Leishmaniasis as an Emerging Infection

Christine M. Choi and Ethan A. Lerner*

Boston University School of Medicine, *Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School

Leishmaniasis is a protozoan disease whose diverse clinical manifestations are dependent both on the infecting species of *Leishmania* and the immune response of the host. Transmission of the disease occurs by the bite of a sand fly infected with *Leishmania* parasites. Infection may be restricted to the skin in cutaneous leishmaniasis, limited to the mucous membranes in mucosal leishmaniasis, or spread internally in visceral leishmaniasis or kala azar. The overall prevalence of leishmaniasis is 12 million cases worldwide, and the global yearly incidence of all clinical forms approaches 2 million new cases (World Health Organization WHO/LEISH/200.42, *Leishmania/HIV Co-Infection in Southwestern Europe 1990–98: Retrospective Analysis of 965 Cases*, 2000). In the last two decades, leishmaniasis, especially visceral leishmaniasis, has been recognized as an opportunistic disease in the immunocompromised, particularly in patients infected with human immunodeficiency virus. *Journal of Investigative Dermatology Symposium Proceedings 6:175–182, 2001*

HISTORY

The increases in travel and the number of immunocompromised individuals allows leishmaniasis to be considered an ''emerging disease''. The purpose of this paper is to highlight selected clinical, histopathologic, and immunologic features of leishmaniasis as well as therapies and vector biology.

The cutaneous afflictions of leishmaniasis have been known since antiquity (Peters, 1988). Descriptions of the cutaneous disease in the Old World are found from the first century AD. New World pottery from Peru and Ecuador dating from AD 400–900 illustrates faces afflicted with a process consistent with leishmaniasis (Lainson *et al*, 1987). The first description in English of a lesion resembling leishmaniasis was made in 1756 by Russell, who described the ''Aleppo evil'' from Syria. In 1885, Cunningham observed organisms in macrophages from lesions of ''Delhi boil'' in India. A Russian army physician named Borovsky noted the protozoal nature of the organism in 1898 in biopsy specimens from skin lesions. In 1903 Leishman published his identification of the parasite in the spleen of an English private who had died of Dumdum fever in Dum-Dum, India in 1900. A few months later Donovan described identical organisms in a splenic puncture specimen from a living child. The distinctive histologic feature of this 2–5 μm parasite was the presence of both a nucleus and a smaller rod-shaped structure consisting of mitochondrial DNA called the kinetoplast. Ross named the parasite ''Leishmania donovani'' later the same year. Other names of leishmaniasis include Oriental sore, Aleppo evil, Delhi boil, Baghdad sore, Rose of Jericho, Chiclero's ulcer, uta, espundia (mucous form), forest yaws, Dumdum fever (visceral form), kala-azar, and black fever.

Manuscript received August 9, 2001; accepted for publication August 9, 2001.

Reprint requests to: Ethan A. Lerner, CBRC/MGH-East, Building 149, 13th Street, Charlestown, MA 02129. Email: ethan.lerner@cbrc2.mgh.harvard.edu

Abbreviations: CL, cutaneous leishmaniasis; HIV, human immunodeficiency virus; ML, mucocutaneous leishmaniasis; VL, visceral leishmaniasis.

CLINICAL FEATURES

Localized cutaneous leishmaniasis (LCL) Leishmaniasis in its various forms is present on all continents except Australia and Antarctica (Lerner and Von Lichtenburg, 1991). LCL is widespread throughout the Old World and is primarily caused by the organisms *Leishmania tropica and Leishmania major*. New world LCL is endemic in Central and South America. Two independent species or ''complexes'' of parasites are responsible for New World LCL: *Leishmania braziliensis* and *Leishmania mexicana*.

LCL usually affects unclothed parts of the body easily bitten by the sand fly vector, including the face, neck, and arms. New World leishmaniasis commonly presents with a solitary primary lesion, whereas multiple primary lesions are often found in Old World disease. After an average incubation period of 1 wk to 3 mo, a red papule appears that enlarges to a plaque or nodule. The lesion often develops into an ulcer, which is well circumscribed with a violaceous border. The ulcer base is granulomatous and crusted, and the margins are hypertrophic but without extensive undermining (**Fig 1**). Painless, rubbery subcutaneous nodules or cords rarely develop around the ulcer due to local lymphangitic spread of the organism. Draining lymph nodes may be enlarged and reveal parasites on biopsy (Al-Gindan and Kubba, 1989). Occasionally, inflammatory satellite papules and subcutaneous induration may develop around the primary lesion representing a reaction to local dissemination of the parasite or its antigenic products (Kubba *et al*, 1987, 1988). Itching and pain are mild, if present. The wound may become superinfected, leading to misdiagnosis. Between 1 and 36 mo, depending upon both the patient and the infecting organism, the ulcer spontaneously regresses leaving a scar with hypo- or hyper-pigmentation. Immunity is considered complete but one study in a Saudi Arabian population found subsequent re-infection in up to 10% of individuals (Killick-Kendrick *et al*, 1985).

Diffuse cutaneous leishmaniasis (DCL) DCL is an anergic variant of LCL in which lesions are disseminated, resembling lepromatous leprosy. Infection may be caused by *Leishmania aethiopica* or, in Central and South America, *Leishmania amazonensis*. The disease usually begins with an initial primary lesion and then disseminates to involve other areas of the skin. The

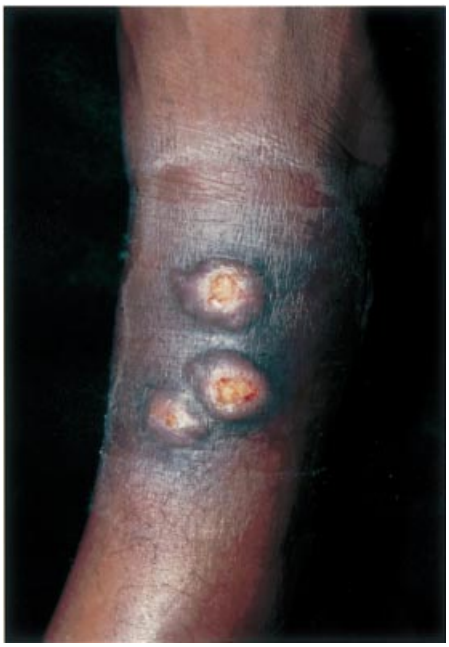

Figure 1. Localized cutaneous leishmaniasis. Ulcer of the lower extremity caused by *L. major*.

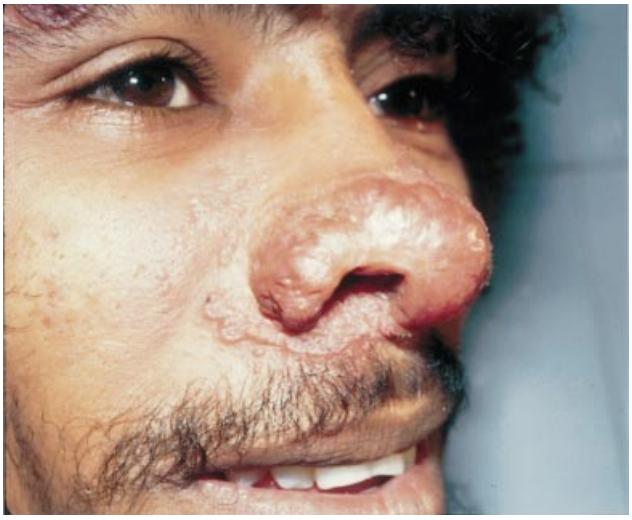

Figure 2. Mucocutaneous leishmaniasis. Young man with healed prior lesions of cutaneous leishmaniasis on the arms caused by *L. braziliensis*, now presenting with new lesions of the nasal mucosa.

lesions are nonulcerative nodules full of parasites, which are often scattered over the limbs, buttocks, and face. Unlike lepromatous leprosy, there is no nerve involvement. The disease does not invade internal organs, but responds only partially to treatment and often relapses, becoming chronic.

