Some Aspects of Protozoan Infections in Immunocompromised Patients - A Review

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Protozoa are among the most important pathogens that can cause infections in immunocompromised hosts. These microorganisms particularly infect individuals with impaired cellular immunity, such as those with hematological neoplasias, renal or heart transplant patients, patients using high doses of corticosteroids, and patients with acquired immunodeficiency syndrome. The protozoa that most frequently cause disease in immunocompromised patients are Toxoplasma gondii, Trypanosoma cruzi, *different* Leishmania *species, and* Cryptosporidium parvum; *the first two species cause severe acute meningoencephalitis and acute myocarditis,* Leishmania *sp. causes mucocutaneous or visceral disease, and* Cryptosporidium *can lead to chronic diarrhea with hepatobiliary involvement. Various serological, parasitological, histological and molecular methods for the diagnosis of these infections are currently available and early institution of specific therapy for each of these organisms is a basic measure to reduce the morbidity and mortality associated with these infections.*

Key words: protozoa - acquired immunodeficiency syndrome-Aids - opportunistic infections

Since the sixties, opportunistic infections, which frequently occur in patients under some kind of immunosuppression, have become very common in daily clinical practice. The number of immunosuppressed patients susceptible to different infectious or neoplastic processes has increased each decade and culminated in the advent of the acquired immunodeficiency syndrome (Aids) at the beginning of the eighties. The increasing use of transplants (kidney, bone marrow, liver, heart, etc.) and the appearance of new immunosuppressive drugs, nowadays extensively used in these patients as well as in patients with neoplasias and autoimmune diseases, has led to the occurrence of a large number of individuals with chronic immunosuppression in our community, who in turn are predisposed to developing opportunistic infections. Tens of pathogens have been described as etiologic agents of these infections, including viruses, bacteria, protozoa, fungi, and helminths. Immunosuppression, either at the humoral or cellular level, may have different consequences for the host depending on its magnitude, including facilitation of the occurrence of these infections, an increased disease/infection rate, alterations in the clinical manifestation of the infection or exacerbation of its course, among others (Young 1981). Aids and lymphoproliferative neoplasias are examples of conditions that lead to abnormalities in practically all compartments of the immune system; however, T lymphocyte-mediated immune defects predominate. The therapy to which these individuals are submitted (e.g., corticosteroids) and malnutrition caused by the base disease itself represent important cofactors in the predisposition to developing infections. Humoral

immunity defects (hypogammaglobulinemia) and neutropenia (number of serum neutrophils $< 1000/mm^3$) usually predispose to the occurrence of bacterial infections caused by Gram-positive cocci, Gram-negative bacilli and some fungi (*Candida*, *Aspergillus*), while infections caused by intracellularly multiplying agents such as mycobacteria, endemic mycosis-inducing fungi and protozoa are rare. These last pathogens are frequent agents of infectious processes that occur in Aids or Hodgkin's disease, where the basic defect is the result of cellular immune depression (Karp & Neva 1999). Aids produces the most severe form of immunosuppression known, and more than one hundred microorganisms causing opportunistic infections in these patients have been identified, many of them intracellular protozoa, which will be discussed in the present review. Infection with human immunodeficiency virus (HIV) leads to important alterations in the clinical course of many disorders caused by these intracellular organisms; curiously, no significant effects of retrovirus-induced immunosuppression on the course of disease have been observed for some protozooses such as malaria. On the other hand, there is evidence that these infections can accelerate the course of HIV infection due to the capacity of many of these organisms to stimulate Th2 type cytokines (IL-4, IL-10, etc.), which favor the progression of the disease caused by HIV (Morrow et al. 1989, Actor et al. 1993, Gilks 1993).

The most frequent protozoan causing opportunistic infections in immunocompromised individuals is *Toxoplasma gondii*. Its association with severe manifestations of immunosuppression has been known for several decades, and the occurrence of encephalitis, myocarditis and disseminated disease has since then been observed in different clinical conditions such as lymphoreticular neoplasias, solid organ transplantation, and, at present, mainly in patients with Aids. Before the advent of this viral infection, opportunistic infections caused by this and other protozoa had rarely been observed in

immunocompromised patients. Other coccidia such as *Isospora belli* and *Cryptosporidium parvum* only gained clinical importance after the recognition of Aids at the beginning of the eighties. Few cases of reactivated Chagas disease in immunosuppressed patients have been reported in the sixties and seventies, but *Trypanosoma cruzi* was never considered to be a true opportunistic agent. The same was the case for protozoa of the genus *Leishmania* whose association with immunosuppressive diseases represented a scientific curiosity in past decades. This panorama changed after the Aids epidemic, and *T. cruzi*, and especially *Leishmania* species, are currently gaining great importance as agents of severe opportunistic diseases in patients affected by this retroviral infection. The advances made during the last few years in the chemotherapy of neoplasias, in the use of transplants and in the therapy of autoimmune diseases have led to the reactivation of protozooses under these conditions, thus confirming the opportunistic character of these pathogens (Ferreira et al. 1997, Borges et al. 1999, Ferreira 1999, Rosenthal et al. 2000, Morgado et al. 2000).

The objective of this review was to update and comment upon some aspects of diseases caused by protozoa in immunosuppressed patients. Four parasites which are important causative agents of severe disease in these individuals were chosen, and within this context, we basically focus on pathogenic, clinical, diagnostic and therapeutic aspects of the infections caused by *T. gondii*, *T. cruzi*, *Leishmania* sp. and *C. parvum*.

OPPORTUNISTIC DISEASE CAUSED BY *TOXOPLASMA GONDII*

About 50% of the world population is infected with *T. gondii*, a protozoan of the coccidian family, an obligate intracellular parasite that multiplies in any nucleated cell of the vertebrate host and that shows universal distribution (Cesbron-Delauw 1994). Felines are the definitive host, with the domestic cat being the most important. This protozoan is found in nature in three forms: the tachyzoite or intracellular proliferative form, present during the acute phase of infection; tissue cysts, responsible for the latent infection of multiple organs and important in disease transmission, and oocysts found only in felines and eliminated in their feces, with this form representing the source of infection for susceptible hosts (Krick & Remington 1978). Human infection occurs through the ingestion of cysts in raw or uncooked meat, mainly pork and beef, ingestion of mature oocysts in food or water contaminated with cat feces, or through transplacental passage of the parasite from the mother to the fetus, with acute infection of the latter (Frenkel 1973). Transmission through blood transfusion, laboratory accidents, and organ transplantation is less common, and epidemiological evidence exists indicating hematophagous insects as biological vectors of *Toxoplasma* (Amato Neto 1970, Herwaldt & Juranek 1993, Amendoeira 1995).

Toxoplasmosis represents an important public health problem, considering that 0.25 to 5 cases of congenital infection occur per 1,000 livebirths, that approximately 10 to 20% of uveitis cases are caused by this parasite, and that the prevalence of *T. gondii*-induced encephalitis can reach up to 40% in patients with Aids (Krick & Remington 1978, Luft & Remington 1988). Therefore, toxoplasmosis is of great clinical importance in man in two major situations: as a cause of congenital infection, with 5 to 24% of children becoming ill and dying during the neonatal period, in addition to the high rate of children with severe neurological and visual sequelae who require education and special and costly care (Frenkel 1973), and as an opportunistic infection of high mortality in immunosuppressed individuals (Carey et al. 1973, Ambroise-Thomas & Pelloux 1993).

