Am J Clin Dermatol 2002; 3 (2): 91-105
1175-0561/02/0002-0091/$25.00/0

Leishmaniasis

Recognition and Management with a Focus on the Immunocompromised Patient

Christine M. Choi[1] and *Ethan A. Lerner*[2]

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

Contents

Abstract

Leishmaniasis is a protozoan disease whose clinical manifestations depend both on the infecting species of *Leishmania* and the immune response of the host. Transmission of the disease occurs by the bite of a sandfly infected with *Leishmania* parasites. Infection may be restricted to the skin in cutaneous leishmaniasis (CL), to the mucous membranes in mucosal leishmaniasis or spread internally in visceral leishmaniasis (VL). In the last 2 decades, leishmaniasis, especially VL, has been recognized as an opportunistic disease in immunocompromised patients, particularly those infected with HIV.

Leishmaniasis is characterized by a spectrum of disease phenotypes that correspond to the strength of the host's cell-mediated immune response. Both susceptible and resistant phenotypes exist within human populations. Clinical cutaneous disease ranges from a few spontaneously-healing lesions, to diffuse external or internal disease, to severe mucous membrane involvement. Spontaneously-healing lesions are associated with positive antigen-specific T cell responsiveness, diffuse cutaneous and visceral disease with T cell non-responsiveness, and mucocutaneous disease with T cell hyperresponsiveness. Current research is focused on determining the extent to which this spectrum of host response is genetically determined.

In endemic areas, diagnosis is often made on clinical grounds alone including: small number of lesions; on exposed areas; present for a number of months; resistant to all types of attempted treatments; and usually no pain or itching. Multiple diagnostic techniques are available.

When evaluating treatment, the natural history of leishmaniasis must be considered. Lesions of CL heal spontaneously over 1 month to 3 years, while lesions of mucocutaneous and VL rarely, if ever, heal without treatment. Consequently, all the latter patients require treatment. Therapy is not always essential in localized CL, although the majority of such patients are treated. Patients with lesions on the face or other cosmetically important areas are treated to reduce the size of the resultant scar. In addition, the species of parasite should be identified so that infection with *Leishmania braziliensis* and *Leishmania 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 because of the high relapse rates. Proven treatments include: antimonials, pentamidine, amphotericin B, interferon with antimony. Treatments where current clinical experience is too limited include: allopurinol, ketoconazole, itraconazole, immunotherapy, rifampin, dapsone, localized heat, paromomycin ointment and cryotherapy. Investigational treatments include: WR6026, liposomal amphotericin and miltefosine. In addition, vaccines for leishmaniasis are being investigated in clinical trials.

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 sandfly infected with *Leishmania* parasites. Infection may be restricted to the skin in cutaneous leishmaniasis (CL), limited to the mucous membranes in mucosal leishmaniasis, or spread internally in visceral leishmaniasis (VL) or kala-azar. Three rare clinical variants of CL include diffuse cutaneous leishmaniasis (DCL), leishmaniasis recidivans (LR) and post-kala-azar dermal leishmaniasis (PKDL). The overall prevalence of leishmaniasis is 12 million cases worldwide, and the global yearly incidence of all clinical forms approaches 2 million new cases.[1] In the last 2 decades, leishmaniasis, especially VL, has been recognized as an opportunistic disease in the immunocompromised, particularly in patients infected with HIV.

The primary focus of this article is on CL but certain aspects of visceral disease are included because this latter form is important in the immunocompromised host.

1. History

The cutaneous afflictions of leishmaniasis have been known since antiquity.[2] Descriptions of the cutaneous disease in the Old

Table I. Histopathologic changes in the various forms of leishmaniasis

Form of disease	Epidermal findings	Dermal findings	Presence of organisms (dermis)	Organization of infiltrate	Montenegro test
LCL	Hyperkeratosis, parakeratosis, follicular plugging, necrosis, basal cell degeneration, rare parasites within epidermal cells	Lymphocytes + histiocytes, occasional plasma cells, rare eosinophils and neutrophils	+++ early lesions ± late lesions	Diffuse	Positive in 92%
DCL	No necrosis	Macrophages full of amastigotes predominate, many free organisms, minimal cellular reaction	+++	Diffuse	Negative; may become positive during treatment which implies a better prognosis
LR	Atrophy, follicular plugging, hyperkeratosis parakeratosis, no necrosis	Langhan's giant cells, granulomas made up of epithelioid cells	±	Well organized granulomas	Strongly positive in 98%
PKDL	Decreased number of pigment granules in hypopigmented macules	Same as for LR but with perivascular fibrous tissue	±	Well organized granulomas	Most patients positive
MCL	Atrophy necrosis	Abundant plasma cells, otherwise, paucity of infiltration	±	Diffuse	Positive in 97%
VL	Increased melanin in basal layer, increased melanoblastic activity	*Leishmania* in macrophages around sweat glands, hair follicles or blood vessels	± organisms present in skin, lymph node, spleen bone marrow, blood (rarely)	Diffuse	Most patients positive 12 months after treatment

DCL = diffuse cutaneous leishmaniasis; **LCL** = localized cutaneous leishmaniasis; **LR** = leishmaniasis recidivans; **MCL** = mucocutaneous leishmaniasis; **PKDL** = post-kala-azar dermal leishmaniasis; **VL** = visceral leishmaniasis; ± = none or few organisms present; **+++** = organisms are abundant.

World are found from the first century AD. New World pottery from Peru and Ecuador dating from AD 400 to 900 illustrates faces afflicted with a process consistent with leishmaniasis.[3] Russell, who described the 'Aleppo evil' from Syria, made the first description in English of a lesion resembling leishmaniasis in 1756. 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 to 5μm 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 for 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.

2. Clinical Features

2.1 Localized Cutaneous Leishmaniasis

Leishmaniasis in its various forms is present on all continents except Australia and Antarctica (see table I).[4] Localized cutaneous leishmaniasis (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, including *Leishmania braziliensis* and *Leishmania mexicana*.

LCL usually affects unclothed parts of the body easily bitten by the sandfly vector, including the face, neck and arms. New World leishmaniasis commonly presents with a solitary primary lesion, while multiple primary lesions are often found in Old World disease. After an average incubation period of 1 week to 3 months, a red papule appears which 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 (figure 1). Painless, rubbery subcutaneous nodules or cords rarely develop around the ulcer as a result of local lymphangitic spread of the organism. Draining lymph nodes may be enlarged and reveal parasites on biopsy.[5] 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.[6,7] A generalized papular eruption may also develop, representing a hypersensitivity reaction. Areas of trauma may koebnerize. Itching and pain are mild, if present. The wound may become superinfected, leading to misdiagnosis. After approximately 1 to 36 months, depending upon both the patient and infecting organism, the ulcer spontaneously regresses leaving a scar with hypo- or hyperpigmentation. Immunity is generally thought to be complete but one study in a Saudi Arabian population found subsequent re-infection in up to 10% of individuals.[8]

