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Pneumocystis carinii and Parasitic Infections in the Immunocompromised Host

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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 presence, progression, and manifestations of some of the common parasitic diseases are altered by immune compromise. 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. Recently, however, the pattern of parasitic infection has been altered by a number of important trends in clinical medicine (see Table 1):

- The growing population of immunocompromised individuals.
- Prolonged survival with immune deficits.
- Increased use of immunosuppressive therapies in underdeveloped regions.
- Highly active antiretroviral therapies (HAART) for human immunodeficiency virus (HIV) infection have resulted in immune reconstitution in many individuals with reduced susceptibility to common parasites and to *Pneumocystis carinii.*
- New immunosuppressive agents are employed in a broader range of patients including generic cyclosporine, tacrolimus, mycophenylate mofetil, sirolimus, costimulatory blockade, antilymphocyte antibodies, and broader application of intensive chemotherapy and hematopoietic transplantation for malignancy.
- Broader use of routine prophylactic strategies for common infections in compromised individuals.

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 parasitic infections are defined by the organisms' "natural" mode of evasion/interaction with the host's immune system and by the nature of the immune lesion(s) (see Table 1). Thus, an organism of low native virulence (e.g., *P. carinii*) causes great morbidity in patients with AIDS or following organ transplantation.

1.1. Parasite-Specific 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 malnutri-

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TABLE 1. Mechanisms for the Evasion of 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]	*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.

tion, by the epidemic of AIDS in developing regions, 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 should suggest acute or reactivated infection in symptomatic individuals. Some infections are prevalent in subgroups of immunologically normal hosts in developed regions. Thus, homosexual males have an increased incidence of infections with intestinal parasites (including *Trichuris* and pathogenic *Entamoeba histolytica, Giardia lamblia, Strongyloides stercoralis*) and nonpathogenic protozoa. Infection with other pathogens (cytomegalovirus, *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* species.

A few features of parasitic infections merit emphasis.

- The life cycle of the parasite 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.
- Immune suppression has the greatest effect on the life cycles of organisms that are normally suppressed or regulated by the host's immune response.
- In general, only those infections due to organisms that can complete their life cycle within the human host are amplified in the immunocompromised host. *Strongyloides stercoralis* is unusual as a nematode because of the ability to complete its life cycle within the human host. Thus, the inflammatory response to all of the helminths are reduced in patients treated with corticosteroids, but only *Strongyloides* infection is significantly exacerbated by such treatment.
- The protozoa as a group have the capability to complete their life cycles within the human host and are common pathogens in the setting of immune deficiency. Accelerated growth of protozoans derepressed by immune dysfunction produces systemic diseases that reflect both the initial sites (organs) of infection and the metastatic spread of infection. Thus, 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 as may be seen in leishmaniasis in AIDS or solid organ transplantation.
- Latency is a critical feature of most parasitic infections of compromised hosts. Primary infections (e.g., due to *Strongyloides, Leishmania donovani, T. cruzi,* and *T. gondii*) may be observed in endemic regions, but are generally less common than reactivation syndromes due to more distant exposures. Transplanted organs are commonly impli-

cated as the source of latent infection in immunologically naive allograft recipients.

- The impact of infections due to organisms that require maturation outside the host and subsequent penetration (often ingestion) into the host is limited by the size of the initial inoculum. The burden of this group of organisms cannot increase during the course of disease. As a result, helminths (worms) tend to cause mechanical obstruction due to size, location, and nonspecific inflammatory responses (fibrosis) and are generally limited to the gastrointestinal (GI) tract. These pathogens (e.g., *Schistosoma* or the liver flukes) may cause organ failure (renal or hepatic), which necessitate transplantation, but are not significantly exacerbated by immune suppression.
- Coinfection is a critical feature of these infections. Thus, invasive disease of the gastrointestinal tract or lungs is more common in the setting of simultaneous viral infection (particularly cytomegalovirus) of these organs. In the compromised individual, all active infections must be treated for successful clinical resolution.

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., *Schistosoma, Trypanosoma cruzi*).[1–3] Others vary or shed surface antigens to avoid detection. Malarial parasites may alter the surface of host cells and release toxins, which induce the production of cytokines, enhancing display of receptors needed for cellular penetration. 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.

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, Cyclospora*

[a]This table does not list common parasitic infections that are not increased in severity or frequency in immunocompromised hosts.
[b]*Pneumocystis carinii* is included for purposes of this discussion, but is considered to be a fungus.

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. In normal hosts, secondary *Leishmania* infections produce smaller lesions and lower parasite burdens than primary infections. The intensity of inflammation in Chagas' disease is similarly dictated by the intensity of the host response. The role of

autoimmunity in the clinical manifestations of Chagas' disease remains unresolved.

1.3. Missing Infections in Compromised Hosts

Immunity to parasites is complex. Generally, multiple components of the immune system contribute to the prevention or resolution of infection. Thus, common knowledge about the nature of immune deficiency in individuals is often incorrect. Patients with AIDS who undergo organ transplantation have been shown to require immune suppression to prevent graft rejection. This observation suggests that although the prime immune deficiencies of AIDS and in transplantation are "T-cell mediated," some aspects of immune function are well preserved in HIV infection. Thus, it is predictable that specific infections would occur with differing frequencies in transplantation, neutropenia, and AIDS. A subgroup of common parasitic infections has not increased substantially in frequency or severity in individuals infected with HIV.[5] These infections include *S. stercoralis,* malaria, *E. histolytica,* and trypanosomiasis. Reports of strongyloidiasis in AIDS generally have been in individuals also receiving immunosuppressive therapies or with underlying malignancy. Given the relative absence of disseminated strongyloidiasis in this population, the immune deficits of AIDS must not include some of the relevant host defenses (e.g., of the intestinal mucosa) that are altered in organ transplantation or in neutropenia. Perhaps of greater importance is the relative absence of *Strongyloides* in transplant recipients on corticosteroid-sparing regimens. It has been suggested that steroids may mimic the effect of naturally occurring ecdysteroids that accelerate the maturation (molting) of rhabditiform larvae.

Multiple potential pathogens often are found in diarrheal stools from individuals infected with HIV.[6,7] The organism(s) causing disease (e.g., *Microsporidia* or *Cryptosporidia* species) may be demonstrated microscopically in the small intestine but often are undetected in stool samples. These diarrheal pathogens are now being recognized in hematopoietic and solid organ transplant recipients, particularly in agricultural areas subject to episodic flooding of potable water supplies. Gastrointestinal cytomegalovirus is an important cofactor to many of these agents.

2. *Pneumocystis carinii*

Pneumocystis carinii was described 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] *Pneumocystis* also has emerged as a major pathogen in solid organ transplant recipients.[29,30] The approach to the prevention of *Pneumocystis* pneumonia has evolved based on experience in AIDS and due to the large number of individuals intolerant of first-line therapies (due to sulfa drug intolerance).[29,30] Recommendations for the prevention of *Pneumocystis* infection have changed with the development of highly active antiretroviral therapies (HAART) for HIV infection.

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). Antimicrobial agents 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. Molecular studies of the organism suggest that *Pneumocystis* is more closely related to the fungi than to the protozoan parasites.[31–34] 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

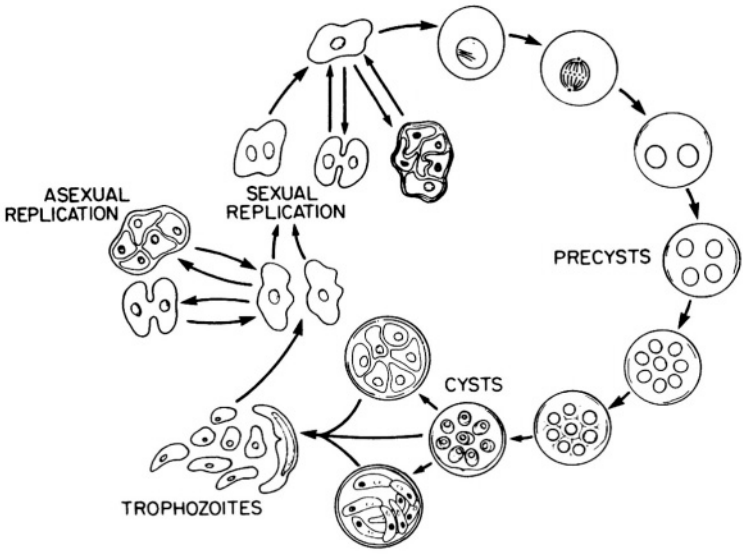

FIGURE 1. Life cycle of *Pneumocystis carinii*. Both sexual and asexual reproduction have been postulated. Glucan synthesis is necessary for cyst wall formation. Only the cyst form stains with methenamine–silver stains.

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. 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* suggests 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 internal "sporozoites." The cysts rapture 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

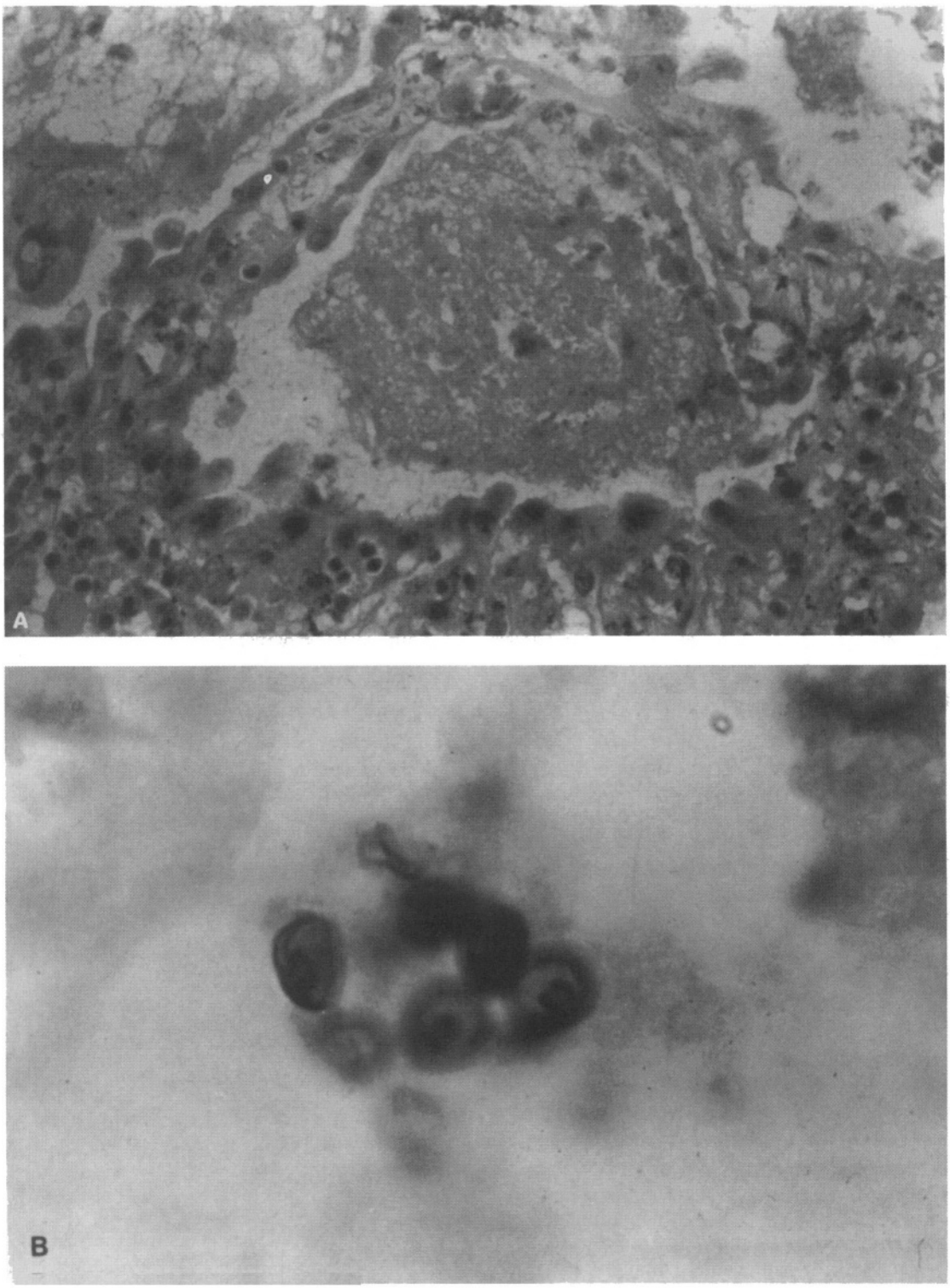

FIGURE 2. (A) Hematoxylin–eosin stain of *Pneumocystis*-infected lung demonstrating pathognomonic intra-alveolar "frothy" material, interstitial widening, and minimal inflammatory cell infiltration. The biopsy was obtained from a patient with AIDS. (B) Induced sputum examination reveals multiple cyst 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.

first *in vitro* cultivation by Pifer *et al.*[41,43] and Latorre *et al.*[44] in 1977.[45,46] Continuous axenic culture of organisms has not been achieved.[42] The culture systems have assisted in "cleaning up" preparation of organisms that are contaminated by host lung cells and proteins and have been useful in drug screening assay systems. Viability tests for *P. carinii* are in their infancy. Reviews of the biology of *P. carinii* have been published.[25,26,40,45]

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.[40,46–48] 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.*[49] in 1966.[50,51] 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. Hughes and others also have demonstrated aerosol transmission of the organism in the animal model. 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. However, mice deficient in GM-CSF have enhanced susceptibility to infection. 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 alternative agents available for treatment of *P. carinii* (aerosolized pentamidine, clindamycin–primaquine, dapsone–trimethoprim, atovaquone, echinocandin and other 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. 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.[60,63,64] 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.[65,66] Solubilized (and particulate) glycoproteins of the 55–60 kDa and 100–116 kDa ranges stimulate T-lymphocyte proliferation from sensitized hosts.[60,65] 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* also is 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 pa-

TABLE 3. Conditions Associated with *Pneumocystis carinii* Pneumonia

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

tients (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 or neutropenia, (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 tend to have greater susceptibility than do patients with other induced immune deficiencies.[70–72] The manifestations of disease are frequently muted in patients receiving a range of immunosuppressive agents or in AIDS patients with advanced disease. Symptoms may emerge with the cessation of immune suppression or with the return of immune function in neutropenic, HIV-infected, or bone marrow transplant or organ transplant recipients.

Serologic studies suggest that seroconversion to *P. carinii* usually occurs some time after the third 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] The absence of immunity to *P. carinii* in babies is illustrated in children with congenital HIV infection. In the pre-HAART era, these patients would be expected to survive for less than 1 year. Patients developing AIDS or HIV infection after 1 year of age do somewhat better with pneumocystosis. 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 [cytomegalovirus (CMV), HIV] infection, or the possibility that coinfection with certain agents may enhance the virulence of infection due to *P. carinii.* The association of CMV with *Pneumocystis* is commonly observed.[73,74] This association is largely due 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. *In vitro,* CMV infection enhances adhesion of organisms to the feeder cell monolayer. Asymptomatic CMV is often found in respiratory secretions from compromised individuals, but does not appear to increase the morbidity or mortality due to *P. carinii* pneumonia in AIDS. Invasive CMV pneumonitis may increase the severity of *Pneumocystis* pneumonia and requires treatment of both entities.[75]

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 suggest 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 have 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. Polymerase chain reaction (PCR) assays have not been applied routinely for clinical diagnosis.