In immunocompetent hosts, the infection is frequently benign, with parasitemia being self-limited, resulting in an asymptomatic clinical form of the disease in most cases. However, in about 20% of cases acute infection is accompanied by febrile lymphadenopathy, asthenia and lymphomonocytosis, with the course of infection being self-limited (Feldman 1968, Darcy & Santouro 1994). After this period, *T. gondii* remains viable in the form of tissue cysts, which reproduce slowly throughout the life of the host, thus characterizing the chronic phase of infection. During this phase, the tissue cysts are controlled by the humoral and cellular immune system, involving T lymphocytes and macrophages which are continuously stimulated by parasite antigens, a fact that protects against reinfection. As a result, parasite multiplication is more active and persists for longer periods of time in less immunologically active tissues such as the central nervous system (CNS) (Johnson 1981, Sims & Talbot 1989, Darcy & Santoro 1994).

Immunocompromised hosts, especially those with deficient cellular immunity, are at risk of recrudescence of the chronic infection and dissemination, with the occurrence of fulminating disease. The preferential reactivation of *T. gondii* in the CNS has been demonstrated clinically and experimentally (Van Thiel 1966, Frenkel et al. 1975, Ruskin & Remington 1976). This fact is probably due to low local immunity, as well as to the presence of the blood-brain barrier which impairs the flow of substances such as specific antibodies and interferon-γ (IFN-γ) that would inhibit parasite multiplication (Ambroise-Thomas & Pelloux 1993). After the CNS, the heart and lungs are the most frequently affected organs (Frenkel 1957, Tschirhart & Klatt 1988, Jautzke et al. 1993).

After the occurrence of Aids, toxoplasmosis became the most common cause of encephalitis in the United States (Luft & Remington 1988). Until then, this protozoosis was only sporadically observed in patients with neoplasias, collagen disease or transplant recipients under immunosuppressive therapy. In these patients, the disease resulted in the reactivation of chronic infection, although cases of acute infection, including patients with disseminated disease, have been described for previously seronegative organ recipients whose donors presented serological evidence of a past infection (Frenkel et al. 1975, Luft et al. 1983, Gray et al. 1989, Bertoli et al. 1995).

The clinical presentation of toxoplasmosis ranges from asymptomatic reactivation, commonly observed in previously infected organ recipients and demonstrable by increased post-transplant antibody titers, to severe disseminated disease. The disease manifests as diffuse encephali-

tis, meningoencephalitis or, more common, tumor lesions with a mass effect. Motor syndrome, consciousness disturbances, seizures and focal signs are common manifestations that are clinically indistinguishable from other CNS complications such as reactivated Chagas disease, primary CNS lymphoma, viral or fungal encephalitis, neurotuberculosis and others, diseases also frequently observed among these patients (Hakes & Armstrong 1983, Luft & Remington 1988, Ferreira et al. 1997).

The variety of immune system defects found in individuals with Aids, such as CD4+ T lymphocyte deficiency, reduced activity of cytotoxic T and NK cells, and the low production of immunoregulatory lymphokines such as IFN-γ, may explain the high frequency of reactivated *T. gondii* infection (Fauci 1984).

The new impact of *T. gondii* infection on public health has raised great interest in the understanding of its immunopathogenesis. The infection triggers both humoral and cellular immune responses, with the latter being more important for the development of protective and persistent immunity (Darcy & Santoro 1994). The role of humoral immunity has been extensively studied. It is known that *T. gondii* infection produces IgM, IgA, IgE and IgG class antibodies, with the first three being detected early during the course of infection. IgM class antibodies are the first to occur and can be detected 7 to 15 days after infection, with maximal concentrations being observed during the second month, followed by a progressive decline and disappearance of these antibodies within a few months. However, low antibody titers may persist for a prolonged period of time, i.e., months or years. IgA antibodies can be detected after the first month of infection in about 95% of cases, reaching a peak concentration between the second and third months, followed by a decline, which, in most cases, is observed between the fourth and seventh month before the decrease in IgM antibodies. IgA antibody detection is therefore of great diagnostic value in acute toxoplasmosis, since these antibodies are rarely found during the chronic phase of infection (Huskinson et al. 1990, Gross et al. 1992).

Cellular immunity seems to be the main mechanism of defense in the control of toxoplasmosis (Johnson 1981). The role of T lymphocytes in *T. gondii* infection was first emphasized by Frenkel in 1957. In 1988, the same author observed that athymic rats do not develop protective immunity and that depletion of CD4+ cells eliminates any previously acquired protection (Frenkel 1988). Since then, a series of experimental studies have demonstrated the importance of CD4+ and CD8+ T lymphocytes, which act synergistically, in the control of this disease and in the prevention of its reactivation (Araujo 1991, Gazzinelli et al. 1991, Parker et al. 1991). This group of lymphocytes represents the major source of IFN-γ, which, in turn, is responsible for resistance during the chronic phase of disease through the activation of macrophages that promote intracellular death of the parasite (Kaufmann 1995). Reactivation of infection is therefore due to a reduction in the expression of IFN-γ as well as IFN-α, which in turn results in the lack of activation of macrophages and microglial cells (Gazzinelli et al. 1993).

Activated macrophages, NK and LAK cells, and the production of different cytokines, such as IL-2, IL-12 and IFN-γ, are of crucial importance for the immune response against *T. gondii* both during the acute and chronic phase of the disease (Gazzinelli et al.1992, 1994).

The diagnosis of acute toxoplasmosis is mainly based on a combination of clinical and laboratory data. In clinical practice, serological tests are routinely employed to detect IgM- and IgG-specific antibodies, including indirect immunofluorescence and immunoenzymatic tests (ELISA), with the latter showing higher sensitivity and specificity (Fuccillo et al. 1987). However, in immunosuppressed patients, particularly those with Aids, the early diagnosis of neurotoxoplasmosis has been limited due to the lack of noninvasive diagnostic tests of high sensitivity and specificity. The clinical picture is nonspecific and cerebrospinal fluid (CSF) findings are found to be normal or show nonspecific alterations such as lymphocytic pleocytosis and discrete CSF hyperproteinorraquia. Imaging analyses such as cranial computed tomography and magnetic resonance are of great value, demonstrating isodense or hypodense, single or multiple lesions with a mass effect, and taking up the contrast dye in a ring-like or nodular manner in more than 90% of cases. Such findings are highly suggestive of toxoplasmosis reactivation granuloma, but are not pathognomonic (Figueiredo et al. 1983, Wanke et al. 1987, Luft & Remington 1988). The serological pattern found in these patients is similar to that observed for the general population with inactive infection. Since in basically all cases the disease results in the reactivation of latent and non-acute infection, IgM antibodies are not habitually detected and IgG antibodies do not discriminate between latent and active infection (Luft et al. 1984, Wong et al. 1984, Weiss et al. 1988, Grant et al. 1990), and are even not detectable in a minority of cases with reactivated disease (Zangerle et al. 1991, Porter & Sande 1992, Garly et al. 1997).