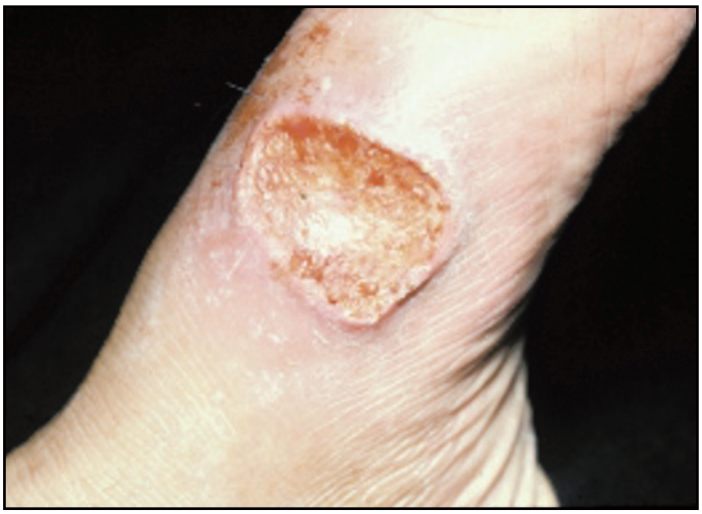

Fig. 1. Localized cutaneous leishmaniasis: ulcer of the lower extremity caused by *Leishmania braziliensis*.

2.2 Diffuse Cutaneous Leishmaniasis

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 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.

2.3 Leishmaniasis Recidivans

LR or 'recurrent leishmaniasis' refers to the development of what is most likely a recurrence near the periphery of a scar of a healed acute lesion of leishmaniasis. Clinically, LR commonly presents as scaly, erythematous papules within scars of healed

lesions, although ulcers, psoriaform lesions and verrucous forms have also been described. The causative organism is usually *L. tropica* in the Middle East and less commonly *L. braziliensis* in South America. Lesions tend to resist treatment and become chronic. The mechanism postulated for recurrent disease in the Old World is reactivation of 'dormant' organisms, with the period of dormancy ranging from 1 to 15 years. Characterization of the species in initial and recurrent lesions, however, has revealed that 50% of the recurrent strains differed from the initial strain, suggesting either a change in the immune status of the host or exogenous reinfection as a mechanism in some cases, rather than reactivation.[9]

2.4 Post-Kala-Azar Dermal Leishmaniasis

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 VL.[10] The rash generally consists of discrete skin-colored or hyperpigmented papules on the cheeks, chin, ears and extensor aspects of forearms. Except in severe cases, lesions heal spontaneously over a few months, perhaps via an acquired cellular immunity that eliminates remaining parasites in the skin.

In contrast to African PKDL, Indian PKDL occurs in 20% of patients approximately 1 to 2 years after recovery from VL.[11] Hypopigmented macules are usually the first manifestation of PKDL in India, beginning as very small macules, which enlarge to form large irregular patches. Lesions are often bilateral and symmetrical, appearing primarily on the chest and back, anterior thighs, arms and neck. The pigmentary loss is never complete, and there is no pigment change in the hair overlying the lesions (table I). 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 more resistant to treatment, requiring longer duration of systemic medication than VL. Hypopigmented areas almost never completely repigment.

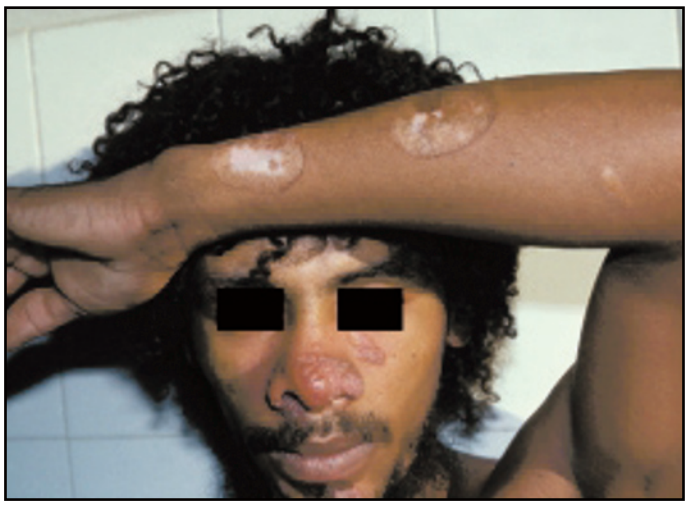

Fig. 2. Mucocutaneous leishmaniasis: a young man with prior healed lesions of cutaneous leishmaniasis on the arms caused by *Leishmania braziliensis*, now presenting with new lesions of the nasal mucosa.

2.5 Mucocutaneous Leishmaniasis

Mucocutaneous leishmaniasis (MCL) is most commonly reported in the New World.[12,13] *L. braziliensis* is the most common etiologic agent, although infections resulting from other *Leishmania* subspecies have been reported. In particular, there have been several reports of mucocutaneous disease as a result of *Leishmania panamensis*, increasing the risk that travelers to Central America could potentially develop MCL.[14] 50% of patients develop mucocutaneous lesions within 2 years of the initial cutaneous lesions, and 90% within 10 years (figure 2). Approximately one-third of patients has 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.[15] Mucous membrane involvement probably develops as a result of hematogenous or lymphatic dissemination, or occasionally from direct extension of nearby skin lesions. Factors associated with the development of MCL include male sex, large or multiple primary lesions, persistent lesions lasting longer than 1 year, and inadequate treatment for the primary skin lesions.

The disease often begins in the nasal septum, which becomes inflamed and infiltrated and subsequently perforates. MCL has a predilection for the distal cartilaginous part of the nose, and the resulting deformation has been named 'tapir's nose', 'parrot's beak' and 'camel's nose'. Mutilation of the nasal septum, palate, pharynx, tonsils, gums and/or lip may ensue, whereas bony structures remain intact. On rare occasions dissemination to the mucous membranes of the eye and genitals is seen. Invasion of the respiratory tract, including the larynx, trachea and bronchi may lead to compromised respiration and swallowing. Malnutrition and pneumonia are the leading causes of death in patients with MCL.

2.6 Visceral Leishmaniasis (VL)

Kala-azar or VL is a systemic disease caused by the dissemination of *L. donovani* or *Leishmania infantum*. The advent of HIV has increased the population of individuals with 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 which is the origin of the name 'kala-azar', meaning 'black fever'. This hyperpigmentation is secondary to increased melanoblastic activity as well as an enhancement of the natural skin color as a result of xerosis. Impairment in wound healing is often seen. After an incubation period of 2 to 4 months, VL runs an insidious chronic course until treated. Although the disease is associated with an increase in serum immunoglobulin (Ig) G and IgM, there is a depression of cell-mediated immunity that predisposes the host to multiple secondary infections.[16] Treatment is usually successful in the absence of immunodeficiency but relapses have been reported.[17] One source of such relapses is the observation that parasites have been demonstrated to persist up to 11 years following clinical cure.[18]

2.7 Viscerotropic Leishmaniasis

L. 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.[19] The clinical presentations were distinct from VL caused by *L. donovani* and included high fever, malaise, intermittent diarrhea and abdominal pain. As skin lesions were not found, this entity is not discussed further in this review.

