be given at a dose of 1 or 2 tablets per week in many patients. The incidence of toxoplasmosis is reduced in AIDS patients receiving cotrimoxazole prophylaxis. Breakthrough infection has occurred during therapy with this antibiotic combination.

Illustrative Case 2

A 37-year-old man was brought to the Massachusetts General Hospital Emergency Ward because of progressive inability to care for himself. The patient was a homosexual whose single sexual partner had died of AIDS 15 months previously. At that time, the patient was serum HIV-negative by ELISA. By history, the patient had been well until 3 months prior to admission, holding full-time employment as a computer programmer. He had complained of some decreased "ability to concentrate" and of mild fatigue. These symptoms were attributed by the patient to depression following the anniversary of his partner's death. Two weeks before presentation, he had seen his personal physician for a general examination. This physician had detected mild diffuse and nontender lymphadenopathy and noted that the patient seemed "tired" but otherwise normal. A serum HIV screening test had been positive by ELISA and confirmed by "Western blot"; the patient had not yet been notified. A serologic test for toxoplasmosis revealed a positive IgG titer (1 : 1024) and a negative IgM titer. On the day of admission, the patient had not appeared at work and had been found comfortable but somewhat confused at home. He had no pets and no other known infectious exposures.

On examination, the patient was thin, but in no acute distress. He complained of a mild headache, but denied fevers, photophobia, or meningismus. On neurologic examination, the patient's short-term memory was impaired, and he forgot the ends of some sentences. His speech was fluent. He was not able to recognize some objects or written words. His general examination revealed mild lymphadenopathy and a palpable spleen tip, but was otherwise unremarkable. Laboratory evaluation revealed a total white blood cell count of 2300 with a normal differential and 137 CD4+ lymphocytes. His hematology, chemistries, and chest radiograph were otherwise within normal limits. A head CT scan was performed that demonstrated multiple (at least three), bilateral, contrast-enhancing, ring-shaped lesions with surrounding edema (Fig. 9). A lymph node biopsy revealed only reactive hyperplasia.

On the basis of the presumed diagnosis of toxoplasmosis, the patient was treated empirically with pyrimethamine and sulfadiazine and switched to clindamycin and pyrimethamine when a drug rash developed on day 5 of therapy. Because of progressive neurologic deterioration in the absence of improvement by CT scan, a stereotactic brain biopsy of one of the brain lesions was performed. *Toxoplasma gondii* was demonstrated (Fig. 8). On antibiotics, the patient improved only slightly over 6 weeks of therapy. A repeat CT scan revealed some improvement in some of the lesions, but a large frontal lesion remained unchanged. Biopsy of the anterior lesion revealed B-cell lymphoma. This tumor progressed rapidly despite therapy. The patient died 3 months later.

Comment. The presentation of toxoplasmosis of the CNS can be subtle. The HIV-positive patient is often well-appearing, without fever or headache. Up to 60% will have focal neurologic deficits, fever, altered mental status, or seizures. The geographic distribution of infection varies with the incidence of *T. gondii* infection in the general population. While toxoplasmosis is an uncommon presenting mani-

festation of AIDS in the United States (3–5%), up to a third of seropositive AIDS patients will eventually develop disease without prophylaxis. The clinical presentation of toxoplasmosis in the AIDS patient is often indistinguishable from "HIV-encephalitis," CNS lymphoma, or progressive multifocal leukoencephalopathy (PML).

The CT scan is often used diagnostically for *T. gondii* infection. The presence of multiple and bilateral contrast-enhancing (nodular or ring) lesions by CT or MRI scan is highly correlated with toxoplasmosis. However, lymphoma can have the same appearance or, as in this case, can coexist with *T. gondii* infection. These tumors are often aggressive and poorly responsive to treatment. By MRI scan, PML lesions usually cause multiple or diffuse subcortical changes of high intensity without gadolinium enhancement. A response to therapy for CNS toxoplasmosis (encephalitis or brain abscess) is often seen clinically within a week and radiographically in 2–3 weeks. Failure to improve on empiric therapy may necessitate further investigation.

Toxoplasmosis is generally seen in AIDS patients with CD4 lymphocyte counts of less than 200/ml blood. The presentation can be similar in non-AIDS immunocompromised patients, although fever and systemic signs are common. PML and lymphoma are also seen in organ transplantation recipients and following intensive chemotherapy for carcinoma. Infection occurs almost exclusively in (IgG)-seropositive individuals. An IgM response is often absent. Lifelong suppressive therapy is needed in AIDS patients after the initial treatment. Breakthrough infection has been seen in AIDS patients on prophylaxis for *P. carinii*.

4. *Cryptosporidium* Species

Cryptosporidium is a protozoan parasite that can cause severe and persistent diarrhea in patients with AIDS or neutropenic hosts and milder, generally self-limited gastroenteritis in less severely immunodeficient individuals and in the immunocompetent host.[6,237–244] Despite the description of this organism in mice by Tyzzer in 1907, the organism was not linked to significant disease in animals until the 1950s. It is recognized as a major pathogen of turkeys, calves, and lambs. Severe cryptosporidial diarrhea was detected early in the AIDS epidemic.[7,245] In Africa and Haiti, AIDS-associated diarrhea presents as the constellation of findings referred to as "slim disease": diarrhea, weight loss, fatigue, fever, and, eventually, death.[6,246–248] Up to 40% of these patients' clinical manifestations may be due to *Cryptosporidium*.[7,240,249] In the United States, carriage of *Cryptosporidium* in AIDS patients is common (up to 5%).[238] Some 10–15% of patients with AIDS and diarrhea will have cryptosporidiosis.[250] More recently, it has been recognized as a common cause of enteritis worldwide in immunocompetent hosts.[244,251–255] *Cryptosporidium* has been detected in up to 5% of immunologically normal individuals experiencing gastroenteritis or chronic malabsorption.[244] In the individual with diarrhea containing *Cryptosporidium*, other organisms are often detected, including amebae, CMV, *Giardia lamblia*, *Isospora belli*, and adenovirus.[250] Outbreaks of cryptosporidial infection have occurred in day care centers and subsequently in the families of affected children. Immunocompromised individuals, including bone marrow transplantation recipients, renal transplant recipients, individuals with primary immunoglobulin deficiencies, as well as patients with AIDS are candidates for severe, and generally unremitting, gastrointestinal and gallbladder infection.[254–256] Despite its growing importance, the pathogenesis of cryptosporidiosis has not been clarified.[257]

4.1. The Organism

Many species of *Cryptosporidium* have been identified, based on the animal species in which they were identified. However, the ability to transmit these infections between species, most notably between animals and man, indicates that this zoonotic organism lacks host specificity.[258] Two species appear to cause significant disease in mammals: *C. parvum* and *C. muris*. *C. parvum* is somewhat smaller (2- to 3-μm diameter oocysts) than *C. muris* (5 to 8-μm oocysts). *C. parvum* is primarily responsible for the diarrheal disease of humans and of cattle, while *C. muris* infects primarily the stomach of nonhuman mammals. Some additional subspecies have been identified recently in stools of patients with AIDS. Additional antigenically distinct subspecies are beginning to be identified in animals.

The coccidia have generally similar life cycles. Infection is initiated by ingestion (fecal–oral route) or, occasionally, by inhalation of oocysts. It is likely that the oocyst can also initiate an autoinfectious cycle. Excystation releases four motile sporozoites that attach to and penetrate the host epithelial cell where they mature into trophozoites (Fig. 10). Asexual (schizogeny) division produces up to eight merozoites that can either reinfect host epithelial cells or initiate a sexual cycle (gametogeny). Sexual reproduction completes the cycle by producing mature oocysts. A fraction of the oocysts have thin walls and rupture within the intestinal lumen to reinitiate the cycle within the host. The majority are excreted in feces.

Little is known about the pathogenesis of this infection. Symptomatic disease is generally associated with infection of the proximal small bowel. The hepatobiliary tree may serve as a reservoir for reinfection in the immunocompromised host. Carriage in the gall bladder has been associated with failure to clear infections in both AIDS patients and in children with hypogammaglobulinemia (Fig. 10). Shedding of cysts occurs in individuals with and without immune compromise or symptomatic diarrhea.

4.2. Epidemiology

Improvements in the identification of *Cryptosporidium* have demonstrated the presence of organisms in up to 32% of the population in some underdeveloped

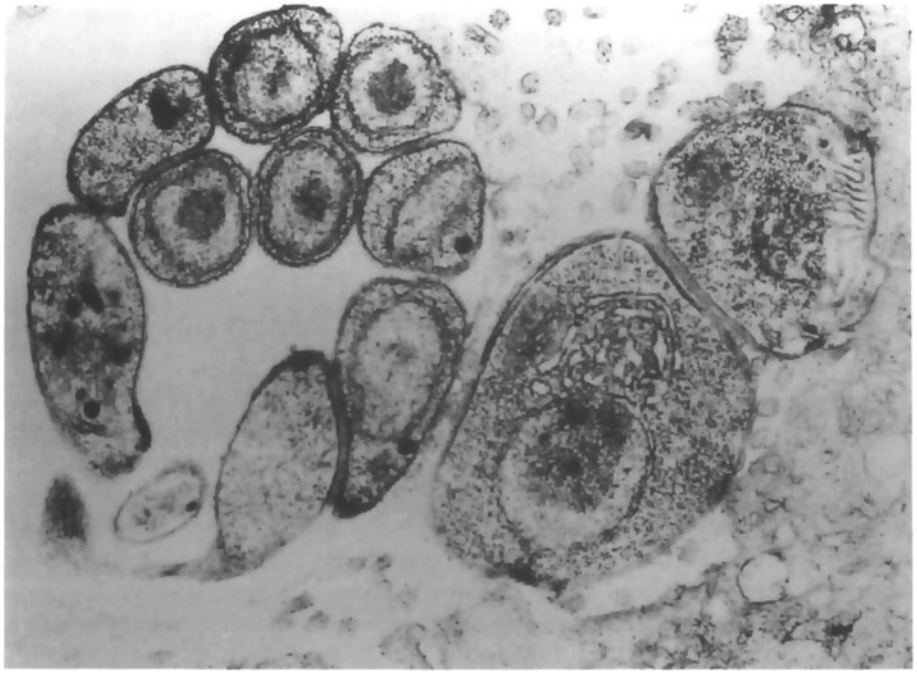

FIGURE 10. Cryptosporidia from the gallbladder of a patient with AIDS. Electron micrograph. ×7500.

nations and up to 20% of people in more developed countries. The worldwide distribution of this disease is confirmed by the high incidence of this infection in patients with AIDS: up to 20% will have identifiable organisms and up to 5% will develop cryptosporidial enteritis.[250,251] Cryptosporidia have also been detected in symptomatic and asymptomatic patients with hematologic malignancies and other immune deficits.[259,260] *Cryptosporidium* is probably a common cause of travelers' diarrhea.[261] Organisms are generally transmitted by fecal–oral contamination. Waterborne transmission has also been demonstrated.[262,263] Spread occurs between animals and between man and animals. Human-to-human spread causes the epidemics seen occasionally in day-care centers and in families.[253,264] Studies in immunocompetent patients demonstrated a range of 1.5–10% of diarrheal diseases is caused by cryptosporidiosis.[252] The Massachusetts General Hospital experience has noted that about 3% of individuals with significant diarrheal illness will have *Cryptosporidium* identified in their stools in the absence of other known pathogens. There is no gender preference, but about half are children younger than 5 years of age. Clustering of cases appears to occur during later summer and the fall. *Cryptosporidium* oocysts have been found in rivers throughout the western United States and in sewage. It is likely that the contamination is due in part to the relative resistance of this organism to traditional means of sterilization of common source water supply, including chlorination, iodophors, hypochlorite, and formaldehyde. Animal-to-person spread is common in animal handlers. The pathogenicity of zoonotic infections may be of lesser severity than those spread person-to-person.