Individuals under immunosuppressive therapy such as organ transplant recipients or patients with malignant diseases, who had been previously infected with *T. gondii*, might show an altered serological profile of this protozoan compatible with reactivation, such as increased IgG antibody titers or, less frequently, increased titers of acute phase antibodies, i.e., IgM, 4 to 13 weeks after the beginning of immunosuppression, and the presence or absence of clinical manifestations, a fact not observed in patients with Aids. On the other hand, seronegative patients receiving organs from seropositive donors may show seroconversion 4 to 6 weeks after transplantation, generally accompanied by disseminated infection whose clinical manifestation usually coincides with the occurrence of antibodies, although late manifestations, i.e., about 10 months after immunosuppressive therapy, have been reported (Luft et al. 1983).

The detection of IgA antibodies has also shown conflicting results. IgA-specific antibodies were detected by immunoblotting in a group of patients with Aids and neurotoxoplasmosis in 91.6% of serum and CSF samples analyzed (Gross et al. 1992). Brazilian investigators have demonstrated the presence of this antibody in serum in 84% of 54 patients with this diagnosis (Borges & Figueiredo 2000a). In contrast, IgA-specific antibodies were detected in sera from HIV-infected patients with and without neurotoxoplasmosis (Darcy et al. 1991).

In cases of reactivation in the CNS, the detection of anti-*Toxoplasma* antibodies in CSF should be interpreted with caution, since their presence may only indicate passive passage from serum to the CNS. However, the demonstration of local production of specific antibodies, whose titers were found to be increased in CSF irrespective of a serum increase, has proved to be of diagnostic value in some situations, such as meningoradiculitis caused by *Borrelia burgdorferi* (Wilske et al. 1986), neurosyphilis (Müller & Moskophidis 1983), encephalitis caused by herpesvirus and paramyxovirus (Reiber 1980, Felgenhauer 1982), congenital toxoplasmosis (Pinon et al. 1986), and neurotoxoplasmosis itself (Wong et al. 1984, Orefice et al. 1990). In a series of 37 patients with Aids and *T. gondii*-induced encephalitis, antibodies were detected in CSF in 23 patients. Among 16 patients evaluated, 70% showed evidence of local production of specific antibodies (Potasman et al. 1988). In another group of patients, indirect immunofluorescence revealed that IgG titers in CSF at a dilution higher than 1/64 show 100% specificity for the differential diagnosis of neurotoxoplasmosis in Aids. In contrast, IgA antibodies detected in CSF by ELISA showed 72.7% specificity. In the same study, the detection of antibody production in the CNS using radial immunodiffusion was not useful for the diagnosis of toxoplasmosis, with a specificity of only 70.8% (Borges & Figueiredo 2000b).

Several clinical and experimental studies have reported different results regarding the usefulness of circulating *T. gondii* antigen as a diagnostic marker of acute infection, congenital toxoplasmosis and disease reactivation in immunocompromised patients. However, its value in routine clinical practice is still controversial due to the divergent results obtained with animals models and in human infection (Araujo & Remington 1980, Dannemann et al. 1991, Fachado et al. 1994, Hafid et al. 1995, Letillois et al. 1995).

Parasite identification in blood through culture media or inoculation into laboratory animals provides questionable sensitivity, the techniques are difficult to carry out and isolation of the parasite is time consuming (Derouin et al. 1987, Tirard et al. 1991, Dannemann et al. 1992).

The use of more sensitive and specific methods, such as the polymerase chain reaction (PCR), has been shown to be effective for the diagnosis of congenital and ocular toxoplasmosis (Dupouy-Camet et al. 1993), but PCR detection of parasitemia in patients with Aids and toxoplasmic encephalitis is only useful in cases of disseminated infection (Khalifa et al. 1994). The usefulness of PCR was also assessed in another study using CSF samples obtained from 14 patients with Aids. Among 9 cases with a diagnosis of neurotoxoplasmosis, PCR was positive in 4 patients only, with no false-positive result being detected (Parmley et al. 1992).

In most cases, the diagnosis of neurotoxoplasmosis is presumptive and based on clinical presentation, tomographic or magnetic resonance findings, and the presence of IgG-specific antibodies in serum. The diagnosis is confirmed based on the therapeutic response which generally occurs between 7 and 14 days (Wanke et al. 1987, Luft et al. 1993). The definitive diagnosis has been made thus far upon demonstration of the parasite, more precisely tachyzoites, in brain tissue obtained by biopsy. However, in addition to being an invasive method which is subject to complications, histopathological analysis confirms the diagnosis in only 50% of clinically diagnosed cases. Access to the lesion is often impaired or routine staining techniques (Wanke et al. 1987, Cohn et al. 1989), and even immunohistochemistry (Conley & Jenkins 1981, Hofflin & Remington 1985), do not detect the parasite in the material obtained. In a histopathological study evaluating a series of 85 cases of neurotoxoplasmosis and Aids, more than 50% of immunoperoxidase-positive cases were negative when standard techniques were employed (Luft et al. 1984).

In contrast to immunocompetent patients in whom the course of disease is mainly self-limited, toxoplasmosis is fatal in immunosuppressed individuals if not recognized and treated early. The drugs routinely employed in the treatment of toxoplasmosis basically act against the proliferative forms, or tachyzoites, present during the acute phase of infection or during reactivation of latent foci in immunocompromised hosts, but do not eradicate the encysted form of the parasite, the bradyzoites (Israelski & Remington et al. 1993, Luft et al. 1993). The combination of pyrimethamine and sulfadiazine is considered to be the most effective scheme, with therapy consisting of two phases: attack or acute treatment and maintenance therapy. The regimen of choice is sulfadiazine at the dose of 4 to 6 g/day combined with pyrimethamine at the dose of 50 to 75 mg/day for 4 to 6 weeks. Maintenance therapy consists of a 50% reduction in the initial dose of both drugs, i.e., 2 g/day sulfadiazine plus 25 mg/day pyrimethamine throughout immunosuppression, i.e., in the case of patients with Aids, throughout life. A response is observed in 70 to 95% of cases and 91% of patients show objective signs of improvement within the first 10 to 14 days (Luft et al. 1993). Clindamycin at the dose of 2.4 to 4.8 g/day or 100 mg/day dapsone, plus pyrimethamine at the doses reported above, are alternative options for patients who develop sulfa intolerance (Katlama et al. 1996). New macrolides such as clarithromycin (1.5 g/day) and azithromycin (2 g/day) in combination with pyrimethamine have provided satisfactory results and even demonstrated some action on the cystic form of this protozoan (Saba et al. 1993). The use of clarithromycin plus minocycline and of azithromycin plus sulfadiazine has also been evaluated. A 75% cure rate was achieved with atovaquone, a hydroxynaphthoquinone acting against cysts and trophozoites, administered at the dose of 3 g/day, and combination with pyrimethamine may improve this response (Kovacs 1992). Folic acid should be added to sulfa- and pyrimethamine-containing schemes at the dose of 10 to 15 mg/day to prevent the myelosuppression provoked by these drugs.