3. Pathology

Histopathologic studies reveal epidermal and/or dermal changes, depending on the type and stage of the disease (table I).[20] Epidermal changes include hyperkeratosis, parakeratosis and follicular plugging. Epidermal atrophy or acanthosis with occasional intraepidermal abscesses may also be seen. Ulcerated areas show widespread necrosis and often secondary bacterial infection. The basal layer may be degenerated. Increased melanin in the basal layer is seen in VL, while pigment granules are decreased in PKDL.

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 to 4μm in length, round- or oval-shaped 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 LR. The lymphohistiocytic infiltrate is arranged diffusely in cutaneous forms of leishmaniasis, except in PKDL and LR where well-organized granulomas are seen. Langhan'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 (IL)-2 (CD25+), transferrin receptors (CD71+), or major histocompatibility complex class II molecules on their surface.[21] Equal numbers of CD4+ and CD8+ lymphocytes are present in LCL lesions. In addition, while the majority of T cells in control skin biopsies bear the T cell receptor αβ complex, 20 to 30% of the T cells in early LCL lesions bear the T cell receptor γδ complex.[22,23]

4. Pathogenesis and Etiology

The arthropod vector of all forms of leishmaniasis is the female sandfly.[24] In the Old World the disease is transmitted by flies of the genus *Phlebotomus*, 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 non-flagellated obligate intracellular form called the amastigote. During the course of probing the skin, the sandfly 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 sandfly 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 sandflies, but in other areas less than 1% of sandflies are infected.[25] Humans are usually accidental hosts of leishmaniasis, because they live in endemic zones and are thereby exposed to infected sandflies. The zoonotic reservoir includes sloths, anteaters, rodents, foxes and

dogs. Transmission of *Leishmania* infection occurs almost exclusively via the bite of an infected sandfly; however, other possible modes of transmission (e.g. direct contact) have been reported.[26-28]

5. Immunology

Leishmaniasis is characterized by a spectrum of disease phenotypes that correspond 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. Clinical cutaneous disease ranges from a few spontaneously-healing lesions, to diffuse external or internal disease, to severe mucous membrane involvement. Spontaneously-healing lesions are associated with positive antigen-specific T cell responsiveness, diffuse cutaneous and visceral disease with T cell non-responsiveness, and mucocutaneous disease with T cell hyperresponsiveness.[29] Current research is focused on determining the extent to which this spectrum of host response to leishmaniasis is genetically determined.

Analysis of the cytokine profiles in three different clinical forms of CL suggests that the host's immune system plays an immunoregulatory role in disease expression. In LCL the primary cytokines are IL-2 and interferon-γ, whereas in MCL and DCL IL-4 and IL-10 predominate.[30] These data correlate well with studies of leishmaniasis in murine models, where the production of IL-2 and interferon-γ mediates healing of disease while IL-4 and IL-10 are associated with disease progression and dissemination.[31,32] Two distinct subsets of helper T cells in the murine immune system are critical in inducing resistance or susceptibility to infection. One subset of cells called T helper (Th) cells type 1 produce IL-2, interferon-γ, tumor necrosis factor (TNF)-α and TNFβ, and augment cell-mediated immune responses by activating macrophages. Th1 cells thus mediate a clinically mild or spontaneously resolving disease. IL-12 develops the cell-mediated immune response by inducing naïve T cells to differentiate into Th1 cells and by inducing both T cells and natural killer cells to produce interferon-γ.[33] In contrast, Th2 cells, which secrete IL-4, IL-5 and IL-10, augment humoral responses and inhibit some cell-mediated immune responses, resulting in disseminated infection. This Th1/Th2 paradigm stresses that the nature and magnitude of the early T cell and cytokine responses to parasitic infection determine the outcome of disease. The use of interferon-γ with antimony in the treatment of VL and DCL is based on data showing that exogenous interferon-γ enhances *in vivo* leishmanial resistance.[34] Although initially promising, this immunotherapeutic approach was shown to have only a modest effect in later studies.[35]

Distinct lymphokine patterns have also been associated with the response to infection in humans.[36] These patterns are more complex and less polarized than in murine models. It is likely that in humans, Th2 cells predominate during the early course of infection while Th1 cells are more important in the healing phase. However, experiments have challenged the Th1/Th2 paradigm in humans with active cutaneous lesions. Researchers detected the synthesis of messenger RNA coding for TNFα, IL-1, IL-6, IL-10 and transforming growth factor-β, but not IL-3 or IL-4. Another interesting finding was that administration of IL-2 and IL-4 into established lesions could induce resistance to some *Leishmania* spp.[37] Overall these results demonstrate that, although disease expression largely depends on the species of infecting parasite, the course of infection is also based on the specific host immune response.

Superoxide molecules produced by macrophages have been considered important in the host response to *Leishmania*. The interest in nitric oxide (NO) as a mediator of numerous biological processes from neurotransmission to immunological responses prompted examination of the effect of NO as a mediator of killing of *Leishmania* both *in vitro* and *in vivo*.[38,39] These studies reveal that the inducible form of NO synthase is associated with resistance to *Leishmania* infection in mice and that NO itself is leishmanicidal. In a study of human CL, cross-linking of the low-affinity receptor for IgE (CD23) on the surface of macrophages was shown to promote intracellular killing of the parasites by the generation of TNFα and NO.[40]

Recent evidence has prompted investigations of other cell types and cellular mediators of disease susceptibility and resistance towards leishmanial infection. Some researchers have suggested a protective role of natural killer cells and CD8+ T cells proliferating in response to *Leishmania* stimulation in Ethiopian CL.[41] Others have studied the immunomodulation of leishmanial infection by focusing on the significance of increased serum levels of TNFα, as well as observed eosinophilia in diseased and recently cured individuals.[37] It is clear that future immunological research will influence both the prevention and treatment of leishmanial infection and disease.

6. Diagnosis

The differential diagnosis for leishmaniasis is extensive (table II). In endemic areas, the diagnosis is often made on clinical grounds alone including: (i) small number of lesions;[1-3] (ii) on exposed areas; (iii) present for a number of months; (iv) resistant to all types of attempted treatments; and (v) usually no pain or itching. Multiple diagnostic techniques are available. Historically, microscopy and culture have been the standard for diagno-