2.3. Changing Patterns of *Pneumocystis*

Prior to the use of prophylactic antimicrobial agents, *P. carinii* pneumonia was the major complication and diagnostic manifestation of AIDS and a common complication of immune suppression of transplantation and cancer therapies. Without prophylaxis, over 80% of individuals

infected with HIV and up to 15% of other immunocompromised hosts would be expected to develop significant *Pneumocystis* pneumonia. Four factors have contributed to the reduction in morbidity associated with *Pneumocystis* infection in developed regions: (1) the incidence of *Pneumocystis* pneumonia and of other opportunistic infections have been reduced in AIDS patients receiving successful antiretroviral therapies (HAART); (2) recognition of *P. carinii* infection as a common presentation of AIDS; (3) improvements in the treatment of viral coinfections (i.e., CMV); and (4) routine use of anti-*Pneumocystis* prophylactic therapies. The incidence of pneumocystosis in AIDS patients is greatest in individuals with fewer than 200 CD4+ lymphocytes/mm^3 or in whom fewer than 20% of circulating lymphocytes are CD4+.[54,69] It is likely that alveolar macrophage activity against the organism also is decreased by HIV and CMV infections. Recent data suggest that primary prophylaxis may not be needed in individuals with persistent improvement in immune functions during HAART therapy as indicated by the absence of detectable HIV and CD4+ lymphocyte counts above 200/ml for 6 months or more. This observation has led to modification of the guidelines for prophylaxis. By contrast, prophylaxis is generally underutilized in patients receiving immune suppressive therapies for autoimmune and connective tissue diseases resulting in preventable infection in these individuals.

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

Clinical signs	Non-AIDS	AIDS (low CD4+ count)
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 (variable)	Asymmetric or bilateral interstitial infiltrate (often normal)
Response: initial Rx	Rapid (3–5 days)	Slow (5–9 days)
Recurrence	Unusual	Common[b]
Side effects of therapy	Usually mild	Common; some severe

[a]Altered by type and duration of immunodeficiency. More common/severe with CMV coinfection.
[b]Patients responding to HAART therapies may have fewer or less severe infections.

2.4. Clinical Manifestations of *Pneumocystis* Infection

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. Patients may report low-grade fevers, sweats, or systemic flulike symptoms. 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 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. Pleurisy and pneumothorax may occur acutely. 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 or tacrolimus therapy may 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 obstruction, and embolic phenomena have been seen in virtually every organ system including retinal lesions.

In the organ transplant recipient, *P. carinii* pneumonia will occur approximately 6–8 weeks after the initiation of immunosuppressive therapy or during periods of increased immunosuppression for treatment of episodes of graft rejection. Unprophylaxed liver transplant recipients treated with corticosteroids for autoimmune hepatitis prior to surgery, may develop *Pneumocystis* infection within days of transplantation. The incidence of *Pneumocystis* pneumonia depends on the center where

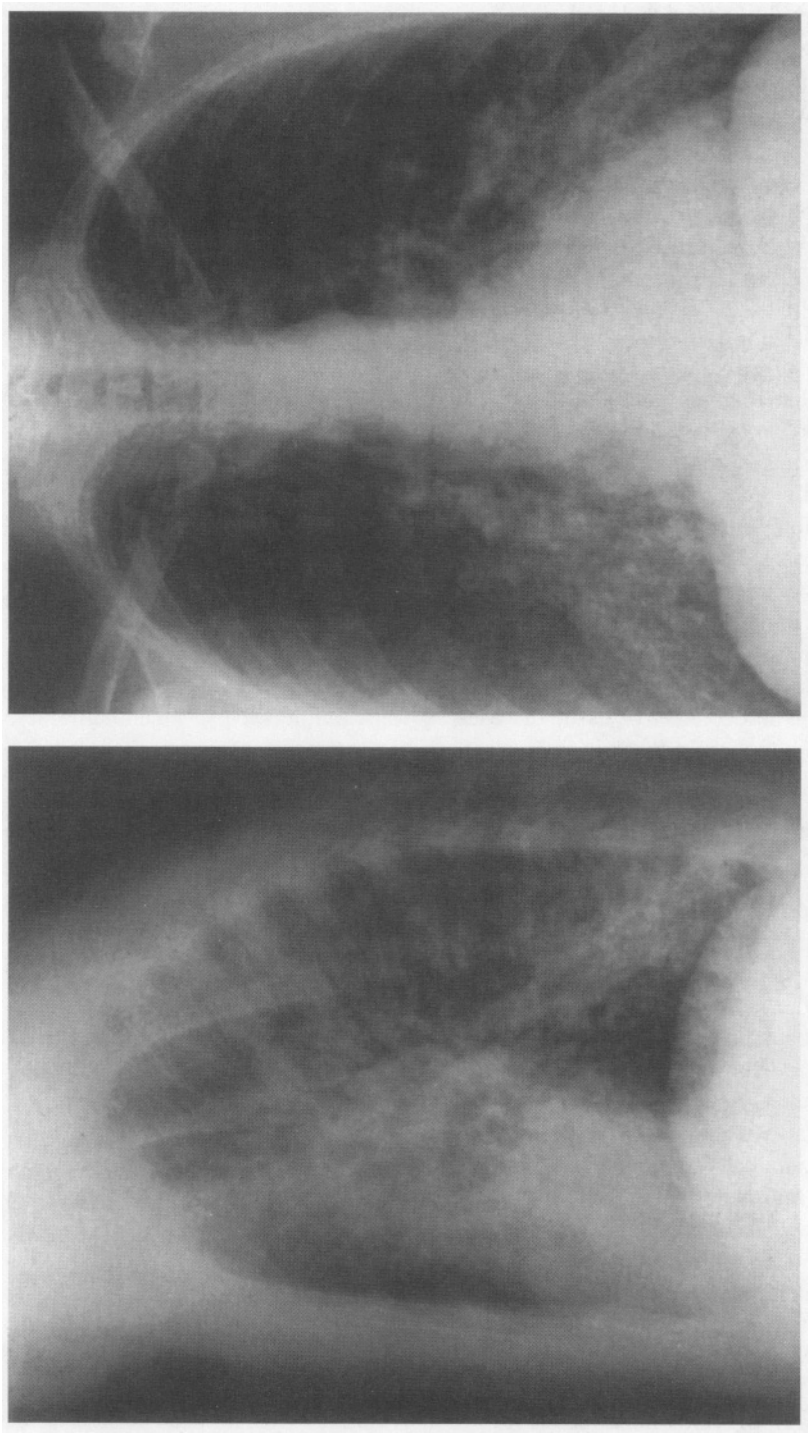

FIGURE 3. Chest radiograph of a 36-year-old man with *P. carinii* pneumonia following chemotherapy for a non-Hodgkin's lymphoma. A complete clinical history appears in Illustrative Case 1 (Section 2.9.2). Typical diffuse and bilateral, fine, interstitial infiltrates are observed.

transplantation is performed and the immunosuppression regimens and prophylactic regimens employed. 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. Lung transplant recipients are 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 also will have a secondary bacterial or viral infection. Heart and heart–lung transplant recipients are particularly susceptible to coinfection with CMV. The cytotoxic T-lymphocyte-mediated response to pulmonary CMV may be difficult to separate from organ rejection. 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

Variability of the radiographic picture matches that of the clinical presentation of *P. carinii.* Like many of the "atypical" pneumonias (pulmonary infection without sputum production), no diagnostic pattern exists for *Pneumocystis* pneumonia on routine chest radiograph. 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, pneumothoraces, 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 these patients. The intravenous drug abuser often will 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 also may 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 must be differentiated 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 6–8 weeks after transplantation. Infection in these hosts is more often due to CMV or CMV with *P. carinii* or other agents than to any other single pathogen.

Children with epidemic "interstitial plasma cell pneumonitis" on the basis of malnutrition, crowding, and institutional living quarters have a more gradual progression of the chest radiograph. Vascular markings and atelectasis are commonly seen, with hyperinflation and intercostal widening preceding consolidation. In AIDS,

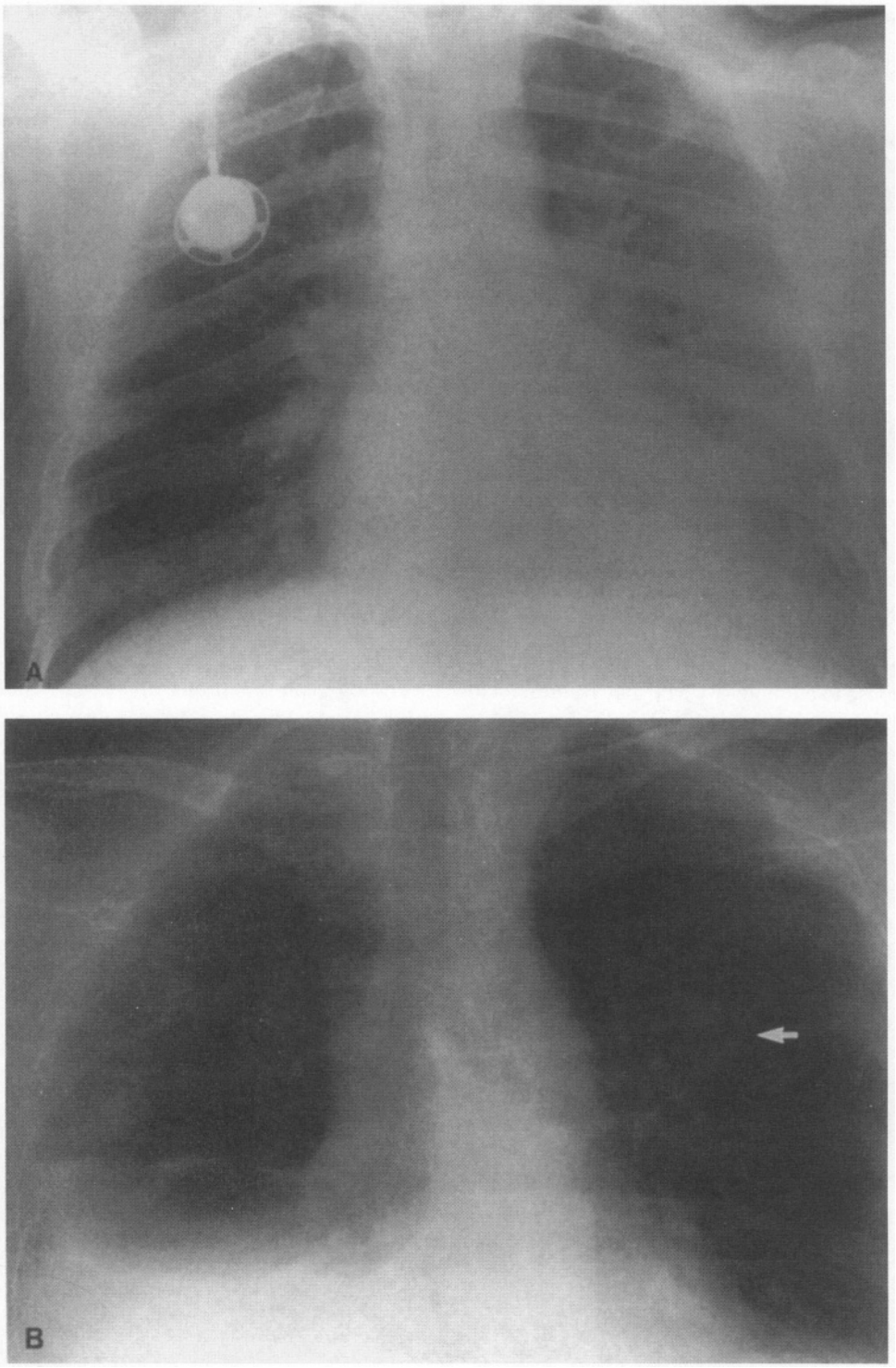

FIGURE 4. (A) Chest radiograph of a 38-year-old man with AIDS who presented with fever, cough, and malaise of 3 weeks' duration. His CD4+-lymphocyte count was 87 at the time of admission. An abscess cavity was seen in the left upper lobe. Bronchoscopic biopsy revealed only *P. carinii*. (B) Chest radiograph 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.

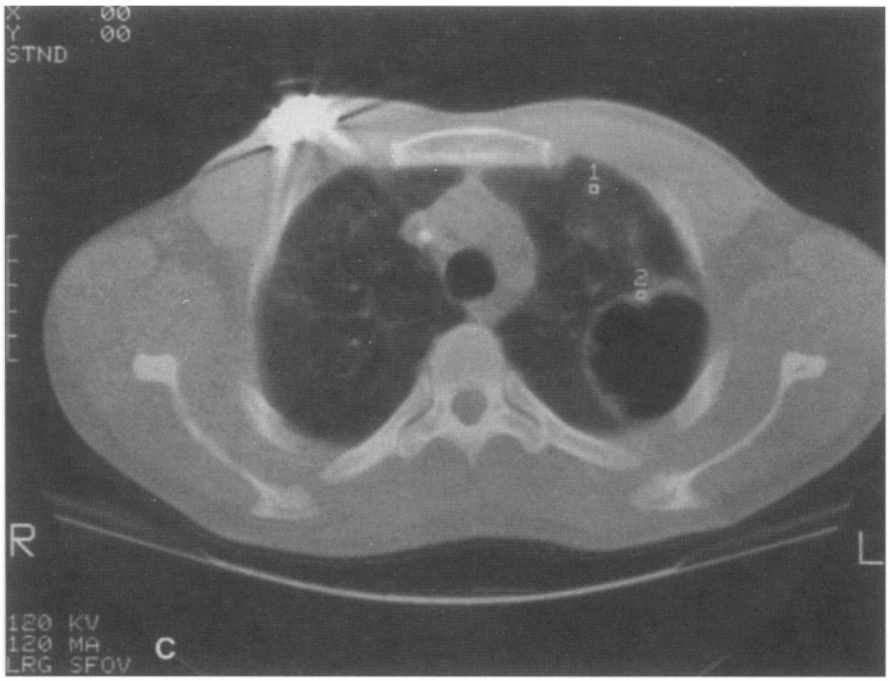

FIGURE 4. (*Continued*)

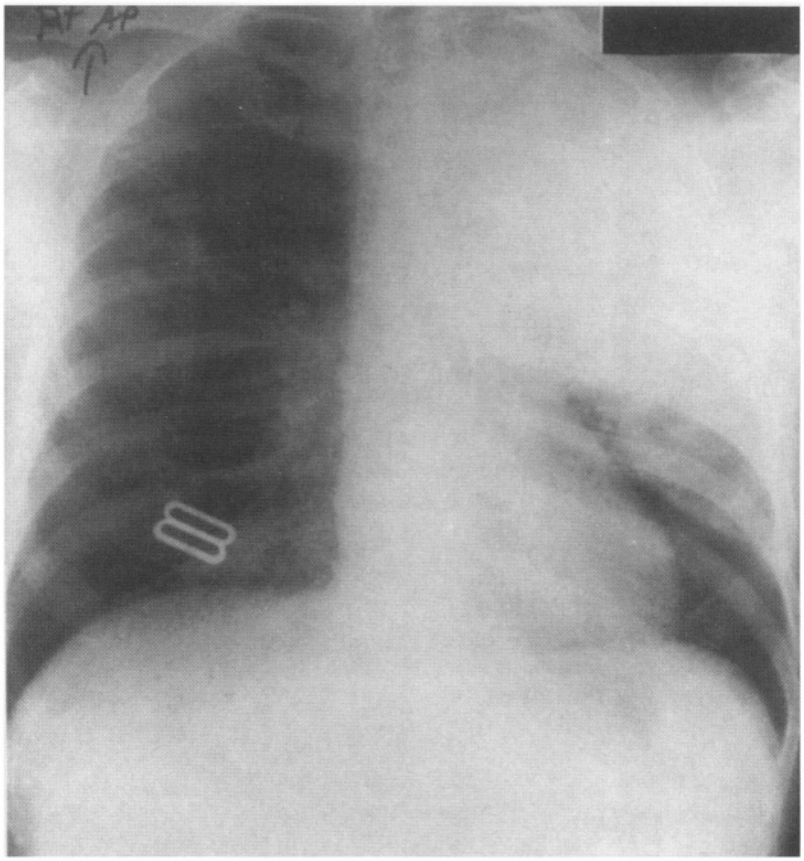

FIGURE 5. Simultaneous upper lobe infection with *P. carinii* and *Legionella pneumophila* in a 46-year-old patient with AIDS while on prophylaxis with aerosolized pentamidine.