OPPORTUNISTIC DISEASE CAUSED BY *TRYPANOSOMA CRUZI*

Chagas disease still represents one of the most important endemic diseases of the American continent and has been diagnosed from the south of the United States to the south of Argentina, with an estimated number of 16 to

18 million chagasic patients. In Brazil, 3 to 5 million people are estimated to be infected with *T. cruzi* and, in contrast to what was observed 2 to 3 decades ago, most of these individuals live today at the periphery of large cities as a consequence of the migration of infected individuals of low socioeconomic level from rural areas to urban centers (Pan American Health Organization 1990, Rocha et al. 1994). Even in non-endemic countries such as the United States, more than 100,000 people are estimated to be infected as a result of the massive migration of Latin Americans to this country (Navin et al. 1985).

Since the end of the sixties, several authors have observed the occurrence of severe forms of Chagas disease, notably meningoencephalitis and myocarditis, in patients under severe immunosuppression. Some cases have been described in Brazil (Amato Neto et al. 1968, França et al. 1969, Queiroz 1973), others in Latin-American countries where the parasitosis is endemic (Rivero et al. 1974, Monte Verde et al. 1976, Pizzi et al. 1982), and one case was documented in the United States (Kohl et al. 1982). Usually, severe manifestations of trypanosomiasis that occurs in immunosuppressed patients result in the reactivation of chronic, previously asymptomatic or oligosymptomatic infection, although severe acute forms resulting from blood transfusions have sporadically been reported in the literature (Corona et al. 1988, Wanderley et al. 1988, Grant et al. 1989, Nickersosn et al. 1989). Localized or systemic exuberant clinical manifestations have been observed in patients with lymphoreticular neoplasias, renal, heart and bone marrow transplant patients, and in patients with Aids (Ferreira et al. 1997). Experimental studies conducted on mice and rats chronically infected with *T. cruzi* and submitted to different immunosuppressive drugs have demonstrated reactivation of the disease, accompanied by an increase in parasitemia, aggravation of myocarditis and increased mortality, thus confirming the opportunistic character of this parasite (Brener & Chiari 1971, McCabe et al. 1985, Sinagra et al. 1993). The use of trypanosomicidal drugs in animals with reactivated infection reduced the magnitude of disease manifestations and prevented further reactivation, findings that are relevant for clinical practice (Meckert et al. 1988).

The understanding of the morphological alterations that occur in immunocompromised chronic chagasic patients has been based on the study of biopsies and autopsy material carried out in a few cases described in the literature. The most frequent anatomopathological finding related to the association between Chagas disease and immunosuppression is acute meningoencephalitis, which is characterized by the presence of generalized cerebral edema and hemorrhagic soft pseudotumoral areas of undefined limits measuring several centimeters; these lesions are generally localized at the brain periphery, affecting the gray and, predominantly, the white substance; lesions can also occur in the brain stem and in the cerebellum. Histologically, the presence of a parenchymatous and perivascular exudate consisting of macrophages, lymphocytes, plasma cells and, more rarely, neutrophils is noted; necrotic foci, hemorrhage and microglial nodules are also commonly found. *T. cruzi* amastigotes are abundant and parasitize glial cells and, rarely, neurons.

Lymphomonocytic meningitis, with the presence of parasites in the meninges, is a constant finding in these cases and permits the in vivo diagnosis upon detection of *T. cruzi* in CSF. Acute myocarditis is another important anatomopathological finding in immunosuppressed chagasic patients, which is characterized by cardiomegaly and focal or diffuse infiltration of the myocardium by mononuclear cells, accompanied by intense parasitism of cardiac fiber cells. Epicarditis and endocarditis can also be observed in these patients. Injury of other organs has been rarely documented; panniculitis might be observed in heart and renal transplant patients in the presence of the parasite; parasitism of the musculature of the esophagus, stomach, colon, ocular globe and uterine cervix has also sporadically been reported (Almeida et al. 1974, Stolf et al. 1987, Oddó et al. 1992, Rocha et al. 1993, 1994).

Few cases of an association between Chagas disease and lymphoreticular neoplasias have been described in the literature, most of them occurring in patients with acute lymphocytic leukemia or Hodgkin's disease. In half these cases, Chagas disease was in fact acute after transfusion and the course of disease was severe since the patients were under marked immunosuppression which was not only the result of the base disease but also of the concomitant use of antineoplastic chemotherapy associated or not with high doses of corticosteroids. The remaining cases were certainly the results of reactivation of chronic infection, since the patients were found to be seropositive upon diagnosis. Among 15 cases selected from the literature, meningoencephalitis was observed in 7/15 cases (46%), myocarditis in 9/15 (60%), and the concomitant occurrence of both manifestation was observed in 3/15 cases (20%). The diagnosis was in general confirmed by the presence of parasites in blood, pericardial fluid, or tissue, particularly brain, heart and esophageal tissue. The mortality rate was very high in these cases (8/15, 53%) and survival of the patients directly depended on the early diagnosis and the rapid institution of specific therapy (Ferreira et al. 1997).

Transplants are increasingly being performed in patients with Chagas disease. Many of them receive kidney or heart transplants, the latter for the treatment of dilated myocardiopathy caused by the parasitosis itself. In renal transplantation, two modalities of trypanosomiasis can be discussed: (1) the transmission of *T. cruzi* from living donors or cadavers to non-chagasic kidney recipients through the transplanted organ itself (therefore, the donor has Chagas disease) or through the transfusion of blood or blood derivatives; (2) reactivation of chronic parasitosis after transplantation as the result of immunosuppression due to the use of corticosteroids, cyclosporin or tacrolimus. In the case of the first modality, the clinical manifestations are similar to those observed during the acute phase of infection and consist of fever, hepatosplenomegaly, myalgias and signs of myocarditis, including the presence of cardiac arrhythmias. The time interval between transplantation and the onset of clinical manifestations can be as short as 30 days or as long as 14 months. In all of these patients, *T. cruzi* is present in peripheral blood and anti-*T. cruzi* IgM antibodies are also detectable in serum by indirect immunofluorescence. In

cases of reactivated chronic infection, involvement of the CNS might be observed in the form of severe meningoencephalitis, which can lead to death in some cases. It is important to note that positive serology for Chagas disease of the organ donor or recipient does not represent a contraindication for transplantation, since long-term follow-up of chagasic transplant recipients has shown that in most patients the disease is not reactivated and that there is no need for prophylactic pre- or post-transplant anti-*T. cruzi* therapy; this treatment only needs to be initiated if there is evidence of acute or reactivated infection. Treatment of the clinical disease of any modality caused by *T. cruzi* consists of oral benznidazole at the dose of 5 mg/kg per day (in two doses) for 60 days; alternatively, nifurtimox, currently not available in Brazil, may be used. The anti-parasitic treatment has been shown to be effective in these cases (Jost et al. 1977, Chocair et al. 1985, Figueiredo et al. 1990, Cantarovith et al. 1992, De Arteaga et al. 1992, Lopez Blanco et al. 1992, Luders et al. 1992).