Table II. Differential diagnosis of leishmaniasis

Localized cutaneous leishmaniasis: acute	Localized cutaneous leishmaniasis: chronic and leishmaniasis recidivans	Mucocutaneous leishmaniasis
Infections	**Infections**	**Infections**
Fungal	**Bacterial**	**Fungal**
Paracoccidioidomycosis	Lupus vulgaris	Paracoccidioidomycosis
Chromoblastomycosis	Leprosy	Sporotrichosis
Sporotrichosis	Syphilitic gummata	Histoplasmosis
Blastomycosis	**Inflammatory**	Blastomycosis
Bacterial	Sarcoidosis	**Bacterial**
Mycobacterioses	Discoid lupus erythematosus	Tuberculosis
Leprosy	Psoriasis	Rhinoscleroma
Lupus vulgaris	Keloids	Gummatous syphilis
Tuberculosis verrucosa cutis	Jessner's lymphocytic infiltrate	Cancrum oris
Other mycobacterioses	**Neoplastic**	Yaws
Treponematoses	Lymphocytoma cutis	**Inflammatory**
Pinta	**Unknown cause**	Lethal midline granuloma
Yaws	Granuloma faciale	**Neoplastic**
Syphilis		Carcinoma
Staphylococcal/streptococcal pyodermas		
Impetigo, ecthyma, furunculosis		
Insect bite		
Parasitic		
Amebiasis		
Viral		
Orf		
Inflammatory		
Sarcoidosis		
Foreign body granuloma		
Pyogenic granuloma		
Neoplastic		
Cutaneous T cell lymphoma		
Basal cell carcinoma		
Squamous cell carcinoma		
Keratoacanthoma		
Cutaneous metastases		

sis. 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 patients with 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 days and 2 weeks. Alternatively, biopsy or aspirate specimens may be inoculated into hamster footpads for *in vivo* culture. In addition, blood samples may be examined for anti-*Leishmania* antibodies by direct agglutination test, enzyme-linked immunoabsorbance agglutination, or by indirect fluorescent antibody test. These serological tests are highly sensitive for the diagnosis of VL but less sensitive for CL.

The intradermal leishmanin (Montenegro) test is useful in non-endemic areas as a diagnostic tool, and in endemic areas as a test for survey work. This test consists of the intradermal injection of 0.10 to 0.20ml of a suspension of antigen prepared from dead promastigotes (approximately 2 to 3 million promastigotes in 1ml of phenolized saline solution). The reaction is read at 48 to 72 hours, and a positive reading consists of induration and erythema over 10mm in diameter sometimes associated with an inflammatory halo. A positive test indicates that a patient has, or

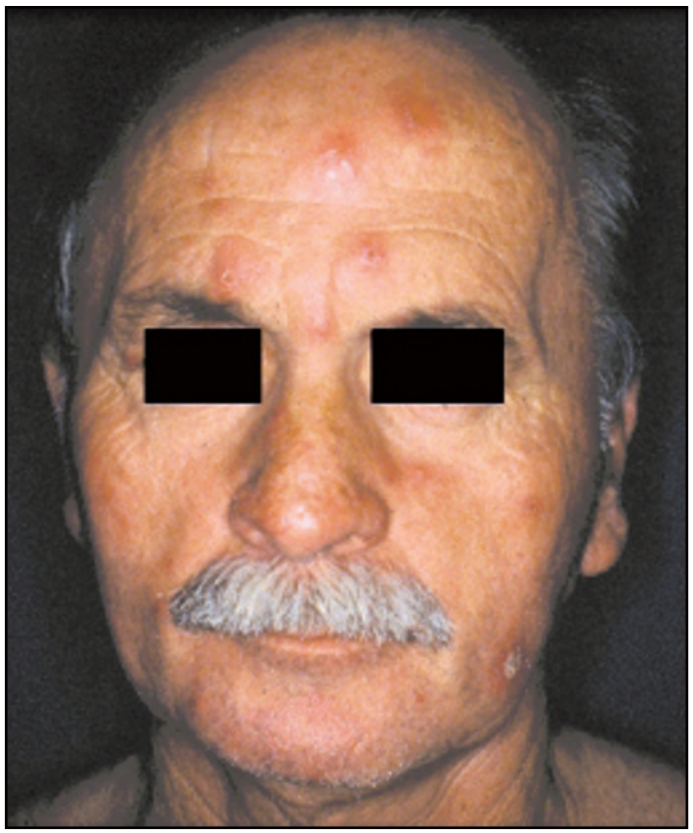

Fig. 3. Generalized cutaneous leishmaniasis caused by *Leishmania braziliensis* in a patient immunocompromised by diabetes mellitus. The term 'generalized' is used to distinguish this case from 'diffuse' which is caused by *Leishmania amazonensis*.

has had, leishmaniasis, but cannot distinguish between active and quiescent disease. The test is positive in 90 to 98% of patients with LCL and MCL, and is negative in patients with disseminated disease. A survey in an endemic area revealed that two-thirds of people had a positive skin test, and that the risk of developing a positive skin test was directly related to the amount and duration of exposure to heavily forested areas. In addition, 50% of people without any signs of past or present disease had positive skin tests, suggesting past or active subclinical infection.[42] The test is not available in the US.

The identification of the specific species of parasite responsible for infection is important for the diagnosis of disease, evaluation of therapy, and prognosis. In addition to monoclonal antibodies[43] and isoenzyme analysis,[44] a number of molecular biology techniques have been developed that are designed for species-specific identification of parasites within the genus *Leishmania*. The polymerase chain reaction (PCR) shows much promise for distinguishing past from current infection and subspeciating clinical isolates. An extensive literature exists in which investigators have used this powerful technique to amplify different parts of the *Leishmania* genome including kinetoplastid DNA, mini-exon repeat sequences, and the internal transcribed spacer of the ribosomal operon.[45-47] Species identification has been achieved in a variety of ways such as multiplexing and using nested PCR.[48] However, the widespread clinical usefulness of these techniques remains to be implemented. These molecular techniques can sometimes be performed on miniscule amounts of material, such as tissue sections or touch preparations, rather than on the cultured organisms needed for the other analyses.

7. Leishmania and HIV Co-Infection

Over the past decade, leishmaniasis has been increasingly documented in immunocompromised patients, namely those infected with HIV.[49-56] Although leishmaniasis has been reported in other immunodepressed states, such as diabetes mellitus (figure 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.[57,58] Co-infection is most frequently manifested as VL.[1]

7.1 Epidemiology

The rising interface between leishmaniasis and developed countries can be attributed to several factors: increased overseas travel, recent US Gulf War veterans, and the simultaneous encroachment of leishmaniasis into urban areas and of HIV infection into rural areas.[33,59] In the Mediterranean, where *L. infantum* is endemic, the number of cases of VL in patients with HIV has increased dramatically since the mid-1980s.[56] The World Health Organization (WHO) estimates that 25 to 70% of adult patients with VL in southern Europe are related to HIV, while between 2 and 9% of patients with AIDS are at risk of experiencing newly acquired or reactivated VL.[52,60,61] 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.[33] 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*.[62] In fact, preliminary data suggest that *Leishmania* may be a cofactor in the pathogenesis of HIV infection: the lipophosphoglycan surface molecule of *L. donovani* has been shown to induce transcription of HIV in CD4+ T cells.[33,63] The WHO estimates that AIDS increases the risk of VL by 100 to 1000 times in endemic regions.[1] Despite the strong connection between leishmaniasis and HIV, *Leishmania* infection is still not included on the list of AIDS-defining criteria.[60,62]

The typical clinico-epidemiological 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/μl. It is clear that IVDUs are the population most at risk for co-infection in southwestern Europe.[1] Some researchers have proposed an anthroponotic mode of transmission via the sharing of contaminated needles by IVDUs to explain the high frequency of HIV and *Leishmania* co-infection in this risk group.[59,62,64,65] 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.[65] Two troubling reports noted parasitemia in asymptomatic blood donors, detected by PCR and/or culture, from endemic regions.[66,67] Blood transfused from such donors into HIV-positive individuals could contribute to the incidence of co-infection.