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. In patients on corticosteroids and in AIDS, the CT scan often will reveal diffuse interstitial 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. Thus, CT also is useful to direct biopsy procedures. 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 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. 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

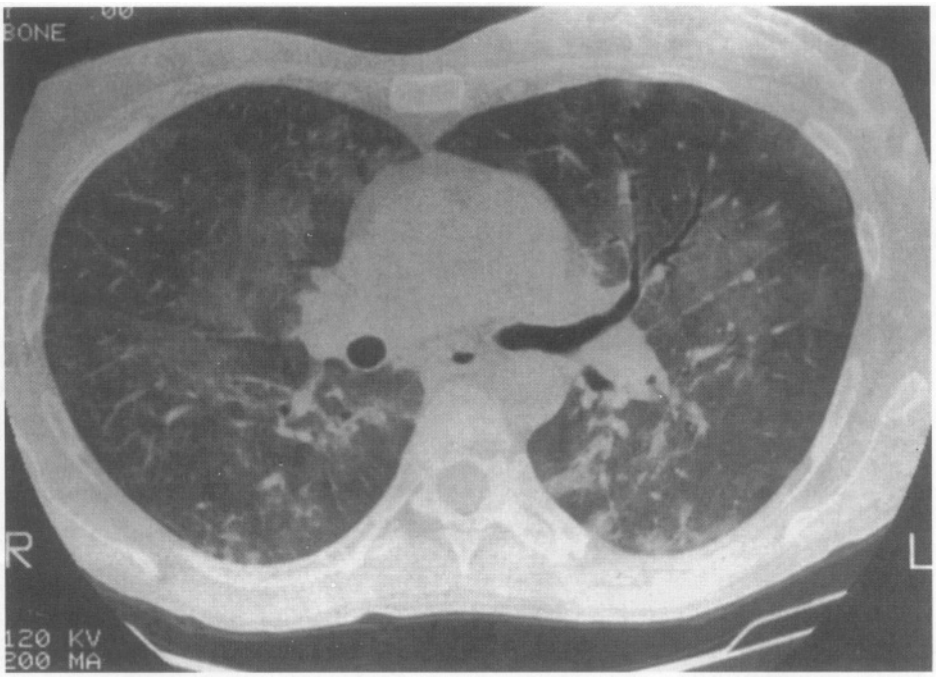

FIGURE 6. CT scan of the chest of an AIDS patient with *P. carinii* pneumonia and a normal chest radiograph. Multiple small interstitial densities and areas of parenchymal consolidation are seen.

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 (as seen in AIDS-associated adenopathy) 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. 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 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 LDH 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 antimicrobial agents. 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 also may be given to the use of colony-stimulating factors to augment the host response. There are few prospective clinical data to support use of these agents.

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 frequent coexistence of multiple processes or infections, the potential toxicity of the agents currently available for the treatment of *Pneumocystis* pneumonia, and the impor-

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	Common; bleeding, subcutaneous air	Rarely worthwhile
Gallium scan, D_LCO	Nonspecific	Injection site	Positive in >95% of infected patients
BAL[b]	>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	
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.
[b] BAL, bronchoalveolar lavage.

tance of a specific etiologic diagnosis in the compromised host, 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 contribute significantly to failures of clinical therapy, the absence of a clinical response to first-line 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 (TMP-SMX) may delay or obscure the ability to make an alternative diagnosis. It may be undesirable in some patients to use invasive techniques to obtain samples for the diagnosis of *Pneumocystis* pneumonia. However, in compromised hosts, empiric therapy must be balanced against the possibility of misdiagnosis, suboptimal or delayed therapy, and/or the avoidable toxicities of antimicrobial agents. In AIDS patients with depressed CD4+ lymphocyte counts, the frequency of *Pneumocystis* pneumonia in patients not receiving prophylaxis may make a therapeutic trial more appropriate than invasive diagnostic tests. The optimal approach therefore must be based on the patient's clinical condition. For patients 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 other immunocompromised hosts. 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 attempted 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. The yield of diagnostically useful material is generally greater from tissue biopsies than from induced sputa or bronchoscopy specimens. Suspicion of *Pneumocystis* pneumonia should lead to early, invasive diagnosis in the non-AIDS-compromised host. The response to therapy decreases over time and empiric therapy (e.g., TMP-SMX-induced nephrotoxicity and hematopoietic suppression in patients receiving cyclosporine) may introduce avoidable toxicities.

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 is of uncertain diagnostic value in an asymptomatic individual or without a history of prior PCP. 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 some nonimmunocompromised 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 children with AIDS.

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 occasionally have 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.[99] 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 do not grow *in vitro* 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 also has been 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 cm^3 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 probably unnecessary given the advantages of immunofluorescent staining coupled to induced sputum or bronchoscopy. The incidence of complications outweighs the potential benefit of the rapid production of a diagnostic specimen. Even in experienced hands, the diagnostic yield of tracheal aspiration is lower than that achieved by induced-sputum examination when both are coupled to immunofluorescence 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 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 and Video-Assisted Thoracoscopy

Surgical open lung biopsy remains the "gold standard" for the evaluation of pulmonary processes in the immunocompromised host.[114] When skilled surgeons perform the procedure, the complication rate of thoracotomy and biopsy should be low. This approach provides the best specimen for cultures and histopathology. The use of video-assisted thoracoscopic biopsy (VATS) directed by radiologic CT scanning generally provides adequate samples for both histologic and microbiologic assessment with minimal morbidity in experienced hands. 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.[25,29,30,115] For most of the available antimicrobial agents, 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.[29,30,116] 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-SMX).[117–120] This consideration includes such factors as the rapidity of clinical response and the ease of administration (oral bioavailability).
2. The use of adjunctive therapies (colony-stimulating factors, immune modulators, aerosolized pentamidine, corticosteroids, antibodies) must be tailored to the individual patient. Reduction in immune suppression is key.
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 antimicrobial given. Antimicrobial side effects are reduced in individuals treated with corticosteroids during antimicrobial therapy. These guidelines are not universally applicable to non-AIDS patients, in part because adverse reactions are less common but persistent despite dose adjustments.
4. *Pneumocystis carinii* that is resistant to anti-

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

Agent(s) (route)[b]	Dose	Options/comments[b]
First line[b]		
Trimethoprim and sulfamethoxazole (TMP-SMX) (IV/PO)	15–20 mg/kg per day TMP 75–100 mg/kg per day SMX	Treat through rash with reduced dose or desensitize in AIDS; alternate agents in non-AIDS
Second line		
Dapsone (PO) with TMP (PO/IV)	100 mg/day 15–20 mg/kg per day	Methemoglobinemia; G6PD; *may* be tolerated in sulfa allergy
Atovaquone, suspension	750 mg liquid PO tid	Variable absorbance, with fatty food; few side effects
Pentamidine isethionate (IV)	4 mg/kg per day 300 mg/day maximum	Lower dose (2–3 mg/kg); IM not advised; breakthrough in transplant and with CMV
Third line[b]		
Trimetrexate (IV)	30–45 mg/m^2 per day	Efficacy = pentamidine; anemia
with folinic acid	80–100 mg/m^2 per day	Marrow toxicity; early relapse
Clindamycin (IV/PO)	450–600 mg q6h	Methemoglobinemia; diarrhea (pyrimethamine for primaquine)
and primaquine	15–30 mg base qd	
Others		
Pyrimethamine	Load 50 mg bid ×2d, then 25–50 mg qd	Not studied fully
with sulfadiazine	Load 75 mg/kg, then 100 mg/kg per day	Maximum 4 g in two doses; up to 8 g (maximum)
Fansidar	Not standardized	Long half-life; not in sulfa allergy
Piritrexim/folinic acid	Under study	Like trimetrexate
8-aminoqunoline	Under study	
Macrolide/sulfonamide	Under study	Synergy; macrolides alone inactive

[a]Adjunctive therapies (see text); corticosteroids (high dose with rapid taper); possibly interferon gamma; granulocyte-macrophage colony-stimulating factor.
[b]Based on clinical judgement of physician; some agents not FDA approved for this indication (ranking of therapies based on author's experience).

microbial agents has been described by a number of authors but in the absence of standardized microbiologic assays has not yet been demonstrated as a cause of clinical therapeutic failure. The apparent failure of an individual patient to respond to therapy may reflect either inadequate serum or tissue levels of antimicrobial, greater degrees of lung injury, or concomitant processes. However, resistance to sulfa drugs has been indicated by mutations in the dihydropteroate synthase gene, which appear to be more common in individuals failing sulfa or sulfone prophylaxis. Switching agents for reasons other than toxicity are not generally recommended unless adequate time (minimum 7 days) with appropriate serum drug levels has been achieved.[116,120,121] While there are patients who appear to "do better" on one agent instead of another, it is much more common to recognize a second process (infection, tumor, allergy, ARDS) complicating *Pneumocystis* pneumonia than "resistant" infection.

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 use of 14 days of therapy in non-AIDS patients and 21 days in AIDS patients is arbitrary. Shorter courses may well be effective, especially in the setting of antimicrobial agents with long serum half-lives, but only if secondary prophylaxis is initiated for patients with persistent immune deficiency. 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. Trimethoprim–Sulfamethoxazole (TMP-SMX, Cotrimoxazole)

Trimethoprim–sulfamethoxazole is the drug of first choice for the treatment of *P. carinii* pneumonia in patients who tolerate this agent.[24,26,29,30,118–120,122–125] 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 3–4 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 SMX divided into four 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 SMX. Serum SMX 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-SMX evokes many fewer adverse reactions than does pentamidine. The rates of adverse reactions in the AIDS patient population are roughly equivalent and amount to 30–50% 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-SMX in adults has not been studied.[121,123] 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-SMX covers a broad spectrum of organisms, including *Listeria,* many *Nocardia,* and many 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-SMX 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 probably should not be used in patients with acute leukemias. Solid organ transplant recipients, particularly with renal and hepatic transplants, frequently suffer nephrotoxicity with intravenous TMP-SMX treatment (and often with pentamidine) and may not recover normal renal function subsequent to drug-induced renal injury. In the HIV-infected, transplant, or chemotherapy patient receiving a variety of hematopoietic-suppressive therapies, drug-related toxicities are common and may necessitate the switching of antimicrobial agents.

2.8.2. Pentamidine

Pentamidine isethionate was the first agent employed for the successful therapy of *P. carinii* pneumonia.[10,40,117,126,127] 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, pentamiine 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-SMX.

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. Pentamidine should be avoided in pancreas transplant recipients due to the potential for islet cell necrosis. 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.

28.3. Alternative Regimens

Multiple new drugs and drug combinations are available for the treatment of *Pneumocystis.* 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-SMX and to pentamidine. Dapsone (100 mg PO per day) has been used in combination therapy with trimethoprim (15 mg/kg per day PO divided into three doses) as an effective alternative oral therapy.[129–132] Many AIDS patients intolerant of sulfamethoxazole will tolerate dapsone (4,4′-diaminodiphenyl sulfone), which is metabolized by the liver ($t_{1/2} \geq 30$ hr). However, the long half-life and side-effect profile (neutropenia in 19%, anemia, fever, hemolysis in G6PD-deficiency, rash, hepatitis) may be particularly disadvantageous in the marrow or organ transplant recipient. The absorption of dapsone from the GI tract may be reduced by antiviral therapy with DDI (2′,3′-dideoxyinosine).

Atovaquone (750 mg suspension PO tid) is FDA-approved for the treatment of mild to moderately severe *Pneumocystis* pneumonia. Side effects of atovaquone are relatively uncommon and are generally mild. Comparative trials between atovaquone (tablets) and TMP-SMX suggest that TMP-SMX should be preferred in patients who tolerate this therapy.[141] Bioavailability of atovaquone has been improved by reformulation as a suspension. Up to 7% of HIV-infected patients develop limiting toxicity on atovaquone during therapy (vs. 20% for TMP-SMX); however, significantly more patients failed therapy due to lack of response in the atovaquone group than in the TMP-SMX group. When pentamidine was compared to atovaquone for therapy of mild to moderate infection, lack of response was observed in 29% of atovaquone patients and 19% of pentamidine patients. However, atovaquone was better tolerated with treatment-limiting side effects in 9% versus 24% for pentamidine. The incidence of rash, the most common side effect of atovaquone, correlates with increasing serum drug levels.[141] Other toxicities include diarrhea, nausea, vomiting, fever, and increased liver function tests.[61,141] Atovaquone will irreversibly stain clothes (yellow), which limits use for prophylaxis in children. Preliminary data in stable organ transplantation patients suggest that there is no interaction between atovaquone and cyclosporine or tacrolimus. Atovaquone is useful for both therapy and prophylaxis in the BMT and solid organ transplant populations. Animal data suggest the possible presence of an interaction between atovaquone and erythromycin that merits further study.

Trimetrexate (45 mg/m^2 per day) *with* folinic acid (80 mg/m^2 per day) has been approved for use in moderately severe pneumonia.[137–140] Trimetrexate is a dihydrofolate reductase inhibitor and is lipid soluble with a serum half-life up to 34 hr. It will produce severe neutropenia in the absence of folinic acid supplementation (which should be continued for 3 to 5 days after cessation of trimetrexate) and in some patients with simultaneous infections due to HIV, CMV, or during therapy with antiviral antimicrobial agents. Side effects include fever, rash, leukopenia, and transaminase elevation. Relapsed infection in AIDS patients has been somewhat more frequent than with other therapies. The survival rate following therapy in AIDS patients is higher with TMP-SMX than with trimetrexate for moderately severe *Pneumocystis* pneumonia. Piritrexim is pharmacologically similar to trimetrexate but has been most useful in combination with a sulfonamide. A variety of chemical modifications of the methotrexate molecule may lead to more selective activity against *P. carinii.*[61]

The combination of clindamycin (600–900 mg IV or PO q6–8h) and primaquine (15–30 mg of base/day PO) is effective in mild to moderate infection.[133–135] No significant differences were observed among treatment groups receiving TMP-SMX, dapsone–trimethoprim, or clindamycin–primaquine for mild-to-moderate *Pneumocystis* pneumonia in AIDS in terms of survival, dose-limiting toxicity, therapeutic failure, or the ability to complete 21 days of therapy.[131] For all three regimens, dose-limiting toxicity was experienced by 30.9% of patients and 6.1% were considered to be therapeutic failures by day 7.[131] Thus, for an individual patient, the side-effect profile is the main determinant of the choice of therapy. The main toxicities of clindamycin include rash (16%), methemoglobinemia, anemia, neutropenia, and the development of *Clostridium difficile* colitis. Pyrimethamine (50–100 mg/day PO after 100–200 mg load) with sulfadiazine or trisulfapyrimidines (4–8 g/day) also are effective but require folinic

acid (10 mg/day) supplementation. Pyrimethamine will decrease the renal clearance of creatinine without altering the glomerular filtration rate. The macrolides (azithromycin, clarithromycin) have little efficacy as monotherapy, but appear to enhance the efficacy of sulfamethoxazole. However, this combination provides little benefit over TMP-SMX.

The clinical utility of DFMO (α-difluoromethylornithine) has not been well established. The presence of the target enzyme in *P. carinii* (ornithine decarboxylase, ODC) and activity against polyamine biosynthesis *in vitro* have been demonstrated.[142] Because humans and *P. carinii* share the target enzyme ODC, the differential sensitivity of the organism to DFMO with rapid depletion of polyamines in *P. carinii in vitro* suggests a mechanism of action beyond ODC inhibition. Clinical experience with DFMO as primary therapy for *Pneumocystis* pneumonia has not been encouraging. Newer agents under study include the echinocandins (glucan synthase inhibitors) which block formation of cysts, the 8-aminoquinolines, which have entered clinical trials, the dicationic substituted *bis*-benzimidazoles (antimicrotubule pentamidine derivatives), terbinafine, isoprinosine, bilobalide (a sesquiterpene from Gingko biloba leaves), biguanide inhibitors (PS-15) of dihydrofolate reductase, quinghaosu, albendazole, proguanil, terbinafine, guanylhydrazones, and some nonquinolone topoisomerase inhibitors.

2.8.4. Adjunctive Therapies

Many patients with *P. carinii* pneumonia will suffer disease progression despite appropriate antimicrobial therapy. In this setting, the initial delay of 4–7 days before responding to therapy may necessitate intubation with mechanical ventilation. It may be that the successful killing of intra-alveolar organisms may contribute to the local inflammatory process and further diminish oxygenation. These patients often will 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. 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 (Po_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 antimicrobial agents 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 also may 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 colony-stimulating factors on local immunity may require T-lymphocyte function. There is controversy regarding the use of colony-stimulating factors 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. G-CSF has been used without complication to support leukocyte counts in solid organ transplant recipients in the presence of CMV infection or drug-induced neutropenia.