Post kala-azar dermal leishmaniasis (PKDL) PKDL is primarily caused by *Leishmania donovani* and is endemic in East Africa and India. In Africa, PKDL occurs in up to 50% of patients recovering from visceral leishmaniasis (Zijlstra *et al*, 1994) The rash generally consists of discrete skin-colored or hyperpigmented papules on the cheeks, chin, ears, and extensor aspects of forearms. Most lesions heal spontaneously over a few months.

In contrast to African PKDL, Indian PKDL occurs in 20% of patients approximately 1–2 y after recovery (Rees and Kager, 1987). Small hypopigmented macules are usually the first manifestation of PKDL and these enlarge to form large irregular patches. Lesions are often bilateral and symmetrical. The pigmentary loss is never complete, and there is no pigment change in the hair overlying the lesions. Erythematous macules develop next, often on the face in a malar distribution, but may also develop in other areas, especially in the hypopigmented patches. Finally, soft, painless, nonulcerative, yellowish-pink nodules replace the hypopigmented and erythematous macules, and sometimes develop *de novo*. Nodules most commonly affect the face, earlobes, trunk, and genitalia, and less frequently occur on the hands and feet. Cases of PKDL are difficult to treat, requiring longer duration of systemic medication. Hypopigmented areas almost never completely repigment.

Mucocutaneous leishmaniasis (MCL) MCL is most commonly reported in the New World (Abdalla *et al*, 1975; Marsden, 1986). *Leishmania braziliensis* is the most common etiologic agent although there have been several reports of mucocutaneous disease due to *Leishmania panamensis*, increasing the risk that travelers to Central America could potentially develop MCL (Melby *et al*, 1992). Fifty per cent of patients develop mucocutaneous lesions within 2 y of the initial cutaneous lesions, and 90% within 10 y (**Fig 2**). Approximately one-third of patients have no prior history of skin lesions. MCL typically affects a small percentage (approximately 3%) of individuals previously infected with *L. braziliensis* subspecies, although a prevalence as high as 34% in endemic areas has been reported (Kerdel-Vegas, 1982). Mucous membrane involvement probably develops due to hematogenous or lymphatic dissemination, or occasionally from direct extension of nearby skin lesions. The disease often begins in the nasal septum which becomes inflamed and infiltrated and subsequently perforates. Malnutrition and pneumonia are the leading causes of death in patients with MCL.

Visceral leishmaniasis (VL) Kala-azar or VL is a systemic disease caused by the dissemination of *Leishmania donovani* or *Leishmania infantum*. The advent of human immunodeficiency virus (HIV) has increased the population of individuals afflicted by VL and is discussed later in this report. Characteristic signs and symptoms include fever, splenomegaly, lymphadenopathy, emaciation, pancytopenia, and hyperglobulinemia. The primary lesion of VL is rarely seen but consists of a small erythematous papule sometimes referred to as a ''leishmanioma''. During the active period of VL a diffuse blackening of the skin appears that is the origin of the name ''kala-azar'', meaning ''black fever''. After an incubation period of 2–4 mo, VL runs an insidious chronic course until treated. Although the disease is associated with an increase in serum IgG and IgM, there is a depression of cell-mediated immunity that predisposes the host to multiple secondary infections (Haldar *et al*, 1983). Treatment is usually successful in the absence of immunodeficiency but relapses have been reported (Berman, 1997). One source of such relapses is the observation that parasites have been demonstrated to persist up to 11 y following clinical cure (Schubach *et al*, 1998).

Viscerotropic leishmaniasis *Leishmania tropica*, which had been thought to cause cutaneous disease exclusively, was found to be the causative organism in several cases of VL reported in soldiers of Operation Desert Storm returning from Saudi Arabia (Magill *et al*, 1993). The clinical presentations were distinct from visceral leishmaniasis caused by *L. donovani* and incuded high fever, malaise, intermittent diarrhea, and abdominal pain.

PATHOLOGY

Histopathologic studies reveal epidermal and/or dermal changes, depending on the type and stage of the disease (Kurban *et al*, 1966). The diagnostic histopathologic changes of leishmaniasis, however, are usually present in the dermis. There is a predominantly mononuclear dermal infiltrate consisting primarily of lymphocytes and histiocytes. The histiocytes may be filled with Leishman-

Donovan (L-D) bodies, which are 2–4 μm oval encapsulated protozoa with a large peripheral nucleus and a smaller rod-shaped kinetoplast of mitochondrial DNA. L-D bodies are numerous in early lesions of LCL and PKDL, very abundant in DCL, but scanty in MCL, VL, and leishmaniasis recidivans, an unusual form of the disease not discussed here in detail. The lymphohistiocytic infiltrate is arranged diffusely in cutaneous forms of leishmaniasis, except in PKDL and leishmaniasis recidivans where well-organized granulomas are seen. Langerhan's epithelial giant cells may be present within the granulomas. Plasma cells may also be abundant, especially in MCL. The infiltrate extends from the upper to lower dermis, sometimes around a central necrotic zone where fibrinoid degeneration of vessels may be present.

Immunophenotypic analysis of cell subsets in LCL lesions reveals an abundance of T cells with an activated phenotype, expressing interleukin-2 (CD25+), transferrin receptors (CD71+), or major histocompatibility complex class II molecules on their surface (Esterre *et al*, 1992). Equal numbers of CD4+ and CD8+ lyphocytes are present in LCL lesions. In addition, whereas the majority of T cells in control skin biopsies bear the TCR αβ complex, 20%–30% of the T cells in early LCL lesions bear the TCR γδ complex (Modlin *et al*, 1989; Lima *et al*, 1994).

PATHOGENESIS AND ETIOLOGY

The arthropod vector of all forms of leishmaniasis is the female sand fly (Swaminath *et al*, 1942). The disease is transmitted by flies of the genus *Phlebotomus* in the Old World and in the New World it is transmitted primarily by *Lutzomyia* and rarely by *Psychodopygus*.

The transmission cycle of the leishmania organisms requires an arthropod vector and a mammalian reservoir. The parasite assumes two distinct forms during the life cycle: an extracellular, flagellated promastigote form, and a nonflagellated obligate intracellular form called the amastigote. During the course of probing the skin, the sand fly injects saliva containing the promastigote form of leishmania. The promastigote is taken up by macrophages and transformed into the round amastigote form. The parasite then resides in the phagolysosome of the macrophage and proliferates despite the presence of lysosomal enzymes. When a sand fly bites an infected host, amastigotes taken up during the blood meal transform back to the promastigote form in the gut of the fly, completing the life cycle. In hyperendemic regions, such as Israel, Jordan, and Saudi Arabia, *L. major* promastigotes are occasionally recoverable from 20% to 50% of female sand flies, but in other areas less than 1% of sand flies are infected (Norton *et al*, 1992). Humans are usually accidental hosts of leishmaniasis, because they live in endemic zones and are thereby exposed to infected sand flies. The zoonotic reservoir includes sloths, anteaters, rodents, foxes, and dogs. Transmission of leishmania infection occurs almost exclusively via the bite of an infected sand fly; however, other possible modes of transmission (e.g., direct contact) have been reported (Nanji and Greenway, 1985; Yadav *et al*, 1989; Eltoum *et al*, 1992).