The susceptible patient includes the entire range of immune dysfunction. Significant cryptosporidial diarrhea has been seen in diabetics after gastrointestinal surgery, in organ transplant recipients, in children with primary immune deficiencies, in AIDS patients, and in normal individuals.[239,256,264,265] Both humoral and cellular immune mechanisms appear to be involved in, and necessary for, protection against *Cryptosporidium*.[266] A syndrome similar to cryptosporidiosis is seen in many patients with AIDS-associated enteropathy who have malabsorption and villous atrophy in the presence *or absence* of identifiable pathogens.[147,238,249] The significance of small numbers of organisms or of HIV itself in the pathogenesis of this syndrome remains unclear.

4.3. Diagnosis

Cryptosporidium infection should be suspected in any patient with profuse watery diarrhea, but primarily in those in whom an underlying immunodeficiency has been identified. It is probably worthwhile to exclude other possible etiologies of infection. More common infections are those due to toxigenic *E. coli, Salmonella, Campylobacter, Shigella,* and antibiotic-associated *C. difficile*.[6,237] *Cholera* and *Yersinia* may have a similar presentation.[267] Viral agents are more difficult to identify, but it is likely that adenovirus, rotovirus, and Norwalk virus are more common pathogens.[268] Cytomegalovirus is an important differential consideration in

addition to, or as the primary agent, instead of *Cryptosporidium*.[210] Many parasites will cause diarrheal syndromes, including *Giardia*, amebae, *Microsporidia*, and *Isospora*.[269,270]

Diagnosis may be made noninvasively by stool testing. Because oocysts are similar in size to yeasts, identification of *Cryptosporidium* requires special staining. Oocysts may be concentrated by the Sheather sugar coverslip flotation method that allows quantitation and identification of infectious organisms. This laborious method has largely been replaced by modified acid-fast staining, Kinyoun's carbol-fuchin negative staining, auramine staining, and safranin staining. Also available are indirect immunofluorescent staining with monoclonal antibodies and experimental methods including direct immunofluorescence and ELISA.[271] Serological tests are under development but have not been refined to allow the diagnosis of acute infection. The immunocompromised host with cryptosporidiosis will not develop a significant serological response to the organism. It may be necessary and worthwhile to proceed to small or large bowel biopsy. Organisms and typical histopathology are better seen in the small bowel.[270,272] On biopsy, infection may be patchy. Typically, there is a loss or blunting of the villi; crypt abscesses develop in immunologically normal hosts. There is an acute and chronic inflammatory response in the lamina propria. Standard hemotoxylin–eosin stained specimens will reveal small organisms at the tips and between microvilli. Ultrastructural studies demonstrate the presence of an extracytoplasmic but intracellular parasitophorous vacuole in immunocompromised individuals. The range of histopathology is quite broad. Mild inflammation may progress to focal necrosis. Our experience suggests a synergistic injury between cytomegalovirus and *Cryptosporidium*. This affects colonic mucosa and also the esophagus, stomach, appendix, pancreatic and bile ducts, in the respiratory tract, and the gall bladder. A few AIDS patients have had cryptosporidial cholangitis in the setting of simultaneous cytomegaloviral infection of the gall bladder. When *Cryptosporidium* is detected in the lungs, it is usually found in association with gastrointestinal cryptosporidiosis.[273,274] In our experience, this has been seen only in AIDS patients and probably reflects aspiration of organisms, rather than acute, primary pulmonary infection.[194]

As noted above, *Cryptosporidium* is detected by either wet mount or fixed preparation of stool or other excretions. The oocysts do not stain with iodine and are orange with Truant's auramine–rhodamine stains. Yeasts are brown with iodine and do not stain with Truant's. On fixed smear, oocysts stain red with dense internal granules on Kinyoun stain, while yeasts stain green. The typical morphology of *Cryptosporidium* will also be seen on Giemsa stain, using a light green counterstain. Improvements in direct immunofluorescent antibody staining may enhance detection. Antigen detection in serum is under development. Specimens should be handled carefully due to the possibility of aerosolizing infectious organisms.

4.4. The Patient

The patient presents with watery diarrhea, abdominal pain, anorexia, nausea and vomiting, fever, and myalgias.[275] Stool examination reveals watery stool without blood or white cells, with intermittent shedding of large number of cryptosporidial oocysts. Severe diarrhea may be associated with malabsorption, as measured by D-xylose of vitamin B_{12} malabsorption, and steatorrhea. Mucosal thickening and small bowel dilatation may be noted in radiographic studies. Infection of the gallbladder is common. In the immunocompetent patient, the syndrome should resolve in 1 to 3 weeks. Organisms may continue to be shed after the resolution of symptoms.

In the immunocompromised patient, recurrent disease may occur in the absence of therapy. Diarrhea may be significant enough to require hospitalization for dehydration or wasting. The right upper quadrant localization of abdominal symptoms may suggest acute cholecystitis. The gallbladder may be dilated, with thickened walls and dilated bile ducts. In the absence of cholangitis, the syndrome may be mimicked by a number of enteropathies in AIDS patients, including that of primary HIV infection.[6,7,276] The severe diarrhea associated with cytomegalovirus infection in some patients with AIDS is occasionally bloody and may merit separate therapy.

4.5. Therapy of *Cryptosporidium*

There is no useful therapy for cryptosporidiosis. Support with fluids and electrolytes and added nutrition may be necessary. In immunosuppressed patients, the disease will resolve if immunosuppressive regimens can be reduced or eliminated. Antimotility agents have not been demonstrated to be effective. Preliminary encouraging results with the macrolide spiramycin (2–3 gm/day) have not been consistently reproducible.[277,278] Spiramycin does appear to have some efficacy in cryptosporidiosis in the non-AIDS immunocompromised individuals. The drug is poorly absorbed with food. Anecdotal reports of adverse effects during therapy with high-dose spiramycin (1.5 g every 8 hr IV) suggest that some patients have had increased stool output and volume loss with the development of fecal leukocytes, protein loss, and progressive loss of mucosal folds in the presence of very few organisms. Occasionally, apotosis with loss of columnar epithelium and vacuolization has been ob-

served. In AIDS patients treated with spiramycin, most continue to excrete organisms, but some have had remission of symptoms. Controlled studies of this agent do not exist but are being conducted. Alpha-difluoromethylornithine (DFMO) also has demonstrated some palliative effect on infection, but toxicities (largely bone marrow depression) have limited its use. Preliminary animal studies using hyperimmune sera against *Cryptosporidium* or bovine colostrum are encouraging, but efficacy in humans has not yet been demonstrated.[279,280] Bovine colostrum is inhibitory for cryptosporidial growth *in vitro*. A few patients have been treated with transfer factor, diclazuril and leclazuril, but these agents have not proved useful in clinical trials.

Somatostatin has been useful in reducing the severity of the secretory-type diarrhea in a few patients.[281] Octreotide is a somatostatin analogue (Sandoz Pharmaceuticals) that also has been useful in treating secretory diarrhea.[282,283] In AIDS patients with refractory symptoms, stool frequency and volume often decrease significantly during therapy with octreotide. Unfortunately, patients with no identifiable organisms appear to do better with this agent than do individuals with documented cryptosporidiosis.

It is worth remembering that organisms shed by patients are infectious. Precautions are necessary to prevent spread from infected individuals within the hospital setting.

5. *Isospora belli*

Isospora belli is a coccidian protozoan that infects the GI tract of immunocompromised individuals. Like *Cryptosporidium, Isospora* was described in 1915, but is still poorly understood. Isosporiasis is a common cause of diarrheal disease in tropical regions, but is found worldwide.[284] The common forms of disease are due to *I. belli* and occasionally *I. hominis*. Both are normally of low pathogenicity. In industrialized regions, these organisms can cause or contribute to disease in immunocompromised individuals.

5.1. The Organism: Life Cycle and Epidemiology

The organism completes its life cycle within the human host, and both sexual and asexual cycles can continue indefinitely within the GI tract. The infection is transmitted by an elliptical oocyst approximately 25–30 μm × 10–15 μm in size. There are two internal sporocysts containing four sporozoites each. Ingestion of sporulated oocysts releases infectious sporozoites that invade the intestinal epithelium and undergo asexual and sexual reproduction. Unsporulated oocysts are also formed and are shed intermittently and mature outside the infected individual. *Isospora* is epidemic in tropical and subtropical areas, including parts of South America, Africa, and Southeast Asia. Person-to-person spread probably accounts for outbreaks seen in the institutional setting. *Isospora* has been demonstrated in 0.2–1% of patients with AIDS in the United States, and in up to 15% in Haiti, but its true prevalence is not known. The mechanism of acquisition of *Isospora* infection has not been clarified completely. It is likely that many carriers of the disease are asymptomatic.

5.2. The Patient

Isospora belli causes diarrhea in immunocompetent patients, especially young children, and in AIDS patients and malnourished individuals.[241,285] The patient presents with watery, nonbloody diarrhea with nausea, abdominal pain, and weight loss. Systematic signs (headache, malaise, myalgias, fever) are often present. Prolonged infection may cause malabsorption. The infection is self-limited in the normal host, clearing in 4–6 weeks.

5.3. Histopathology and Diagnosis

Diagnosis of isosporiasis is established by identification of *Isospora* oocysts in fecal specimens in the appropriate clinical setting. Organisms can be identified by the modified acid-fast stain, by sugar flotation, by auramine–rhodamine stain, or by intestinal biopsy. Histopathology of the biopsy specimen reveals mucosal atrophy, villous blunting, crypt hypertrophy, and inflammation of the lamina propria, primarily with eosinophils, lymphocytes, and plasma cells. The organism is found in vacuoles within the cytoplasm of epithelial cells. Extraintestinal dissemination of the organism is rare. Multiple stool specimens may be necessary to demonstrate organisms because of small numbers of oocysts. Fecal leukocytes may be absent. Charcot–Leyden crystals are often seen in stool samples. A duodenal aspirate may be useful in detecting organisms. Electron microscopy may be necessary to find organisms on colonic biopsy. There are no useful serologic tests at present. A mild leukocytosis and eosinophilia may be seen in the peripheral blood smear.

5.4. Therapy

Isosporiasis responds to therapy with oral trimethoprim–sulfamethoxazole (TMP-SMZ) at a dose of 160 mg TMP component 4 times a day for 10

days.[285,286] In AIDS patients, prolonged additional therapy with TMP-SMZ (twice a day for an additional 3 weeks) has been necessary for clearance of oocysts.[287] Prophylactic therapy with TMP-SMZ (once a day) or pyrimethamine–sulfadoxine (Fansidar) has been useful in preventing relapses. Other "successful" therapies may be useful in part because of the treatment of other concomitant infections.[286] Patients may continue to excrete organisms long after the successful completion of therapy. This observation probably supports the use of prophylactic therapy in AIDS patients or other symptomatic immunocompromised hosts.