The role of aerosolized pentamidine in the acute management of patients with *Pneumocystis* pneumonia remains unclear. 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 the fluoroquinolones and possibly the echinocandins and pneumocandins will also be useful.

2.8.5. Response to Therapy

The initial manifestations of *Pneumocystis* pneumonia have been altered by prophylactic antimicrobial therapy, by successful highly active antiretroviral therapy (HAART) for AIDS, and by new immunosuppressive regimens, including those with calcineurin inhibitors, mycophenylate mofetil, and antithymocyte globulins. The response to therapy also has changed with these new regimens. While the incidence of *Pneumocystis* pneumonia has declined in response to HAART, when infected, AIDS patients are presenting with more 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 response of the non-AIDS immunosuppressed patient is determined by the ability to reduce exogenous immunosuppressive regimens. Most non-AIDS immunosuppressed patients do better with initial therapy for *P. carinii* pneumonia than do patients with low CD4+ lymphocyte counts and HIV infection: 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 more often is due to other factors than it is to failure of a given antimicrobial agent. Adding pentamidine to TMP-SMX 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 can 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 atovaquone. The echinocandins (β-1,3 glucan synthase inhibitors) inhibit cell wall synthesis, preventing cyst formation. This class of agents may prove to be useful adjuncts to therapy. The clinical assessment of the patient is usually the best guide to subsequent therapy. Patients who fail to respond to antimicrobial agents 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. In the pre-HAART era, 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 appeared to diminish somewhat over time, reflecting viral resistance. Complications are more common in the AIDS population, including a tenfold 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 may be reversed by continuing the drug at reduced levels. It is worth checking serum SMX levels if side effects occur. Serious toxicity requires switching to an alternative regimen.

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

2.9.1. Patients Requiring Prophylaxis

The spectrum of patients who will require anti-*Pneumocystis* prophylaxis has changed dramatically with newer immunosuppressive regimens for organ transplantation and the treatment of graft-versus-host disease, the use of more intensive chemotherapeutic regimens for malignancy, intensive immune suppression for connective tissue diseases, and conversely, the decreasing incidence of opportunistic infection in AIDS.[392] There are few clear rules regarding patients who "should" be on prophylaxis. However, experience dictates that these might include:

- AIDS patients with CD4+ lymphocyte counts below 200 CD4+ lymphocytes/mm^3 blood or less than 20% CD4+ lymphocytes total, rising HIV viral loads, persistent cytomegalovirus infection, or recurrent opportunistic infections suggestive of persistent T-cell defects despite HAART therapy. In individuals who are noncompliant with HAART therapy or for whom HAART is not available, *anti-Pneumocystis* prophylaxis should be attempted.
- Individuals receiving anti-T-cell therapies or cor-

ticosteroids over 20 mg/day of prednisone for a period of over 2–3 weeks (an arbitrary duration consistent with the life cycle of the organism).

- Solid organ transplant recipients, depending on the incidence of infection in the institution (>5–10% without prophylaxis) but up to lifelong for heart, liver, and liver recipients and 6 months to a year posttransplant for kidney recipients. These recommendations are consistent with the periods of greatest risk due to intensity of immune suppression. Any transplant recipient with a history of *Pneumocystis* pneumonia or frequent opportunistic infections, who is receiving prophylaxis or therapy for CMV infection or treatment of acute rejection, merits consideration of *Pneumocystis* prophylaxis. Individuals with chronic graft dysfunction and who are receiving higher than usual levels of immune suppression also merit prophylaxis.
- Use in neutropenic cancer patients is controversial, given the marrow suppression that may result from TMP-SMX use. However, intensive chemotherapy with neutropenia of more than 7–10 days may justify prophylaxis with alternative agents.

In AIDS patients, the incidence of *Pneumocystis* is greatest in patients with less than 200 CD4+ lymphocytes/mm^3 blood or less than 20% CD4+ lymphocytes total. The incidence of primary *Pneumocystis* pneumonia and other opportunistic infections (e.g., CMV and *M. avium-intracellulare* complex) is reduced in proportion to the control achieved of viral infection. Prophylaxis with TMP-SMX reduces the incidence of pneumonia by over tenfold. While these guidelines are useful, the incidence of *Pneumocystis* pneumonia is also high in patients with rapidly progressive immune deterioration or a prior history of *Pneumocystis* infection, or both. Prophylaxis against *Pneumocystis* and other opportunistic infections has changed due to HAART therapy. Recent data suggest that individuals with CD4+ T-cell counts above 200/mm^3 and with viral replication suppressed to undetectable levels by antiretroviral therapy for a period of 3 or more months do not appear to require primary prophylaxis for *P. carinii* pneumonitis (and toxoplasmosis). The caveats to this recommendation are that the follow-up period is relatively brief. Anecdotal reports and nonrandomized trials suggest that sustained counts over 200 CD4+ lymphocytes/mm^3 in individuals with a history of *Pneumocystis* pneumonia may also be protected, but sufficient data do not yet exist. It does appear that significant immune reconstitution occurs in most individuals with AIDS after more than 12 weeks of successful antiviral therapy. However, it is expected that the degree of immune function achieved is inconsistent and in some individuals recurrence of viral infection and opportunistic infections have been observed. Thus, firm recommendations for secondary prophylaxis with HAART must be individualized. A number of AIDS patients have experienced complications of immune reconstitution (*Pneumocystis* pneumonitis, CMV retinitis, MAC lymphadenitis) upon initiation of HAART. These cases probably reflect subclinical infection with symptoms reflecting recrudescence of the immune response. A similar picture may occur if HAART is introduced before completion of therapy for active infection due to *Pneumocystis,* CMV, or tuberculosis.

2.9.2. Antimicrobial Prophylaxis—Agents

Pioneering studies in children with hematopoietic malignancies, especially those on corticosteroid therapy, led to the development of TMP-SMX for the prevention of *Pneumocystis* pneumonia.[71,72,119,124,147,148] Successful experience with prophylaxis at the Saint Jude's Children's Research Hospital (Dr. Walter Hughes) was broadened to include immunocompromised adults and patients with severe combined immunodeficiency syndrome (SCID).[147] The advantages of daily cotrimoxazole (single or double strength) were the prevention of infection due to *Pneumocystis* as well as most *Toxoplasma gondii, Listeria monocytogenes, Nocardia asteroides,* and common urinary, gastrointestinal, and pulmonary bacterial pathogens. The incidence of side effects was fairly modest with relatively short periods of prophylaxis. 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 for the prevention of *Pneumocystis* to administer cotrimoxazole 3 days each week (either consecutive or alternative) with one double-strength tablet at bedtime. Lower doses of antimicrobial have also been effective.[147] These doses of TMP-SMX are usually well tolerated, with a minimal incidence of side effects and relatively modest bone marrow suppression. It should be noted that we have observed pulmonary and central nervous system infections due to *T. gondii* and *Nocardia* species in individuals receiving 3-day/week regimens in adult solid organ and hematopoietic stem cell transplantation recipients. Alternative regimens are occasionally necessary in non-AIDS patients requiring prophylaxis. These patients will tolerate atovaquone (1500 mg PO qd), pentamidine 300 mg by aerosol or intravenously every 3–4 weeks, dapsone (100 mg orally a day), or weekly Fansidar (pyrimethamine–

sulfadoxine).[149] The use of these latter two agents should be guided by knowledge of their side effects.

TMP-SMX is the agent of choice for the prevention of *Pneumocystis* infection in patients who tolerate this agent.[150,151] Aerosolized pentamidine has become less popular for prophylaxis due to inconvenience of aerosol administration, atypical presentations of pulmonary and occasionally extrapulmonary pneumocystosis, the lack of activity against *T. gondii* and other potential pathogens, and a lower success rate in preventing *Pneumocystis* infection than TMP-SMX. Lower-dose regimens of TMP-SMX are generally well tolerated (in up to 75–80% of AIDS patients); potential side effects are those seen in therapy. In patients completing a course of cotrimoxazole therapy for pneumonia, prophylaxis should be initiated immediately to prevent sensitization. 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 (i.e., apical *Pneumocystis* infection) 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 from the lungs of patients during the nebulizer treatments. 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. 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 during aerosolized treatment but may occur after a total dose of 3 g. Aerosolized pentamidine also has the effect of reducing the number of organisms in sputum such that the sensitivity of microscopic diagnosis on induced sputum samples may be reduced. However, this agent has proved 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 also has 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. Atovaquone is also active against *Toxoplasma gondii* and is used at a dose of 1500 mg PO daily.[392] Concerns about the prevention of *Pneumocystis* also have 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 further study.

Illustrative Case 1

A 36-year-old man with non-Hodgkin's lymphoma was treated with high-dose corticosteroids and cytotoxic chemotherapy. He presented with fevers, a diffuse pulmonary process, and hypoxemia. His chest radiograph [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. Antimicrobial agents included cotrimoxazole (TMP-SMX) 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-SMX and was on a gradual steroid taper. His course had been complicated by a skin rash and fever, probably due to TMP-SMX, and thrombocytopenia due to pentamidine. Notable in his course was that despite therapy for *Pneumo-*

cystis for 21 days, his chest radiograph had failed to improve, with diffuse infiltrates consistent with adult respiratory distress syndrome. The effects of past chemotherapy also were 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-*Pneumocyslis* 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 intracytoplasmic and intranuclear inclusions consistent with CMV infection and rare *P. carinii* cysts. On the basis of these results, he was treated with intravenous ganciclovir, 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 was probably the main factor in the development of infection in this host but it 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 evaluation. Congestive heart failure, pulmonary embolus, and bacterial superinfection [Fig. 5 (Section 2.5)] are common cofactors. Because clinically significant antimicrobial resistance has not been clearly 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 is 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"), particularly in the cyst form, is not indicative of clinical disease ("toxoplasmosis") in the absence of an 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] This has complicated the development of molecular diagnostic tests.

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.

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 retic-

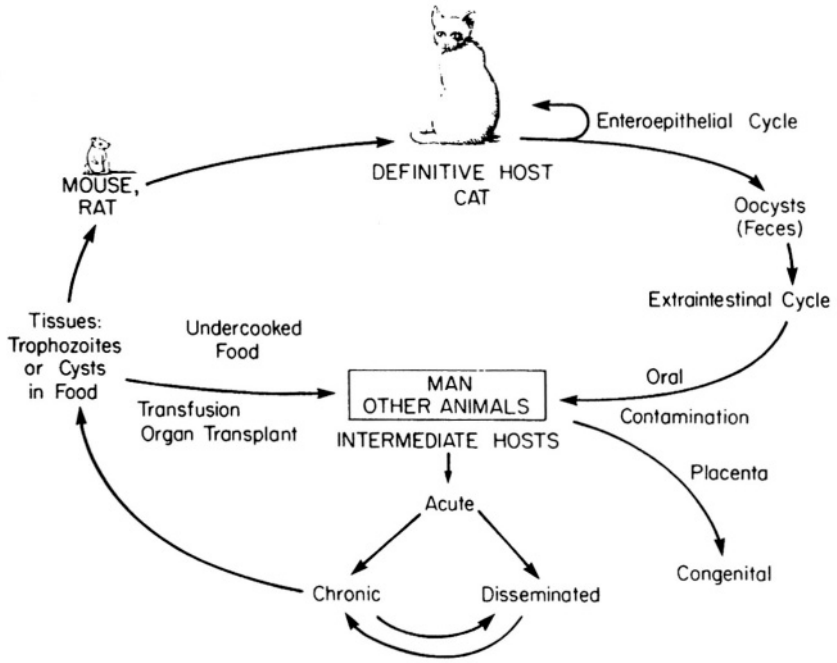

FIGURE 7. Life cycle of *Toxoplasma gondii.*

uloendothelial cells and of other tissues.[155] Trophozoites are 4- to 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 individuals with un-

treated AIDS. While patients on maintenance therapy for toxoplasmosis do not develop *Pneumocystis* pneumonia, cotrimoxazole prophylaxis is largely, but 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 phagolysosomes. 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, colony-stimulating factors) 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) during chemotherapy (especially regimens including corticosteroids) and in organ, particularly heart, and hematopoietic transplantation recipients.[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. Clinical Presentations of Toxoplasmosis

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 infections 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 "flulike" syndrome or infectious mononucleosis and is generally benign and self-limited. 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] Myocarditis, myositis, and polymyositis may occur in normal individuals and in those on modest amounts of immune suppression, occasionally in association with dermatomyositis. Because of the broad differential diagnosis of common lymphadenopathy syndromes, including viral illnesses and cat-scratch disease, 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 with visual loss, glaucoma, photophobia, and pain. 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. Eye infection in the immunologically normal host produces severe inflammation and necrosis. Granulomatous inflammation of the choroid accompanies retinal injury. 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. AIDS-associated *Toxoplasma* chorioretinitis is uncommon; it will often occur (~30–60%) in patients with concurrent CNS lesions. These patients present with acute loss of visual acuity, eye pain, and with panophthalmitis and areas of coagulative necrosis containing cysts and tachyzoites. Occasionally, retinal vascular thrombosis may occur. The characteristic retinal lesions often are raised with healing and may be unilateral or bilateral. The posterior uveitis of toxoplasmosis may be confused with that of syphilis, tuberculosis, histoplasmosis, or leprosy.

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 and recipients, particularly cardiac, should be assessed prior to transplantation.

Because of the 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 display systemic manifestations of toxoplasmosis similar to those seen in the immunocompetent host than is the individual with AIDS. 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 may be that of focal mass lesions (e.g., seizures), "diffuse" infection with widespread microglial nodules of the gray matter, or encephalitis with diffuse CNS impairment or multiple focal neurologic deficits.[186] The diffuse form suggests a degree of immune impairment inadequate for abscess formation and often progresses rapidly to death. Some 10% (United States) to 25% (Europe) of acutely infected immunocompromised individuals will have a neurologic presentation.

Toxoplasmosis in AIDS may also present with pneumonitis, myositis, myocarditis, diffuse gastrointestinal involvement with pain, diarrhea, hepatic dysfunction, ascites, orchitis, panhypopituitarism, diabetes insipidus, and SIADH. Pulmonary infection may also be significant in both AIDS and non-AIDS compromised hosts.[193,194] The clinical onset of pneumonia is associated with the gradual onset of fever, dyspnea, cough, and occasionally hemoptysis. Chest X rays reveal a diffuse bilateral pulmonary infiltrate with atypical features including interstitial infiltrates and small nodules. The serum LDH is often elevated. In this setting, it should be possible to isolate organisms from lung washings.[195] In both AIDS and transplant patients, asymptomatic myocarditis and myositis are common, associated histologically with myocyte necrosis.

In the organ transplant recipient, the clinical presentation is similar, with the exception that the disease is generally due to dissemination from the transplanted organ.[157,168] In contrast to AIDS, allograft recipients who develop infections are generally seronegative. The greater severity of disease in the seronegative recipient of a seropositive organ than in reactivation disease in the seropositive individual suggests that antibody is partially protective. Given the predilection of the organism for myocardium, toxoplasmosis occurs most often in heart

transplantation. Transmission in large series exceeds 80% in cardiac transplantation into seronegative individuals. In part, this may reflect the intensity of infection (i.e., number of cysts) from regions with high endemicity (e.g., France, Haiti) as well as immune compromise. Such individuals may present with heart failure or arrhythmia, which may be indistinguishable from graft rejection or primary cardiac CMV infection. Identification of organisms, including tissue cysts, in a seronegative individual should raise the specter of acute disseminated disease.

