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CONTINUING MEDICAL EDUCATION

SOME RECENT OBSERVATIONS IN CUTANEOUS LEISHMANIASIS

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Leishmaniases are a group of protozoal infections caused by many species of the genus *Leishmania* (family *Trypanosomatidae*). They occur in zoonotic cycles. Each cycle has its own animal as reservoir, its own insect vector and its own spectrum of disease. Rarely, transmission occurs from man to man (anthroponotic cycle). Because of mass tourism and influx of refugees and migrant workers, the disease is no longer confined to the known endemic areas. Cases are being seen in all parts of the world. WHO estimates that there are 400,000 new cases of leishmaniasis in the world each year.[1]

Since 1976, when leishmaniasis was included as one of the six diseases in the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, there has been a great deal of new information on the subject. The review highlights those findings that are of special interest to practicing dermatologists.

The Parasite: Leishmania have two morphological forms. In the infected tissues, they are present inside the macrophages as amastigotes or Leishman-Donovan bodies. In the sandfly, they have a flagellum and are called promastigotes. The transformation of the inoculated promastigotes in the skin to amastigotes occurs inside the macrophages and is temperature dependent.[2] Only mature promastigotes with a special surface antigen (m.w. 116, 000 daltons) are infective; immature promastigotes are susceptible to complement mediated cytotoxicity.[3]

The taxonomy of *Leishmania* has greatly expanded in recent years. Many new species have been recognised and the pathogenic role of the established species has been questioned.[4] For example, *L. infantum*, a subspecies of *L. donovani*, is the principal parasite of cutaneous leishmaniasis in Spain, France, Italy, Greece and North Africa.[4] *L. major* and *L. aethiopica* are the main parasites of zoonotic cutaneous leishmaniasis (wet rural type) in the Old World. *L. tropica* causes anthroponotic cutaneous leishmaniasis (dry urban type). *L. mexicana* and *L. braziliensis* are species complexes and cause cutaneous leishmaniasis in the New World. Some subspecies of *L. braziliensis*, namely, *L.b. braziliensis* and *L.b. panamensis* cause mucocutaneous leishmaniasis.

Species identification and differentiation is an area of major thrust in leishmaniasis research. New techniques have become available. The most widely used is starch gel isoenzyme electrophoresis.[5] In this technique, the electrophoretic mobility of upto 13 isoenzymes (malate dehydrogenase, phosphogluconate dehydrogenase and aspartate aminotransferase, etc) generates an enzyme profile that is compared with reference strains.

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Species have been further characterised into zymodemes.[5] Monoclonal antibodies have been successfully utilised to distinguish between the New World species of *Leishmania*.[6] Kinetoplast DNA hybridization is another technique.

Vector and Animal Reservoir: There are some 600 species of sandfly of which some 70 species serve as vectors for leishmaniases. Only the female sandfly is haematophagus and, therefore, the vector, *Phlebotomous papatasi* is the principal vector of zoonotic cutaneous leishmaniasis and *Paraphlebotomous sergentii* for anthroponotic cutaneous leishmaniasis. Information about the habitat, biological behaviour and population dynamics is now available for many endemic foci and is essential in understanding the disease patterns and in formulating the control programmes.

Rodents, especially gerbils, are the main animal reservoir of cutaneous leishmaniasis in the Old World. Domestic animals such as dogs also become infected but comparative pathology has shown that the disease in such instances is more like human disease and is self-healing. In the rodents the disease is non-healing and an infected animal transmits the infection for all its life.

Clinical Features: All leishmaniases are cutaneous infections commencing at the site of parasite inoculation. Many infections terminate sub-clinically and such cases are recognised in epidemiological surveys by the positive leishmanin test. Others develop into self-healing cutaneous leishmaniasis. Many different types of leishmaniases i.e. muco-cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, leishmaniasis recidivans, lymphatic leishmaniasis, visceral leishmaniasis (Kala-azar) and post kala-azar dermal leishmaniasis (PKADL) are sequelae occurring in a small number of patients. The clinical outcome of any infective inoculum depends on the parasite and host factors.