7.2 Immunology of Leishmaniasis in HIV

As described in section 5, 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.[49] 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 serological 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/μl) 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.[52]

Several studies have proposed that patients with HIV 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.[68] 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.[64,69]

Despite the development of more sensitive techniques for serological 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. Furthermore, 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. However, the overall dysfunction of the immune system in patients with HIV, especially in the advanced stages of AIDS, may also explain the decrease in specific antibody production.[52,62] It is therefore recommended that two or more serological tests and antigens freshly prepared in the laboratory be used to increase sensitivity.[1] 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.[70]

7.3 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.[59,61] 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 patients amastigotes were discovered in unusual locations such as the digestive tract (16 patients), lungs (7), skin

(7) and tonsil (1). 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/μl.[60] 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 micro-organisms (i.e. mycobacteria), opportunistic infections concurrent with leishmaniasis, or drug therapy-induced adverse effects.[64] Furthermore, as mentioned above, serological 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.[62] The median survival of HIV-infected patients with VL is 13 to 19 months.[35] In the study of 54 Spanish co-infected patients, 18.5% died during their first episode of VL.[59] A significant predictor of mortality was the fulfillment 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 months after VL diagnosis, while the group of patients without AIDS (n = 28) reached similar survival levels at approximately 30 months 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.

In contrast to the reliable cure of VL in immunocompetent hosts, who have relapse rates of less than 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 occurring within 12 months.[35] 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.[59,62] 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 section 8.

7.4 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.[62] In one report, a child in the advanced stages of AIDS presented with an unusual clinical picture of DCL caused by *L. major*.[51] 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 CL in a patient with AIDS.[71] 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 CL.[55] 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.[49] However, this finding 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 patients with 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.[64]

8. Therapy

The natural history of leishmaniasis must be considered when evaluating therapeutic agents. Lesions of CL heal spontaneously over 1 month to 3 years, while lesions of mucocutaneous and visceral disease rarely, if ever, heal without treatment. Consequently, all patients with MCL or VL require treatment. Therapy is not always essential in LCL, although the majority of patients with LCL are treated. There are two important indications for therapy. 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. Finally, treating patients with *Leishmania* and HIV co-infection requires close monitoring for effectiveness of treatment, especially because of the high relapse rates.

The pentavalent ammonial compound sodium stibogluconate (Pentostam™[1]), introduced after World War II, is still the

1 Use of tradenames is for product identification purposes only and does not imply endorsement.

conventional therapeutic agent.[72] Ironically, the exact chemical structure of this drug remains unknown. The drug appears to inhibit amastigote glycolytic activity and fatty acid oxidation. It is available from the Centers for Disease Control (CDC) to treat patients diagnosed in the US. The CDC will also aid in making a diagnosis. The accepted dosage for the treatment of leishmaniasis is 20 mg/kg intravenously or intramuscularly daily, with an upper limit of 30 mg/kg/day.[73] CL is treated for 20 days and visceral and mucosal disease for 28 days. Adverse 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 electrocardiogram (ECG) commonly develop, and occur more frequently the higher the daily dose and the longer the duration of therapy. The ECG abnormalities include T wave inversion, ST segment elevation or depression, and prolongation of the QT interval.

A review on the use of sodium stibogluconate for the treatment of leishmaniasis generated several recommendations with regard to these adverse effects.[74] All patients receiving 20 mg/kg/day of sodium stibogluconate should ideally have an ECG, serum chemistries and complete blood count pre-treatment, and weekly during treatment. However, healthy children receiving only 20 days of therapy may not require ECG monitoring. If more than 20 days of therapy are required, an ECG should be performed biweekly. Elderly patients or patients with underlying cardiac, hepatic or renal disease should be monitored more frequently. Therapy should be discontinued if one of the serum aminotransferases reaches 5 times the upper limit of normal, or if patients develop concave ST segments, prolongation of the QT interval to >0.5 seconds, or significant arrhythmias. Chemical pancreatitis is very common, with almost invariable rises in amylase and lipase to abnormal levels in patients treated with therapeutic doses of sodium stibogluconate. Sudden deaths resulting from antimonials are mainly caused by arrhythmias or pancreatitis.

In the US, most patients are admitted for cardiac monitoring during treatment with sodium stibogluconate. In South America, where a slightly different pentavalent antimonial, meglumine antimonate (Glucantime™), is available, the drug is often administered at home. Meglumine antimonate is administered at 85% of the dose of sodium stibogluconate, 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.[73]

A wide variety of alternative systemic and topical treatments have been utilized to treat leishmaniasis (table III). Most of these treatments have not been analyzed with randomized, placebo-controlled trials, making it difficult to accurately evaluate their effectiveness. A few conclusions, however, may be drawn: pentamidine has been used successfully as a second-line agent for VL and New World CL,[75] and amphotericin B has some effectiveness in mucosal disease.[76] The combination of antimony and interferon-γ has been used effectively in the treatment of refractory VL in Brazil. However, the improvement in cure rates has been deemed only modest.[35] 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 than treatment with antimonials alone.[35] 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 lidocaine/prilocaine anesthesia, as a practical means of treating LCL in young patients.

Table III. Therapeutic options for leishmaniasis

Therapeutic agent	LCL	DCL	MCL	VL	References
Antimonials (IV/IM)	E	E	E	E	1,74
Antimonials (IL)	E				73
Pentamidine	E			E	1,75
Amphotericin B	E		E	E	1,76,77
Interferon with antimony		?	E	E	35
Allopurinol	?			?	79
Ketoconazole	E				78
Itraconazole	?	?	?		80
Immunotherapy	?				81
Rifampin		?			73
Dapsone	?				73
Localized heat	?	?			82
Paromomycin ointment	E				83
Cryotherapy	?				84
WR6026				I	85
Liposomal amphotericin B	E			E	57,58
Miltefosine				?	86

DCL = diffuse cutaneous leishmaniasis; **E** = effective; **I** = investigational; **IL** = intralesional; **IM** = intramuscular; **IV** = intravenous; **LCL** = localized cutaneous leishmaniasis; **MCL** = mucocutaneous leishmaniasis; **VL** = visceral leishmaniasis; **?** = current clinical experience too limited.

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.[79] 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 mucosal leishmaniasis.[80] Trials with allopurinol have shown mixed results.[78] 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.[81] With regard to topical therapy, paromomycin ointment, localized heat, and cryotherapy have all been tried with variable results.[82-84] Combinations of therapeutic strategies may be attempted more 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 US. AmBisome® therapy is rapidly effective and less toxic than conventional formulations of amphotericin B.[57] However, the liposomal formulation is also more than 50 times more expensive than the conventional formulation.[58] Liposomal amphotericin B should be used for the treatment of VL when costs of hospitalization would exceed drug costs.

WR 6026 is a primaquine analogue that has shown effectiveness in animal models of VL, and underwent a phase II clinical efficacy trial in the treatment of 16 patients with VL. Adverse effects included gastrointestinal distress, headache and methemoglobinemia.[85] 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 CL and showed dose-dependent cure rates up to 96.9%.[87] 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.[86] Gastrointestinal adverse 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.