The incidence of toxoplasmosis in AIDS appears to be decreasing with the introduction of more effective antiviral therapies. Without HAART, *Toxoplasma* encephalitis has been a common presentation for patients with AIDS. In untreated AIDS patients, as in most immunocompromised individuals, toxoplasmosis is usually disseminated at the time of presentation.[196,197] In the AIDS population, lymphadenopathy may be seen in primary HIV infection and with opportunistic infections including mycobacteria and CMV. Lymph node biopsies are generally nondiagnostic. In AIDS, the occurrence of disseminated *Toxoplasma* infection is usually correlated with low circulating CD4+ lymphocyte levels (<50). Between 5% and 50% of *Toxoplasma*-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 commonly diagnosed. Signs of meningitis are uncommon, while seizures, confusion, depression, visual changes, and hydrocephalus are more common. For HIV-infected individuals whose CD4+ counts remain above 200 lymphocytes/mm^3 for at least 3 months, studies suggest that primary prophylaxis may be discontinued without short-term (less than a year) detriment. Definitive data are lacking regarding the long-term persistence and efficacy of immune reconstitution during HAART.

The presentation of CNS toxoplasmosis 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 CNS lesions of toxoplasmosis must be distinguished from other infections or tumor (Fig. 8).[175,197–205] The infection can present as focal abscesses or diffuse meningoencephalitis, based on the distribution of the preexisting latent lesions. Acute, primary toxoplasmosis has also been reported. The progression of *Toxoplasma*-induced mental status changes is subacute when compared to that due to HIV alone. HIV-associated leukoencephalopathy or progressive multifocal leukoencephalopathy forms part of the "AIDS dementia complex," which also can present

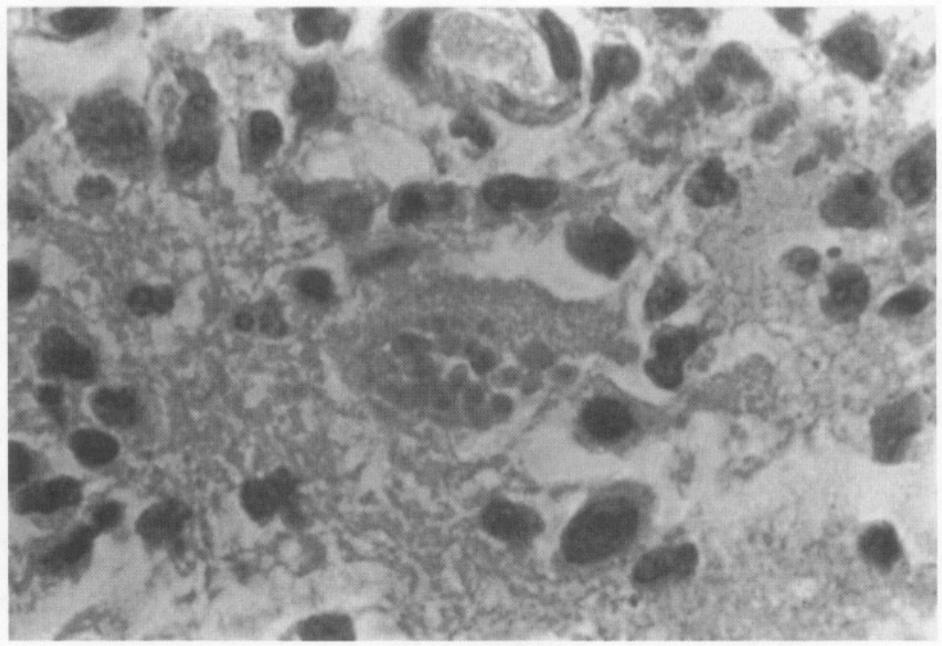

FIGURE 8. Hematoxylin-eosin stain of a brain biopsy specimen from the edge of a large anterior brain abscess from a patient with AIDS. A tissue cyst contains multiple intracystic bodies or bradyzoites consistent with a diagnosis of toxoplasmosis.

with focal or 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 is not often helpful in establishing a diagnosis of toxoplasmosis, biopsies of infected areas may be required to confirm the diagnosis.

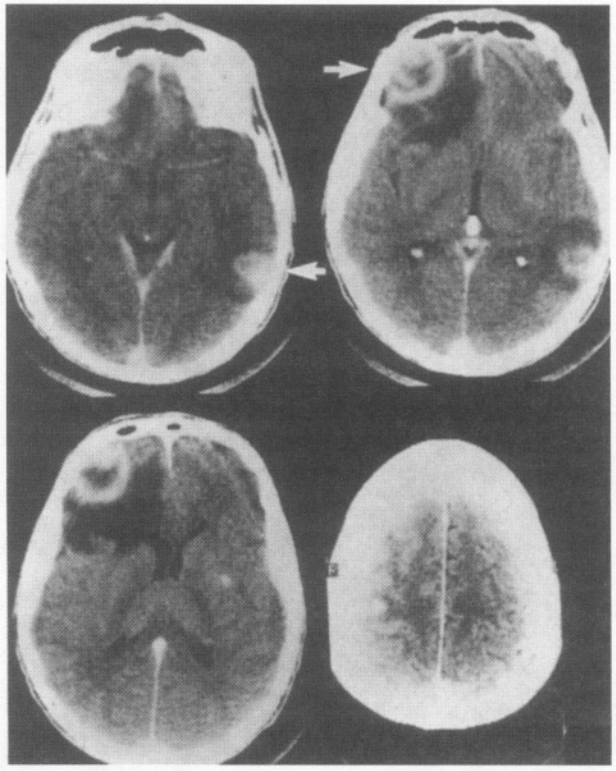

FIGURE 9. CT scan from the patient in Fig. 8 reveals multiple and bilateral contrast-enhancing brain abscesses surrounded by edema. This presentation is seen in *T. gondii* infection more often than the large solitary lesions often associated with CNS lymphoma.

3.3. 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 ongoing disease, i.e., 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, or the amplification of *T. gondii-specific* DNA from body fluids 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 antimicrobial agents (notably pyrimethamine or sulfa), which approaches 50% with the antimicrobial agents 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; hypoglycorrhachia 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.

In AIDS, empiric therapeutic trials frequently are the best approach in stable patients. In organ or hematopoietic transplant recipients, given the risk of the rapid progression of fungal infection of the CNS, the threshold for invasive diagnosis to distinguish potential etiologies of infection must be lower. In either group, 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 may be found in lung lavage or lung biopsy sample from patients with pneumonitis.

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 asymmetric 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, and less commonly other infectious or malignant processes.[208] Non-AIDS-immunocompromised patients also will have multiple CNS lesions, but the differential diagnosis must be broadened and invasive diagnosis considered earlier. Up to 20% of patients will have single lesions without contrast enhancement. Occasionally, uptake of contrast dye may be delayed, particularly in neutropenic patients and in those on corticosteroids. 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 have ring enhancement with 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 often reveal small, bilateral lesions not seen by CT. Similar lesions of the posterior fossa also may be seen with cryptococcal and mycobacterial infections. Large focal abscesses may be seen that cannot be distinguished from necrotic tumor or abscesses due to other pathogens. Serologic testing and CSF antibody levels may be useful in this group. Radiologic evidence of encephalitis is seen less frequently. Thallium single photon emission computed tomography (SPECT) scanning or positron emission tomography (PET) generally demonstrate greater uptake of methionme or glucose than lymphomatous lesions, but overlap (false-positive and -negative scans) does occur.

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. Touch preparations of tissues and cytocentrifuged fluid samples may be stained with Wright–Giemsa. 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 also have 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 monolayers 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 (often with significant nonspecific staining of tissues), or ELISAs using unfixed tissues. All have proved useful clinically, although the immunoperoxidase is the most consistently sensitive and specific.

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, in the periphery of abscesses and 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 also can 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. Skin Testing and Cellular Immunity

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 *Toxoplasma* antigens also is an indicator of previous exposure to *T. gondii.* Inversion of the T-lymphocyte helper/suppressor ratio (an excess of suppressor T cells) occasionally is 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 and Polymerase Chain Reaction. 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

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 fluorescent 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)	1:1000–1:20	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)	1.7–3.0	Remains elevated; early onset; sensitive
Immunosorbent (IgM) (latex bead)	Positive	Positive	Simple, sensitive (vs. IFA); fewer false-positives

[a]A two-tube or fourfold 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.

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 tilers 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-coaled 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 (two-lube) rise from a low chronic liter to a high acute tiler 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. Panels of serologic tests are often more useful than single assays.

Polymerase chain reaction (PCR) assays are available on both a research and commercial basis. These nonstandardized tests have been useful in the diagnosis of intrauterine infections, ocular, congenital, acute disseminated, and CNS infections particularly when brain biopsy is not feasible. The tests are highly specific, but have lacked sensitivity (varying from 11 to 85%) in part because some patients with *Toxoplasma* encephalitis or ocular infection lack parasitemia or dissemination outside the blood–brain barrier. Tests based on amplification of genomic repetitive elements and nested PCR systems have been somewhat more sensitive than those based on the Bl element.

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 commercial solid-phase IgM immunosorbenl assay. The failure of serologic testing points out the importance of obtaining baseline tilers 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 liters are typically low. In some hosts, notably in newborns and in recent organ transplant (especially cardiac) recipients, elevated liters of anti-*Toxoplasma* antibody may be normal. IgG antibody tilers in newborns may reflect maternal antibody liters. In congenilal infection, the child may not produce specific antibodies before 2–9 months of age. Serial tests may be helpful in mis situation. Cardiac and heart-lung transplant recipients may demonstrate significant tilers even without acute infection. Elevated CSF IgM levels should always be taken as indication of possible brain involvemenl. PCR testing of blood or buffy coal may detect cases of disseminated infection in advance of seroconversion. PCR of CSF also may be useful if positive. Negative PCR tests do not prove the absence of toxoplasmosis.

3.4. Therapy of *Toxoplasma gondii* Infection

3.4.1. Acute Infection

Initial therapy for *T. gondii* infection should include a reduction in the immunosuppressive therapy whenever 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 with the possible exception of atovaquone. Empiric therapy should be initialed in patients with the appropriate clinical syndrome and who are at risk for disseminated toxoplasmosis. Such conditions include CNS, pericardial, pulmonary, hepatic, splenic, or bloodborne disease in patients with AIDS, patients receiving high-dose corticosteroid and/or calcineurin-inhibitor therapies, cardiac transplant recipients at risk for primary infection, bone marrow transplantation recipients, or acute hematopoietic malignancy.[206,209,225–229]

Antimicrobial therapy is outlined in Table 8. A synergislic combination of antibiotics including pyrimethamine (200 mg load, then 25–75 mg/day with folinic acid 5–15 mg/day) and sulfonamide or clindamycin is favored for *Toxoplasma* infection in all patients. Date in AIDS patients suggest that oral clindamycin (600 mg PO q6h) may be as effective as high-dose intravenous drug (i.e.,

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

Condition/duration	Drug[b]	Dose	Alternatives	Comments
Disseminated/CNS acute encephalitis; 6 weeks	Pyrimethamine	100–200 mg PO load; then 25–50 mg PO qd or qod	Pyrimethamine/folinic acid plus clindamycin (900–1200 mg mg/d IV q6h *or* 300–600 PO qid), *or* azithromycin (1200–1500 mg/d); or clarithromycin (1 g bid), *or* atovaquone (750 mg tid-qid)	Bone marrow suppression; decrease dose for neutropenia; may avoid folinic acid in leukemia; lifelong suppression; role of HAART in prevention of CNS relapse not clear; sulfa allergy common; *C. difficile* common
	with folinic acid and	10 mg/day		
	sulfadiazine *or*	4 g PO; then 1–1.5 g PO qid		
	trisulfapyrimidine	75–100 mg/kg per day		
Suppression (after acute infection to 6–12 months after Toxo + heart transplant); lifelong	Pyrimethamine	25–75 mg/day; folinic acid; *and* sulfadiazine 500–1000 mg/d *or* clindamycin 300–450 mg PO qid	Replace clindamycin with atovaquone 750 mg PO tid-qid *or* dapsone 100 mg/d *or* azithromycin 600 mg/d PO	Alternatives without documented efficacy; no data to support discontinuation of HAART
Prophylaxis (AIDS with + serology); lifelong	TMP-SMX	DS PO qd	SS qd or DS tiw *or* dapsone 50 mg/d + pyrimethamine 50 mg/wk + folinic acid *or* atovaquone 750 mg bid	Breakthroughs in nondaily regimens in transplant; secondary prophylaxis essential; need for primary prophylaxis after recovery of CD4+ counts (HAART)?
Congenital (1st 18 weeks of gestation to term)	Spiramycin (FDA)	1 g PO tid or qid		In pregnancy or sulfa allergy with pyrimethamine; Rx needed for neonate
Ocular or transfusion	As for CNS infection			Steroids for inflammation in meningitis/eye infections
Acute normal host	None			

900–1200 mg IV q6h).[227–231] The most common sulfonamide used is sulfadiazine (6–8 g/day after a 4-g load), but it is interchangeable with trisulfapyrimidine for this purpose.[228] Other sulfonamides are not equally effective. It must be noted that solid organ and hematopoietic transplant recipients (renal, or those on calcineurin inhibitors) will rarely tolerate high-dose intravenous sulfa or TMP-SMX. Hydration and adequate urine flow are critical. As an alternative to pyrimethamine–sulfa or clindamycin–pyrimethamine, pyrimethamine may be used with atovaquone (750 mg PO tid or qid), an effective oral agent in individuals with normal gastrointestinal function, and which has unique activity against the intracystic form of *T. gondii.* Alternate strategies include pyrimethamine with azithromycin (1000–1500 mg/day), trimetrexate, or minocycline (100 mg PO bid).[232,233] The newer macrolides roxithromycin, azithromycin, and clarithromycin in combination with sulfonamide or pyrimethamine may have the advantage of excellent tissue penetration in excess of serum levels.

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 patient must be well hydrated to prevent crystalluria. Alternative therapies are less toxic and less active.[234] Therapy is generally continued at high dose for 6 weeks with reduced dosing thereafter for chronic suppression. 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).[178,235] For CNS infection, no significant changes are seen in radiologic studies before 2–3 weeks; the neurologic examination is more sensitive to progression or response to therapy. Non-AIDS immunodeficient patients do better in general than AIDS patients suffering from disseminated toxoplasmosis. The main limitation of therapy in AIDS has been drug toxicity, which occurs in at least half of individuals. Discontinuation of therapy in the absence of effective HAART for at least 3 months is generally associated with a relapse of brain disease. Acute neurologic deterioration may occur

with the initiation of HAART or reduction in immune suppression.

3.4,2. Prophylaxis

In seropositive AIDS patients with CD4+ lymphocyte counts $<200/mm^3$ and in seropositive neutropenic patients or those on high-dose corticosteroids, primary prophylaxis is recommended using TMP-SMX (DS qd). The incidence of toxoplasmosis is reduced in AIDS patients receiving cotrimoxazole prophylaxis. The use of pyrimethamine (50 mg/day) and sulfadiazine (2 g/day) also prevents both diseases.[228] Given the lower sensitivity of *T. gondii* to sulfa compared with *P. carinii* and/or the role of latent CNS infection, daily drug regimens are preferred. For seronegative recipients of cardiac transplants, more intensive prophylaxis with pyrimethamine and sulfa (2–4 g/day) is suggested for the first 3 months posttransplantation with lifelong TMP-SMX (1 DS tab/day) thereafter. Alternatives include dapsone (50–100 mg/day) with pyrimethamine (50 mg biw-tiw), and folinic acid (10 mg/week), atovaquone (1500 mg/day ± pyrimethamine), or one of the macrolide regimens (none well studied). 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. Breakthrough infection has occurred during therapy with this antimicrobial combination. In solid organ transplant recipients, atovaquone (1500 mg/day) prevents both infections.

After acute infection, lifelong suppressive therapy is recommended in the absence of return of normal immune function.[206] Data on secondary prophylaxis for *T. gondii* in HAART or after transplantation are lacking. Success is directly proportional to the penetration of the agent into the CNS and the patient's ability to tolerate drug side effects. Relapse in AIDS has been observed during prophylactic therapy with pyrimethamine, trimethoprim-sulfamethoxazole, and spiramycin.[206,231,236] Higher-dose pyrimethamine (50–75 mg/day) has been used in some AIDS and transplant patients for therapy and prophylaxis to increase serum levels. In general, folinic acid supplementation is needed. In AIDS patients with toxoplasmosis, some early relapses have been seen with the macrolide prophylactic regimens.[236] In combinations with pyrimethamine, skin rash (38%), GI (38%), liver function test (77%), or hematologic (54%) toxicities are seen. 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 needing 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 antimicrobial agents in clearing intracellular organisms.