6. Microsporidia

The Microsporidia make up a phylum consisting of approximately 80 genera and over 700 species of organisms. These are obligate, intracellular, spore-forming, protozoal parasites that were first identified in 1857 as causing disease in insects, fish, rodents, and some primates. These organisms were rarely implicated in clinical disease prior to the advent of AIDS.[288–290] Case reports describe a series of children with seizure disorders and children and adults with corneal ulcerations, keratitis, or iritis. Keratitis appears to be a rare manifestation of microsporidiosis of the immunocompetent host. Disseminated disease has been described in a young boy with thymic aplasia. Autopsy of this child revealed disseminated Microsporidia involving the lungs, stomach, colon, kidneys, adrenal glands, heart, liver, and other muscles. The organism was identified as *Nosema connori*. Cases of microsporidiosis have been elicited by treatment with corticosteroids as well as in other immunodeficient states. Corneal infections with Microsporidia have been identified in both AIDS and non-AIDS immunocompromised patients. Microsporidial infection has been identified in patients with AIDS since the mid-1980s as a cause of chronic diarrhea with weight loss.[267,289] In general, the cause of this form of infection has been identified as *Encephalitozoon bieneusi*, for which man is the definitive host. Up to 30% of AIDS patients with weight loss and chronic diarrhea have been found to be infected with Microsporidia in some series. Disseminated disease in AIDS patients has involved skeletal muscle, kidney, liver, eye, and intestinal wall as well as intestinal epithelial cells. In this population, other genera have also been identified. These include *Pleistophora* (a pathogen of fish and insects), *Encephalitozoon* species (found in many mammals), and *Enterocytozoon cuniculi*. Further genera have been identified in small numbers of patients.

6.1. The Organism

Microsporidia have spores ranging in size from 1 to 20 μm.[291] Spores infecting mammals are generally 1–2 μm in diameter, requiring electron microscopy for identification, using biopsy specimens or aspirates of affected large or small bowel. These spores have thick walls, allowing them to persist outside the host and also making them difficult to stain. They are generally gram-positive and contain PAS-positive granules. Birefringent spores will be found throughout the fibrous stroma, but will not stain on hemotoxylin–eosin stain. Organisms are found in the supranuclear cytoplasm of cells as binucleated spores containing a coiled polar tube. Small-bowel biopsy reveals the greatest number of organisms. All the developmental forms of the organism's life cycle occur within the epithelial cells of the small bowel.[292] Infection of the liver produces a granulomatous hepatitis. Organisms can spread via blood, lymph, or infected macrophages throughout the body. Significant accumulations have been found most often in brain or kidney. Brain infection results in focal seizure disorders, while kidney involvement may produce interstitial nephritis of some severity. In non-AIDS patients, a granulomatous vasculitis of cerebral vessels may produce a meningoencephalitis. It appears that cell-mediated immunity is critical for protection against significant disease due to the Microsporidia.[288]

6.2. The Patient

Like individuals with cryptosporidiosis and isosporiasis, patients with microsporidial infection present with chronic watery, nonbloody, nonmucoid diarrhea without fever. Patients have weight loss despite continuing to eat well. Intestinal mucosa are normal to minimally inflamed endoscopically. Enterocyte degeneration accompanies partial villous atrophy and modest inflammation in the lamina propria. By contrast, patients with "AIDS-associated enteropathy" without identifiable infection are more likely to have malabsorption, including lactase deficiency, with minimal changes in villous morphology. The possible mechanisms of this syndrome includes bacterial overgrowth secondary to local immune deficiency.

6.3. Diagnosis and Therapy

Suspicion of the presence of Microsporidia may be raised by the presence of organisms seen on Brown–Brenn-stained tissue sections (Fig. 11). The organism is occasionally seen on Giemsa-stained impression smears and on thin sections stained with methylene blue–azure

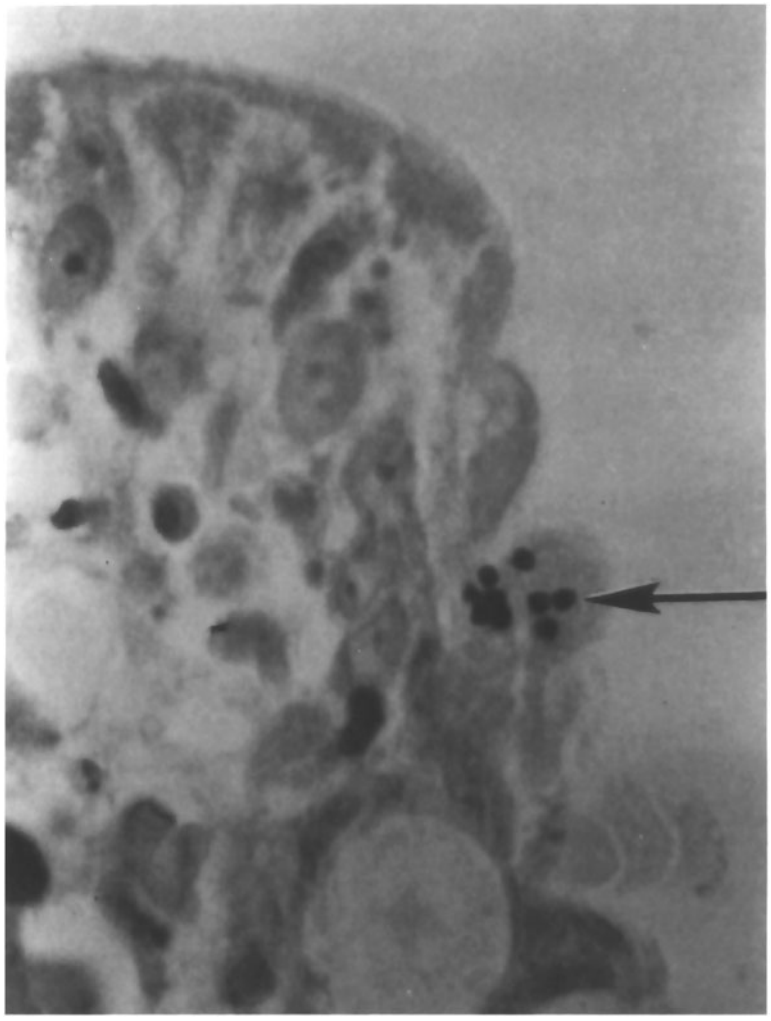

FIGURE 11. *Microsporidia* within the jejunal epithelium of a patient with AIDS (←) are gram-positive spores by Brown–Brenn stain. ×1750. Courtesy of Drs. R. Weber and R. T. Bryan, Parasitic Diseases Branch, Centers for Disease Control.

II with a basic fuschin or toluidine blue counterstain. Species identification requires electron microscopy.[291] Unconcentrated stool specimens or duodenal aspirates can be screened after fixation in 3 volumes of 10% formalin or on thin smears made with methanol fixation. Staining with a mixture including chromotrope 2R (Harleco, Inc.) has been used by some workers in place of Giemsa, toluidine blue O, Gram's stains, stool concentration, or electron-microscopic examination.[293] Non-AIDS patients have a more severe inflammatory response and greater destructive ulceration of corneal tissues during microsporidial infection. The diagnosis of microsporidium infection can be made by electron microscopy of corneal or conjunctival scrapings. A variety of serologic tests have been developed to detect antibodies binding to spores and extracts of organisms. These tests are being refined for clinical use.

There is no known therapy for microsporidiosis. Albendazole (400 mg PO bid for 4–6 weeks) has anecdotal efficacy. Symptomatic therapy is similar to that for cryptosporidiosis (see Section 4.5). The increasing frequency of isolation of *Microsporidia* from AIDS patients (0–30%) varies with the region from which the patients are derived and with travel to tropical regions. It is likely that improved diagnostic techniques will further increase recognition of this phylum in many immunocompromised hosts.

7. *Strongyloides stercoralis*

Since the original association of *Strongyloides stercoralis* with "Cochin China diarrhea" in 1876, strongyloidiasis has been recognized as an important human intestinal pathogen. This nematode currently infects almost 100 million people worldwide.[294,295] This parasitic helminth can complete its entire life cycle within the human host, allowing for persistent and occasionally

lifelong infection. In the immunocompromised host, dissemination of worms beyond the GI tract produces the life-threatening "hyperinfection syndrome."[296,297] The presence of a persistent carrier state greatly enlarges the at-risk population for severe disease during periods of immune suppression.

7.1. The Organism

Strongyloides stercoralis is a nematode that completes its complex life cycle within the human host.[295] The infective form is the filariform larvae that live in soil contaminated with feces and penetrate exposed skin areas that come into contact with the soil. These larvae follow the venous circulation to the right heart and to the pulmonary alveolar capillary bed (Fig. 12). The worms then penetrate into the alveolar space, are carried up the bronchial tree, and are swallowed. Some larvae migrate through other tissues, especially muscles, producing local symptoms. In the small intestine, the larvae mature through two molts, producing adult female worms that produce fertilized eggs through parthenogenesis. This process occurs within the intestinal mucosa, where eggs mature to first-stage rhabditiform larvae. Maturation of the rhabditiform larvae occurs over 24–48 hr. These larvae are passed in the stool or enter an autoinfective cycle within the GI tract. The autoinfective reproductive cycle within the GI tract allows for enhanced growth in the absence of immunologic controls. Some of the rhabditiform larvae mature within the intestinal lumen and penetrate into the vascular tree via the wall of the bowel or perianal skin (external autoinfection) to reinitiate the cycle. Larvae passed with the stool may become infective either via direct maturation or via intermediate sexual development into male and female forms. The filariform larva is approximately 600 μm long, while the rhabditiform larva is approximately 200–300 μm long.

FIGURE 12. *Strongyloides stercoralis* larva isolated from the sputum of a patient with disseminated strongyloidiasis due to an ACTH-secreting tumor.

The free-living female worm is 1 mm long; the adult male worm is slightly shorter.

7.2. Epidemiology

Strongyloides is an unusual parasite in that the human is the major host, although some other animals, including cats, dogs, and subhuman primates, may harbor active infections. The frequency of infection of these animals in endemic areas is not known. The parasite is found worldwide and is most common in the tropics and subtropics. Approximately 1% of dogs in the eastern United States may be infected. Patients may have no history of travel to an endemic region. Chronic strongyloidiasis is a condition of relatively low worm burden restricted to an autoinfectious cycle between the skin and the intestinal tract, but without a sufficient immune response to clear the infection. Chronic infection has persisted for over 30 years in some patients.

The exact components of the immune system responsible for prevention of disease or the reduction of the severity of infection are not known.[295] Disseminated infection has been reported in people with a broad array of immune defects.[298] This population includes individuals with hematopoietic malignancies or connective tissue disease being treated with immunosuppressive therapies (notably corticosteroids); hosts with congenital or acquired hypogammaglobulinemia, chronic malignancies, malnutrition, severe burns, or alcoholism with hepatic cirrhosis; and persons with occupational exposure to contaminated feces.[299–306] Increased corticosteroid dosages used for the treatment of *Strongyloides*-induced bronchospasm or for organ graft rejection have been associated with the development of disseminated infection.[307–312] Abdominal surgery or steroid therapy for apparent ulcer disease, ulcerative colitis, or Crohn's disease can exacerbate underlying infections.[309,313,314] The hyperinfection syndrome has also been reported in normal individuals without apparent predisposing immune defects.