IMMUNOLOGY

Leishmaniasis is characterized by a spectrum of disease phenotypes that corresponds to the strength of the host's cell-mediated immune response. Both susceptible and resistant phenotypes exist within human populations. For example, many people in endemic regions develop positive skin tests without ever manifesting signs of clinical disease. Spontaneously healing lesions are associated with positive antigen-specific T cell responsiveness, diffuse cutaneous and visceral disease with T cell nonresponsiveness, and mucocutaneous disease with T cell hyperresponsiveness. (Blackwell, 1992) Current research is focused on determining the extent to which this spectrum of host response to leishmaniasis is genetically determined. An extensive literature exists on the immunology of leishmaniasis. The reader is referred to this literature with respect to cytokines, T cells of the Th1 and Th2 subtypes, killer T cells, humoral responses, and nitric oxide. (Pirmez *et al*, 1993; Bardaró and Johnson, 1993; Kemp *et al*, 1993; Assreuy *et al*, 1994; Stenger *et al*, 1994; Davidson, 1998; Lezama-Davila *et al*, 1998; Maasho *et al*, 1998; Etges and Muller, 1998; Jones *et al*, 1998; Herwaldt, 1999; Mossalayi *et al*, 1999).

DIAGNOSIS

In endemic areas, the diagnosis is often made on clinical grounds alone, based on the following observations: (a) small number of lesions (1–3); (b) lesions situated on exposed areas; (c) lesions present for a number of months; (d) lesions resistant to all types of attempted treatments, and (e) usually no pain or itching. Multiple diagnostic techniques are available. Historically, microscopy and culture have been the standard for diagnosis. A punch or wedge biopsy may be performed, preferably from the indurated border of a lesion and not from a necrotic center. A touch prep or Giemsa-stained tissue impression slide may be done on excised tissue, scalpel scrapings, slit skin smears, or dermal cells obtained using a root canal file. Fine needle aspiration of a lesion may be done after injection of sterile saline. In cases of suspected VL, splenic aspirates are used. Specimens from a biopsy or aspirate may be cultured on NNN blood agar (Nicolle's modification of Novy and McNeal's medium) or rabbit blood agar, with the growth of promastigotes apparent between 2 d and 2 wk.

The identification of the specific species of parasite responsible for infection is important for the diagnosis of disease, evaluation of therapy, and prognosis. Consequently, a number of molecular biology techniques have been developed that are designed for species-specific identification of parasites within the genus *Leishmania*. Monoclonal antibodies (McMahon-Pratt *et al*, 1982) and isoenzyme analysis (Kilgour *et al*, 1974) have been used. Oligonucleotide primers based on kDaNA sequences have been used in the polymerase chain reaction (PCR) (de Bruijn and Barker, 1992; Katakura *et al*, 1998; El Tai *et al*, 2001). Species identification has been achieved in a variety of ways, such as multiplexing and using nested PCR (Aransay *et al*, 2000). The widespread clinical usefulness of these techniques remains to be implemented.

LEISHMANIA AND HIV CO-INFECTION

Over the past decade, leishmaniasis has been increasingly documented in immunocompromised patients, namely those infected with HIV (Machado, 1992; Gillis *et al*, 1995; Coutinho *et al*, 1996; Kubar *et al*, 1998; Mattos *et al*, 1998; Castellano *et al*, 1999; Lachaud *et al* 2000; Santos-Gomes *et al*, 2000). Although leishmaniasis has been reported in other immunodepressed states, such as diabetes (**Fig 3**), PKDL in renal transplant recipients, or in those taking corticosteroids, the greater magnitude of the former problem compels us to focus our discussion on concomitant *Leishmania* and HIV infections (Alrajhi *et al*, 1998; Roustan *et al*, 1998). Co-infection is most frequently manifested as VL (WHO, 2000).

Epidemiology The increased appearance of leishmaniasis in developed countries can be attributed to several factors: increased overseas travel, U.S. Gulf War veterans, and the simultaneous encroachment of leishmaniasis into urban areas and of HIV infection into rural areas (Lopez-Velez *et al*, 1998; Herwaldt, 1999). In the Mediterranean, where *L. infantum* is endemic, the number of cases of VL in HIV-infected patients has increased dramatically since the mid-1980s (Santos-Gomes *et al*, 2000). The World Health Organization (WHO) estimates that 25%–70% of adult VL cases in southern Europe are related to HIV, while between 2% and 9% of AIDS patients are at risk of experiencing newly acquired or reactivated VL (Sulahian *et al*, 1997; Kubar *et al*, 1998; Rosenthal *et al*, 2000). Surveillance data for southern Europe recorded 1461 cases of co-infection from January 1990 through June 1998, with the majority (57.2%) reported in Spain (Herwaldt, 1999). In some regions of Spain and Portugal, VL is the third most common opportunistic infection in HIV-infected patients after *Toxoplasma gondii* and *Cryptosporidium parvum* (Alvar *et al*, 1997). In fact, preliminary data suggest that *Leishmania* may be a cofactor in

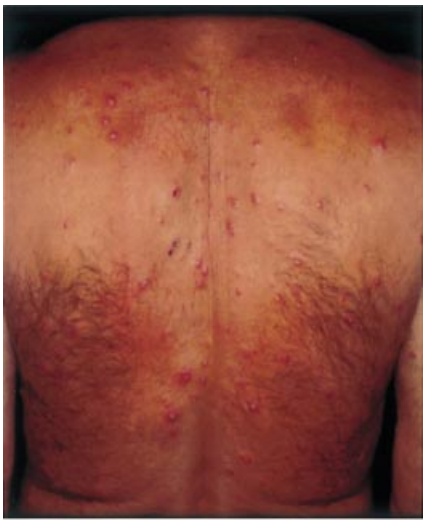

Figure 3. Generalized cutaneous leishmaniasis believed to be caused by *L. braziliensis* in a patient immunocompromised by diabetes. The term "generalized" is used to distinguish this case from "diffuse" caused by *L. amazonensis*.

the pathogenesis of HIV infection: the lipophosphoglycan (LPG) surface molecule of *L. donovani* has been shown to induce transcription of HIV in CD4+– T cells (Pineda *et al*, 1998; Herwaldt, 1999). The WHO estimates that AIDS increases the risk of VL by 100–1000 times in endemic regions (WHO, 2000). Despite the strong connection between leishmaniasis and HIV, *Leishmania* infection is still not included on the list of AIDS-defining criteria (Alvar *et al*, 1997; Rosenthal *et al* 2000).

The typical clinico-epidemiologic profile of a co-infected individual is a young male intravenous drug user (IVDU) infected with *L. infantum*, with a CD4+ T cell count below 200 per ul.· It is clear that IVDU are the population most at risk for co-infection in south-western Europe (WHO, 2000). Some researchers have proposed an anthropoonotic mode of transmission via the sharing of contaminated needles by IVDU to explain the high frequency of HIV and *Leishmania* co-infection in this risk group (Alvar *et al*, 1997; Hall, 1997; Agostoni *et al*, 1998; Lopez-Velez *et al*, 1998). Although there is no direct evidence of the spread of *L. infantum* through this vehicle, the generation of such a hypothesis demonstrates the heightened awareness of the medical community for the role of leishmaniasis in HIV-positive patients (Hall, 1997). Two troubling reports noted parasitemia in asymptomatic blood donors, detected by PCR and/or culture, from an endemic region (le Fichoux *et al*, 1999; Otero *et al*, 2000). Blood transfused from such donors into HIV-positive individuals could contribute to the incidence of co-infection.