Illustrative Case 2

A 37-year-old man was brought to the Massachusetts General Hospital emergency department 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 antimicrobial agents, 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 infec-

tion varies with the incidence of *T. gondii* infection in the general population. While toxoplasmosis is an uncommon presenting manifestation 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; however, the MRI scan is more sensitive. 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 t 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 also are 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.* Clinically, patients with CNS toxoplasmosis may worsen with the initiation of HAART therapy. This is thought to reflect the recrudescence of immune function with declining HIV viral loads. Such patients, as for reconstitution syndrome in *Pneumocystis* infection, may benefit from the transient addition of corticosteroids for suppression of the inflammatory response. However, other potential pathogens also should be excluded.

4. *Cryptosporidium* Species

Cryptosporidium is a protozoan parasite that can cause severe and persistent diarrhea in immunocompromised patients, particularly those with AIDS, but also in individuals with immunoglobulin deficiency, solid organ and hematopoietic transplant recipients, and neutropenic hosts. This is a common form of self-limited gastroenteritis in less severely immunodeficient individuals and in the immunocompetent host.[6,237–244] The organism also has been associated with biliary disease and uncommonly with respiratory infection in some individuals. The relative absence of effective therapy for cryptosporidiosis increases the impact of this pathogen. 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, organ 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

Cryptosporidium is a coccidian protozoan parasite (phylum Apicomplexa, class Sporozasida, subclass Coccidiasina) of which two other members (*Isospora belli* and *Eimeria* sp.) cause significant infection of gastrointestinal and respiratory epithelia in compromised hosts. Many species of cryptosporidia have been identified in most vertebrate species. The ability to transmit infection 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. The variability of the ability of clinical isolates of C. *parvum* to cause disease in humans and to respond to therapy suggests that further subspecies of *C. parvum* exist. Some additional subspecies have been identified in stools of patients with AIDS. Additional antigenically distinct subspecies are beginning to be identified in animals. The coccidia complete their life cycle within the

human host. The exacerbation of infection by immune suppression reflects the role of the immune system in controlling replication. Infection is initiated by ingestion (fecal–oral route) or occasionally by inhalation of oocysts with an average of 7–10 days elapsing before clinical symptoms emerge. Disease may be delayed for up to a month following exposure. It is likely that the oocyst can also initiate an autoinfectious cycle. Excystation, usually in the presence of bile or digestive enzymes, releases four motile sporozoites that attach to and penetrate the host epithelial cell. These motile sporozoites mature within a unique four-layered parasitophorous vacuole where they mature into trophozoites (Fig. 10). Asexual (schizogeny) division produces up to eight meronts, which mature into 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. Shedding of cysts occurs in individuals with and without immune compromise or symptomatic diarrhea.

Little is known about the pathogenesis of this infection. Symptomatic disease is generally associated with infection of the proximal small bowel. The voluminous water diarrhea suggests a hypersecretory mechanism the basis of which remains unknown. No toxin production has been demonstrated and the degree of villous injury is modest compared with the intensity of the diarrheal illness. The hepatobiliary tree may serve as a reservoir for reinfection in the immunocompromised host. Carriage in the gallbladder has been associated with failure to clear infections both in AIDS patients and in children with hypogammaglobulinemia (Fig. 10). The unique intracellular vacuole places the organism in an extracytoplasmic location, which may contribute to the inability of antimicrobial agents to clear infection.

4.2. Epidemiology

Seroprevalence studies for *Cryptosporidium* have demonstrated the presence of organisms in over 65% of individuals in rural and urban slum areas of underdeveloped nations and 20–30% of people in more developed countries. In regions of the United States with high levels of dairy and cattle farming, seroprevalence approaches 50%. 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 also have been detected in symptomatic and asymptomatic patients with hemato-

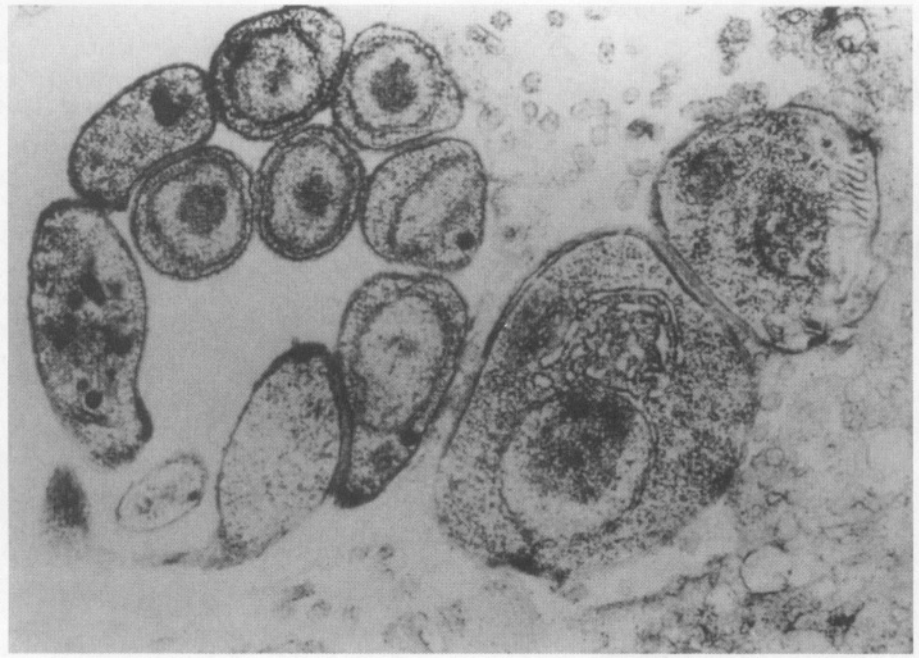

FIGURE 10. Cryptosporidia from the gallbladder of a patient with AIDS. Electron micrograph. ×7500.

logic malignancies and other immune deficits.[259,260] Shedding is sporadic, making epidemiologic studies less reliable. *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. Animal-to-person spread is common in animal handlers. *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. Water filtration appears to be more effective than is treatment with disinfectants. The relative role of person-to-person spread compared with contamination of public water supplies is unclear. However, water-borne outbreaks have been documented with compromised hosts serving as sentinels for inadequacy of the treatment of public water supplies. Clusters of cases have been documented in rural areas after flooding of well water and with cattle contaminating public water supplies. An outbreak, which may have affected 403,000 residents of Milwaukee, Wisconsin in 1993, was attributed to heavy rain and snow runoff carrying contaminants into the rivers supplying Lake Michigan prior to water treatment that met federal standards. Outbreaks also have been associated with fruits and vegetables washed in contaminated water. Organisms survive freezing and require heating above 72°C for 1 min for inactivation.

Susceptible patients are those with the entire range of immune dysfunction. Young children and newborns may be more susceptible due to immaturity of immune function or to fecal–oral contamination. Infections have been reported after viral infections (measles, chicken pox, infectious mononucleosis). Elderly individuals may be similarly at risk. 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 antimicrobial-associated *Clostridium 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, rotavirus, and Norwalk virus are more common pathogens.[268] In the immunocompromised host, cytomegalovirus is an important differential consideration as a predisposing factor for *Cryptosporidium* as well as causing primary invasive gastrointestinal infection.[210] Coinfection with Microsporidia is occasionally seen. Mycobacterial infection and histoplasmosis also may cause diarrheal illness in these patients. Many parasites will cause diarrheal syndromes, including *Giardia lamblia, Entamoeba histolytica, Cyclospora cayetensis,* Microsporidia, and *Isospora belli*.[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. Modified acid-fast staining, Kinyoun's carbol–fuchin negative staining, auramine staining, and safranin staining have largely replaced this laborious method. Also available are indirect immunofluorescent staining with monoclonal antibodies and rapid methods including direct immunofluorescence and ELISA.[271] Serologic 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 serologic 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 hematoxylin–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, the respiratory tract, and the gallbladder. A few AIDS patients have had cryptosporidial cholangitis in the setting of simultaneous cytomegaloviral infection of the gallbladder. 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* also will be seen on Giemsa stain, using a light green counterstain. Improvements in direct immunofluorescent antibody staining may enhance detection. Antigen detection and PCR assays on serum samples have been reported. 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] They often have been treated for diarrhea in the recent past with incomplete resolution. Less commonly, cholangitis, pancreatitis, reactive arthritis (Reiter's syndromelike illness), and pneumonia may be recognized.[276] Stool examination reveals watery stool without blood or white cells, with intermittent shedding of a large number of cryptosporidial oocysts. Severe diarrhea may be associated with malabsorption, as measured by D-xylose absorption studies, 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. The ability to resolve infection depends on reversal of immune compromise in addition to therapy. Immunity seems to include both B- and T-cell responses, although humoral immunity is incompletely protective. CD4+ lymphocytes appear to control susceptibility and duration of infection while interferon-γ reduces the intensity of infection in animal models.

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] The severe diarrhea associated with cytomegalovirus infection in AIDS patients or after transplantation may be bloody and merits separate therapy.

4.5. Therapy of Cryptosporidiosis

There is no consistently useful therapy for cryptosporidiosis (Table 9). 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, apoptosis with loss of columnar epithelium and vacuolization has been observed. 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. Other macrolides have been studied including clarithromycin, which appears to be useful in prophylaxis, and azithromycin, which was

TABLE 9. Therapy for Common Intestinal Parasites of Compromised Hosts

Infecting organism	Primary	Alternative	Comments
Cryptosporidia No therapy proven efficacious. Self-limited in immunocompetent patients	Paromomycin 500–750 mg tid or qid to 100 mg bid with food × 14–28 days, ± azithromycin 600 mg/day × 4 weeks, followed by paramomycin for 8 weeks (*JID* **178**:900, 1998), *or* nitrazoxanide 500 mg bid-qid	Octreotide 50–500 μg tid SC or IV at μg/hr *or* azithromycin 1200 mg PO qd × 28, then 500 mg/day (azithromycin alone not very effective); atovaquone 750 mg bid-tid with food	HAART is most effective (*Lancet* **351**:256, 1998); nitrazoxanide up to 2.0 g/day promising but not FDA approved; Unimed: 1-800-864-6330; diclazuril (not FDA approved) Janssen 800-521-2437; symptomatic Rx; nutritional support; hyperimmune colostrum; benefit of rifabutin? water: boiled 1μ filter
Isospora belli	TMP-SMX 2 DS tab PO bid or 1 DS tid × 10 days, then bid × 2–4 weeks	Pyrimethamine 50–75 mg/d PO + folinic acid 10 mg/d PO × 14–28 days ± sulfadiazine if resistant	Chronic suppression in AIDS patients without HAART response; 1 TMP-SMX 3×/wk *or* (pyrimethamine 25 mg/d PO + folinic acid 5 mg/d PO)
Microsporidiosis (*CID* **27**:1, 1998) Ocular: *Encephalitozoon hellum* or *cuniculi*, *Vittaforma corneae* or *Nosema* sp. Intestinal: *E. bieneusi*, *E. (Septata) intestinalis*. Disseminated: *E. hellum* or *cuniculi*, *E. intestinalis*, *Pleistophora* sp.	Albendazole 400–800 mg PO bid × 21 days to 3 months	Metronidazole 500 mg tid; atovaquone 750 mg PO tid; thalidomide ?(HIV, males) 100 mg qd (teratogen); in HIV+ patients, ?*E. hellum* with fumagillin eyedrops; for *V. corneae*, may need keratoplasty	To obtain fumagillin: 1-800-547-1392. Thrombocytopenia with fumagillin in *E. bieneusi*; HAART most successful (*Lancet* **351**:256, 1998); Dx: Most labs use modified trichrome stain; need electron micrographs for species identification; FA and PCR methods in development

not effective in clinical trials. Diclazuril sodium, a benzeneacetonitrile derivative, and letrazuril, which has greater bioavailability, did not prove effective in clinical trials. Paromomycin has reduced parasite carriage in some patients but does not appear to produce cure in trials. Nitrazoxanide also is in trials with some data suggesting clinical improvement and reduction in oocyte shedding in small numbers of patients. 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 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. *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 native virulence. These organisms also can cause or contribute to disease in immunocompromised individuals.

5.1. The Organism: Life Cycle and Epidemiology

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 is not known. It is likely that many carriers of the disease are asymptomatic.

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 also are formed and are shed intermittently and mature outside the infected individual. Oocysts can remain viable in the environment for months.

5.2. The Patient

Isospora belli causes diarrhea in immunocompetent patients, especially young children and residents of chronic psychiatric care facilities. *I. belli* also causes chronic diarrhea in malnourished individuals and in AIDS patients who are not receiving cotrimoxazole prophylaxis for *Pneumocystis* and in those without access or response to immune reconstitution with antiviral therapies (HAART).[241,285] In the immunocompetent patient, *I. belli* infection has an incubation period of 7–10 days and causes self-limited diarrheal illness. The patient presents with watery, nonbloody diarrhea with nausea, abdominal pain, and weight loss. Systematic signs (headache, malaise, myalgias) are often present, although high-grade fever is unusual. The patient continues to secrete infectious oocysts for weeks after acute infection. Some individuals have presented with a more chronic abdominal pain or diarrhea syndrome. 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, sugar flotation, auramine–rhodamine stain, or 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 shed oocysts. Fecal leukocytes may be absent. Charcot–Leyden crystals are often seen in stool samples. String tests, duodenal aspirates, and small bowel biopsies have been 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-SMX) at a dose of 160 mg TMP component four times a day for 10 days (Table 9) [285,286] In AIDS patients, prolonged additional therapy with TMP-SMX (twice a day for an additional 3 weeks) has been necessary for clearance of oocysts.[287] Prophylactic therapy with TMP-SMX (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. In transplant patients with isosporiasis and in AIDS patients, reduction in immune suppression or antiviral therapy with immune reconstitution is needed for complete resolution.

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, snails, rodents, and some primates. They are occasionally found in irrigation and drainage ditches and in surface water. 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 *Enterocytozoon bieneusi,* for which man is the definitive host. Other common forms in humans include *Encephalitozoon hellum, Enc.* (formerly *Septata*) *intestinalis, Pleistophora* spp., *Trachipleistophora hominis* and *T. anthropopththera, Vittaforma corneae* (formerly *Nosema corneum*), and *Brachiola vesicularum.* Further genera have been identified in small numbers of patients. These relatively undefined species have been attributed to groupings called either "*Nosema-like*" or "collective groups" of Microsporidia based on the region of origin. Up to 30% of AIDS patients with weight loss and chronic diarrhea are 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 solid organ transplant recipients (heart, lung, liver, kidney–pancreas) and BMT recipients, microsporidial diarrhea, pneumonitis, keratitis, and encephalitis have been observed.

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 hematoxylin–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. Organisms have been identified in the prostate and urethra of infected individuals although formal demonstration of sexual transmission has not been made. 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 mucosae are normal to minimally inflamed endoscopically. Enterocyte degeneration accompanies partial villous atrophy and modest inflammation in the lamina propria. Person-to-person spread seems likely. 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 include bacterial overgrowth secondary to local immune deficiency. Some patients have presented with myositis (*Pleistophora* sp.), bronchiolitis and nephritis (*Enc. helium*), sinusitis (*Enc. intestinalis*), or hepatitis (*Enc. cuniculi*). The mechanism for dissemination remains uncertain.