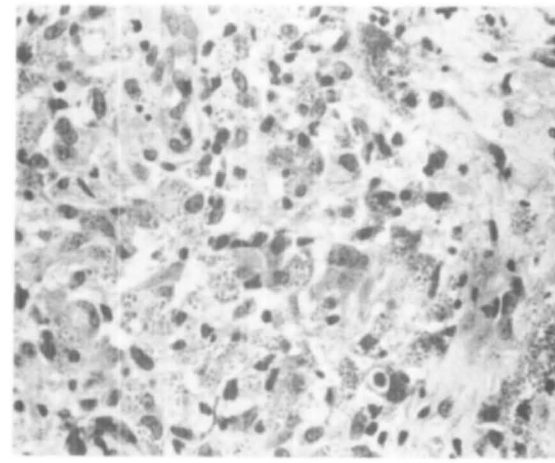

Fig. 1. Heavy load of amastigotes in intact macrophages. The differential diagnosis of parasitized macrophages includes *Histoplasma, Rhinoscleroma* and granuloma inguinale. This pathological picture corresponds with group 1. (H&E X400).

Several phenomena have come to light in recent years. For example, the tropism of *L. major* to skin and of *L. donovani* to the viscera is in part explained by the relative temperature sensitivity of the species concerned. In in vitro studies using human macrophages, it was observed that *L. tropica* (old classification) multiplied best at 35 degree C and was completely eliminated at 39 degree C, whereas *L. donovani* grew well at 35 degree C and 37 degree C and was only partially eliminated at 39 degree C.[8] The amastigotes of *L. donovani* are able to evade complement mediated cytotoxicity and this too facilitates survival and contributes to visceralization.[9] *L. major* and *L. tropica* are dermotropic in man only; in balb/C mice and hamsters respectively, they regularly visceralize.[4]

The generic lesion of cutaneous leishmaniasis is oriental sore. It has a characteristic morphology and lends itself to easy clinical diagnosis.[10] Each lesion represents an infective bite; it evolves slowly from a non-descript insect-bite like lesion into an inflammatory nodule that frequently ulcerates. A

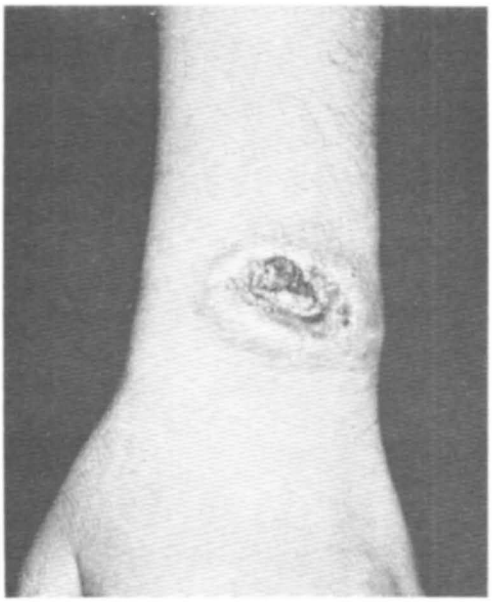

Fig. 2. Typical oriental sore. Note volcanic configuration, exposed site and skin crease orientation.

fully evolved, asymptomatic lesion showing two or more of the following morphological features is diagnostic of cutaneous leishmaniasis: exposed site location, pairing or clustering of lesions, skin crease orientation, volcanic configuration, satellite papules, lymphatic subcutaneous nodules, and iceberg nodules.[10]

Some of the afore-mentioned morphological features are produced by the dissemination of the infection from the site of parasite inoculation i.e. the primary lesion. Subcutaneous nodules representing lymphatic dissemination were seen in 10% of the patients in cutaneous leishmaniasis due to *L. major* in Saudi Arabia.[11] These nodules were always proximal to the primary skin lesion and, when multiple, showed a sporotrichoid configuration or appeared as beaded cords. Only 64% of the lymphatic nodules were positive for amastigotes. The parasite negative nodules, especially those showing vasculitis and increased mast cells, raised the possibility of dissemination of leishmanial antigen rather than viable parasites in some cases. Occurrence of satellite papules, sometimes after anti-leishmanial treatment, also represented dissemination phenomenon.[12] It was suggested that the dissemination in the case of satellite papules was vascular and, further, asymmetrically occurring sparse satellite papules were parasite inhabited and represented dissemination of the parasite, whereas, symmetrically distributed, confluent satellite papules were parasite uninhabited and represented dissemination of parasite antigens only; the two types were named satellite cutaneous leishmaniasis and reactive satellite papules, respectively.[12] Lymmphatic nodules and satellite papules were also noted in Lebanese patients infected in Saudi Arabia.[13] Another manifestation of dissemination is the finding of regional lymphadenitis. This was observed in 8% of patients with cutaneous leishmaniasis in a field study in Saudi Arabia.[14] In the majority, epitrochlear lymph nodes were involved and this was surprising as skin lesions occurred with equal frequency on the upper and lower extremities. The enlarged