Immunology of leishmaniasis in HIV The host's immune system plays an important role in disease resistance and susceptibility. The T cell-mediated response directed at infected macrophages is of prime significance in leishmanial destruction (Castellano *et al*, 1999). It follows that the depressed CD4+- T cell levels of HIV-infected patients render them particularly vulnerable to leishmaniasis. Furthermore, because their deficient cell-mediated immunity allows the parasites to disseminate, it is not surprising that the most commonly reported clinical form of infection is VL. One study involving 236 HIV-positive patients living in an *L. infantum* endemic region used serologic analysis and western blotting to follow the evolution of CD4+– T cell counts and to study the relationship between *L. infantum* infection and VL disease. Overall, the ratio of VL disease to *Leishmania* infection in HIV-positive patients was high (1:10) and comparable with the statistics reported by the WHO. Nevertheless, 18 of the 32 HIV-positive, *L. infantum*-seropositive patients did not develop VL, despite severe and prolonged immunosuppression (CD4+– T cell counts lower than 250 cells $\times 10^6$ per liter) in eight of these individuals. An unexpected finding was that VL patients with very low CD4+– T cell counts could maintain specific anti-leishmanial antibody production. Finally, because clinical manifestations appeared at significantly higher ($p = 0.028$) CD4+– T cell levels at the time of diagnosis for primary VL patients than for reactivation of a latent *Leishmania* infection, the researchers suggested that supplementary control mechanisms besides the T cell-mediated response may operate once *Leishmania* infection and parasite–host interaction have been well established (Kubar *et al*, 1998).

Several studies have proposed that HIV-infected patients are particularly susceptible to certain variants of *L. infantum*. Rapid identification of causative *Leishmania* species using PCR techniques in 33 patients revealed that *L. infantum* strains from the immunocompromised patients with VL had three different DNA sequences, whereas all strains from the immunocompetent patients shared an identical sequence (Minodier *et al*, 1997). Isoenzyming typing of *L. infantum* in both immunocompetent and immunocompromised patients revealed both a higher variability of zymodemes and the exclusive presence of specific zymodemes in the immunocompromised patients, which suggests that these hypovirulent strains are normally contained by the intact cellular immune systems of immunocompetent hosts (Jimenez *et al*, 1995; Agostoni *et al*, 1998).

Despite the development of more sensitive techniques for serologic diagnosis, the relatively common lack of a humoral immune response often leads to delayed diagnosis of VL in the setting of HIV infection. In those co-infected individuals capable of mounting a humoral response, it is estimated that specific anti-leishmanial antibody levels are 50 times lower than in individuals with intact immune systems. Seropositivity may depend on which infection the patient acquired first. It has been proposed that anti-leishmanial titers may be higher when *Leishmania* infection precedes HIV infection, in which case clinical VL would be a reactivation of latent infection, while seronegativity results from primary VL acquired after HIV infection. The overall dysfunction of the immune system in patients with HIV, however, especially in the advanced stages of AIDS, may also explain the decrease in specific antibody production (Alvar *et al*, 1997; Kubar *et al*, 1998). It is therefore recommended that two or more serologic tests and antigens freshly prepared in the laboratory be used to increase sensitivity (WHO, 2000). Investigators have used PCR as a reliable tool to diagnose VL in HIV- positive patients and have also demonstrated that a positive PCR result is predictive of clinical relapse (Pizzuto *et al*, 2001).

Clinical manifestations of VL in HIV-infected individuals Patients who are co-infected with leishmaniasis and HIV manifest clinical signs and symptoms that are often unique and can be either more or less severe than usual. These include greater rates of recurrence, an atypical course of infection, and localization of *Leishmania* amastigotes in unusual sites (Sulahian *et al*, 1997; Lopez-Velez *et al*, 1998). The severity of VL in HIV-infected individuals spans a broad spectrum from completely asymptomatic cases to those manifesting classic VL symptoms. In a retrospective study conducted in France on 91 co-infected patients, the classic VL symptoms of fever (87% of patients), splenomegaly (74%), and hepatomegaly (49%) were common. In 31 (34%) of these cases amastigotes were discovered in unusual locations such as the digestive tract (16 patients), lungs (seven), skin (seven), and tonsil (one). These sites of parasitation, which would be considered atypical in immunocompetent patients, were only detected in the most severely immunocompromised patients, with a mean CD4+ T cell count of 20 per μl (Rosenthal *et al*, 2000). In conjunction with the unique presentations of VL and HIV co-infection, clinical diagnosis can be delayed or complicated by the similar features that characterize HIV infection alone, HIV co-infection with other opportunistic microorganisms (i.e., mycobacteria), opportunistic infections concurrent with leishmaniasis, or drug therapy-induced

side-effects (Agostoni *et al*, 1998). Furthermore, as mentioned above, serologic tests are often falsely negative.

Mortality during the first VL episode in HIV-infected patients is between 10% and 19%, depending on the toxicity of antiparasitic treatment, concomitant opportunistic infections, and other complications (Alvar *et al*, 1997). The median survival of HIV-infected patients with VL is 13–19 mo (Davidson, 1998). In the study of 54 Spanish co-infected patients, 18.5% died during their first episode of VL. A significant predictor of mortality was the fulfilment of AIDS-defining criteria at the time of VL diagnosis: the relative mortality risk was 2.42 times higher in patients with an AIDS diagnosis than in patients who did not meet AIDS-defining criteria. The group of patients with AIDS (n = 26) experienced a dramatic decrease in percent survival to less than 60% within the first 5 mo after VL diagnosis, whereas the group of patients without AIDS (n = 28) reached similar survival levels at approximately 30 mo after VL diagnosis. The survival curve of this latter group showed no significant differences compared with the survival curve of the control group of patients who had AIDS but not VL (Lopez-Velez *et al*, 1998).

In contrast to the reliable cure of VL in immunocompetent hosts who have relapse rates < 5%, more than 80% of VL and HIV co-infected patients are expected to experience a relapse following effective treatment of their first episode, with almost all relapses occuring within 12 mo (Davidson, 1998). The parasite can remain quiescent in several organs and can be reactivated when the host's immune system becomes depressed, a predictable event during the progression from asymptomatic HIV infection to AIDS (Alvar *et al*, 1997; Lopez-Velez *et al*, 1998). In addition, the parasite may develop drug resistance, resulting in incomplete treatment of the initial VL episode or unresponsiveness to a previously effective drug during a subsequent relapse of VL. Secondary prophylaxis has been recommended to minimize these relapses and will be discussed in *Therapy*.