6.3. Diagnosis

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 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. Small bowel biopsy (distal duodenum) provides the optimal specimen for histologic demonstration of enteric infection. Staining

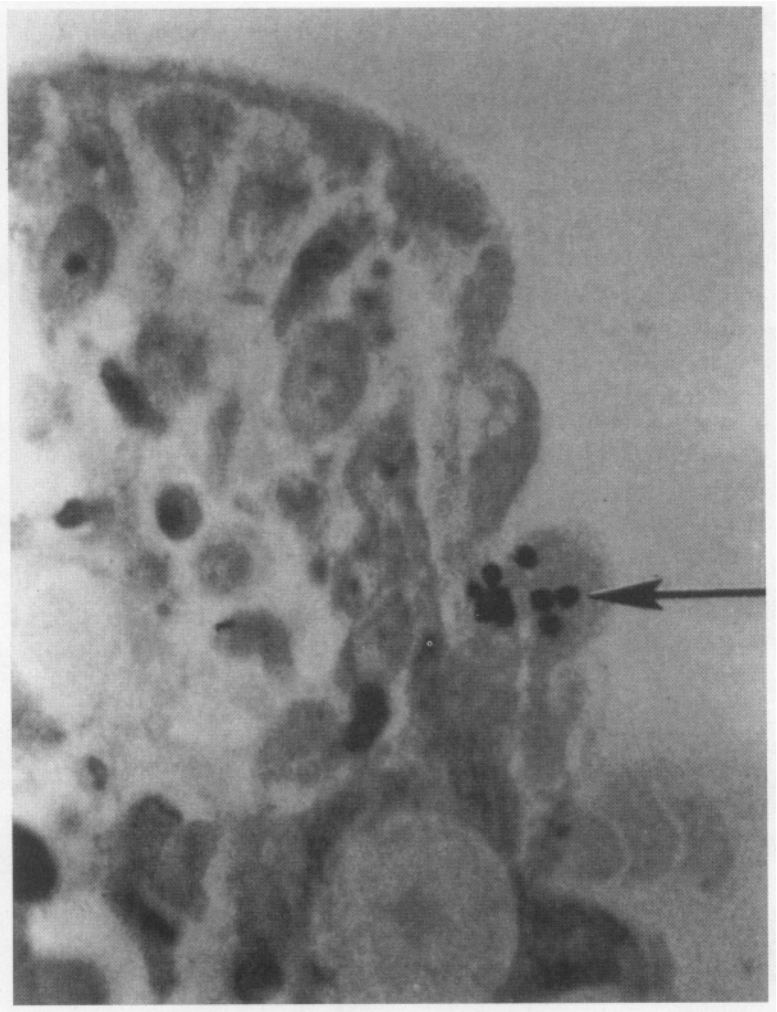

FIGURE 11. Microsporidia within the jejunal epithelium of a patient with AIDS (←) are gram-positive spores by Brown–Brenn stain. ×1750. Courtesy of Drs. R. Weber and R. T. Bryan, Parasitic Diseases Branch, Centers for Disease Control.

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] Intestinal biopsies reveal villus atrophy, crypt hyperplasia, and variably increased numbers of intraepithelial lymphocytes usually with minimal neutrophilic infiltration. *Enc. intestinalis* is often more widespread and may be found in macrophages of the lamina propria. 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. Immunofluorescence microscopy is not generally useful for stool diagnosis but may be useful with intestinal biopsies and with tissues in disseminated disease. Molecular studies of these organisms have also led to the development of a variety of nucleic acid tests that are available on a research basis. These tests are being refined for clinical use.

6.4. Therapy

Therapy for microsporidiosis depends on reversal of immune deficits (Table 9). In AIDS, immune reconstitution in response to HAART therapy has been associated with remission of chronic disease. Albendazole (400 mg PO bid for 3–6 weeks) has had some efficacy in reducing disease activity and reducing or eradicating parasites from stool, sputum, eye scrapings, sinuses, and other sites. These infections generally have been due to *Enc. intestinalis, Enc. hellum,* or *Enc. cuniculi* but not *Ent.*

bieneusi. Thalidomide, 100 mg per day (a teratogen), has been reported to be useful in some HIV-infected patients infected with *Ent. bieneusi.* Fumagillin (for *Enc. cuniculi*) and an analogue TNP-470 have limited activity against *V. corneae* and *Enc. intestinalis* in cell cultures. Furizolidone and fumagillin have been used in small numbers of AIDS and organ transplant patients with *Ent. bieneusi.* Many agents including thiabendazole, 5-fluorouracil, sparfloxacin, nifedipine, and itraconazole have limited activity. Other agents with reported efficacy may have activity against co-pathogens: azithromycin, atovaquone, metronidazole, and thiabendazole. Symptomatic therapy and nutritional support are important adjuncts to therapy. The frequency of isolation of Microsporidia (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.

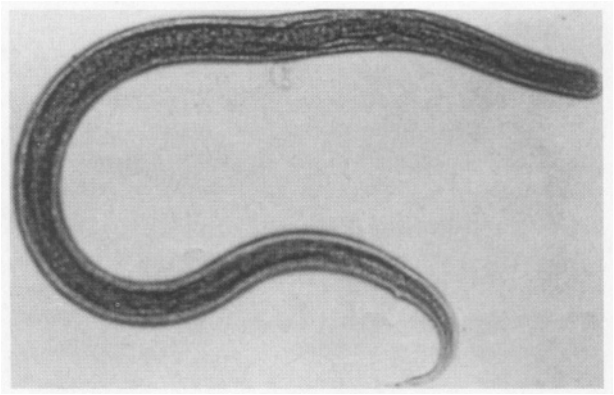

FIGURE 12. *Strongyloides stercoralis* larva isolated from the sputum of a patient with disseminated strongyloidiasis due to an ACTH-secreting tumor.

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, with increased numbers of larvae completing the autoinfection cycle, large numbers of worms enter the systemic circulation producing the "hyperinfection syndrome."[296,297] When organisms are found in organs not generally associated with the life cycle of *Strongyloides*, "disseminated" or overwhelming infection is said to have occurred. 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 found worldwide in tropical and subtropical regions in warm, moist soil often contaminated by human feces. The organism completes a complex life cycle within the human host.[295] The infective form is the filariform larvae that 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. Male worms cannot penetrate the mucosa and are excreted. Beneath the small intestine mucosa, female larvae mature through two molts, producing adult female worms that produce fertilized eggs through parthenogenesis. Eggs mature to first-stage rhabditiform larvae within the intestinal mucosa. Maturation of the rhabditiform larvae occurs over 24–48 hr. These larvae are passed in the stool or enter an autoinfective cycle within the gastrointestinal tract. The autoinfective reproductive cycle 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. The free-living female worm is 1 mm long; the adult male worm is slightly shorter.

7.2. Epidemiology

Strongyloides has been found in temperate and tropical regions. It 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. Many give a remote history of rash associated with febrile or diarrheal illness. 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] Renal and hepatic transplantation have been associated with strongyloidiasis. Disseminated disease has been reported only in renal transplant recipients and anecdotally in hepatic transplant recipients receiving tacrolimus. All reported cases were in patients receiving corticosteroid therapy. Cyclosporin A, but not tacrolimus, has been reported to exert an inhibitory effect on *Strongyloides.*[313] Whether corticosteroids play a direct role in the maturation of rhabditiform larvae (via mimicking of parasite ecdysteroid hormones) is uncertain. The graft may also transmit the organism. Abdominal surgery or steroid therapy for apparent ulcer disease, ulcerative colitis, or Crohn's disease can exacerbate underlying infections.[309,314] The hyperinfection syndrome has also been reported in normal individuals without apparent predisposing immune defects.

Strongyloidiasis has not been associated with AIDS in the absence of other factors contributing to susceptibility (e.g., chemotherapy, malignancy, malnutrition). 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.

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 Lieberkiihn 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. Gram-negative bacteremia, pneumonia, and meningitis are common features of disseminated disease. Bacteria are thought to escape via mucosal breaks or attached to migrating worms. We also have observed recurrent episodes of polymicrobial sepsis and peritonitis in renal transplant patients with hyperinfection syndrome.

7.4. The Patient

Acute GI infection generally will 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

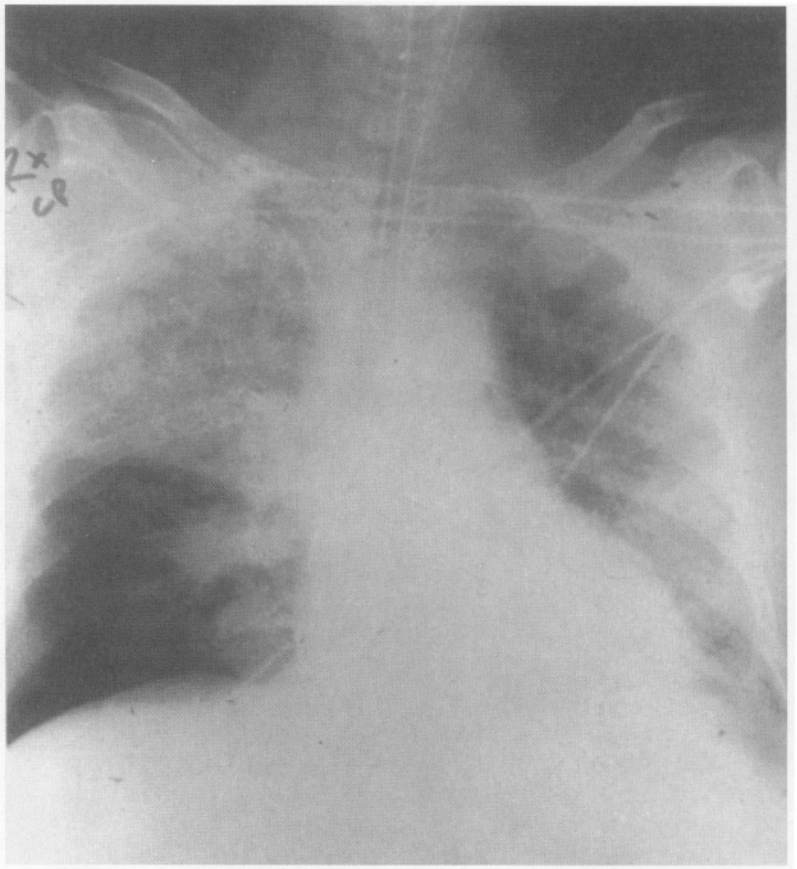

FIGURE 13. Chest radiograph of a patient with disseminated strongyloidiasis. The patient had fever, hemoptysis, hypoxemia, and gram-negative bacteremia with fluctuating, dense pulmonary infiltrates.

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 strongyloidiasis 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. Most 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. "Hyperinfection Syndrome" and Disseminated Strongyloidiasis

The predilection of this organism for the lungs and 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 "pas-

senger" 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 instituted prior to elective immune suppression in patients with exposures to endemic regions, even without identification of organisms.[294] Disseminated infection has been observed up to 3 years after solid organ transplantation, but generally during periods of prolonged neutropenia following chemotherapy or hematopoietic transplantation.

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 and in patients receiving corticosteroids.

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 also may 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 also will be seen by Papanicolaou stain or by experienced observers on Gram's and acid-fast stains of concentrated specimens. A modified agar plate is useful in stool examination. Serologic testing is rarely available in a time frame useful for clinical diagnosis, but should be used for screening of patients from endemic regions. Molecular diagnostics are under development.

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 immunofluorescence 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 5–7 days. In our experience, thiabendazole is

effective but poorly tolerated in cirrhosis or transplant recipients. A number of drugs are being studied but are of less certain efficacy, including mebendazole, cambendazole, and albendazole in high doses. Ivermectin (200 μg/kg per day for 1–2 days) is useful and better tolerated than thiabendazole in clinical trials and our experience but is not FDA approved for this indication.[317] In most centers, ivermectin is considered the drug of choice for disseminated disease. Albendazole (400 mg PO qd × 3 days) also has been useful in some individuals. Side effects of therapy may include hypotension, neurotoxicity, and leukopenia with mild elevations of liver function tests. 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, DNS, 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). Reports of failures have been made with each of these regimens. Therapeutic failures with thiabendazole may be successfully treated with ivermectin. Patients at risk for dissemination due to immune suppression should be evaluated for the presence of the carriage state.

Illustrative Case 3

A 66-year-old man immigrated to the United States from the Dominican Republic approximately 30 years prior to this admission. He had a history of heavy smoking and alcohol abuse and had 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 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 anxious man with bilateral lower extremity edema and increased abdominal girth with progressive dyspnea on exertion. His appetite was increased but he had lost 7 kilograms in weight over the past few weeks. 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 antimicrobial agents. 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 frank perforation of his colon. 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, aggressive 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. Ivermectin has been used successfully in many such patients and is generally preferred over thiabendazole. The presence of *P. carinii* speaks to the chronicity of immune suppression. CMV is an important cofactor to many systemic infections, including *Pneumocystis,* and may contribute to bowel ulceration and/or perforation in the compromised host. In general, such patients will have a negative CMV antigenemia assay in the peripheral blood and will require endoscopy with biopsy for diagnosis.

8. *Entamoeba histolytica* and Amebiasis

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 with disease occurring in about 10% of exposed individuals. 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 also is not understood. Invasive amebae (*E. histolytica*) can be distinguished from nonpathogenic forms (*E. dispar*) using antibody staining, zymodeme analysis, genetic probes (e.g., for adhesion molecules and enzymes), and restriction mapping of amebic strains. The significant clinical features of amebiasis include the separation of nonpathogenic from pathogenic amebae, the distinction of lumenal infection from invasive disease, and the consideration of this diagnosis prior to the initiation of immunosuppressive therapies.

8.1. The Organism

E. histolytica is a pseudopod-forming protozoan of the family Endamoebidae, order Amoebida, class Lobosea, which also includes other human parasites: *E. hartmanni* ("small amebae"), *E. polecki, E. coli,* 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, enzymatic, and genetic (cDNA) differences between pathogenic and nonpathogenic strains of amebae. Characteristic electrophoretic patterns of isoenzymes or "zymodemes" may distinguish pathogenic strains.[319] *In vitro,* it was thought to be possible to change a nonpathogenic organism into a pathogenic organism by altering the bacterial flora surrounding the organisms in culture. However, these studies have not been reproducible and it is not clear that this conversion occurs *in vivo.* The determinants of pathogenicity are not clear. *E. histolytica* and *E. dispar* are indistinguishable morphologically.

8.2. Life Cycle

The life cycle of the organism begins with the ingestion of food contaminated by infective cysts, which can survive for weeks in the environment. Excystment in the small bowel releases motile trophozoites covered with filopodia used for epithelial attachment in the colon. The organism grows in a low-oxygen environment. Encystment of trophozoites occurs only in the large bowel. 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. Individuals exposed while traveling usually have resided in endemic areas for a month or longer. Amebiasis is the third leading cause of mortality due to parasitic infection, following malaria and schistosomiasis.

Infection is most common in persons of lower socioeconomic status, with crowded living conditions and poor sanitation, in institutionalized individuals, and in promiscuous male homosexuals. 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] AIDS patients do not appear to be at increased risk for the development of amebiasis. 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 probably are not relevant to GI symptoms. Invasive disease is most common in children, pregnant women and immediately postpartum, malnutrition, and immunocompromised individuals. Among compromised hosts, patients receiving chemotherapy, corticosteroid therapy, or immunosuppression for organ transplantation are at a greater risk for the development of fulminant colitis.[325,326]

Serologic assays are useful for the detection of inva-

sive 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.

8.4. Immunology

Antibodies against *E. histolytica* that develop during acute infection do not appear to be protective.[327,328] Such antibodies may persist for up to 10 years after acute infection. The presence of serum antibodies correlates with the presence of both invasive amebiasis and hepatic disease. Antibody can lyse trophozoites *in vitro.* Complement also can 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. Amebae isolated from liver abscesses appear to be resistant to antibody-mediated lysis. The cellular immune response appears to be depressed acutely during infection and returns after treatment of disease.[326,327] Sera from infected individuals may contain a factor that is suppressive for cell-mediated immune responses. Recurrent infection is uncommon and resistance to subsequent infection appears to be mediated by the cellular immune response activating killing by macrophages.

8.5. Pathogenesis

The pathology of amebic disease is important. The characteristics of inflammatory changes in the bowel may be easily 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. Trophozoites are found in tissues in an amorphous, eosinophilic matrix. Given the ability of the organism to lyse neutrophils, leukocytes are found only at the edge of established amebic ulcers. 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. Periportal inflammation and fibrosis may be seen with few if any organisms in the ports. 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] Immune compromise does not appear to affect the development of either invasive or noninvasive disease. Since local inflammation usually is 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 complica-

tions 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 socioeconomic groups, men than in women, and alcoholics than in nondrinkers. In addition to poor hygiene, host immunity also may 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 often will 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. Amebic brain abscess is generally catastrophic. Genitourinary disease is described in the form of rectovaginal fistulae or as ulceration with granulomatous disease of the vagina or penis.