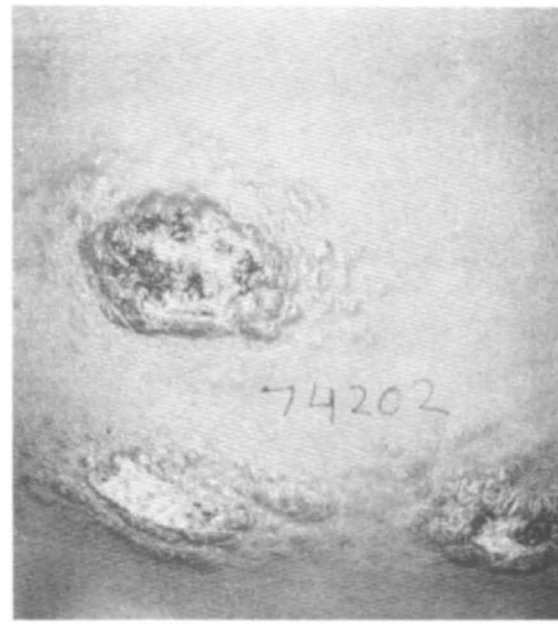

Fig. 3. Reactive satellite papules around clustered, partially healed, cutaneous leishmaniasis lesions over the right lower quadrant of the abdomen.

lymph nodes showed a pathology ranging from non-specific to granulomatous inflammation; amastigotes were only occasionally seen. However, what established the leishmanial nature of these enlarged lymph nodes was the consistent finding of leishmanial antigen with an immunoperoxidase technique. One reason for not finding the amastogotes in the enlarged lymph nodes is the higher temperature in which the dermotropic parasite failed to survive. It was also noted that affected lymph nodes remained enlarged and palpable for periods of several months after clinical resolution of the skin lesions.

Neural involvement may not be unique to leprosy. It may sometime occur in cutaneous leishmaniasis.[15] Assorted neural changes including neuritis were seen in 14 skin biopsies amongst 288 specimens from patients with cutaneous leishmaniasis due to *L. major*.[15] In the same study, sensory testing of 50 consecutive cutaneous leishmaniasis patients identified two patients with diminished sensations over the lesions in whom skin biopsies showed marked perineural inflammation and in one of the two patients presence of amastigotes in the nerve.[15] Further studies are required to confirm this finding. There is, however, an earlier report of a possible neurological manifestation (tremors) in a patient with kala-azar in Kenya.[16]

Leishmaniasis has also gained a place on the list of opportunistic infections occurring in immune-suppressed patients.[17]

Cutaneous leishmaniasis is believed to evoke permanent immunity. This is not entirely true. Reinfection, even with the same strain, can occur in upto 10% of the patients. This was well documented in a recent case report;[18] however, it was also observed that in reinfection, the incubation period was shorter and the disease was milder.

Pathology and Immunopathology: The pathology of cutaneous leishmaniasis shows a heterogeneous range of patterns with some merging. Ridley[2] classified the pathology into 5 groups: Group I is predominantly macrophages with the highest parasite load; Group II is focalised granulomas consisting of macrophages, lymphocytes and plasma cells, central necrosis and moderate number of parasites; Group III is the least specific consisting of mixed inflammatory cells and scanty or absent parasites; Group IV is predominantly lymphocytic with giant cells and occasional parasite in the latter cells; Group V shows epithelioid cell granulomas with Langhans giant cells and only a few plasma cells and lymphocytes and no parasites. Only Groups II and III are associated with necrosis and ulceration of the lesion.[2] In another study,[19] it was noted that the parasite index showed a