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 US. The treatment of choice, the best dosage, and the duration are still unknown for this group of patients. However, based on the knowledge that longer treatment periods tend to lead to higher cure rates in immunocompetent patients, it follows that HIV-infected patients with VL should be treated for longer periods or at higher dosages than immunocompetent patients. A recent randomized trial comparing meglumine antimonate with amphotericin B in HIV-infected patients with VL showed similar efficacy and toxicity rates.[77] Serious adverse effects of meglumine antimonate included cardiotoxicity and chemical pancreatitis, whereas amphotericin B more frequently induced nephrotoxicity. Other investigators reported a high frequency of acute pancreatitis, acute renal failure, leukopenia and death in HIV-infected patients with VL who were treated with meglumine antimonate at doses recommended by the CDC.[88]

The management of HIV-infected patients with VL warrants special attention for several reasons. First, it is estimated that 60 to 90% of patients with AIDS experience a relapse of the disease after responding well to initial treatment, making post-therapeutic follow-up essential.[53] In other patients, response to treatment may be incomplete or may be interrupted by drug toxicity. Although reports of severe pancreatitis, myocarditis and renal insufficiency as a result of 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.[62] The optimal regimen for secondary prophylaxis and management of harmful drug-drug interactions while on concomitant antiretroviral therapy are areas to be investigated further.

Clinical evaluation of the response to therapeutics can be difficult because of the overlap of symptoms between VL and concomitant AIDS-related diseases. Furthermore, clinical improvement is not always indicative of parasitological cure. New techniques such as optimized PCR, a highly sensitive (97% for peripheral blood and 100% for bone marrow) assessment tool for diagnosis and follow-up of VL, can facilitate monitoring of disease status.[53] Finally, education and awareness of those involved in the treatment of HIV-infected patients is crucial. Because VL usually develops in the advanced stages of AIDS at CD4+ T cell counts below 200/μl, clinicians in endemic areas should be vigilant for signs and symptoms of leishmaniasis so that therapy can be started early.[52]

9. 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. Vaccination

with virulent promastigotes, a practice called 'leishmanization', has been performed on at least 10 000 individuals in USSR, 5000 in Israel and over 200 000 civilians and 1.25 million soldiers in Iran.[89,90] The 'vaccine' produces a lesion in approximately 77% of recipients, which is 5 to 10mm in diameter and lasts an average of 4 to 6 months. In general, inoculated individuals had lesions that were smaller and healed sooner than lesions from non-vaccinated populations. Reduced rates of subsequent infection have been noted in the vaccinated populations. Adverse effects include large, non-healing lesions that persist for years and eventually require treatment, and immediate-type hypersensitivity reactions, which may last for a few hours. In addition, since leishmanization may introduce individuals with an active lesion to non-indigenous areas (soldiers returning home), this practice may theoretically produce new foci of transmission.

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 *Leishmania* homolog of receptors for activated C kinase antigen of *L. major* can induce an IL-12-mediated, protective Th1 response.[91]

The spread of HIV infection and the common use of immunosuppressive drugs make the development of a live vaccine less attractive. One study revealed that live parasites could be recovered from the site of immunization as well as from draining lymph nodes of animals inoculated with promastigotes rendered 'non-pathogenic' via high doses of gamma radiation.[92] A recent phase II study in Colombia of a killed *L. amazonensis* vaccine, using BCG as adjuvant, against CL showed a good safety profile, and a phase III efficacy trial may soon follow.[93] Because cross-species protection in human beings with different species of *Leishmania* is rare, vaccines using the killed etiologic agents of VL are warranted for the immunocompromised patient.

The development of an effective, non-infectious 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.[94] 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, non-toxic therapeutic agents.

10. Conclusion

Leishmaniasis is a fascinating protozoal disease with varied clinical and pathological manifestations. The increasing incidence of *Leishmania* and HIV co-infection during the last 2 decades has focused attention on the unique clinical presentations of leishmaniasis in HIV-infected patients. It is hoped that this excitement will lead to better therapeutic agents to treat, and vaccines to prevent, leishmaniasis in immunocompromised populations.

Acknowledgments

This work was supported by grants from the National Institutes of Health and the Cutaneous Biology Research Center at Massachusetts General Hospital. We thank Ana Maria Roselino, M.D. for figure 2. The authors would like to thank the publishers of the Journal of the American Academy of Dermatology, Mosby Inc., for granting permission to use sections of material previously published in an article in that Journal.

References

1. World Health Organization. Leishmania/HIV co-infection in southwestern Europe 1990-1998: retrospective analysis of 965 cases [online]. Available from URL: http://whqlibdoc.who.int/hq/2000/WHO_LEISH_2000.42.pdf [Accessed 2001 Nov 1]
2. 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 1988; 82: 179-84
3. Lainson R, Shaw JJ. Evolution, classification and geographical distribution. In: Peters W, Killick-Kendrick R, editors. The leishmaniases in biology and medicine. San Diego: Academic Press, 1987: 1-120
4. Lerner EA, Von Lichtenburg FC. Case records of the Massachusetts general hospital: a 21 year old woman with a persistent rash on the elbow after a sojourn in Central America (leishmaniasis). N Engl J Med 1991; 324: 476-85
5. Al-Gindan Y, Kubba R, el-Hassan AM, et al. Dissemination in cutaneous leishmaniasis, 3: lymph node involvement. Int J Dermatol 1989; 28: 248-53
6. Kubba R, Al-Gindan Y, el-Hassan AM, et al. Dissemination in cutaneous leishmaniasis, 2: satellite papules and subcutaneous induration. Int J Dermatol 1988; 27 (10): 702-6
7. Kubba R, El-Hassan AM, Al-Gindan Y, et al. Dissemination in cutaneous leishmaniasis, 1: subcutaneous nodules. Int J Dermatol 1987; 26: 300-4
8. Killick-Kendrick R, Bryceson AD, Peters W, et al. Zoonotic cutaneous 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 1985; 79 (3): 363-5
9. Saravia NG, Weigle K, Segura I, et al. Recurrent lesions in human Leishmania braziliensis infection: reactivation or reinfection? Lancet 1990; 336: 398-402
10. Zijlstra EE, El-Hassan AM, Ismael A, et al. 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 1994; 51 (6): 826-36
11. Rees PH, Kager PA. Visceral leishmaniasis and post kala-azar dermal leishmaniasis. In: Peters W, Killick-Kendrick R, editors. The leishmaniases in biology and medicine. London: Academic Press, 1987: 848-907
12. Abdalla RE, El hadi A, Ahmed MA, et al. Sudan mucosal leishmaniasis. Trans R Soc Trop Med Hyg 1975; 69: 443-9
13. Marsden PD. mucosal leishmaniasis ('espundia' Escomel, 1911). Trans R Soc Trop Med Hyg 1986; 80: 859-76
14. Melby PC, Kreutzer RD, McMahon-Pratt D, et al. Cutaneous leishmaniasis: review of 59 cases seen at the National Institutes of Health. Clin Infect Dis 1992; 15: 924-37
15. Kerdel-Vegas F. American leishmaniasis. Int J Dermatol 1982; 21: 291-303
16. Haldar JP, Ghose S, Saha KC, et al. Cell-mediated immune response in Indian kala-azar and post kala-azar dermal leishmaniasis. Infect Immun 1983; 42: 702-7
17. Berman JD. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin Infect Dis 1997; 24 (4): 684-703