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. Individuals with positive serologies and negative stool examinations or with a syndrome consistent with amebiasis (liver abscess, mass lesions, acute colitis) should undergo endoscopic evaluation (unless the patient has toxic megacolon and fulminant colitis). Radiologic evaluation will reveal the presence of thickened intestinal walls in severe amebic colitis, with narrowing and loss of folds.[331] Ameboma may be confused with chronic infection due to tuberculosis, *Yersinia* infection, carcinoma, lymphoma, or regional enteritis. Infection by *E. histolytica* must be excluded before initiating corticosteroid therapy for colitis.

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 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 antimicrobial agents or of anticipated rupture or in the presence of mixed bacterial and amebic infection may be indicated. Occasionally, percutaneous 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 is 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). Agents with actions within the lumen of the bowel include diiodohydroxyquin, 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, ornidazole, secnidazole, and nimorazole). High-dose metronidazole (750 mg tid for 5–10 days) is needed and often poorly tolerated. Emetine and dehydroemetine must be given intramuscularly and have depressant effects on the myocardium. Erythromycin and tetracycline have activity only in the bowel wall. Chloroquine works only in hepatic disease.

Treatment for asymptomatic cyst passers is controversial due to the frequency of infection with nonpathogenic strains of amebae.[329] 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 and surgery should be avoided if possible.[332] Similarly, liver abscess is rarely an indication for surgery. Antimicrobial 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* and rarely *Balamuthia mandrillaris.*[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. A number of *Acanthamoeba* species are pathogenic for humans including: *A. castellani, A. hatchetti, A. griffini, A. divonensis, A. palestinensis, A. culbertsoni, A. astronyxis,* and *A. rhysodes.* 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. *B. mandrillaris* trophozoites are 30 μm in diameter and are

indistinguishable from those of *Acanthamoeba. B. mandrillaris* cysts have a wavy, three-layered outer wall which averages 15 μm in diameter. 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. *Acanthamoeba* has been isolated in pharyngeal swabs of normal individuals with respiratory viral illnesses and positive serologic assays found in healthy individuals. However, other than corneal disease, granulomatous amebic encephalitis due to *Acanthamoeba* is generally restricted to debilitated individuals or those with immune defects. These defects include individuals with AIDS, diabetes mellitus, cirrhosis, corticosteroid therapy, and cancer chemotherapy and in renal, hepatic, and bone marrow transplant recipients. Corneal infection by *Acanthamoeba* is often associated with contamination of contact lens solutions. *B. mandrillaris* causes infection in both normal and immunocompromised individuals. 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 acute and rapidly progressive meningitis that is fatal in less than 4 days without therapy. The inflammatory cell response is primarily with 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 meningoencephalitis due to *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. *Acanthamoeba* keratitis is usually associated with corneal trauma and the use of contact lenses. Both cysts and trophozoites are found in affected corneas in the center of an inflammatory ring. Giant cells may be present in any inflammatory exudate. Involvement of the posterior segment of the eye may occur. 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. *B. mandrillaris* causes a subacute-to-chronic granulomatous meningoencephalitis similar to that of *Acanthamoeba* in both compromised and normal hosts. Involved areas may contain cysts and trophozoites in a perivascular location, with vasculitis and hemorrhagic necrosis of meninges and brain tissues. Organisms also may be found in other tissues (kidney, skin, adrenals). The inflammatory exudate includes lymphocytes, monocytes, plasma cells, and giant cells.

9.4. The Patient and Diagnosis

While acute PAM will present in a fashion similar to cases of severe bacterial meningitis, the patient will rapidly progress from headache, fever, and vomiting to seizures, coma, or paralysis. *Naegleria* infection will generally present within 1 week after exposure to contaminated water or possibly to inhaled cysts. *Naeglaria* may produce false sensations of taste or smell similar to those seen in some patients with heart failure and early brain stem 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 also may present with headache, fever, and meningismus. The infectious exposure is generally uncertain. Nodular or ulcerative skin lesions may be present for months before clinical disease emerges. Infections involving the sinuses, bone, and lungs have also been described. 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 de-

pressed. 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.

While infection due to *B. mandrillaris* occurs in normal individuals, individuals with AIDS, diabetes, and renal failure are notably susceptible. Disease has been reported as acute necrotizing meningoencephalitis or as subacute, granulomatous meningoencephalitis. *B. mandrillaris* may be seen in wet mounts of CSF that generally has a mononuclear pleocytosis. Imaging studies of the brain demonstrate multiple hypodense mass lesions. The organism can be grown on tissue culture cells or in axenic media.

9.5. Therapy

Amebic keratitis can be cured with early diagnosis, generally before the characteristic corneal ring develops. Corneal scrapings are often contaminated with common bacterial pathogens; *Acanthamoeba* must be considered in the differential diagnosis and appropriate parasitologic evaluation made. Motile trophozoites may be observed in wet mounts of corneal scrapings. Cysts and trophozoites may be seen in Giemsa, hematoxylin and eosin, periodic acid–Schiff, and Calcofluor white-stained specimens. Molecular assays are under development.

Few individuals have survived primary amebic meningoencephalitis.[333,334] Early diagnosis and therapy of *Naeglaria* infection are critical to survival. Those few patients who survived received intrathecal and systemic treatment with amphotericin B at high dose ($\geq$1 mg/kg per day). Given the rapid progression of PAM, there is no opportunity for gradual escalation of dosing. Passive immunotherapy may be useful when antisera become available clinically. There is no useful therapy for *Acanthamoeba,* although *in vitro* sensitivity *of Acanthamoeba* to polymyxin, pentamidine, propamidine, ketoconazole, miconazole, 5-fluorocytosine, paromomycin, neomycin, and ivermectin has been demonstrated. These agents, coupled with debridement, have had some efficacy in the treatment of keratitis. Effective therapies for *B. mandrillaris* have not yet been described.

10. Leishmaniasis

Leishmaniasis encompasses a variety of syndromes, including cutaneous, mucocutaneous, and visceral disease. The manifestations of infection vary 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. In endemic regions and travelers to such areas, leishmaniasis has been recognized in a broad spectrum of both normal and immunocompromised individuals.

10.1. The Organism

Species of *Leishmania* can be separated by geographic distribution and the usual 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.* However, most species may be associated with more invasive disease also. *Leishmania tropica* can cause chronic, relapsing cutaneous disease (recidivans form) and was associated with visceral disease in U.S. soldiers in the Middle East after Operation Desert Storm. Similarly, *L. mexicana* has been found in patients with visceral disease. *Leishmania donovani* (India and Africa), *L. infantum* (Mediterranean), and *L. chagasi* (South America) can cause visceral leishmaniasis. Consensus about species differentiation is being addressed using sequence and hybridization analysis using kinetoplast DNA probes.

Leishmania exists in two forms. Within the sand fly (phlebotomus) vector or in culture, they develop from amastigotes into single-celled, flagellated extracellular promastigotes. The promastigotes migrate to the sand fly pharynx in a nondividing or stationary phase, a form that has high infectivity for macrophages. Human infection is initiated by the bite of an infected female phlebotomine sand fly. Within the cells of vertebrate hosts, the organisms become small, rounded amastigotes (without flagellae) of 2–3 μm in diameter. Organisms enter 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. Complement activation may assist in the penetration of promastigotes into macrophages.

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 immunocompromised individuals, especially those receiving corticosteroids, with hematologic malignancies, or after organ transplants, disease may progress more rapidly and be more difficult to diagnose.[336,340–345] Leishmaniasis may not present for years after exposure and/or initiation of immune suppression. 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] Some 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. In solid organ transplant recipients, pulse-dose steroids, antilymphocyte antibodies, and intensified immune suppression used to treat graft rejection may accelerate disease. At least one renal allograft recipient with exposure in the pretransplant period developed clinical disease more than 7 years after transplantation, although the average time is closer to 8 months.[345,348] 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 and Immunology

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 mucosal 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.

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 level of immune response determines the manifestations of the disease. In cutaneous leishmaniasis, patients lacking immune responsiveness to the parasite may develop tissue 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.

10.4. The Patient

Infection in normal and immunocompromised individuals share most clinical characteristics. 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. Im-

mune 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. Pancytopenias and hypergammaglobulinemia are common. Complications are the results of anemia, thrombocytopenia, liver failure, or secondary bacterial infection. Atypical manifestations include involvement of the gastrointestinal tract and peripheral neuropathies (axonal degeneration and demyelination) described in patients with AIDS.

10.5. Diagnosis

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 is often diagnostic. Parasites are occasionally seen in peripheral blood smears or sputum samples. 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]

10.6. Therapy

Leishmaniasis is treated with 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. Mortality approaches 28% in immunocompromised individuals. Occasional abnormalities in liver function tests and electrocardiograms may be seen, with elevations of the total white blood count as side effects of therapy. Pancreatitis is common after pentavalent antimony therapy.[348] Patients also may 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 four 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. Antimony resistance has been documented in India. Interferon has been used with antimony as primary therapy in some patients. Amphotericin B and lipid-associated formulations of amphotericin B have been used as alternatives for therapy of cutaneous, mucocutaneous, and visceral leishmaniasis. Amphotericin should not be given with antimony compounds. Pentamidine is another 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] The role of T lymphocytes may be in enhancing the production of mucosal IgA. 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 daycare 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. Antigen detection tests allow the detection of *Giardia* by immunofluorescence or ELISA. Serologic testing is useful in epidemiology. Patients are generally treated with metronidazole, 250 mg three or four times a day for 7–10 days. This drug should be used with caution in children or 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 three times a day for 5 days (2 mg/kg in young children). Furazolidone, paromomycin, and tinidazole have also been used successfully. Albendazole has some activity but is not recommended for routine use.

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 immunocompromised hosts. Malaria has been transmitted with renal, heart, and liver allografts derived from endemic regions. It is unclear whether infection acquired other than at the time of transplantation is due to new primary infection (mosquito bite) or recrudescence of old infection (e.g., hepatic *Plasmodium vivax*) in an immunocompromised host. While immunity to *P. falciparum* develops in individuals from endemic regions, the association of immune suppression with enhanced severity or incidence of infection has not been made.

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] Corticosteroids administered for renal transplantation tend to normalize serum creatinine values, perhaps by reversal of graft rejection, but do not affect the characteristic fevers associated with malaria.

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 (1.2 g bid IV or 600 mg PO tid × 7 days) and quinine (650 mg PO tid) has been effective in some patients.[385,386] Alternative therapies include TMP-SMX with pentamidine or quinine with azithromycin. 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.

11.4. American Trypanosomiasis (Chagas' Disease)

Trypanosoma cruzi is a protozoan that infects man during a blood meal by the reduvid bug. Endemic regions for Chagas' disease (*T. cruzi*) and for African sleeping sickness (caused by *T. brucei brucei* and *T. brucei gambiense*) do not overlap. *T. cruzi* develop in the bloodsucking Triatominae or kissing bug, releasing metacyclic trypamastigotes, which are discharged with feces during blood meals. The parasite infects a variety of cell types and transforms intracellularly as amastigotes, differentiates into trypamastigotes, and enters new cells after rupture of the host cell. The vectors are commonly found in dilapidated housing and in the wild in endemic regions where the organism is maintained in many mammalian host intracellularly as trypomastigotes. *Trypanosoma cruzi* have a particular predilection for muscle (including cardiac) and neuroglial cells and produce local inflammation with lymphocytes, macrophages, and plasma cells. Within heart muscle, pseudocysts are formed that are clumps of intracellular amastigotes, often in association with myocarditis. In addition to reduvid infection, infection has occurred as a result of organ transplantation and

via blood transfusion. Heart failure due to Chagas' disease is associated with biventricular enlargement, thinning, aneurysms, and mural thrombi and is a common basis for transplantation in endemic regions. Conduction abnormalities also are common. Dilatation of the esophagus and colon also is associated with loss of innervation of the mesenteric plexi in association with local inflammation.

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.

Approximately 10–30% of infected individuals develop clinical disease. 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] Immunosuppression of individuals with chronic infection due to *T. cruzi* often is of greater severity than the original disease. In renal allograft recipients, cases of central nervous system involvement have been described. Activation of Chagas' disease has occurred in the setting of AIDS. In both groups, brain abscesses have developed, which is not a part of the syndrome in normal hosts. In Brazil and elsewhere in South America, heart transplants have been performed in patients with known Chagas' disease, some of whom developed cardiac allograft infection. In some, disease progressed despite treatment with benznidazole. Reduced levels of immune suppression with tacrolimus or cyclosporine and prophylaxis with nifurtimox have allowed successful transplantation in some of these individuals. Disease may relapse with cessation of therapy or due to CMV infection. Transfusion-associated Chagas' disease is reported in renal and hepatic allograft recipients.

Diagnosis is based on detection of circulating parasites in blood or buffy coat and later the detection of IgM antibody to the parasite. Chronic carriers may have elevated IgG titers; these antibodies may cross-react with those for syphilis, malaria, leishmania, and autoimmune antigens. We have seen parasites in pericardial fluid from a liver transplant recipient, who also had parasites detected in bone marrow and lymph node biopsies. Molecular, axenic, and murine (xenodiagnosis) cultivation also have been achieved. Treatment is with nifurtimox or benznidazole. Side effects are common and treatment often fails to eradicate the parasite.

11.5. African Trypanosomiasis

African trypanosorniasis 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 "antigenie variation." A hemolymphatic 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 trypanosorniasis 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 also may 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 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*

Cyclospora was first described in Papua New Guinea in 1997.[387] In the interim this group of organisms has been attributed to the blue-green algae, cyanobacteria, and fungi and has been termed a "large cryptosporidium"; they have been implicated as the causative agent of a severe diarrheal illness in travelers and in patients with AIDS.[388–391] Electron microscopic studies have suggested that these "cyanobacteriumlike" bodies represent a new protozoan pathogen of the coccidian genus *Cyclospora,* and named *Cyclospora cayetanensis.* Cyclospora and *Eimeria* species appear to belong to the same genus and are found in the gastrointestinal tracts of snakes, moles, rodents, chickens, and humans. Like *Cryptosporidium,* this organism causes self-limited but relapsing diarrheal illness in travelers, children, and other normal hosts (up to 3 months) and possibly persistent diarrhea in the cases described in patients with AIDS. Infection is most often described in travelers after contact with contaminated water supplies in tropical regions. *Cyclospora* does not appear to be a major cause of travelers' diarrhea; it is found in many (up to 0.5%) urban water supplies worldwide. Person-to-person spread was suggested by an outbreak occurring among medical house staff in Chicago. Asymptomatic infection has been demonstrated.

The organism is 8–10 μm in diameter with a cluster (morula) of refractile, membrane-bound globules within a limiting membrane. The organism is a thick-walled sphere with a fibrillar coat and granular cytoplasm. The organism contains two sporocysts that contain sporozoites with nuclei and micronemes. The organism is variably acid-fast, although the organism can be identified often in wet mounts of diarrheal stool or by recognizing the characteristic blue autofluorescence under the ultraviolet epifluorescence microscope. Safranin staining and sporulation tests also may be used for identification. In jejunal biopsies, *Cyclospora* are found in a characteristic supranuclear location of the cytoplasm of jejunal epithelial cells. Oocysts are long-lived in the environment and are resistant to chlorination, formalin, and freezing. Sporulation occurs in the environment, as for *Isospora,* suggesting that the replication of this organism may not be intensively regulated by the hosts' immune system.

Infected individuals develop symptoms within 7–10 days of exposure. Patients present with a flulike illness with the abrupt onset of watery diarrhea accompanied by prominent systemic signs including myalgia, headache, nausea, vomiting, and low-grade fever. The intensity of diarrhea fluctuates over 4–8 weeks without therapy. Treatment is limited to cotrimoxazole (DS bid), which should be followed with prophylaxis in immunodeficient individuals.

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