Other clinical forms of *Leishmania* and HIV co-infection Although the majority of AIDS-related cases involve *L. infantum*- induced VL, the total clinical spectrum of leishmaniasis has been reported in HIV-infected patients. Cutaneous lesions may occur before, after, or at the same time as visceral lesions; however, exclusive cutaneous involvement is rare (Alvar *et al*, 1997). In one report, a child in the advanced stages of AIDS presented with an unusual clinical picture of DCL caused by *L. major* (Gillis *et al*, 1995). Unlike the localized papular or nodular lesions typical of *L. major*-induced LCL, the child's lesions were diffusely disseminated, scaly plaques resembling the characteristic appearance of xeroderma pigmentosum or chronic graft *versus* host disease. Antimonial therapy rendered clinical improvement in a case of generalized cutaneous leishmaniasis in a patient with AIDS (Rosatelli *et al*, 1998). Although the association between MCL and HIV infection is rare, one study documented the presence of multiple cutaneous lesions with mucosal involvement in young HIV-infected males with American cutaneous leishmaniasis (Mattos *et al*, 1998). The discovery of a dermatofibroma colonized by Leishmania parasites in an HIV-positive man with VL has prompted the speculation of a new variety of dermal Kala-Azar, the fibrohistiocytic type (Castellano *et al*, 1999). This finding, however, could have also represented a secondary leishmanial parasitation of a previously existing dermatofibroma, or even the casual coexistence of both entities. Leishmanial parasitation of cutaneous Kaposi's sarcoma in HIV has also been described. In a study of 22 cases of Mediterranean leishmaniasis and HIV co-infection, the parasite was recovered from the skin lesions of Kaposi's sarcoma in two patients who had first been diagnosed through bone marrow aspiration (Agostoni *et al*, 1998).

THERAPY

The natural history of leishmaniasis must be considered when evaluating therapeutic agents. Lesions of cutaneous leishmaniasis heal spontaneously over 1 mo to 3 y, whereas lesions of mucocutaneous and visceral disease rarely, if ever, heal without treatment. Consequently, all cases of MCL and VL require treatment. Therapy is not always essential in LCL. Patients with lesions on the face or other cosmetically important area should be treated to reduce the size of the resultant scar. In addition, the species of parasite should be identified so that infection with *L. braziliensis* and *L. panamensis* can be treated to reduce the risk of development of mucocutaneous disease. Treating patients with *Leishmania* and HIV co-infection requires close monitoring for effectiveness of treatment, especially due to high relapse rates.

The pentavalent ammonial compound sodium stibogluconate (Pentostam) remains the conventional therapeutic agent after 50 y (http://www.medletter.com; WHO, 2000). The drug appears to inhibit amastigote glycolytic activity and fatty acid oxidation. It is available from the Centers for Disease Control to treat cases diagnosed in the U.S.A. by calling (770) 488–7760. The CDC will also aid in making a diagnosis. The accepted concentration for the treatment of leishmaniasis is 20 mg per kg IV or IM daily, with an upper limit of 30 mg per kg per daily dose (Berman, 1988). Cutaneous leishmaniasis is treated for 20 d and visceral and mucosal disease for 28 d. Side-effects associated with parenteral antimonial administration include arthralgias, myalgias, abdominal discomfort, reversible elevations of hepatocellular enzymes, chemical pancreatitis, and occasional anemia, leukopenia, or thrombocytopenia. Changes in the EKG commonly develop, and occur more frequently the higher the daily dose and the longer the duration of therapy. The EKG abnormalities include T wave inversion, ST segment elevation or depression, and prolongation of the QT interval. A review on the use of Pentostam for the treatment of leishmaniasis generated several recommendations with regard to these side-effects (Herwaldt and Berman, 1992).

In the U.S.A., most patients are admitted for cardiac monitoring during treatment with Pentostam. In South America, where a slightly different pentavalent antimonial, meglumine antimoniate (Glucantime), is available, the drug is often administered at home. Glucantime is administered at 85% of the dose of Pentostam, and both drugs have similar efficacy. The antimonials have a reported efficacy of > 90% in most studies, although cure rates ranging from 34% to 100% have been reported, depending on the parasite species and the dose and duration of treatment.

A wide variety of alternative systemic and topical treatments have been utilized to treat leishmaniasis (**Table I**). Most of these treatments have not been analyzed with randomized, placebo-controlled trials, making it difficult to accurately evaluate their

Table I. Therapeutic options for leishmaniasis[a]

Therapeutic agent	LCL	DCL	MCL	VL	Reference
Antimonials (IV/IM)	E	E	E	E	1,72
Antimonials (IL)	E				71
Pentamidine	E			E	1,73
Amphotericin B	E		E	E	1,74
Interferon w/antimony		?	E	E	34
Allopurinol	?			?	75
Ketoconazole	E				77
Itraconazole	?	?	?		76
Immunotherapy	?				78
Rifampin		?			71
Dapsone	?				71
Localized heat	?	?			81
Paromomycin ointment	E				80
Cryotherapy	?				79
WR6026				I	82
Liposomes	E			E	55,56
Miltefosine				?	84

[a]LCL, localized cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; VL, visceral leishmaniasis; E, believeable effectiveness; ?, current clinical experience too limited; I, investigational.

effectiveness. A few conclusions, however, may be drawn: Pentamidine has been used successfully as a second-line agents for VL and New World CL (Soto-Mancipe *et al*, 1993). Amphotericin B has some effectiveness in mucosal disease (Sampaio *et al*, 1971). The combination of antimony and interferon-γ has been used effectively in the treatment of refractory VL in Brazil but the improvement in cure rates has been deemed only modest (Davidson, 1998). Granulocyte macrophage colony-stimulating factor in combination with antimonials for the treatment of VL can induce a quicker rise in leukocytes and fewer secondary infections (Davidson, 1998). The combination of immunotherapy with chemotherapy for leishmaniasis remains experimental, and the cost of such regimens precludes their routine use. Some clinicians favor the use of cryotherapy, especially combined with EMLA anesthesia, as a practical means of treating LCL in young patients.

Current clinical experience with other agents is too limited to draw any firm conclusions. A placebo-controlled clinical trial showed some effectiveness of ketoconazole in treatment of LCL in South America (Navin *et al*, 1992). Interestingly, ketoconazole may be more effective than antimony in treating *L. mexicana* infections, but less effective in *L. braziliensis* infections. Itraconazole has shown some promise in uncontrolled trials, most recently in a pilot study for the treatment of ML (Amato *et al*, 2000). Trials with allopurinol have shown mixed results (Velez *et al*, 1997). Finally, immunotherapy consisting of three vaccinations of live Bacille Calmette Guerin (BCG) with killed leishmania promastigotes was comparable in efficacy with three standard courses of antimony in Venezuelan LCL (Convit *et al*, 1987). With regard to topical therapy, paromomycin ointment, localized heat, and cryotherapy have all been tried with variable results (Bassiouny *et al*, 1982; El-On *et al*, 1992; Levine, 1992). Combinations of therapeutic strategies should be researched in the future.

The administration of drug-containing liposomes in theory delivers the therapeutic agent of choice directly to macrophages in which amastigotes reside. Three liposomal formulations of amphotericin B are available, but only one (''AmBisome'') is licensed for the treatment of VL in Europe and the U.S.A. AmBisome therapy is rapidly effective and less toxic than conventional formulations of amphotericin B (Alrajhi *et al*, 1998); however, the liposomal formulation is also more than 50 times more expensive than the conventional formulation (Roustan *et al*, 1998). Liposomal amphotericin B should be used for the treatment of VL when costs of hospitalization would exceed drug costs.

WR 6026 is a primaquine analog that has shown effectiveness in animal models of VL, and underwent a phase II clinical efficacy trial in the treatment of 16 patients with kala azar. Adverse effects included gastrointestinal distress, headache, and methemoglobinemia (Sherwood *et al*, 1994). We have not been able to identify any reports since this one from 1994 on the use of WR 6026 in the treatment of leishmaniasis.