18. 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 1998; 58 (6): 824-7
19. Magill AJ, Grogl M, Gasser RAJ, et al. Visceral infection caused by leishmania tropica in veterans of Operation Desert Storm. N Engl J Med 1993; 328 (19): 1383-7
20. Kurban AK, Malak JA, Farah FS, et al. Histopathology of cutaneous leishmaniasis. Arch Dermatol 1966; 93: 396-401
21. Esterre P, Dedet JP, Fremay C, et al. Cell populations in the lesion of human cutaneous leishmaniasis: a light microscopical, immunohistochemical and ultrastructural study. Virchows Archiv A Pathol Anat 1992; 421: 239-47
22. Lima H, Vasconceles W, David JR, et al. American cutaneous leishmaniasis: in situ characterization of the cellular immune response over time. Am J Trop Med Hyg 1994; 50: 743-7
23. Modlin RL, Pirmez C, Hofman FM, et al. Lymphocytes bearing antigen-specific gd T cell receptors accumulate in human infectious disease lesions. Nature 1989; 339: 544-8
24. Swaminath CS, Shortt HE, Anderson L. Transmission of Indian kala-azar to man by bites of phlebotomus argentipes, ann. and brun. Indian J Med Res 1942; 30: 473-7
25. Norton SA, Frankenburg S, Klaus SN. Cutaneous leishmaniasis acquired during military service in the middle east. Arch Dermatol 1992; 128: 83-7
26. Eltoum IA, Zijlstra EE, Ali MS, et al. Congenital kala-azar and leishmaniasis in the placenta. Am J Trop Med Hyg 1992; 46: 57-62
27. Nanji AA, Greenway DC. Leishmania braziliensis infection of the nipple. BMJ 1985; 290: 433-4
28. Yadav TP, Gupta H, Satteya U, et al. Congenital kala-azar. Ann Trop Med Parasitol 1989; 83: 535-7
29. Blackwell JM. Leishmaniasis epidemiology: all down to the DNA. Parasitology 1992; 104 Suppl.: S19-34
30. Pirmez C, Yamamura M, Uyemura K, et al. Cytokine patterns in the pathogenesis of human leishmaniasis. J Clin Invest 1993; 91 (4): 1390-5
31. 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 1998; 76 (6): 372-90
32. Jones DE, Elloso MM, Scott P. Host susceptibility factors to cutaneous leishmaniasis. Front Biosci 1998; 15 (3): D1171-80
33. Herwaldt BL. Leishmaniasis. Lancet 1999; 354: 1191-9
34. Bardaró R, Johnson WDJ. The role of interferon-g in the treatment of visceral and diffuse cutaneous leishmaniasis. J Inf Dis 1993; 167 (1 Suppl.): S13-7
35. Davidson RN. Practical guide for the treatment of leishmaniasis. Drugs 1998; 56: 1009-18
36. 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 1993; 61 (3): 1069-73
37. Lezama-Davila CM, Isaac-Marquez AP, Padierna-Olivos J, et al. Immunomodulation of Chiclero's ulcer: role of eosinophils, T cells, tumour necrosis factor and interleukin-2. Scand J Immunol 1998; 47 (5): 502-8
38. Assreuy J, Cunha FQ, Epperlein M, et al. Production of nitric oxide and superoxide by activated macrophages and killing of Leishmania major. Eur J Immunol 1994; 24: 672-6
39. Stenger S, Thuring H, Rollinghoff M, et al. Tissue expression of inducible nitric oxide synthase is closely associated with resistance to Leishmania major. J Exp Med 1994; 180: 783-93
40. Mossalayi MD, Arock M, Mazier D, et al. The human immune response during cutaneous leishmaniasis: NO problem. Parasitol Today 1999; 15: 342-5
41. 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 1998; 66: 2698-704
42. Marrano NM, Mata LJ, Durack DT. Cutaneous leishmaniasis in rural Costa Rica. Trans Roy Soc Trop Med Hyg 1989; 83: 340
43. McMahon-Pratt D, Bennett E, David JR. Monoclonal antibodies that distinguish subspecies of Leishmaniasis braziliensis. J Immunol 1982; 129: 926-7
44. Kilgour V, Gardener PJ, Godfrey DG, et al. Demonstration of electrophoretic variation of two aminotransferases in leishmania. Ann Trop Med Parasitol 1974; 68: 245-6
45. 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 1992; 52: 45-58
46. 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 2001; 97 (1): 35-44
47. 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 1998; 36 (8): 2173-7
48. 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 2000; 66 (5): 1933-8
49. Castellano VM, Rodriguez-Peralto JL, Alonso S, et al. Dermatofibroma parasitized by leishmania in HIV infection: a new morphologic expression of dermal Kala Azar in an immunodepressed patient. J Cutan Pathol 1999; 26: 516-9
50. Coutinho SG, Da-Cruz AM, de Oliveira MP, et al. CD4+ and CD8+ T cell immune responses of immunocompetent and immunocompromised (AIDS) patients with American tegumentary leishmaniasis. Mem Inst Oswaldo Cruz 1996; 91: 381-4
51. Gillis D, Klaus S, Schnur LF, et al. Diffusely disseminated cutaneous Leishmania major infection in a child with acquired immunodeficiency syndrome. Pediatr Infect Dis J 1995; 14: 247-9
52. Kubar J, Marty P, Lelievre A, et al. Visceral leishmaniasis in HIV-positive patients: primary infection, reactivation and latent infection. Impact of CD4+ T-lymphocyte counts. AIDS 1998; 12: 2147-53
53. Lachaud L, Dereure J, Chabbert E, et al. Optimized PCR using patient blood samples for diagnosis and follow-up of visceral leishmaniasis, with special reference to AIDS patients. J Clin Microbiol 2000; 38: 236-40
54. Machado ES, Braga MP, 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 1992; 87: 487-92
55. Mattos M, Caiza A, Fernandes O, et al. American cutaneous leishmaniasis associated with HIV infection: report of four cases. J Eur Acad Dermatol Venereol 1998; 10 (3): 218-25
56. Santos-Gomes G, Gomes-Pereira S, Campino L, et al. Performance of immunoblotting in diagnosis of visceral leishmaniasis in human immunodeficiency virus-leishmania sp.-coinfected patients. J Clin Microbiol 2000; 38: 175-8
57. Alrajhi AA, Saleem M, Ibrahim EA, et al. Leishmaniasis of the tongue in a renal transplant recipient. Clin Infect Dis 1998; 27: 1332-3
58. Roustan G, Jiminez JA, Gutierrez-Solar B, et al. Post kala-azar dermal leishmaniasis with mucosal involvement in a kidney transplant recipient: treatment with liposomal amphotericin B. Br J Dermatol 1998; 138: 526-8
59. Lopez-Velez R, Perez-Molina JA, Guerrero A, et al. Clinicoepidemiologic characteristics, prognostic factors, and survival analysis of patients coinfected with human immunodeficiency virus and leishmania, in an area of Madrid, Spain. Am J Trop Med Hyg 1998; 58: 436-43
60. Rosenthal E, Marty P, le Ficoux Y, et al. Clinical manifestations of visceral leishmaniasis associated with HIV infection: a retrospective study of 91 French cases. Ann Trop Med Parasitol 2000; 94: 37-42
61. Sulahian A, Garin YJ, Pratlong F, et al. Experimental pathogenicity of viscerotropic and dermotropic isolates of Leishmania infantum from immunocompromised and immunocompetent patients in a murine model. FEMS Immunol Med Microbiol 1997; 17: 131-8
62. Alvar J, Canavate C, Gutierrez-Solar B, et al. Leishmania and human immunodeficiency virus coinfection: the first 10 years. Clin Microbiol Rev 1997; 10: 298-319