Strongyloidiasis has been reported to be more common in sexually active homosexual men with normal immune function. Direct transmission of the parasite occurs via rectal intercourse, by oral–anal exposure, or by contact with skin in the perianal area. Few patients with AIDS are reported to have developed disseminated strongyloidiasis.[7,302,306] This finding contradicts the perception that cellular immunity is solely responsible for protection against *Strongyloides*. The relative absence of this finding in AIDS may reflect underdiagnosis or unreported cases of disease, but it is apparent that this infection is still more common in other classes of immunocompromised individuals.

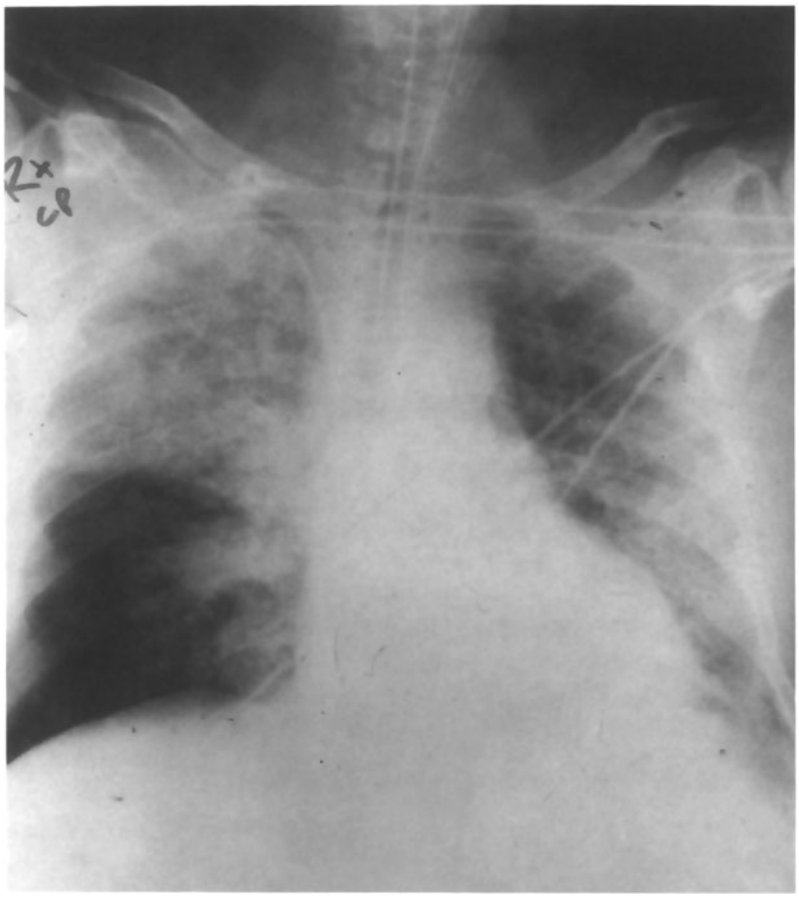

FIGURE 13. Chest radiograph of a patient with disseminated strongyloidiasis. The patient had fever, hemoptysis, hypoxemia, and gram-negative bacteremia with fluctuating, dense pulmonary infiltrates.

7.3. Pathogenesis

The role of the immune system in the modulation of infection due to *Strongyloides* is demonstrated by changes in the course of disease in the presence of immune suppression (Figs. 12 and 13). In the normal host, repenetration of the gut wall by maturing filariform larvae is limited. Long-standing infection may be associated with minimal fibrosis, with adult worms in the crypts of Lieberkühn, and with eggs and larvae in the bowel lumen. Chronic infection produces minimal inflammation of the bowel wall with some villous blunting. With immune suppression, especially in the setting of corticosteroids, penetration by the larvae through the gut wall increases. In this setting, worms accumulate in the lungs in significant numbers. This penetration produces bowel wall edema with mucosal ulceration and mucous secretion. Depending on the level of immune suppression, the inflammatory response on the wall of both the small and large intestine may be severe or minimal. The inflammatory response is both acute and chronic, and includes plasma cells, eosinophils, histiocytes, giant cells, lymphocytes, and neutrophils. Granuloma formation may occur around degenerating larvae, but is often absent during immune suppression. Bacterial or fungal infection carried from the lumen of the GI tract may cause acute infection of the bowel wall, peritonitis, or sepsis. Involvement of the CNS is common, with meningitis due to *Strongyloides* and to accompanying organisms.

7.4. The Patient

Acute GI infection will generally produce epigastric fullness or pain and, in some individuals, diarrhea and malabsorption.[315] Passage of larvae through the lungs may produce eosinophilic pneumonia or "Loeffler's syndrome" or milder manifestations of dyspnea, cough, bronchospasm, and fever.[316] Most of these individuals will have a peripheral blood eosinophilia. Gram-negative septicemia and necrotizing pneumonia may occur numerous times without specific treatment of the underlying worm infection. Gram-negative meningitis is a common complication of strongyloidiasis; larvae are infrequently detected in the meninges or CSF of affected patients. Bacterial superinfection as a complication of strongy-

loidiasis is equally common in normal and in immunocompromised individuals, supporting the presumption that bacterial infection is a function of worm penetration, rather than of the underlying immunodeficiency state. The mechanism of such superinfection is unknown, but superinfection occurs in one third to one half of individuals with disseminated infection.

Many individuals with chronic infection are asymptomatic. Others have abdominal pain, diarrhea, and urticaria.[301] In chronic infection, respiratory symptoms are less prominent; however, immune complex disease may cause arthritis. The two common skin manifestations of strongyloidiasis include (1) a migratory, pruritic, raised, linear rash called "creeping eruption" or "larva currens" and (2) crops of urticarial eruptions that appear to be manifestations of immediate hypersensitivity reactions to migrating worms. Two thirds of individuals with chronic infection will develop transient and recurrent urticarial eruptions on the skin of the waist and buttocks. The migratory rash may move across the skin at a rate of up to 10 cm/hr. It is unclear whether these eruptions are a reaction to the worms themselves, secreted antigens, or components of the skin or gut flora that are carried along with the organism. Visceral or cutaneous migration appears to be much slower, but may produce a similar rash.

7.5. Disseminated Strongyloidiasis: "Hyperinfection Syndrome"

The predilection of this organism for the lungs and for the CNS is manifested most impressively with disseminated infection in immunocompromised individuals (Figs. 12 and 13).[296,303] The complications associated with dissemination reflect both a large worm burden and the effects of organisms accompanying the migrating nematodes. Local and systemic infections and allergic responses may be seen to both the worms and the "passenger" bacteria from the gut. Unlike the transient eosinophilic pneumonia that may be seen with acute infection, the hyperinfection syndrome is accompanied by significant pneumonitis.[312] Pulmonary bacterial superinfection occurs in the setting of small-airway obstruction secondary to entrapped worms. Pneumonitis is generally accompanied by abdominal crisis: severe abdominal pain with ileus, small-bowel obstruction, and occasionally, septic shock. Hepatic failure has been reported; CNS involvement may include eosinophilic meningitis, altered mental status, coma, or focal neurologic deficits. Polymicrobial bloodstream infection may be seen and includes the entire range of gut flora, including *Candida*. Cavitary pulmonary lesions may develop, and transient rashes or skin swelling of the buttocks or lower abdomen may be noted. Peripheral blood eosinophilia is variably observed. Mortality with disseminated infection generally exceeds 75% and is usually due to gram-negative sepsis. Because the consequences of disseminated *Strongyloides* are so grave, *preemptive* treatment should be considered prior to elective immune suppression in patients with exposures to endemic regions, even without identification of organisms.[294]

7.6. Diagnosis

Early diagnosis and therapy are the main determinants of outcome. The diagnosis of strongyloidiasis should be suspected in the presence of GI symptoms accompanied by urticaria or eosinophilia or in individuals who have lived in endemic areas. Because systemic manifestations may be altered by the presence of immunodeficiency, clinical suspicion in the presence of rhabditiform larvae found in stool or in duodenal aspirates should be considered sufficient for the diagnosis in the appropriate host. Strongyloidiasis needs to be considered in the appropriate patient whose pneumonia does not respond to therapy based on sputum examination and culture data. GI symptoms are frequently missed because of the preeminent pulmonary or CNS symptoms.[315] Negative stool examinations may be misleading. Eosinophilia is frequently absent in disseminated infections.

The diagnosis of strongyloidiasis is based on the demonstration of filariform larvae in stools, sputum, or CSF. In chronic infection, small numbers of larvae may be hard to find. Larvae may be found by use of the Enterotest capsule ("string test"), which contains a long nylon thread. The swallowed capsule releases the thread, which is withdrawn after several hours and may be coated with mucus containing larvae. Duodenal aspiration or purged stool specimens may also reveal organisms not detected in routine specimens. Sputum examination may reveal bacterial or fungal pneumonia in the absence of identifiable larvae. Diagnosis is occasionally made on unstained wet-mounts of bronchoalveolar lavage specimens or transtracheal aspirates from infected lungs.[306] Formal–ether concentrates of sputum may be of use if the larvae are few in number. The worm will also be seen by Papanicolau stain or by experienced observers on Gram's and acid-fast stains of concentrated specimens.

Neither chest nor abdominal radiologic studies are diagnostic of infections. Chest X rays show patchy or diffuse bilateral pulmonary infiltrates (Fig. 13). Pulmonary processes may be transient or progress to consolidation, especially in the presence of bacterial superinfection. These processes clear with appropriate therapy. Barium swallows demonstrate duodenal and jejunal dilatations and bowel wall edema with narrowing in areas of

fibrosis. Disseminated disease is frequently accompanied by dilatation of the small bowel with air–fluid levels.

In the immunologically normal individual with active infection, higher titers to *Strongyloides* antigens have been demonstrated. The utility of these tests in individuals with disseminated infection has not been established. Both immunofluorescent assays and ELISAs have been described. These assays may become useful in detecting at-risk populations before the initiation of immunosuppressive therapy. Antigen detection tests are under development.

7.7. Therapy

All patients infected with *S. stercoralis* should be treated. Uncomplicated GI infections may be treated successfully with thiabendazole, 50 mg/kg per day (PO), divided into two doses, to a maximum dose of 3 g/day over 2 or 3 days. Thiabendazole has many toxicities, including nausea, vomiting, dizziness, and, occasionally, a sense of disembodiment and of urine odor. In uncomplicated disease, lower doses are likely to be effective, and confirmation of the clearance of worms should be made by stool examination at 6 and 12 months after therapy. Disseminated disease is treated with the same drug for a period of 5 days. A number of drugs are being studied, but are of less certain efficacy, including mebendazole, cambendazole, and albendazole in high doses. Ivermectin has proven useful and less toxic and is in clinical trials.[317] Of interest, the immunosuppressive agent cyclosporin A may have some efficacy in the treatment of strongyloidiasis. However, we have seen strongyloidiasis in organ transplant recipients receiving cyclosporin A to prevent graft rejection.

Patients at risk for dissemination due to immune suppression should be evaluated for the presence of the carriage state. Patients with disseminated infection are treated for 5–7 days for strongyloidiasis, but may require more prolonged therapy for secondary bacterial infections of the lungs, CNS, or abdomen. Immunosuppressive agents should be reduced as much as possible and areas of focal infection drained. Repeated courses of therapy may be necessary in chronically immunosuppressed individuals (i.e., those with AIDS or organ transplants). Therapeutic failures with thiabendazole may be successfully treated with ivermectin. Side effects of therapy may include hypotension, neurotoxicity, and leukopenia with mild elevations of liver function tests.