Currently, there is no effective oral medication for the treatment of leishmaniasis. In a small clinical trial, oral zinc sulfate was used to treat cutaneous leishmaniasis and showed concentration-dependent cure rates of up to 96.9% (Sharquie *et al*, 2001). Miltefosine, a phosphocholine analog that interferes with cell-signaling pathways and membrane synthesis, was recently tested in phase II clinical trials for the treatment of Indian VL in a large population. Gastrointestinal side-effects were frequent, but final cure rates ranged between 93% and 97%. Therefore, miltefosine is the first oral agent that appears to be both highly effective and well tolerated for the treatment of VL (Jha *et al*, 1999).

The treatment of VL in HIV-infected patients is based on the therapy recommended for treating VL in immunocompetent patients. AmBisome is currently the only drug licensed for VL in the U.S.A. The treatment of choice, the best dosage, and the duration are still unknown for this group of patients. The management of HIV-infected patients with VL warrants special attention for several reasons. First, it is estimated that 60%–90% of AIDS patients experience a relapse of the disease after responding well to initial treatment, making post-therapeutic follow-up essential (Lachaud *et al*, 2000). In other cases, response to treatment may be incomplete or may be interrupted by drug toxicity. Although reports of severe pancreatitis, myocarditis, and renal insufficiency due to antimonial therapy in co-infected patients have been described, it has not been established that drug tolerance in HIV- infected patients is poorer than in immunocompetent patients (Alvar *et al*, 1997). The optimal regime for secondary prophylaxis and management of harmful drug–drug interactions while on concomitant antiretroviral therapy are areas to be investigated further.

VACCINATION

Methods used for prevention or control of leishmaniasis have included eradication of the vector or its habitat, destruction of animal reservoirs, treatment of human reservoirs, and vaccination. Technical difficulties such as drug resistance, drug toxicity, insecticide resistance, financial constraints, and operational difficulties have impaired progress toward effective control of leishmaniasis.

Vaccination trials in animal models and/or humans have been performed using virulent promastigotes, promastigotes attenuated or killed with either gamma irradiation, heat, or a mutagen, and specific antigens purified from promastigotes (Higashi, 1988; Nadim and Javadian, 1998). Reduced rates of subsequent infection have been noted in vaccinated populations. Side-effects include large, nonhealing lesions that persist for years and eventually require treatment, and immediate-type hypersensitivity reactions, which may last for a few hours.

The use of cytokines as adjuvants to vaccines is being investigated. IL-12 has been shown to be an effective adjuvant in experimental vaccination against *L. major* infection. Also, vaccination with the DNA that encodes the LACK antigen of *L. major* can induce an IL-12- mediated, protective Th1 response (Herwaldt *et al*, 1993).

The development of an effective, noninfectious vaccine is problematic. The difficulty associated with the development of a vaccine directed against the parasite suggests that evaluation of a vaccine based on the vector should be attempted. As vaccines for leishmaniasis are being taken to clinical trials, long-term follow-up will be necessary to determine the degree and duration of protection. Control and prevention of leishmaniasis in the future depends on the development of more efficacious vaccines and convenient, nontoxic therapeutic agents.

CONCLUSION

Leishmaniasis is a fascinating protozoal disease with varied clinical and pathologic manifestations. The increasing incidence of travel and co-infection with *Leishmania* and HIV during the last two decades has focused more attention on leishmaniasis. It is hoped that this attention will lead to better therapeutic agents to treat, and vaccines to prevent, this emerging infection.

We thank Ana Maria Roselino for ***Fig 3****. This work was supported by grants from the NIH and an agreement between the Cutaneous Biology Research Center at Massachusetts General Hospital and Shiseido Cosmetics Ltd.*

REFERENCES

Abdalla RE, El hadi A, Ahmed MA, El Hassan AM: sudan mucosal leishmaniasis. *Trans R Soc Trop Med Hyg* 69:443–449, 1975

Agostoni C, Dorigoni N, Malfitano A, *et al*: Mediterranean leishmaniasis in HIV-infected patients: epidemiological, clinical, and diagnostic features of 22 cases. *Infection* 26:93–99, 1998

Al-Gindan Y, Kubba R, el-Hassan AM, Omer AH, Kutty MK, Saeed MB: Dissemination in cutaneous leishmaniasis, 3. Lymph node involvement. *Int J Dermatol* 28:248–253, 1989

Alrajhi AA, Saleem M, Ibrahim EA, Gramiccia M: Leishmaniasis of the tongue in a renal transplant recipient. *Clin Infect Dis* 27:1332–1333, 1998