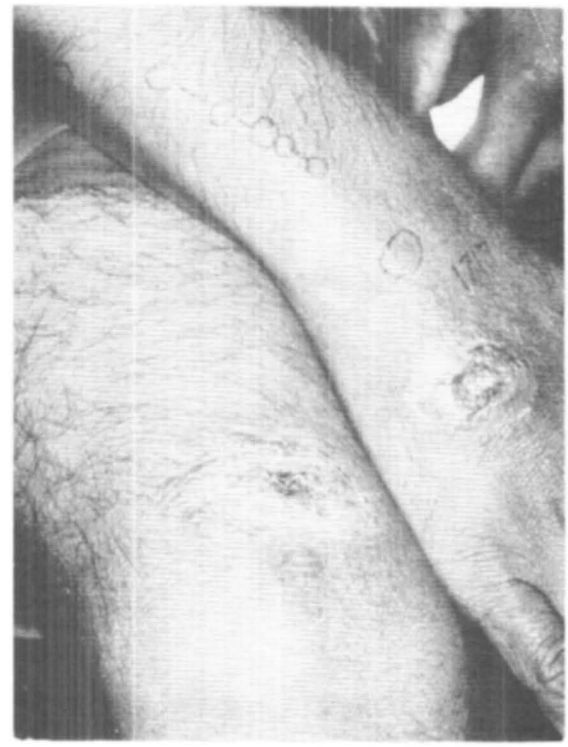

Fig. 4. Lymphatic, subcutaneous nodules proximal to a volcanic nodule on the dorsum of right hand. The nodules showed sporotrichoid configuration but the additional finding of thickened lymphatic suggests a "beaded cord". Another lesion on the knee was not associated with similar nodules.

direct correlation with the number of macrophages, and an inverse correlation with the number of epithelioid cells and lymphocytes; no correlation was noted with plasma cells. A strong epithelioid response with inconspicuous plasma cells is likely to be associated with poor prognosis,[19] whereas necrosis either in the granuloma or in the collagen is associated with good prognosis.[2]

Studies of T-cell phenotypes using monoclonal antibodies have so far yielded conflicting results. Jaroskova et al from Kuwait[20] found reduced number of helper/inducer cells in cases with persistence of cutaneous leishmaniasis, whereas El-Hassan et al from Saudi Arabia[21] found reduced number of suppressor/cytotoxic cells in such cases. One possible explanation for this discrepancy may be in the selection of patients studied. Epidermis is immunologically involved in cutaneous leishmaniasis as shown by positive staining for HLA-DR antigen.[21] Direct immunofluorescence showed the presence of leishmanial antigen on the surface of the keratinocytes.[21] Inflammatory cells in the dermis also expressed HLA-DR antigen.[21]

Immunology: The immunology of leishmaniasis is no less complex than leprosy. There are four main immune mechanisms that operate in varying combinations. Local factors such as complement are concerned with resisting initial infection and containing the disease to the site of inoculation. The terminal membrane attack complex (C5b-C9) mediates the cytotoxicity for the protozoa and is antibody independent.[22] All *Leishmania* species are able to escape this destruction by being able to bind C3 and enhancing their phagocytosis by macrophages bearing C3b receptors.[23]

The second stage of host defence is killing and elimination of intracellular amastigotes. This is achieved in one of two ways: activation of the macrophages and production of an oxidative burst, or by lymphocyte mediated cytotoxicity. Factors such as acid phosphatase produced by *L. donovani* can decrease intracellular killing by blocking peroxidase reaction or decreasing superoxide anions.[24,25] On the other hand, gamma interferon (gIFN)[26] and lymphokines derived from helper/inducer T-cells[27] activate infected macrophages and enhance killing of intracellular parasites. Lymphocyte mediated cytotoxicity requires class II or Ia antigen compatibility and recognition of certain surface antigens on the infected macrophages.[28] Helper/inducer T-cells (Lyt 1+2−) can also cause cytotoxicity by direct contact with the infected macrophages.[29]

Specific antibodies seem to contribute little to elimination of infection. Highest levels of antibodies are seen in non-healing leishmaniases e.g. diffuse cutaneous leishmaniasis and visceral leishmaniasis.[27] However, these may play a role in the prevention of reinfection. In a study of New World cutaneous leishmaniasis, circulating species-specific IgE was found in 48% of the patients and it was postulated that this IgE may prevent reinfection by causing mast cell degranulation and eosinophil chemotaxis.[30]

The status of the immune-response in a given subject can be assessed by a variety of parameters e.g. leishmanin skin test, histopathological findings, in vitro responses to leishmanial antigens, serum antibody and T-cell phenotypes. However, unfortunately, it seems that these immunological parameters are neither indicative of the state of protective immunity nor predictive of the outcome of the infection.[31]