Illustrative Case 3

The patient is a 66-year-old man who immigrated to the United States from the Dominican Republic approximately 30 years prior to this admission. He has a history of heavy smoking and alcohol abuse and a sister with a history of active pulmonary tuberculosis. During an evaluation for hypertension and angina 3 years prior to admission, the patient was noted to have a 5-mm nodule in his left chest on chest X ray. No further evaluation of this nodule was performed at the time. A few days prior to admission to Massachusetts General Hospital, he presented to an outside hospital with chest pain, nausea, and vomiting. He was ruled out for myocardial infarction, but was found to have exercise-induced anginal electrocardiographic changes. His laboratory evaluation included the following values: potassium, 1.9; chloride, 89; bicarbonate, 49; glucose, 150–180; a fasting cortisol of 72.3 (8 AM) and 56.4 (12 PM). A review of systems revealed: an increase in anxiety, bilateral lower extremity edema, increased abdominal girth with progressive dyspnea on exertion, an increased appetite but a 14-pound weight loss. The patient had new-onset diarrhea without abdominal pain and a cough with occasional hemoptysis but with little sputum production. He did note intermittent fevers as high as 101°F and penile paresthesias when urinating. He was allergic (hives) to sulfa drugs.

On physical examination, the patient was a Hispanic man who appeared cushingoid in body habitus. His skin examination revealed hyperpigmented knuckles and nail beds. He had three-plus pitting edema to the knees without clubbing. His chest X ray (Fig. 13) revealed a right middle lobe infiltrate with mediastinal widening and a generalized interstitial pattern. His oral examination revealed thrush.

His white blood count was normal without eosinophils. His ACTH was 665 (normal range 10–56). His amylase was 269 (45–113); his ionized calcium was 0.94 (1.14–1.30). Stool examination was negative for pathogenic bacteria or parasites. Sputum examination and cultures were unremarkable.

The patient developed progressive respiratory distress requiring intubation and transfer to the medical intensive care unit. Deep-suctioned sputum examination was positive for both *P. carinii* and *S. stercoralis* (Fig. 12). He was treated with thiabendazole, intravenous pentamidine, and broad-spectrum antibiotics. His admission blood cultures grew enteric gram-negative rods overnight, and sputum cultures grew CMV. The patient failed to clear his *Strongyloides* infection with thiabendazole and was treated with ivermectin. Nonetheless, he progressed to perforation of his bowel. Liver biopsy during laparotomy revealed a malignant small cell neoplasm consistent with small cell carcinoma of the lung. The patient expired on the 14th hospital day.

Comments. This case demonstrates the importance of a careful epidemiologic history in the management of the immunocompromised patient. Patients from areas endemic for *S. stercoralis* remain at risk for disseminated infection ("hyperinfection") for many years after the initial exposure. This common presentation included a negative stool examination for *S. stercoralis* and a normal sputum Gram's stain. The patient was immunocompromised due to an ACTH-secreting small cell carcinoma presumed to be lung-derived. Pulmonary infection with *Strongyloides* is probably less important than the frequency of bronchial obstruction with subsequent bacterial superinfection. The cataclysmic event in this patient's course was bowel perforation and gram-negative sepsis *despite* appropriate antibiotic therapy. Sustained bacteremia even without perforation is a common complication of strongyloidiasis in the compromised host. Patients may have paralytic ileus with or without perforation, hemorrhagic and fluctuating pulmonary infiltrates progressing to adult respiratory distress syndrome, bacteremia or bacterial pneumonia, and parasitic or bacterial meningitis *or both*. While eosinophilia (>25–30%) is common, this finding is usually absent in patients receiving corticosteroids. Larvae are detected in sputum or pulmonary aspirates, especially when concentrated prior to examination.

In patients known to be at risk for *Strongyloides* infection, aggres-

sive evaluation or preemptive therapy is mandatory *prior to* initiating immunosuppression—e.g., organ transplantation or chemotherapy. Larvae have been detected in purged stools when routine examinations are unremarkable.

The presence of *P. carinii* and of CMV is of uncertain significance. These pathogens speak to the chronicity of immune suppression and the role of corticosteroid excess in the pathogenesis of this patient's illness.

8. *Entamoeba histolytica*

Entamoeba histolytica is a parasite of worldwide distribution that lives in the intestinal lumen of over 500 million people.[318] The organism is usually a benign commensal. Under appropriate conditions, *E. histolytica* invades the intestinal mucosa, causing dysentery, mass lesions (ameboma), or extraintestinal lesions including liver abscesses. Because the basis of the conversion from commensal to invasive parasite is unclear, the mechanism by which amebiasis is exacerbated by immune suppression is also not understood. The significant clinical features of amebiasis are the separation of nonpathogenic from pathogenic amebae, the distinction of lumenal infection from invasive disease, and the consideration of this diagnosis in the setting of a patient with GI symptoms *prior to* the elective initiation of immunosuppressive therapy.

8.1. The Organism

Human amebae include several species of parasites: *E. histolytica, E. hartmanni,* and *E. gingivalis*. Rarely, amebae common in animals have been found in humans; these organisms have not been implicated in disease. The motile form of the organism is the trophozoite, which lives in the colonic lumen. The trophozoites divide and encyst, producing thick-walled cysts with four nuclei. Unlike other parasites, these cysts do not persist in tissues and are found only in the lumen of the human bowel. Trophozoites are fragile and have not been implicated in the transmission of disease. Trophozoites are found in tissues during invasion. Strain differences appear to be common in *E. histolytica*. These differences account for geographic variation in the pathogenicity of the organism. There are antigenic and enzymatic differences between pathogenic and nonpathogenic strains of *E. histolytica*. Characteristic electrophoretic patterns of isoenzymes or "zymodemes" may distinguish pathogenic strains.[319] *In vitro,* it has been possible to change a nonpathogenic organism into a pathogenic organism by altering the bacterial flora surrounding the organisms in culture. It is not clear that this conversion occurs *in vivo*. The determinants of pathogenicity are not clear.

8.2. Life Cycle

The life cycle of the organism begins with the ingestion of food contaminated by infective cysts. Excystment releases motile trophozoites covered with filopodia used for epithelial attachment. The organism grows in a low-oxygen environment. Invasive trophozoites in tissues are large (20–40 μm) in comparison to those found in carriers (10–25 μm). The characteristic of hematophagia is considered diagnostic of invasive amebae and active disease. The nonpathogenic cysts of *E. hartmanni* are much smaller in diameter (10 μm) than those from *E. histolytica* (8–20 μm). Humans are definitive hosts for *E. histolytica*. The development of good animal models has been difficult.[320] However, characteristic intestinal and hepatic disease has been produced by coinfection using pathogenic *E. histolytica* with bacteria and by direct inoculation of organisms into the hepatic parenchyma or circulation.[320,321]

8.3. Epidemiology

Infection due to *E. histolytica* is common in tropical regions, in parts of Central and South America, India, and western and South Africa. Up to 50% of the residents of some areas are infected. People in these hyperendemic regions are subject to constant reinfection. Occasional epidemics of amebiasis are related to contamination of a water supply or to unsanitary conditions surrounding institutionalized individuals. Serologic assays are useful for the detection of invasive disease. These tests generally revert to negative within 6–12 months after the acute invasive disease. However, the indirect hemagglutination assay remains positive for years. Invasive disease is more common in persons of lower socioeconomic condition. Amebiasis is the third leading cause of mortality due to parasitic infection, following malaria and schistosomiasis.

Male homosexuals in the United States have a fixed high incidence of carriage of *E. histolytica,* with some urban areas reporting infection rates approaching 30% of sexually active individuals.[322,323] The incidence of invasive disease has not corresponded to the presence of luminal infection in homosexual AIDS patients in either the United States or Latin America. Reports of the increased incidence of amebiasis in homosexual patients with AIDS in the United States do not demonstrate an increased prevalence of pathogenic strains or of invasive GI disease.[7,324] It appears that most of the parasites recovered from homosexual men are nonpathogenic and are probably not relevant to GI symptoms. AIDS patients do not appear to be at increased risk for the development of amebiasis. By contrast, immunocompromised patients

receiving chemotherapy, corticosteroid therapy, or immunosuppression surrounding organ transplantation are at a greater risk for the development of fulminant colitis.[325,326]

8.4. Immunology

Antibodies against *E. histolytica* that develop during acute infection do not appear to be protective.[327,328] The presence of serum antibodies correlates with the presence of both invasive amebiasis and hepatic disease. Antibody can lyse trophozoites *in vitro*. Complement can also produce partial lysis. Complement-resistant amebae may "cap-off" bound surface antibody or may shed surface antigens to limit the efficacy of the humoral immune response. The cellular immune response appears to be depressed acutely during infection and returns after treatment of disease.[326,327] Resistance to subsequent infection appears to be mediated by the cellular immune response. The absence of an increased incidence of invasive disease in patients with AIDS suggests that other mechanisms are also operative. This absence is particularly notable given the increased carriage of amebae by male homosexuals.

8.5. Pathogenesis

The pathology of amebic disease is important. The characteristics of inflammatory changes in the bowel may easily be confused with other more benign processes. Invasion of the intestinal mucosa produces a local loss of mucin from the surface of epithelial cells, with underlying edema and hyperemia. Superficial ulceration develops with minimal local inflammation. Ulceration progresses superficially, with penetration into the mucosa producing the characteristic "flask ulcer" extending into the submucosa of the intestinal wall. The ulcers themselves are usually small with raised borders and a necrotic base and with normal mucosa between adjacent ulcers. Extensive disease may involve large segments of the intestinal mucosa. Organisms are found superficially at the edge of the epithelial lesion. Chronic amebic infection will result in thickening of the colonic mucosa. Progressive disease within the wall of the intestine can produce a pseudotumor consisting of necrotic tissue with acute and chronic inflammation and granulation and fibrosis. This mass lesion is called an *ameboma*.

Further complications occur locally with perforation of the GI tract or by penetration into the portal circulation, allowing seeding of the liver.[319] Liver involvement may occur in the absence of clinically important intestinal disease. The liver is involved by local inflammation, followed by focal necrosis and granuloma formation. Progressive necrosis and parenchymal reaction provide a thin capsule to the enlarging amebic abscess. Organisms are found primarily at the edge of the abscess. Amebic abscesses are single lesions in over 80% of individuals and are generally found in the right lobe in the posterior portion adjacent to the diaphragm. Large abscesses may decompress into the right or left chest or into the bronchial tree, producing catastrophic disease. The necrotic debris has been characterized as "anchovy paste" exudate, which may be found in the sputum after rupture of an abscess into the bronchi. Rupture of abscesses of the left lobe of the liver may be associated with acute pericardial tamponade. Bacterial superinfection is surprisingly uncommon. With appropriate therapy, pathologic changes in the intestines or the liver resolve without fibrosis.

8.6. The Patient

The presentation of amebic infection depends on the extent of disease and the condition of the host. Clinical presentations of this common disease can be divided into subcategories: asymptomatic cyst carriers; a possible chronic, nondysenteric colitis syndrome; acute rectocolitis; toxic megacolon with fulminant colitis; ameboma; and painless rectal bleeding. The interaction of immune depression with the presentation of disease appears to determine the severity and rapidity of disease progression.[326] Immunocompromise does not appear to alter the differentiation between invasive and noninvasive disease. Since local inflammation is usually modest, fibrosis is not characteristic of disease. The alterations produced by immune suppression may be subtle. It should be anticipated that bacterial superinfection and peritonitis are more common in the setting of broad-spectrum immune suppression such as with corticosteroid therapy. The important factors in infection are the strain of *E. histolytica* and the nutritional status of the host.