A few patients with chagasic heart disease have already received heart transplants; these individuals were submitted to powerful immunosuppression after transplantation consisting of high doses of corticosteroids, immunosuppressors (azathioprine, cyclosporin, tacrolimus, etc.) and anti-lymphocytic globulin. The combination of these drugs, used to prevent organ rejection, markedly favored reactivation of the protozoosis in at least two thirds of cases. The time between transplantation and reactivation might exceed one year. The predominant clinical manifestations were fever, signs of acute myocarditis accompanied by heart failure and arrhythmias, and infiltrating erythematous cutaneous lesions, which are histologically characterized by panniculitis with the presence of a large number of *T. cruzi* amastigotes. The diagnosis was in general made by anatomopathological examination of endocavitary biopsies. Curiously, no parasites were observed in peripheral blood, even after repeated analyses using different parasitological techniques. Benznidazole administered at the recommended doses for 60 days was found to effective in the treatment of reactivated Chagas disease, leading to the disappearance of signs and symptoms of the disease, but without curing the parasitosis. Allopurinol has shown some efficacy in a small series recently studied in Brazil. The prophylactic pre- or post-transplant use of benznidazole is not indicated since this drug does not eliminate the parasite from the organism and, therefore, does not prevent reactivation (Stolf et al. 1987, Bocchi et al. 1993, Kirchoff 1993).

Only one case of fulminating Chagas disease has been described in the literature in a bone marrow transplant recipient (Geiseler et al. 1987).

The advent of the Aids epidemic at the beginning of the eighties opened the possibility of the occurrence of reactivation of this parasitosis in co-infected individuals under profound immunosuppression as a result of this viral infection.

About 80 cases of reactivation in patients with Aids have been documented in the literature, most of them reported in Brazil and Argentina. Only one tenth of these cases have been described in detail, while the remaining cases have been communicated at congresses or specialty symposia (Ferreira et al. 1997, Rocha et al. 1997, Sartori et al. 1998). Curiously, the first Aids case reported in the United States involved an immigrant from El Salvador who carried both infections (Gluckstein et al. 1992). At the beginning of the nineties, about 6% of autopsied HIV-positive patients with reactivated Chagas disease had been recorded in our hospital in Uberlândia, MG, an area endemic for Chagas disease (Borges et al. 1996). Although in most published cases no information regarding the CD4 T lymphocyte count is available, recent findings indicate that practically all patients with reactivated disease have CD4 T cell levels below 200 cells/mm^3, similarly to what is observed for other opportunistic infections affecting these patients (Ferreira et al. 1997, Sartori et al. 1998). In contrast to heart transplant patients with reactivated disease in whom myocardial involvement is predominant, involvement of the CNS is common in patients with Aids, being observed in 75 to 80% of co-infected individuals. CNS involvement manifests as acute, uni- or multifocal meningoencephalitis with fever, headache, vomiting, seizures and focal neurological signs. Analysis of CSF samples demonstrates the presence of mild to moderate pleocytosis (< 100 cells/mm^3), predominantly consisting of mononuclear cells, elevated protein levels and the presence of *T. cruzi* upon direct examination of the fluid (Ferreira et al. 1991, 1997). Parasites are also easily detected in peripheral blood using Giemsa-stained smears, by concentration methods (Strout, microhematocrit, etc.), and even by the quantitative buffy coat (QBC) assay. The identification of parasitemia by other techniques such as blood culture or xenodiagnosis should not be considered as evidence of reactivation, since these methods may yield a positive result during any phase of the disease, including chagasic patients not infected with HIV (Ferreira et al. 1997).

Modern imaging methods are frequently used to diagnose encephalic lesions in HIV-positive patients. In the case of chagasic meningoencephalitis, cranial computed tomography reveals the presence of hypodense, predominantly subcortical, single or multiple lesions which have the shape of a ring after contrast injection. These lesions closely resemble those seen in neurotoxoplasmosis, although the main areas involved in this parasitosis are the base nuclei and the thalamus. Magnetic nuclear resonance seems to be more sensitive than tomography, detecting a larger number of lesions in both infections (Rocha et al. 1994, Ferreira et al. 1997, Ferreira 1999).

Another organ frequently involved in the reactivation of American trypanosomiasis is the heart. The frequency of this occurrence is not known, since no complete autopsy study of cases published in the literature or communicated at congresses exists. Involvement of the heart is often discrete and is certainly not detected clinically or radiologically. Based on the few cases published, about half of the patients are estimated to present myocarditis during disease reactivation. Clinical manifestations, when present, include signs and symptoms of congestive heart failure (dyspnea, tachycardia, edemas, etc.) and the presence of cardiac arrhythmias. An increase in heart volume, together with the presence of sometimes voluminous peri-

cardial hemorrhage, can be documented by two-dimensional echocardiography. Parasites may be present in the pericardial fluid. In biopsy or autopsy studies, the presence of *T. cruzi* should be always confirmed by immunohistochemical techniques or by electron microscopy due to the simultaneous presence of associated infections (for example, *T. gondii*) (Ferreira et al. 1997, Sartori et al. 1998).

The diagnosis of Chagas disease reactivation should be made as early as possible, since anti-parasite treatment can improve the prognosis and survival of the patient. The identification of *T. cruzi* in body fluids (blood, CSF, pericardial or ascitic fluid, etc.) is the key to the diagnosis of Chagas disease and should be carried out as soon as a suspicion exists. Brain or myocardial biopsies should also be performed, if possible. Survival of patients with Chagas disease, even when treated, is low, although the latest cases published showed remission a long time after treatment owing to the concomitant administration of antiretroviral drugs that increase and stabilize serum CD4 T lymphocyte levels (Ferreira et al. 1997, Sartori et al. 1998, Ferreira 1999).

The drugs acting on *T. cruzi* are nifurtimox and benznidazole, with only the latter being available in Brazil. Benznidazole is administered orally at the dose of 5 mg/kg per day in two doses for a period of 60 days. The drug seems to be effective in the treatment of disease reactivation under any type of immunosuppression, including HIV-positive patients. Fever, neurological or cardiac signs, and cutaneous lesions generally regress within the first 2 or 3 weeks of treatment, with no parasitemia being detected upon direct examination, blood culture or xenodiagnosis. Adverse effects commonly occur after the first days of drug use, with cutaneous eruption, peripheral neuropathy and granulocytopenia being the most frequent. In countries where nifurtimox is still commercialized, its use is indicated in situations of reactivation at the dose of 8 to 10 mg/kg per day, orally, for 60 to 90 days. Its efficacy as a trypanosomicidal drug seems to be similar to that of benznidazole. Triazole derivatives (itraconazole, fluconazole) have been used for reactivation treatment by some authors, with apparent success, although they are not recommended as first-line therapy in these situations (Nishioka et al. 1993, Ferreira et al. 1997, Sartori et al. 1998, Ferreira 1999). After remission and treatment discontinuation, the introduction of a second prophylaxis is recommended, since further reactivations may occur later. An expert committee, brought together by the Pan American Health Organization in 2000, recommended the use of benznidazole at the dose of 5 mg/kg per day, three times a week, for an undefined period of time as secondary prophylaxis; nifurtimox might also be used for this purpose. Antiretroviral therapy should be systematically administered to these patients in order to maintain elevated CD4 T lymphocyte levels, thus reducing the chance for disease reactivation. A group of 13 HIV-positive chagasic patients under retroviral therapy have been followed up by our group for months and none of them has shown any clinical signs of trypanosomiasis activity thus far. There is currently no consensus regarding the use of drugs as primary prophylaxis in chagasic patients infected with HIV (Anonymous 1996).