63. 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 1998; 36: 2419-22
64. Agostoni C, Dorigoni N, Malfitano A, et al. Mediterranean leishmaniasis in HIV-infected patients: epidemiological, clinical, and diagnostic features of 22 cases. Infection 1998; 26 (2): 93-9
65. Hall AJ. Report on the 8th European Congress of Clinical Microbiology and Infectious Diseases, Lausanne, Switzerland; 1997 May 25-28. Trop Med Int Health 1997; 2: 719-20
66. le Ficoux 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 1999; 37 (6): 1953-7
67. Otero AC, da Silva VO, Luz KG, et al. Short report: occurrence of Leishmania donovani DNA in donated blood from seroreactive Brazilian blood donors. Am J Trop Med Hyg 2000; 62 (1): 128-31
68. Minodier P, Piarroux R, Gambarelli F, et al. Rapid identification of causative species in patients with Old World leishmaniasis. J Clin Microbiol 1997; 35: 2551-5
69. 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 1995; 131: 197-204
70. 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 2001; 39 (1): 357-61
71. Rosatelli JB, Souza CS, Soares FA, et al. Generalized cutaneous leishmaniasis in acquired immunodeficiency syndrome. J Eur Acad Dermatol Venereol 1998; 10 (3): 229-32
72. Drugs for parasitic infections [online]. Available from URL: www.medicalletter.com/html_files/publicreading.htm#Parisitic [Accessed 2000 Mar 1]
73. Berman JD. Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy, and future strategies. Rev Inf Dis 1988; 10: 560-86
74. Herwaldt BL, Berman JD. Recommendations for treating leishmaniasis with sodium stibogluconate (pentostam) and review of pertinent clinical studies. Am J Top Med Hyg 1992; 46: 296-306
75. Soto-Mancipe J, Grogl M, Berman JD. Evaluation of pentamidine for the treatment of cutaneous leishmaniasis in Colombia. Clin Infect Dis 1993; 16: 417-25
76. Sampaio SA, Castro RM, Dillon NL, et al. Treatment of mucocutaneous (American) leishmaniasis with amphotericin B: report of 70 cases. Int J Dermatol 1971; 10: 179-81
77. Laguna F, Lopez-Velez R, Pulido F, et al. Treatment of visceral leishmaniasis in HIV-infected patients: a randomized trial comparing meglumine antimoniate with amphotericin B. Spanish HIV-Leishmania Study Group. Aids 1999; 13 (9): 1063-9
78. Velez I, Agudelo S, Hendrickx E. Inefficacy of allopurinol as monotherapy for Colombian cutaneous leishmaniasis: a randomized controlled trial. Ann Intern Med 1997; 1 (3): 232-6
79. Navin TR, Arana BA, Arana FE, et al. Placebo-controlled clinical trial of sodium stibogluconate (pentostam) versus ketoconazole for treating cutaneous leishmaniasis in Guatemala. J Infect Dis 1992; 165: 528-34
80. Amato VS, Padilha AR, Nicodemo AC, et al. Use of itraconazole in the treatment of mucocutaneous leishmaniasis: a pilot study. Int J Infect Dis 2000; 4 (3): 153-7
81. Convit J, Castellanos PL, Rondon A, Pinardi ME, Ulrich M, Castes M, et al. Immunotherapy versus chemotherapy in localised cutaneous leishmaniasis. Lancet 1987:1(8530);401-5
82. Levine N. Cutaneous leishmaniasis treated with controlled localized heating. Arch Dermatol 1992; 128: 759-61
83. El-On J, Halevy S, Grunwald MH, et al. Topical treatment of old world cutaneous leishmaniasis caused by Leishmania major: a double-blind control study. J Am Acad Dermatol 1992; 27: 227-31
84. Bassiouny A, Meshad M, Talaat M. Cryosurgery in cutaneous leishmaniasis. Br J Dermatol 1982; 107: 467-74
85. 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 1994; 19: 1034-9
86. Jha T, Sundar S, Thakur C, et al. Miltefosine, and oral agent, for the treatment of Indian visceral leishmaniasis. N Engl J Med 1999; 341: 1795-800
87. Sharquie KE, Najim RA, Farjou IB, et al. Oral zinc sulphate in the treatment of acute cutaneous leishmaniasis. Clin Exp Dermatol 2001; 26 (1): 21-6
88. Delgado J, Macias J, Pineda JA, et al. High frequency of serious side effects from meglumine antimoniate given without an upper limit dose for the treatment of visceral leishmaniasis in human immunodeficiency virus type-1 infected patients. Am J Trop Med Hyg 1999; 61 (5): 766-9
89. Higashi GI. Vaccines for parasitic diseases. Ann Rev Public Health 1988; 9: 483-501
90. Nadim A, Javadian E. Leishmanization in the Islamic Republic of Iran. In: Walton B, Wijeyaretne PM, Modabber F, editors. Research on control strategies for the leishmaniases. Ottawa: International Development Research Center, 1998: 336-9
91. Herwaldt BL, Stokes SL, Juranek DD. American cutaneous leishmaniasis in U.S. travelers. Ann Intern Med 1993; 118 (10): 779-84
92. Rivier D, Shah R, Bovay P. Vaccine development against cutaneous leishmaniasis: subcutaneous administration of radioattenuated parasites protects CBA mice against virulent Leishmania major challenge. Parasite Immunol 1993; 15: 75-84
93. Velez ID, del Pilar Agudelo S, Arbelaez MP, et al. Safety and immunogenicity of a killed Leishmania (L.) amazonensis vaccine against cutaneous leishmaniasis in Colombia: a randomized controlled trial. Trans R Soc Trop Med Hyg 2000; 94 (6): 698-703
94. Valenzuela JG, Belkaid Y, Garfield MK, et al. Toward a defined anti-Leishmania vaccine targeting vector antigens: characterization of a protective salivary protein. J Exp Med 2001; 194: 331-42

Correspondence and offprints: Dr *Ethan A Lerner*, Cutaneous Biology Research Center/Massachusetts General Hospital-East, Building 149, 13th Street, Charlestown, MA, 02120, USA.