Only 10–20% of individuals infected with *E. histolytica* will develop clinically significant disease. Most individuals will spontaneously eradicate the parasites. Symptomatic individuals are at risk of further complications of disease if not adequately treated. It is possible that the majority of these infections are with nonpathogenic strains. A syndrome of irritable bowel disease in the presence of *E. histolytica* has been termed *chronic nondysenteric infection*. There is some controversy as to whether or not the organisms play a significant role in the development of the syndrome of chronic intermittent abdominal pain with diarrhea.[329] The basis of this pattern of infection remains unclear. In general, the presentation of acute disease depends on the extent of

colonic involvement and the rapidity with which the disease develops. "Acute rectocolitis" usually involves the gradual onset of diarrhea and acute abdominal pain. Watery stools may become blood-stained. Tenesmus is common. Many small, superficial, mucosal ulcerations with segmental distribution are seen by colonoscopy. The ulcers contain necrotic debris and trophozoites, with underlying hyperemia and some submucosal hemorrhage. Fecal leukocytes are uncommon. While adults are generally well compensated, children may be toxic, with high fevers, and become rapidly dehydrated.[330] Right lower quadrant pain may be due to acute appendicitis (which is uncommon) or to "typhlitis," which usually occurs in the presence of mucosal thickening in the ileocecal region. Complications are common in individuals developing amebic appendicitis. Liver abscesses, bleeding, and perforation with fistula formation are common complications in patients with undiagnosed amebic appendicitis.

Host factors seem to determine the progression of acute dysenteric disease to the fulminant colitis.[326,327] Fulminant colitis is a rapidly progressive syndrome. The patient may present with bloody diarrhea with foul odor and diffuse abdominal pain. High fever is present, with signs of ileus, dehydration, and shock developing early. Tenesmus and rectal bleeding are present, and perforation with peritonitis occurs in up to two thirds of these individuals. Toxic megacolon may occur, but is uncommon. Extraintestinal disease may occur. The syndrome is more common in the presence of malnutrition, in older individuals, and in the immunocompromised patient, particularly those receiving corticosteroids. Up to two thirds of these individuals will die due to complications of intestinal injury. Surgical debridement is of uncertain value in the absence of acute peritonitis or toxic megacolon. Both antiamebic and antibacterial therapies are necessary.

Up to 2% of patients with invasive disease will develop ameboma, usually of the cecum or ascending colon. The diagnosis is frequently incidental to the evaluation of acute dysenteric amebiasis or with an asymptomatic abdominal mass lesion. Diffuse thickening of the gastric wall with mucosal ulceration may give the appearance of Crohn's disease. Treatment of this complication with corticosteroids may have disastrous side effects.[325] The presence of rectal bleeding without pain may be chronic and is a reflection of congestive colitis, usually without ulceration.

Extraintestinal disease is generally restricted to amebic liver abscess. This complication is more common in lower socieconomic groups, in men than in women, and in alcoholics than in nondrinkers. In addition to poor hygiene, host immunity may also be a contributing factor. Amebic liver abscess usually occurs in the absence of acute rectocolitis. Intestinal involvement can be demonstrated in only 38% of individuals with hepatic abscess. The clinical presentation is usually acute, with abdominal pain, fever, and symptoms localizing in the subdiaphragmatic region. Right shoulder pain is common and is increased by coughing. Fever, rigors, and sweats are common, associated with cachexia. Some patients will present with a more gradual evolution with hepatomegaly and anemia. In most individuals, liver function tests are mildly abnormal, and the patients may be slightly jaundiced. The patient will often have an elevated white blood count. Other extraintestinal foci of infection may include the skin of the perianal region or the skin or tissues overlying the involved intestines or thoracic regions.

8.7. Diagnosis

The diagnosis of amebiasis is made by the demonstration of trophozoites of *E. histolytica* in smears made from colonic samples. Motile organisms containing red blood cells in the presence of small superficial ulcers of the colonic mucosa are the most common findings. Differentiation of pathogenic amebae from nonpathogenic organisms can be done on wet-mounted smears using fresh or preserved specimens. The addition of iodine will allow the differentiation of *E. histolytica* cysts from yeasts and other amebic species. The addition of methylene blue will allow the differentiation of cysts from leukocytes, which stain blue. Areas with both mucus and blood are optimal for examination. Cyst morphology is best seen on fixed specimens stained with iron–hematoxylin or a trichrome stain. This morphology is more easily observed after concentration of a specimen using sedimentation or flotation on formalin–ethyl acetate.

Serologic diagnosis is useful for the diagnosis of invasive GI disease or hepatic disease, but is rarely useful acutely. Radiologic evaluation will reveal the presence of thickened intestinal walls in severe amebic colitis, with narrowing and loss of folds.[331] Free air may be observed in the presence of perforation.

In the absence of localizing signs, the diagnosis of a liver abscess may be overlooked. Ultrasonography, CT, or liver–spleen scintigraphy will demonstrate liver abscesses in the majority of cases. The presence of a liver abscess with a positive amebic serology should be considered diagnostic of amebic liver abscess. In immunosuppressed individuals or in malnourished patients with chronic abscesses, negative serologies may be slow to convert to positive. Organisms are infrequently found on stool examination to confirm the presence of *E. histolytica* infection. Serologic tests are particularly useful because they peak 2–3 months after acute infection and generally return to low levels by 1 year after infection. In

TABLE 9. Drugs for the Treatment of Amebiasis

Clinical presentation	Drug	Adult dosage[a]
Asymptomatic cyst passer		
Drug of choice	Iodoquinol[b]	650 mg tid × 20 days
Alternatives	Diloxanide furoate[c]	500 mg tid × 10 days
	Paromomycin	25–30 mg/kg/day in 3 doses for 7 days
Invasive intestinal disease		
Drugs of choice	Metronidazole[c]	750 mg tid × 10 days
	followed by	
	iodoquinol *or*	As above
	diloxanide	As above
Alternatives	Dehydroemetine[d]	1–1.5 mg/kg/day (max 90 mg/day IM for up to 5 days)
	followed by	
	iodoquinol	As above
Hepatic abscess		
Drugs of choice	Metronidazole	As above
	followed by	
	iodoquinol	As above
Alternatives	Dehydroemetine	As above
	followed by	
	chloroquine phosphate	600 mg base (1 g)/day × 2 days, then 300 mg base (500 mg/day × 2–3 weeks)
	plus	
	iodoquinol	As above

[a]Pediatric doses are different.
[b]Maximum dosage of 2 g/day should *not* be exceeded (risk of optic neuritis).
[c]Possible carcinogen; not for use in pregnant women; other nitroimidazoles available outside the United States (tinidazole, ornidazole) may be preferred.
[d]Monitor EKG; probably preferred over emetine (1 mg/kg per day IM × 5 days; maximum 60 mg/day).

endemic areas, positive serologic tests are less useful. Percutaneous drainage of a large abscess carries the risk of rupture and spread of infection. However, percutaneous drainage in the setting of disease unresponsive to antibiotics or of anticipated rupture or in the presence of mixed bacterial and amebic infection may be indicated. Occasionally, percutanous drainage has provided a diagnosis in the setting of negative serologic tests. Catheters placed into liver abscesses should be removed immediately after draining the lesion.

8.8. Therapy

A broad range of antiamebic drugs are available, and their use is based both on their site of action (tissue vs. colonic lumen) and the potential toxicities of each agent (Table 9). Emetine and dehydroemetine must be given intramuscularly and have depressant effects on the myocardium. These agents are effective in tissues. Agents with actions within the lumen of the bowel include diiodohydroxyquine, diloxanide furoate, and paromomycin. The most effective drugs available at present have activity both in the tissue and in the intestinal lumen; they include metronidazole and the nitroimidazole derivatives (tinidazole, ornidiazole, secnidazole, and nimorazole).

Treatment for asymptomatic cyst passers is controversial due to the frequency of infection with nonpathogenic strains of amebae.[329] Some recommended treatments are presented in Table 9. The luminal agents such as diloxanide are more effective than metronidazole in the absence of invasive disease. Invasive disease should be treated with a nitroimidazole with a luminal agent. In the presence of colonic perforation, antibacterial therapy should be added, using metronidazole to cover anaerobic flora as well as the amebic infection. The need for surgery with microperforation is controversial. However, with acute appendicitis or toxic megacolon, surgery will be necessary. Ameboma may be cured with antibiotic therapy alone.[332] Similarly, liver abscess is rarely an indication for surgery. Antibiotic therapy is generally successful, and abscesses resolve without fibrosis. Because treatment failures with metronidazole have been observed, liver abscesses are generally treated with combination therapy using a nitroimidazole agent and dehydroemetine.

9. Primary Amebic Meningoencephalitis

Primary amebic meningoencephalitis (PAM) is an uncommon infection of the CNS produced by the amebae *Naegleria* and *Acanthamoeba*.[333] Depending on the species of organism, the progression of disease may be chronic or acute. PAM is usually fatal.

9.1. The Organism

While secondary involvement of the nervous system with amebic infection can occur with *Entamoebae histolytica,* primary infections are due to *Naegleria fowleri* and a number of species of *Acanthamoeba. Naegleria* trophozoites are amorphous or sluglike in shape. The most easily identifiable form of the organism is the flagellate induced from trophozoites placed in distilled water. Flagellates do not occur during human infection. *Acanthamoeba* trophozoites have pseudopodia surrounding one end. Both *Naegleria* and *Acanthamoeba* may be seen containing ingested red blood cells; both occur in a thick-walled cyst form. The trophozoites of these organisms vary from 7 to 20 μm in size. These organisms are capable of producing PAM in many animal species by intranasal or intravenous inoculation. There does not appear to be an intermediate host. The virulence of a given strain will depend on the organism and on the host's immune status.

9.2. Epidemiology

Amebic meningoencephalitis has been reported worldwide. The majority of cases are due to *Naegleria* species. The natural habitat of these amebae is soil and fresh warm water. Common-source epidemics have been detected as a result of contaminated water supplies. However, these organisms are commonly found in pools of warm water such as aquariums or hospital hydrotherapy tanks. Reported cases of PAM have come from throughout the United States and Europe, central Africa, India and other parts of Asia, and Australia. Pathogenic organisms are easily missed in the evaluation of acutely ill patients.

9.3. Pathogenesis

Naegleria generally enters the CNS via the olfactory neuroepithelium when water or dust particles enter the nose. By contrast, *Acanthamoeba* causes infection of other organ systems, including skin, lung, or eye, and then spreads to the CNS via the bloodstream. The clinical presentation of *Naegleria* infection is an acute and rapidly progressive meningitis that is fatal in less than 4 days without therapy. The inflammatory cell response is primarily polynuclear leukocytes. As *Naegleria* invades the superficial cortex, the olfactory and frontotemporal areas of the brain quickly develop hemorrhagic necrosis. Trophozoites are prominent.

The pace of infection with *Acanthamoeba* is generally slower, producing ultimately fatal disease in 2–3 weeks. The inflammatory response is usually lymphocytic, with macrophages, granuloma formation, giant cells, and vasculitis. The pathology and clinical picture are those of multiple space-occupying brain abscesses. These abscesses are located in the white matter in deep midline and midbrain structures. Organisms are found in tissues, but, in general, not in CSF. Because no therapy is available, this infection is uniformly fatal. Because of the effects on deep structures, *Acanthamoeba* may cause a picture of subacute or chronic encephalitis before focal deficits develop related to brain abscesses.