OPPORTUNISTIC DISEASE CAUSED BY *LEISHMANIA* SP.

Leishmaniasis is a parasitic disease widely distributed throughout the world, which is endemic in the tropical and subtropical regions of 88 countries. About 2 million cases are estimated to occur annually worldwide, most of them presenting the cutaneous or mucosal form. *Leishmania* is an obligate intracellular parasite and control of this infection involves a vigorous Th1-dependent cellular immune response. Therefore, the observation of the opportunistic character of this protozoosis during the last few years was no surprise, with this parasite causing mucocutaneous or visceral disease in immunosuppressed patients, particularly those infected with HIV (Alvar et al. 1994, 1997). Co-infection with this retrovirus emerged as a new and interesting pathology, whose frequency is currently increasing. Cases of HIV-*Leishmania* co-infection have been reported in more than 25 countries, particularly those located at the margins of the Mediterranean Sea such as Portugal, Spain, France, Italy, Greece and North-African countries, and, to a lesser extent, in Equatorial African, Asian and South American countries (Alvar 1994, Dedet et al. 1995, Gradoni et al. 1996, Borges et al. 1999). More than 2,000 cases of visceral leishmaniasis associated with HIV infection have been reported in European countries alone, a fact due to the simultaneous dissemination of both diseases and the overlapping geographic distribution of the two infections as the result of the urbanization of leishmaniasis and the ruralization of Aids (WHO 1995, Ferreira 1996, Rabelo et al. 1998). In Brazil, about 80 cases of HIV-*Leishmania* co-infection have been documented, most of them with the mucocutaneous form of the disease and few cases with the visceral form. This number will probably increase during the next few years, since infectologists are currently more aware of the diagnosis of this co-infection (Rabelo et al. 1998, Borges et al. 1999).

Today, it has been demonstrated that Aids and leishmaniasis, particularly the visceral form, can interact in a vicious cycle of mutual aggravation. Aids leads to the dissemination of the parasites to practically all organs and body systems, while, on the other hand, leishmaniasis accelerates the course of HIV infection by decreasing the latent period of viral infection and thus the life expectancy of the patient. Various other opportunistic infections such as tuberculosis or systemic mycoses are found in co-infected patients, demonstrating the marked immunosuppression generated in these individuals (Albrecht 1998, Rosenthal et al. 1999).

The epidemiology of visceral leishmaniasis in the Mediterranean region suffered profound changes since the advent of Aids. It has been known for decades that this infection frequently affects children, but this picture changed radically after the occurrence of Aids, since most cases diagnosed at present in these countries refer to young adults suffering from immunosuppression due to HIV. Leishmaniasis has sporadically been detected in individuals with other types of immunosuppression, such as renal transplant patients and patients with lymphoreticular neoplasias, among others (Fernandez-Guerrero et al. 1987, Alvar 1994). Most HIV-positive co-

infected patients are addicted to injectable drugs. Thus, when an individual with leishmaniasis shares syringes and needles used for drug injection with other partners, leishmaniasis might be transmitted through blood remaining on the material, with this fact representing an alternative form of transmission of this protozoosis between individuals. Amastigotes are demonstrable in peripheral blood smears in more than 60% of co-infected patients, thus confirming the existence of this form of transmission. In addition, these individuals can serve as true reservoirs of the parasite by easily infecting phlebotomine flies feeding on their blood (Medrano et al. 1993, Alvar 1994, Alvar et al. 1997).

Leishmaniasis is currently known to frequently manifest in the form of subclinical infections which can persist in individuals for months or years. Most cases of visceral leishmaniasis that occur in HIV-positive patients are believed to result from reactivation of latent infections, although primary infections with *L. infantum* have been reported among patients in Italy and Spain (Alvar 1994, Gradoni et al. 1996, Alvar et al. 1997).

Any *Leishmania* species can cause disease in immunocompromised patients. Parasites isolated from co-infected patients included strains previously undetected in immunocompetent individuals, including some species restricted to lower animals. Visceral dissemination of species that commonly cause the cutaneous form of the disease (e.g., *L. braziliensis*) has also been observed by us and others (Coura et al. 1987, Machado et al. 1992, Alvar et al. 1997).

The clinical-laboratory presentation of simultaneous HIV and *Leishmania* infections has been described in detail in the literature. Many cases show an asymptomatic or oligosymptomatic course and parasites are casually detected during routine blood and bone marrow examination. Cutaneous forms of the disease with disseminated lesions, involving the nasal, oral or pharyngeal mucosa, have been observed in patients with advanced stage Aids, sometimes accompanied by extensively destructive lesions; lesion recurrence after treatment has been a common event among patients with an apparent therapeutic response to a first course of antimonial drugs or amphotericin B. For the visceral form, classical clinical manifestations include fever, weight loss, hepatosplenomegaly and peripheral pancytopenia, which are observed in about 75 to 80% of cases; adenopathies and signs of pulmonary involvement with interstitial pneumopathy have also been documented. The gastrointestinal tract is frequently found to be involved (in about 30% of cases), and amastigotes have been isolated from the esophagus, stomach, small and large bowel, pancreas and, obviously, liver. In these cases no clinical signs and symptoms might be noted, although many patients present abdominal pain, diarrhea, vomiting and dysphagia; biopsies obtained from any part of the digestive tract easily detect the presence of parasites. Hemorrhagic diathesis due to thrombocytopenia and hepatic involvement may occur in a small percentage of cases. Most co-infected patients have low CD4 T lymphocyte counts, usually less than 200 cells/mm^3, a fact that might explain the frequent association with other opportunistic infections commonly observed

in these patients, such as esophageal candidiasis, pneumocystosis, tuberculosis, toxoplasmosis, cryptococcosis, and cytomegalovirus infection. Some authors have observed that the clinical presentation of visceral leishmaniasis is influenced by the CD4 T cell count, i.e., the lower the lymphocyte count the more atypical is the clinical picture. Nontypical localization of the disease has been frequently found in patients with CD4 T lymphocyte counts < 50 cells/mm^3 (La Rosa et al. 2001). The clinical presentation of visceral leishmaniasis itself should be differentiated from other infections, particularly disseminated histoplasmosis and miliary tuberculosis, which present highly overlapping clinical manifestations (Dedet et al. 1995, Rosenthal et al. 1995, 2000, Alvar et al. 1997, Borges et al. 1999).