Alvar J, Canavate C, Gutierrez-Solar B, *et al:* Leishmania and human

immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev* 10:298–319, 1997
Amato VS, Padilha AR, Nicodemo AC, *et al*: Use of itraconazole in the treatment of mucocutaneous leishmaniasis: a pilot study. *Int J Infect Dis* 4:153–157, 2000
Aransay AM, Scoulica E, Tselentis Y: Detection and identification of Leishmania DNA within naturally infected sand flies by seminested PCR on minicircle kinetoplastic DNA. *Appl Environ Microbiol* 66:1933–1938, 2000
Assreuy J, Cunha FQ, Epperlein M, Noronha-Dutra A, O'Donnell CA, Liew FY, Moncada S: Production of nitric oxide and superoxide by activated macrophages and killing of Leishmania major. *Eur J Immunol* 24:672–676, 1994
Bardaró R, Johnson WDJ: The role of interferon-g in the treatment of visceral and diffuse cutaneous leishmaniasis. *J Inf Dis* 167 (Suppl. 1):S13–S17, 1993
Bassiouny A, Meshad M, Talaat M: Cryosurgery in cutaneous leishmaniasis. *Br J Dermatol* 107:467–474, 1982
Berman JD: Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy, and future strategies. *Rev Inf Dis* 10:560–586, 1988
Berman JD: Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clin Infect Dis* 24:684–703, 1997
Blackwell JM: Leishmaniasis epidemiology: all down to the DNA. *Parasitology* 104:S19–S34, 1992
de Bruijn MH, Barker DC: Diagnosis of New World Leishmaniasis: specific detection of species of the Leishmania braziliensis complex by amplification of kinetoplast DNA. *Acta Tropica* 52:45–58, 1992
Castellano VM, Rodriguez-Peralto JL, Alonso S, Gomez-De la Fuente E, Ibarrola C: Dermatofibroma parasitized by Leishmania in HIV infection: a new morphologic expression of dermal Kala Azar in an immunodepressed patient. *J Cutan Pathol* 26:516–519, 1999
Convit J, Castellanos PL, Rondon A, *et al*: Immunotherapy versus chemotherapy in localised cutaneous leishmaniasis. *Lancet* 1(8530):401–405, 1987
Coutinho SG, Da-Cruz AM, de Oliveira MP, Mendonca SC, De Bertho AL, Luca P: CD4+ and CD8+ T cell immune responses of immunocompetent and immunocompromised (AIDS) patients with American tegumentary leishmaniasis. *Mem Inst Oswaldo Cruz* 91:381–384, 1996
Davidson RN: Practical guide for the treatment of leishmaniasis. *Drugs* 56:1009–1018, 1998
http://www.medletter.com: Drugs for Parasitic Infections. Available from URL. http://www.medletter.com, click on: public reading room, 2000
El Tai NO, El Fari M, Mauricio I: Leishmania donovani: intraspecific polymorphism of Sudanese isolates revealed by PCR-based analyses and DNA sequencing. *Exp Parasitol* 97:35–44, 2001
El-On J, Halevy S, Grunwald MH, Weinrauch L: Topical treatment of old world cutaneous leishmaniasis caused by *Leishmania major*: a double-blind control study. *J Am Acad Dermatol* 27:227–231, 1992
Eltoum IA, Zijlstra EE, Ali MS, Ghalib HW, Satti MM, Eltoum B, el-Hassan AM: Congenital kala-azar and leishmaniasis in the placenta. *Am J Trop Med Hyg* 46:57–62, 1992
Esterre P, Dedet JP, Fremay C, Chevallier M, Grimaud JA: Cell populations in the lesion of human cutaneous leishmaniasis: a light microscopical, immunohistochemical and ultrastructural study. *Virchows Arch a Pathol Anat* 421:239–247, 1992
Etges R, Muller I: Progressive disease or protective immunity to Leishmania major infection: the result of a network of stimulatory and inhibitory interactions. *J Mol Med* 76:372–390, 1998
le Fichoux Y, Quaranta JF, Aufeuvre JP, *et al*: Occurrence of Leishmania infantum parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. *J Clin Microbiol* 37:1953–1957, 1999
Gillis D, Klaus S, Schnur LF, Piscopos P, Maayan S, Okon E, Engelhard D: Diffusely disseminated cutaneous Leishmania major infection in a child with acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 14:247–249, 1995
Haldar JP, Ghose S, Saha KC, Ghose AC: Cell-mediated immune response in Indian kala-azar and post kala-azar dermal leishmaniasis. *Infect Immun* 42:702–707, 1983
Hall AJ: Report on the 8th European Congress of Clinical Microbiology and Infectious Diseases, Lausanne, Switzerland May 25–28,1997. *Trop Med Int Health* 2:719–720, 1997
Herwaldt BL, Berman JD: Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of pertinent clinical studies. *Am J Top Med Hyg* 46:296–306, 1992
Herwaldt BL: Leishmaniasis. *Lancet* 354:1191–1199, 1999
Herwaldt BL, Stokes SL, Juranek DD: American cutaneous leishmaniasis in U.S. travelers. *Ann Intern Med* 118:779–784, 1993
Higashi GI: Vaccines for parasitic diseases. *Ann Rev Public Health* 9:483–501, 1988
Jha T, Sundar S, Thakur C, *et al:* Miltefosine, and oral agent, for the treatment of Indian visceral leishmaniasis. *New Engl J Med* 341:1795–1800, 1999
Jimenez M, Ferrer-Dufol M, Canavate C, *et al*: Variability of Leishmania (Leishmania) infantum among stocks from immunocompromised, immunocompetent patients and dogs in Spain. *FEMS Microbiol Lett* 131:197–204, 1995
Jones DE, Elloso MM, Scott P: Host susceptibility factors to Cutaneous leishmaniasis. *Frontier Bioscience* 15:D117–D180, 1998
Katakura K, Kawazu S, Naya T, *et al*: Diagnosis of kala-azar by nested PCR based on amplification of the Leishmania mini-exon gene. *J Clin Microbiol* 36:2173–2177, 1998
Kemp M, Kurtzhals JA, Bendtzen K, *et al*: Leishmania donovani-reactive Th1- and Th2-like T-cell clones from individuals who have recovered from visceral leishmaniasis. *Infect Immun* 61:1069–1073, 1993
Kerdel-Vegas F: American leishmaniasis. *Int J Dermatol* 21:291–303, 1982
Kilgour V, Gardener PJ, Godfrey DG, Peters W: Demonstration of electrophoretic variation of two aminotransferases in Leishmania. *Ann Trop Med Parasitol* 68:245–246, 1974
Killick-Kendrick R, Bryceson AD, Peters W, Evans DA, Leaney AJ, Rioux JA: Zoonotic cutanous leishmaniasis in Saudi Arabia; lesions healing naturally in man followed by a second infection with the same zymodeme of *Leishmania major. Trans R Soc Trop Med Hyg* 79:363–365, 1985
Kubar J, Marty P, Lelievre A, Quaranta JF, Staccini P, Caroli-Bosc C, LeFicoux Y: Visceral leishmaniosis in HIV-positive patients: primary infection, reactivation and latent infection. Impact of CD4+ T-lymphocyte counts. *AIDS* 12:2147–2153, 1998
Kubba R, Al-Gindan Y, el-Hassan AM, Omer AH, Kutty MK, Saeed MB. Dissemination in cutaneous leishmaniasis. 2. Satellite papules and subcutaneous induration. *Int J Dermatol* 27:702–706, 1988
Kubba R, El-Hassan AM, Al-Gindan Y, Omer AH, Kutty MK, Saeed MB: Dissemination in cutaneous leishmaniasis, 1. Subcutaneous nodules. *Int J Dermatol* 26:300–304, 1987
Kurban AK, Malak JA, Farah FS, Chaglassian HT: Histopathology of cutaneous leishmaniasis. *Arch Dermatol* 93:396–401, 1966
Lachaud L, Dereure J, Chabbert E, *et al*: using patient blood samples for diagnosis and follow-up of visceral Leishmaniasis, with special reference to AIDS patients. *J Clin Microbiol* 38:236–240, 2000
Lainson R, Shaw JJ: Evolution classification and geographical distribution. In: Peters Wkillick-Kendrick R, eds. *The Leishmaniases in Biology and Medicine*. San Diego: Academic Press, 1987, pp. 1–120
Lerner EA, Von Lichtenburg FC: Case records of the Massacusetts General Hospital: a 21 year old woman with a persistent rash on the elbow after a sojourn in Central America (Leishmaniasis). *New Engl J Med* 324:476–485, 1991
Levine N: Cutaneous leishmaniasis treated with controlled localized heating. *Arch Dermatol* 128:759–761, 1992
Lezama-Davila CM, Isaac-Marquez AP, Padierna-Olivos J, Aguilar-Torrentera F, Chapa-Ruiz R: Immunomodulation of Chiclero's ulcer. Role of eosinophils, T cells, tumour necrosis factor and interleukin-2. *Scand J Immunol* 47:502–508, 1998
Lima H, Vasconceles W, David JR, Lerner EA: American cutaneous leishmaniasis: In *situ* characterization of the cellular immune response over time. *Am J Trop Med Hyg* 50:743–747, 1994
Lopez-Velez R, Perez-Molina JA, Guerrero A, *et al:* Clinicoepidemiologic characteristics, prognostic factors, and survival analsis of patients coinfected with human immunodeficiency virus and leishmania, in an area of Madrid, Spain. *Am J Trop Med Hyg* 58:436–443, 1998
Maasho K, Sanchez F, Schurr E: Indications of the protective role of natural killer cells in human cutaneous leishmaniasis in an area of endemicity. *Infect Immun* 66:2698–2704, 1998
Machado ES, Braga MdP, Da Cruz AM, *et al*: Disseminated American muco-cutaneous leishmaniasis caused by Leishmania braziliensis braziliensis in a patient with AIDS. a case report. *Mem Inst Oswaldo Cruz* 87:487–492, 1992
Magill AJ, Grogl M, Gasser RAJ, Sun WCN: Visceral infection caused by Leishmania tropica in veterans of Operation Desert Storm. *N Eng J Med* 328:1383–1387, 1993
Marsden PD: Muscosal Leishmaniasis (''espundia'' Escomel, 1911). *Trans R Soc Trop Med Hyg* 80:859–876, 1986
Mattos M, Caiza A, Fernandes O, Goncalves AJ, Pirmez C, Souza CS, Oliveira-Neto MP: American cutaneous leishmaniasis associated with HIV infection: report of four cases. *J Eur Acad Dermatol Venereol* 10:218–225, 1998
McMahon-Pratt D, Bennett E, David JR: Monoclonal antibodies that distinguish subspecies of Leishmaniasis braziliensis. *J Immunol* 129:926–927, 1982
Melby PC, Kreutzer RD, McMahon-Pratt D, Gam AA, Neva FA: Cutaneous Leishmaniasis: Review of 59 cases seen at the National Institutes of Health. *Clin Infect Dis* 15:924–937, 1992
Minodier P, Piarroux R, Gambarelli F, Joblet C, Dumon H: Rapid identification of causative species in patients with Old World leishmaniasis. *J Clin Microbiol* 35:2551–2555, 1997
Modlin RL, Pirmez C, Hofman FM, *et al*: Lymphocytes bearing antigen-specific gd T-cell receptors accumulate in human infectious disease lesions. *Nature* 339:544–548, 1989
Mossalayi MD, Arock M, Mazier D, Vincendeau P, Vouldoukis I: The human immune response during cutaneous leishmaniasis: NO problem. *Parasitol Today* 15:342–345, 1999
Nadim A, Javadian E: Leishmanization in the Islamic Republic of Iran. In: Walton B, Wijeyaretne Pmmodabber F, eds. *Research on Control Strategies for the Leishmaniases*. Ottawa: International Development Research Center, 1998, pp. 336–339
Nanji AA, Greenway DC: Leishmania braziliensis infection of the nipple. *Br Med J* 290:433–434, 1985
Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF: Placebo-controlled clinical trial of sodium stibogluconate (Pentostam) versus Ketoconazole for treating cutaneous leishmaniasis in Guatemala. *J Infect Dis* 165:528–534, 1992
Norton SA, Frankenburg S, Klaus SN: Cutaneous leishmaniasis acquired during military service in the middle east. *Arch Dermatol* 128:83–87, 1992
Otero AC, da Silva VO, Luz KG, Palatnik M, Pirmez C, Fernandes O, Palatnik de Sousa CB: Short report: occurrence of Leishmania donovani DNA in donated blood from seroreactive Brazilian blood donors. *Am J Trop Med Hyg* 62:128–131, 2000
Peters W: Royal Society of Tropical Medicine and Hygiene presidential address. Manson House, 15 October, 1987. ''The little sister'' – a tale of Arabia. *Trans R Soc Trop Med Hyg* 82:179–184, 1988