9.4. The Patient and Diagnosis

While acute PAM will present as severe bacterial meningitis, the patient will rapidly progress from headache, fever, and vomiting to seizures, coma, or paralysis. *Naegleria* may produce false sensations of taste or smell similar to those seen in some patients with heart failure and early brainstem herniation. *Naegleria* trophozoites will be found in CSF, with markedly low glucose levels and a neutrophil count as high as 15,000. The CSF protein level may be normal or slightly elevated. Progression is even more rapid in immunocompromised hosts.

Patients with *Acanthamoeba* infection may also present with headache and fever. Focal neurologic deficits or seizures occur early in the course of this subacute disease. The patient will undergo a gradual neurologic deterioration over the course of 2–4 weeks. The clinical picture exceeds what would be expected from a focal brain abscess. The CSF generally does not contain organisms, and the glucose is normal or slightly depressed. The CSF contains 100–400 cells, which include both lymphocytes and neutrophils. Organisms are found in brain biopsy specimens. Serologic tests are useful diagnostically, but not clinically. *Acanthamoeba* infection may produce disease of the eye or respiratory tract in normal hosts. Eye injury has been associated with infections carried by contact lenses or local trauma.

9.5. Therapy

Few individuals have survived primary amebic meningoencephalitis.[333,334] Amphotericin B should be used at a dose of at least 1 mg/kg per day. Given the rapid progression of PAM, there is no opportunity for gradual escalation of dosing. There is no useful therapy

for *Acanthamoeba,* although *in vitro* sensitivity of *Acanthamoeba* to polymixin, pentamidine, ketoconazole, 5-fluorocytosine, and paromomycin has been demonstrated.

10. Leishmaniasis

Leishmaniasis encompasses a variety of diseases, including cutaneous, mucocutaneous, and visceral disease, depending on the species of *Leishmania* and the immune status of the host. Because all species of *Leishmania* are intracellular parasites of macrophages, the functional status of T lymphocytes and of cytokines affecting macrophage function will determine whether or not the organism disseminates locally or systemically.

10.1. The Organism

Species of *Leishmania* can be separated by geographic distribution and the clinical manifestations of infection. The organisms that cause cutaneous and mucocutaneous leishmaniasis include *L. braziliensis, L. major, L. mexicana, L. tropica, L. peruviana,* and *L. aethiopica. Leishmania tropica* can also cause chronic, relapsing cutaneous disease (recidivans form). *Leishmania donovani, L. infantum,* and *L. chagasi* can cause visceral leishmaniasis. Consensus about species differentiation will probably result from sequence and hybridization analysis using kinetoplast DNA probes.

Leishmania exists in two forms. Within the sand fly vector or in culture, they are single-celled, flagellated extracellular promastigotes. Within the cells of vertebrate hosts, they are amastigotes without flagellae, 2–3 μm in diameter. Human infection is initiated by the bite of an infected female phlebotomine sand fly. Organisms are phagocytosed by macrophages at the site of the bite and replicate by binary fission within the macrophage. Each strain of *Leishmania* has a unique and complex interaction with the phagolysosome; in general, the parasite is resistant to the lysosomal acidic pH.

10.2. Epidemiology

The spread of leishmaniasis is dependent on the presence of the appropriate species of sand fly.[335] Other than *L. donovani* and *L. tropica,* the organism is generally maintained in wild animals, including rodents, dogs, marsupials, and other wild animals. *Leishmania donovani* and *L. tropica* appear to be able to use the human as a definitive host. Various forms of *Leishmania* are found in the southern United States, Central and South America, and throughout Africa, southern Asia, Europe, and the Middle East. Virtually all the cases seen in the United States are acquired outside the country. The exact frequency of these infections is unclear, because the pathogen has caused disease as late as 30 years after the initial infection. Distant exposure must be excluded before the diagnosis is excluded. In endemic areas, the annual incidence is 0.1–1% and may go as high as 5% during epidemics. Malnourished or immunocompromised individuals are most susceptible to symptomatic and severe infections.[336]

Malnutrition is probably the most important immunosuppressive mechanism predisposing to severe visceral leishmaniasis.[335,337,338] While many individuals in endemic areas are infected but develop relatively mild disease, individuals with malnutrition are much more likely to develop classic, advanced, visceral disease. This difference is most easily seen in children, in whom untreated symptomatic infection will be fatal in 75–90% of those affected. Only 10–20% of affected individuals will develop clinically apparent disease.[339] In immunosuppressed individuals with hemotologic malignancies, organ transplants, or immunosuppressive therapies, especially corticosteroids, disease may have a more rapid progression and be more difficult to diagnose.[336,340–345] In these individuals, the disease is more often chronic and the response to therapy less rewarding. Chronic, relapsing, visceral leishmaniasis has been described as a complication of AIDS in patients from Spain, France, and Italy.[346,347] AIDS patients do not develop antibodies to *Leishmania,* in contrast to the nearly uniform detection of antibodies in immunocompetent individuals and in non-AIDS immunocompromised patients with visceral disease. It is possible that *Leishmania* may reduce immune responsiveness to infections due to other organisms. Antibodies against a broad range of antigens have been observed in early visceral disease.

10.3. Pathogenesis

Cutaneous and mucocutaneous forms of leishmaniasis begin when promastigotes are injected subcutaneously by the sand fly and enter local host cells. Local inflammation is primarily lymphocytic and granulomatous, with necrosis of the skin occurring early. Organisms spread via the bloodstream or lymphatics to the mucosal surfaces of the nose, mouth, pharynx, and larynx. Inflammation is generally modest. Dermal necrosis is probably due to the local immune reaction. Subsequently, hyperkeratosis and acanthosis may occur. The number of organisms in infected cells is variable.

In visceral leishmaniasis, infected macrophages from the skin serve as a reservoir for organisms that infect spleen, lymph nodes, liver, bone marrow, and intestinal mucosa. This infection causes hyperplasia of focal lymphoid tissue with granulomata. Ulceration of mu-

cosal surfaces may occur, and endothelial proliferation may occur in pulmonary alveolar capillaries and in blood vessels of the renal glomeruli. The spleen and liver are enlarged due to parasitization of macrophages and Kupffer cells. Some areas of skin around the initial bite will have nodules containing parasites; some areas that appear normal will also contain parasites.

10.4. Immunology

Immunity to *Leishmania* is thought to be mediated by CD4+ T lymphocytes, with lymphokines from these cells enhancing the killing of intracellular organisms.[347–349] The manifestations of the disease are determined by the level of immune response. In cutaneous leishmaniasis, patients lacking immune responsiveness to the parasite may develop diffuse cutaneous leishmaniasis (DCL) with little lymphocyte infiltration of areas of involvement. By contrast, "leishmaniasis recidivans" is the result of an exuberant lymphocytic response to low numbers of parasites that persist within macrophages. In visceral leishmaniasis, the host is completely lacking a cellular immune response to leishmanial antigens.[350] Parasitization of the reticuloendothelial system is uncontrolled. Despite high levels of circulating antibodies in immune complexes, disease may spread rapidly without therapy. Suppressor lymphocytes appear to play a role in the loss of antigen reactivity seen in visceral leishmaniasis.

10.5. The Patient: Clinical Manifestations

Cutaneous and mucocutaneous leishmaniasis may cause clinical symptoms weeks to years after initial infection. In cutaneous disease, a small papule at the site of the bite will develop into a nodule. Necrosis leaves a painless ulcer with raised firm edges. Multiple lesions may occur in the same area or along lymphatics. These lesions will generally heal spontaneously. Specific strains of *Leishmania* may involve the ears, upper face, and nose or cause painful lesions. Immune suppression may cause a relapse in previously healed areas. Experience with leishmaniasis in soldiers serving in the Middle East suggests that systemic manifestations of cutaneous disease are common and may reflect organisms found in the bone marrow and in other visceral locations in the immunologically normal host.

Mucocutaneous leishmaniasis ("espundia") is usually a complication of cutaneous disease occurring years after the initial skin lesions have healed. Organisms that spread to mucosal tissues may produce symptoms similar to sinusitis or nosebleeds. Granulomatous inflammation with necrosis may destroy the nasal septum and surrounding tissues. This inflammation is frequently complicated by bacterial superinfection.

Uncommon forms of leishmaniasis include a chronic relapsing or recidivans disease and DCL. Chronic relapsing disease occurs in or surrounding the area of original cutaneous involvement. Diffuse cutaneous disease is associated with lack of immune reactivity to leishmanial antigens. Patients develop nonulcerative nodules across the skin, with little inflammatory reaction. Visceral leishmaniasis will become symptomatic weeks to months after infection, with the gradual onset of fever, sweats, and weight loss. Nonspecific abdominal complaints accompany hepatosplenomegaly and wasting of large muscle groups. Complications are the results of anemia, thrombocytopenia, liver failure, or secondary bacterial infection.

10.6. Diagnosis and Therapy

Patients with symptoms of leishmaniasis are modest problems in differential diagnosis. The cutaneous lesions, especially in the immunocompromised host, might be seen in diseases due to mycobacteria, including *M. leprae* or *M. marinum,* cutaneous diphtheria, sarcoidosis, histoplasmosis, yaws, and fungi including sporotrichosis. Mucocutaneous disease may be confused with the destructive lesions of lethal midline granulomatous disease or blastomycosis. Visceral leishmaniasis with hepatosplenomegaly can mimic or complicate lymphoma, malaria, schistosomiasis, endocarditis, brucellosis, leukemia, immunosuppression for organ transplantation or with corticosteroids, or advanced histoplasmosis.[345,346]

Diagnosis is based on the identification of organisms from tissues. In cutaneous disease, needle aspiration or punch biopsy should be performed at the edge of the lesion. In visceral kala-azar, tissue aspirate from spleen, bone marrow, lymph node, or liver are often diagnostic. Parasites are occasionally seen in peripheral blood smears. Parasites can be cultured in special media or in animals. Serologic tests are useful in non-AIDS patients, but may be negative in the first week to 10 days after infection.[351]

Leishmaniasis is treated wtih pentavalent antimony: stibogluconate sodium (Pentostam) or meglumine antimoniate (Glucantime). Stibogluconate is used at a dose of 20 mg/kg body weight for 2–3 weeks. Controversy exists as to the maximum daily dose, but it should generally not exceed 850 mg to 1 g daily in adults. Relapse with mucocutaneous and visceral disease is not uncommon and may necessitate multiple courses of treatment for cure. Occasional abnormalities in liver function tests and EKGs may be seen, with elevations of the total white blood count as side effects of therapy. Patients may also

complain of arthralgias. Meglumine is given by injection for American cutaneous disease (20 mg/kg per day × 15 days). Allopurinol (20 mg/kg per day in 4 divided doses) may have additive efficacy with pentavalent antimony or in patients with unresponsive disease. Allopurinol monotherapy may be less toxic, less costly, and more effective than the antimony compounds. Amphotericin B is another alternative for therapy of cutaneous or mucocutaneous leishmaniasis. Pentamidine is an alternative for therapy of visceral disease. Relapses are apparently related to failure to develop an immune response during the course of therapy. Newer therapies under study include topical therapies for skin disease and ketoconazole for systemic disease due to *L. mexicana*.[352]

11. Other Parasitic Diseases of the Immunocompromised Host

The presentations and frequencies of some of the most common parasitic diseases of man are relatively unchanged in the patient with altered immunity. Others are important in the presence of specific immune lesions. The failure to detect enhanced infection may relate to variability in the duration or severity of these diseases in normal individuals. More often, the failure of immune suppression to exacerbate infection suggests either a lack of involvement of the immune system in the control of infection in the normal host or the inability of the organism to complete its life cycle in man. A number of important infections are occasionally problematic in the patient with immune dysfunction. These infections are discussed below.