The diagnosis of leishmaniasis in patients with Aids is easy due to the fact that these individuals present a large number of parasites in their tissues. Detection of *Leishmania* in bone marrow or spleen aspirates shows high positivity (70 to 100%), thus permitting a rapid confirmation of the diagnosis; as mentioned above, peripheral blood smears stained with Giemsa can reveal the presence of the parasite in up to 60% of cases. A recent study employing the QBC technique, which is widely used for the parasitological diagnosis of malaria, easily demonstrated the presence of the amastigote forms of the parasite in bone marrow and peripheral blood of non-immunosuppressed patients with kala-azar; this method may therefore be of great diagnostic value in co-infected patients (Liarte et al. 2001). Liver biopsies may also serve as a tool to confirm the diagnosis of the parasitosis. Unfortunately, most co-infected patients do not produce sufficient amounts of antibody, and serological tests (ELISA, immunofluorescence) are positive in only 40 to 50% of cases, thus being of limited value for the diagnosis of this infection. In the mucocutaneous form, biopsy almost always leads to a diagnosis due to the abundance of parasites in the material examined. PCR may be used for the detection of these protozoa in blood and other body fluids, and tissue removed for biopsy (Piarroux et al. 1994).

Treatment of HIV-positive patients co-infected with *Leishmania* has generally been difficult. Pentavalent antimony, preferentially N-methylglucamine antimonate, is still considered the drug of choice for these patients, with 20 mg/kg of antimony base being administered intravenously, daily for 30 days. This recommendation is valid for both the mucocutaneous and the visceral form of the disease. In a Spanish study, treatment with antimonial drugs led to remission in most patients, although recurrence was later observed for most cases (Lopes-Velez et al. 1998). On the other hand, French authors only observed a 50% clinical and parasitological response to antimonial drugs in co-infected patients (Rosenthal et al. 1995). In countries with proven resistance of the parasite to antimonial drugs, such as India, the rates of therapeutic failure and recurrence are probably much higher. An increased rate of side effects upon the use of these drugs has been observed for HIV-positive patients; myocardiotoxicity and elevated serum amylase levels due to pancreatitis have been reported by several authors (Alvar et al. 1997, Lopez-Velez et al. 1998).

Within this context, amphotericin B probably represents a more efficient therapy for all forms of leishmaniasis in HIV-positive patients. In the south of France, practically 100% of patients with visceral disease responded to an initial course of amphotericin B (Rosenthal et al. 1995). Total amphotericin B doses of about 1 to 2 g were sufficient to induce remission in most cases. Liposomal amphotericin B used in these patients initially showed surprisingly good results, although it did not prevent disease recurrence. Unfortunately, its high cost limits its use in Brazil (Lopez-Velez et al. 1998). Classical side effects have been observed with the use of amphotericin B (fever, shivering, hypopotassemia, nephrotoxicity, etc.), with these effects being less frequent when lipid formulations are used, which also permit the use of higher doses (3-4 g/total dose). Pentamidine, allopurinol and paromomycin are other drugs used for the treatment of co-infected patients, although the results are not promising. The use of recombinant IFN-γ in combination with antimonial drugs showed a good response in three Spanish cases of kala-azar associated with HIV infection (De Gorgolas et al. 1993).

Irrespective of the type of drug used for the treatment of this clinical entity, there is a clear tendency towards multiple recurrences after the initial therapeutic course (30% after 6 months and 60-70% after 12 months) (Lopez-Velez et al. 1998). Based on this observation, several authors have recommended long-term secondary prophylaxis as done for other opportunistic infections in patients with Aids. Pentavalent antimony administered once a month seems to be efficient in preventing recurrence in these patients (Ribera et al. 1996). Pentamidine has also been used for this purpose, with the advantage of simultaneously acting on *Pneumocystis carinii* (Lopez-Velez et al. 1998). Amphotericin B deoxycholate and lipid formulations may be employed for prophylaxis at weekly doses, particularly in patients who previously developed systemic mycoses that require long-term maintenance therapy to prevent recurrence (Davidson & Russo 1994).

With respect to the mortality of co-infected individuals, about 20% of patients die during the first episode of visceral disease and about 70% die after one year, during which the disease relapsed one or more times. Factors leading to a poor prognosis include advanced immunosuppression (very low CD4 levels) and thrombocytopenia (Lopez-Velez et al. 1998). The introduction of combined antiretroviral therapy in order to improve the immunological parameters leads to a reduction in the number of reactivation episodes and subsequent recurrences in the case of both the visceral and the mucocutaneous forms of the disease (La Rosa et al. 2001).

OPPORTUNISTIC DISEASE CAUSED BY *CRYPTOSPORIDIUM PARVUM*

Over the last few years, there has been growing interest on the part of doctors and parasitologists in human intestinal coccidia, which can cause disease in both immunocompetent and immunocompromised individuals. Three of these protozoa have been the subject of numerous publications, not only as true opportunistic agents of diseases that occur in immunosuppressed patients but also as the etiologic agent of large epidemics generally associated with contaminated water or food. *C. parvum*, *I. belli* and *Cyclospora cayetanensis* are the main coccidian parasites found in the digestive tract of mammals, including humans. *I. belli* rarely causes infection in immunocompetent individuals but is one of the main causes of diarrhea in patients with Aids. In contrast, *C. cayetanensis*, discovered only some years ago, has been responsible for large epidemics in different countries worldwide, causing prolonged diarrhea in immunocompetent and -suppressed individuals. In the present review we only focus on some aspects of human cryptosporidiosis (Fayer & Ungar 1986, Guerrant 1997, Griffiths 1998).

C. parvum was described for the first at the beginning of the 20th century in the digestive tract of the common mouse. However, its pathogenic role in animals was only recognized many decades later, during the seventies, when it was shown to be an important agent of enteritis in cows and fowl (turkeys). The first human cases were described in 1976, but the disease only gained importance after the occurrence of Aids in 1982. Despite its opportunistic behavior in these patients, *C. parvum* is also known to be an important cause of acute gastroenteritis in immunocompetent individuals (Fayer & Ungar 1986). The evolutive cycle of this parasite is completed within a single host; infection starts with the ingestion of water or food contaminated with oocysts eliminated in the feces of infected individuals or animals. The oocysts, measuring on average 5 μm, contain 4 infectious sporozoites. Upon release into the intestinal lumen, the sporozoites penetrate intestinal cells, where they mature asexually inside extracytoplasmic superficial parasitophorous vacuoles into type 1 meronts, which, in turn, release merozoites that again invade enterocytes, generating a cycle of type 2 meronts (type 2 merogony), thus representing a cycle of internal autoinfection. However, some second generation merozoites may differentiate into macro- and microgametocytes which are fertilized and produce the egg or zygote after a period of maturation. This zygote transforms into an oocyst which divides by sporogony, resulting in the production of sporozoites. Millions of mature oocysts are eliminated in feces, thus leading to broad contamination of the environment where they remain viable for many months (Fayer & Ungar 1986, Guerrant 1997, Griffiths 1998).

Cryptosporidiosis is found worldwide, with the highest prevalence being observed in less developed countries, mainly Latin America and Africa. Seroprevalence studies have shown a positivity rate of the order of 30% in North America and Europe, and of up to 60% in Latin-American countries. The most affected population groups are children aged less than two years and immunocompromised individuals, particularly those with Aids. The prevalence rate varies among these individuals according to the geographical regions studied, with rates ranging from 2-4% in the United States to 50% in Africa. Two Brazilian studies, one carried out in Rio de Janeiro and the other in Uberlândia, MG, found rates of infection with this protozoan of 18.2 and 13%, respectively, considering that all individuals met the criteria for the diagnosis of Aids. Patients with other types of immunosuppression, such as congenital hypogammaglobulinemia, protein-calo-

rie malnutrition, diabetes mellitus and hematological neoplasias, show a high predisposition to developing severe forms of cryptosporidiosis (Moura et al. 1989, Costa-Cruz & Ferreira 1996, Pedersen et al. 1996, Sorvillo et al. 1998).