Pineda JA, Gallardo JA, Macias J, *et al*: Prevalence of and factors associated with visceral leishmaniasis in human immunodeficiency virus type 1-infected patients in southern Spain. *J Clin Microbiol* 36:2419–2422, 1998

Pirmez C, Yamamura M, Uyemura K, Paes-Oliveira M, Conceicao-Silva F, Modlin RL: Cytokine patterns in the pathogenesis of human leishmaniasis. *J Clin Invest* 91:1390–1395, 1993

Pizzuto M, Piazza M, Senese D, *et al*: Role of PCR in diagnosis and prognosis of visceral leishmaniasis in patients coinfected with human immunodeficiency virus type 1. *J Clin Microbiol* 39:357–361, 2001

Rees PH, Kager PA: Visceral leishmaniasis and post kala-azar dermal leishmaniasis. In: Peters Wkillick-Kendrick R, eds. *The Leishmaniases in Biology and Medicine*, vol. 2. London: Academic Press, 1987, pp. 848–907

Rosatelli JB, Souza CS, Soares FA, Foss NT, Roselino AM: Generalized cutaneous leishmaniasis in acquired immunodeficiency syndrome. *J Eur Acad Dermatol Venereol* 10:229–232, 1998

Rosenthal E, Marty P, le Ficoux Y, Cassuto JP: Clinical manifestations of visceral leishmaniasis associated with HIV infection: a retrospective study of 91 French cases. *Ann Trop Med Parasitol* 94:37–42, 2000

Roustan G, Jiminez JA, Gutierrez-Solar B, Gallego JL, Alvar J, Patron M: Post-kala-azar dermal leishmaniasis with mucosal involvement in a kidney transplant recipient: treatment with liposomal amphotericin B. *Br J Dermatol* 138:526–528, 1998

Sampaio SA, Castro RM, Dillon NL, Martins JE: Treatment of mucocutaneous (American) leishmaniasis with amphotericin B. report of 70 cases. *Int J Dermatol* 10:179–181, 1971

Santos-Gomes G, Gomes-Pereira S, Campino L, Araujo MD, Abranches P: Performance of immunoblotting in diagnosis of visceral Leishmaniasis in human immunodeficiency virus-Leishmania sp.-coinfected patients. *J Clin Microbiol* 38:175–178, 2000

Schubach A, Marzochi MC, Cuzzi-Maya T, *et al*: Cutaneous scars in American tegumentary leishmaniasis patients: a site of Leishmania (Viannia) braziliensis persistence and viability eleven years after antimonial therapy and clinical cure. *Am J Trop Med Hyg* 58:824–827, 1998

Sharquie KE, Najim RA, Farjou IB, Al-Timimi DJ: Oral zinc sulphate in the treatment of acute cutaneous leishmaniasis. *Clin Exp Dermatol* 26:21–26, 2001

Sherwood J, Gachihi GS, Muigai RK, *et al*: Phase 2 efficacy trial of an oral 8-aminoquinoline (WR6026) for treatment of visceral leishmaniasis. *Clin Infect Dis* 19:1034–1039, 1994

Soto-Mancipe J, Grogl M, Berman JD: Evaluation of pentamidine for the treatment of cutaneous leishmaniasis in Colombia. *Clin Infect Dis* 16:417–425, 1993

Stenger S, Thuring H, Rollinghoff M, Bogdan C: Tissue expression of inducible nitric oxide synthase is closely associated with resistance to Leishmania major. *J Exp Med* 180:783–793, 1994

Sulahian A, Garin YJ, Pratlong F, Dedet JP, Derouin F: Experimental pathogenicity of viscerotropic and dermotropic isolates of Leishmania infantum from immunocompromised and immunocompetent patients in a murine model. *FEMS Immunol Med Microbiol* 17:131–138, 1997

Swaminath CS, Shortt HE, Anderson L: Transmission of Indian kala-azar to man by bites of Phlebotomus argentipes, ann. & brun. *Indian J Med Res* 30:473–477, 1942

Velez I, Agudelo S, Hendrickx E: Inefficacy of allopurinol as monotherapy for Colombian cutaneous leishmaniasis. A roandomized controlled trial. *Ann Intern Med* 1:232–236, 1997

WHO: *Leishmania/HIV Co-Infection in Southwestern Europe 1990–98: Retrospective Analysis of 965 Cases*. World Health Organization WHO/LEISH/200.42. 2000

Yadav TP, Gupta H, Satteya U, Kumar R, Mittal V: Congenital kala-azar. *Ann Trop Med Parasitol* 83:535–537, 1989

Zijlstra EE, El-Hassan AM, Ismael A, Ghalib HW: Endemic Kala-azar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical infection and post-kala-azar dermal leishmaniasis. *Am J Trop Med Hyg* 51:826–836, 1994