11.1. *Giardia lamblia*

Giardiasis is the most common protozoan disease in the United States and an important cause of enteric disease worldwide. Infection with *G. lamblia* is caused by ingestion of food or water contaminated with cysts. Trophozoites develop in the duodenum after exposure of cysts to acid in the stomach. This free-swimming flagellate causes disease by attachment to intestinal epithelial cells and may cause prolonged infection despite therapy. The patient may develop diarrhea, malabsorption, and weight loss of varying degrees.[353] Children are more frequently infected than adults, and prior infection appears to confer protection against subsequent attacks.[354] Immunocompromised individuals appear to be at greater risk, perhaps most often related to malnutrition.[355] Infection appears to be more common in patients with hypogammaglobulinemia or dysgammaglobulinemia, although circulating antibody does not appear to be protective.[277,356–361] Mucosal IgA may provide a barrier against giardial infection.[362] Secretory IgA of breast milk may also be protective.[363]

11.1.1. Epidemiology

Despite the frequency of *G. lamblia* in the stools of homosexual males, giardiasis is not a common pathogen of unusual severity in patients with AIDS.[364–367] *Giardia* is found in contaminated water, in animals including beavers and dogs, and after person-to-person spread in day care centers.[263,354,368–372] Infection rates are high in institutions for the mentally disabled and in patients with achlorhydria. The cyst is hardy and survives for months in fresh cool water.[373] Small numbers of cysts can cause infection.

11.1.2. Diagnosis and Therapy

Giardia is a common cause of diarrhea and nonspecific abdominal complaints. Unexplained malabsorption or lactose intolerance may be observed.[353,374–376] Colonoscopy will be entirely normal.[377] The detection of organisms in stool or on duodenal aspirate or using the Enterotest vial will provide the diagnosis. Patients are generally treated with metronidazole, 250 mg 3 or 4 times a day for 7–10 days. This drug should be used with caution in children or in pregnant women. Repeat therapy may be necessary in up to 20% of individuals. Malabsorptive symptoms may be slower to resolve than the infection itself. Alternatively, quinacrine hydrochloride can be used for therapy at 100 mg orally 3 times a day for 5 days (2 mg/kg in young children).

11.2. Malaria

Given the importance of malaria as a pathogen worldwide, it is striking that this intracellular protozoan has not emerged as an opportunistic pathogen in patients with AIDS. The life cycle of malaria begins when the female anopheline mosquito inoculates sporozoites into the host during a blood meal. These organisms enter liver cells and proliferate, releasing merozoites that then invade erythrocytes. Immunity occurs in endemic areas after repeated infection and is both species- and strain-specific. Passive transfer of antibodies is protective, blocking infection of red blood cells and killing intracellular organisms via antibody-dependent cellular cytotoxicity (ADCC). ADCC requires normal splenic function. The development of immunity requires normal T-cell function. Immunity to sporozoites appears to be mediated at least in part by T cells and not by antibody.

Malaria is primarily a tropical disease and is caused by four major species, with each having different clinical manifestations. *Plasmodium falciparum* causes the most

rapidly progressive disease, including anemia, renal failure, cerebral disease, pulmonary edema, liver failure, and death. *Plasmodium vivax* causes anemia and splenic rupture in severe cases. *Plasmodium malariae* may persist as an asymptomatic infection for many years and may cause nephrotic syndrome in children. *Plasmodium ovale* causes the acute infectious syndrome seen in all forms of malaria. The acute presentations will be of rigors with high fever and sweats. Headache and nausea and occasionally seizures may be seen in all forms, but are particularly important symptoms with *P. falciparum*. As the life cycle of the organism become synchronized, cycles of chills, fever, and sweats become characteristic for the species of malaria. Most patients will have nonspecific complaints, including myalgias, cough, and diarrhea, and may have anemia, jaundice, or abdominal tenderness. Relapse in malaria (*P. ovale* and *P. vivax*) may present years after initial infection. It is likely that infection is more severe in patients with functional or anatomic asplenia, during pregnancy, and possibly during immune suppression.[378]

Therapy is based on the type of malaria seen on the peripheral blood smear and on the pattern of antibiotic resistance in the area in which it was acquired. If malaria is likely, the blood smears should be repeated after a negative examination, as the level of parasitemia may fluctuate. Significant infection in immunocompromised individuals has been associated with transfusions of infected blood with *P. malariae* into immunocompromised individuals. Transfusional disease will cause significant infection in immunocompromised individuals.[378]

11.3. Babesiosis

The babesiae are protozoan parasites of animals transmitted by the Ixodid tick to humans as an incidental host. There are over 70 species of *Babesia,* several of which cause human disease, including *B. microti* and *B. divergens*. Most of the cases of human babesiosis have been described from the northeastern United States. A few cases of *B. divergens* have been reported from Europe. Occasionally, babesiosis has been seen as a complication of blood transfusion or in organ transplantation. On the basis of serologic studies, it is likely that babesial infections occur worldwide but are asymptomatic or mildly symptomatic.

The organism reproduces within erythrocytes, causing hemolysis and hemoglobinuria. Hypotension may result from the release of a kallikrein activator by the organism. Splenectomy or abnormal splenic function and T-lymphocyte dysfunction have been associated with especially severe disease. Recurrent and apparently relapsing infection has been reported in a single AIDS patient. The level of parasitemia is markedly enhanced by administration of corticosteroids in animal models.

Most patients will present with fever, chills, diarrhea, vomiting, anemia, myalgias, and fatigue.[379–383] The clinical manifestations will depend on the species of *Babesia* causing infection. *Babesia microti* causes mild disease that appears to remit spontaneously.[383] Patients will have mild elevations in liver function tests and parasitemia of less than 10%. Symptoms and parasitemia may exist for up to 4 months and are increased after splenectomy. *Babesia divergens* has been seen in splenectomized hosts, with severe disease culminating in renal failure, hypotension, and severe anemia. These infections have all been fatal. Diagnosis is by blood smear. Antibody-based tests are also available.[384]

Treatment has been controversial, but therapy with clindamycin and quinine has been effective in some patients.[385,386] The high-grade parasitemia seen in splenectomized patients or in those receiving corticosteroids has responded to exchange blood transfusion in a few patients. It appears that pentamidine isethionate also reduces parasitemia. Quinine can be given in a dose of 650 mg orally 3 times a day with clindamycin at 600 mg orally 3 times or intravenously 3 or 4 times a day.

11.4. American Trypanosomiasis (Chagas' Disease)

Trypanosoma cruzi is a protozoan that infects man during a blood meal by the reduvid bug. These organisms develop intracellularly, releasing aflagellates that enter new cells and repeat the cycle. *Trypanosoma cruzi* have a particular predilection for muscle (including cardiac) and neuroglial cells, and produce local inflammation with lymphocytes, macrophages, and plasma cells. In addition to reduvid infection, infection has occurred as a result of organ transplantation and via blood transfusion. Up to 20 million people are infected worldwide, including up to 40–50% of the population of endemic areas of Central and South America. Cases related to blood transfusion have been reported in the United States and Canada, as well as in endemic areas.

The patient will present with fever, lymphadenopathy, hepatosplenomegaly, and headache. A small, painful indurated area (chagoma) or unilateral orbital edema with conjunctivitis (Romana's sign) will be present in many patients. The patient may develop symptoms of myocarditis or meningoencephalitis. Sequelae of the acute infection may be seen many years after symptomatic or asymptomatic initial infection. The major complications of Chagas' disease are cardiac arrhythmias or conduction defects with congestive heart failure. GI involvement may appear as megacolon or megaesophagus.

Latent infection may be reactivated by immune suppression, including individuals who are the recipients of infected organ grafts.[1]

Treatment is with nifurtimox or benznidazole, but may fail to eradicate the parasite completely.

11.5. African Trypanosomiasis

African trypanosomiasis is caused by *Trypanosoma brucei,* which causes African sleeping sickness. Infection is initiated by the bite of the tsetse fly of Africa. After being injected into the human host, the parasites multiply locally, producing a chancre at the site of replication. Once in the bloodstream or within tissues, the parasite evades immune detection through a process called *antigenic variation.* A hemolyphatic phase of the disease occurs, with bloodstream invasion weeks or months after the initial chancre. This phase is characterized by fever, lymphadenopathy, fleeting rashes, edema of face or legs, ascites, or pleural and pericardial effusion. Jaundice and myocarditis may progress to rapidly fatal complications. Trypanosomal invasion of the basal ganglia produces meningeal inflammation extending into the brain cortex with perivascular cuffing. Persistent headache and altered mental function will develop, with a decreased level of consciousness commonly termed "sleeping sickness."

This disease affects over 20,000 people a year. In contrast to most of the pathogens of importance to the immunocompromised individual, African trypanosomiasis *causes* immune suppression sufficient to allow the development of opportunistic infection, especially pneumonia. Because the treatment for infection is often toxic (suramin or melarsoprol), malnourished patients in endemic areas need to have their nutrition and general clinical status optimized before they will tolerate treatment. Patients will often relapse after therapy. Many of the manifestations of the disease appear to be immune-mediated. Generalized B-cell activation results in an increase in serum immunoglobulins (including autoantibodies) and immune complexes. Patients with African trypanosomiasis may also have diminished reactivity to vaccination or to skin testing.

Diagnosis is based on the detection of parasites in blood, on aspiration of chancres, from lymph nodes, or from organisms found in CSF. Therapy is effective if meningoencephalitis has not developed. The drugs used in therapy include suramin and pentamidine. Both these drugs have toxic side effects. Late-stage disease is treated with melarsoprol. Lethal encephalopathy occurs in up to 10% of patients treated with melarsoprol. Some success with α-difluoromethylornithine (DFMO) has been reported.

11.6. *Cyclospora*

A group of blue-green algaelike organisms (cyanobacteria) has been implicated as the causative agent of a severe diarrheal illness in travelers and in patients with AIDS.[387–391] Electron microscopic studies have suggested that these "cyanobacteriumlike" bodies represent a new protozoan pathogen of the coccidian genus *Cyclospora.* Like *Cryptosporidium,* this organism causes self-limited relapsing diarrheal illness in the normal host (up to 3 months) and, possibly, persistent diarrhea in the few cases described in patients with AIDS. Infection is most often described in travelers after contact with contaminated water supplies in tropical regions.

The organism is 8–10 μm in diameter with a cluster (morula) of refractile, membrane-bound globule within a limiting membrane. The organism is a thick-walled sphere with a fibrillar coat and granular cytoplasm. There is no nucleus but a series of internal lamellar-type structures similar to chloroplasts. Organisms stain with modified acid-fast and phenosafranin stains. Sheather's sugar flotation has been used to concentrate stool specimens. Organisms can be excysted or sporulated *in vivo* in potassium dichromate. *Cyclospora* have been detected following exposure to contaminated water and in travelers in tropical regions (up to 11% of nonnative individuals with diarrhea). Person-to-person spread was suggested by an outbreak occurring among medical housestaff in Chicago. Infection is most prominent in small bowel, often in combination with other parasitic infections. Malabsorption (as measured by D-xylose testing) is often present. No effective therapy has yet been described for *Cyclospora* infection, based on controlled clinical trials.

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