The main route of transmission of cryptosporidiosis is through the ingestion of water or food contaminated with mature oocysts, with the ingestion of only 10 oocysts being sufficient to produce infection, and with the parasitosis persisting due to the cycle of internal autoinfection (Fayer & Ungar 1986). Thousands of people have been infected in urban epidemics that occurred in England and in the United States due to contamination of the water sources with fecal material containing oocysts, which are resistant to the usual chlorination of drinkable water sources available to the population. Smaller outbreaks were observed in day-care centers, hospitals and rural areas, where contact with contaminated animals is common (Richardson et al. 1991, Moore et al. 1993, Goldstein 1996, Hoxie et al. 1997).

The clinical manifestations of cryptosporidiosis vary according to the host's immunological status. Symptomatic or oligosymptomatic infections are common in endemic areas and can be identified through serological population surveys (Fayer & Ungar 1986, Guerrant 1997). The most common clinical form of this protozoosis in immunocompetent individuals is acute gastroenteritis; this modality of the disease is self-limited and the incubation period ranges from 1 to 2 weeks. The patient in general presents with low fever, colic abdominal pain, headache, loss of appetite, nausea, vomiting and diarrhea, which is secretory and disabsorptive leading to the loss of various liters of fluid per day. The duration of the clinical manifestations ranges from 3 to 12 days in most cases, although in some it may persist for more than 4 weeks. Clinical cure does not coincide with parasitological cure, since oocyst elimination can last for several weeks (Fayer & Ungar 1986, Cardell & Adiss 1994).

In immunosuppressed patients, including patients with Aids, the clinical presentation of this protozoosis is severe and consists of intense diarrhea, with several daily bowel movements, accompanied by voluminous loss of fluid, dehydration and marked weight loss. These manifestations last for several weeks, and cryptosporidiosis can be considered incurable in HIV-infected patients in the advanced phase of the disease (Pozio et al. 1997, Griffiths 1998, Manabe et al. 1998).

Similar to other opportunistic infections that occur in HIV-infected patients, severe cryptosporidiosis also tends to affect patients with low CD4 T lymphocyte levels (below 100 cells/mm^3) (Flanigan et al. 1992). Extraintestinal manifestations of this coccidiosis are also observed in patients with Aids, and particularly affect the biliary tract, liver, pancreas, joints and the respiratory system. Biliary tract infections include alithiasic cholecystitis, sclerosing cholangitis, papillitis and terminal bile duct stenosis (Bonacini 1992, Manabe et al. 1998, Griffiths 1998).

The digestive presentation of cryptosporidiosis should be differentiated from other causes of acute gastroenteritis in immunocompetent individuals, such as infections caused by bacteria (*Escherichia coli*, *Cam-*

pylobacter, *Salmonella* sp., etc.), viruses (rotavirus) and other protozoa (*Giardia lamblia*, *I. belli* and *C. cayetanensis*). In patients with Aids, the differential diagnosis should also be made with isosporiasis and cyclosporiasis, with diarrhea caused by microsporidia (*Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*), and with chronic diarrhea induced by HIV itself (Griffiths 1998).

The most practical and easiest way of diagnosing cryptosporidiosis is the detection of parasite oocysts in feces or other body fluids. Various techniques have been used to visualize these oocysts, such as Ziehl-Neelsen staining, modified, Kinyoun carbolfuchsin staining, modified, and staining with safranin-methylene blue and methenamine silver. Concentration methods, such as formalin-ether sedimentation or Sheather's sugar flotation method, increase considerably the sensitivity of oocyst detection. The oocysts of this protozoon can be visualized in histological sections of intestinal mucosa obtained by biopsy and stained with hematoxylin-eosin as single cells or grouped on the mucosal surface, i.e., at the brush border of the intestinal epithelium. More recent techniques using fluorescent dyes such as acridine orange and auramine carbolfuchsin provided results comparable to those obtained with the staining methods described above for diarrheic feces, and are able to detect parasites even in formed feces. Immunoenzymatic techniques (ELISA) using monoclonal antibodies have shown a high degree of sensitivity and specificity for the detection of oocysts in feces and contaminated water (Costa-Cruz et al. 1996, Hoxie et al. 1997, Guerrant 1997, Griffiths 1998). Serological tests for the detection of antibodies represent an alternative to parasitological methods for the diagnosis of cryptosporidiosis. Immunoenzymatic assays (ELISA) using crude extract of ruptured oocysts as antigen have shown good sensitivity, although some studies have demonstrated that immunoblotting is much more sensitive and specific for antibody detection. In these cases, IgA type responses are observed which are directed against antigens found on the surface of sporozoites, the infectious form of the parasite; such antibodies seem to exert a protective effect against symptomatic infection (Mooss et al. 1998, Eisemberg et al. 2001). A specific PCR has been developed and may represent a better option for the diagnosis of this infection in the future (Griffiths 1998).

The disease is self-limited in immunocompetent patients who do not require specific treatment in addition to hydration and adequate water-electrolyte replacement. In patients with Aids, hydration should be vigorous, particularly in cases with the severe and debilitating form of the disease. Antidiarrheal agents such as diphenoxylate and loperamide should be used to reduce the loss of water through feces and the number of evacuations, thus improving the quality of life of these patients. A somatostatin analog, octreotide acetate, inhibits intestinal secretion of fluids and improves diarrhea in these patients, but, obviously, without a parasitological cure (Cello et al. 1991). Various drugs have been tested for the treatment of cryptosporidiosis, but none of them has shown a complete curative effect thus far. The orally administered macrolides spiramycin, roxithromycin and azithromycin

have demonstrated partial efficacy, but without eradication of the parasite in most of the individuals treated (Soave et al 1990). Paromomycin, an aminoglycoside administered orally at the dose of 2 g/day, has shown good activity on this coccidium. In some series, symptomatic improvement was observed in most patients and eradication of the infection was even reported in a few individuals (Clezy et al 1991, Fichtembaun et al. 1993). However, a recent controlled study did not demonstrate the expected efficacy of this drug in the treatment of human cryptosporidiosis (Hewitt et al. 2000).

Since patients with cryptosporidiosis eliminate large amounts of oocysts in their feces, they might easily contaminate the environment, the persons in contact with them, water and food. Universal precautions, such as washing the hands and adequate destination of dejecta and contaminated material, are fundamental for the prevention of this protozoosis and epidemic outbreaks. However, in the case of a cryptosporidiosis outbreak, particularly that transmitted through contaminated water reservoirs, the population should be advised to consume only boiled or bottled water, a recommendation that should be also made to patients with Aids. It is important to remember that chlorination does not eliminate *Cryptosporidium* oocysts from the public water reservoirs, which eventually become contaminated through the feces of domestic animals present in nearby rural areas (Hayes et al. 1989, Moore et al. 1993).

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