Diagnosis: Demonstration of the parasite in direct smears and skin biopsy remain the easiest and the most convenient methods of diagnosis. The smears are positive in only 50-70% of the patients. These results depend on the age of the lesion i.e. young lesions are more likely to yield the parasite than older lesions. The investigator's skills also influence the results. Some imagination has been applied to smear techniques in recent years. The most notable is the use of a dental nerve broach which is claimed to give 100% positive

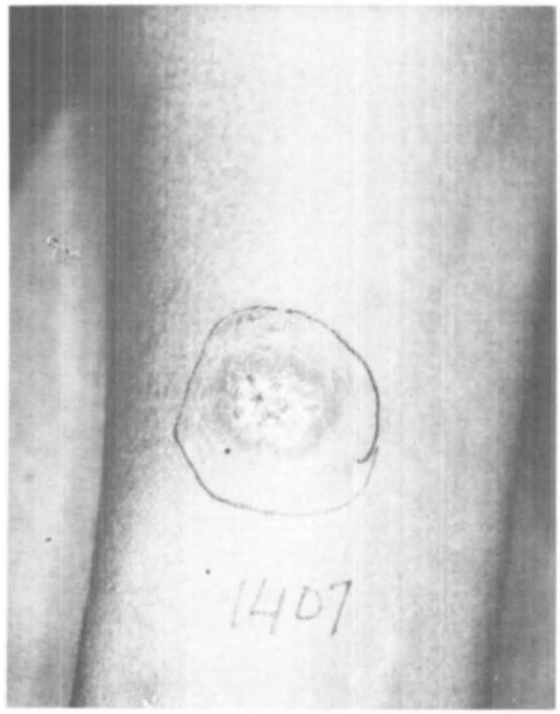

Fig. 5. An example of iceberg nodule. The ink mark around the skin lesion shows the extent of the subcutaneous (endophytic) component. Such lesions are diagnostic of cutaneous leishmaniasis and are unsuitable for local treatment.

results.[32] The dental broach technique is particularly recommended when repeated smears are desired for monitoring parasite positivity.[32] Needle aspiration, sand-papering the surface of the lesion, and the use of sharpened matchsticks[33] are some of the other techniques for obtaining material for smears. The promastigote form of the parasite can be isolated from culture of the infected tissue on NNN (Novy-MacNeal medium, modified by Nicolle) or Schneider's insect culture medium. However, contamination is a major limiting factor and can be overcome by passaging the infected material through the nose of a hamster or the rear foot of a balb/C mouse.[4] A culture is a prerequisite for species identification and large quantities of the isolates are needed for procedures such as isoenzyme electrophoresis. The technique of species identification using the sequence homology of kinetoplast DNA requires fewer than 100 organisms[7] whereas monoclonal antibodies will do the same in tissue sections.[6]

Animal inoculation is an invaluable procedure when the parasite load in the infected tissue is low. Balb/C mouse is best for *L. major* and *L. mexicana*, the golden hamster for all the other species.[34] The period of parasite recovery can be shortened to as little as 3 days by injecting the infected material in the peritoneal cavity instead of the foot.[35]

Leishmanin skin test becomes positive within 1-3 months of onset of the skin lesion in most cases of cutaneous leishmaniasis in the Old World as well as the New World.[34, 36] Visceral leishmaniasis patients are anergic. The antigen consists of washed promastigotes of any species of *Leishmania* suspended in 0.5% phenol saline.[34] An intradermal injection of 0.1 ml of the antigen (containing 1 million organisms) is given along with a control and the result is read at 48-72 hours. A positive result is graded according to the size of the induration on the following scale: grade I: 5-6 mm, grade II: 7-8 mm, grade III: over 8 mm, grade IV: blister.[34] Leishmanin test is positive in some cases of glandular tuberculosis and lepromatous leprosy but not Chagas disease.[34] A positive leishmanin test predicts a more favourable response to antileishmanial treatment.[14]

Serology is valuable mainly in visceral leishmaniasis. Complement fixation test (CFT), haemagglutination test (HI), indirect fluorescent antibody test (IFAT), counter-current immunoelectrophoresis (CIEP), and enzyme-linked immunosorbent test have been successfully employed.[34] In cutaneous leishmaniasis in the Old World, CF is invariably negative but IFAT is positive in the acute state and negative in leishmaniasis recidivans and diffuse cutaneous leishmaniasis.[34] In a study of cutaneous leishmaniasis due to *L. major* in Saudi Arabia, IFAT titres correlated with the number and the size of the lesions.[37] In another study of

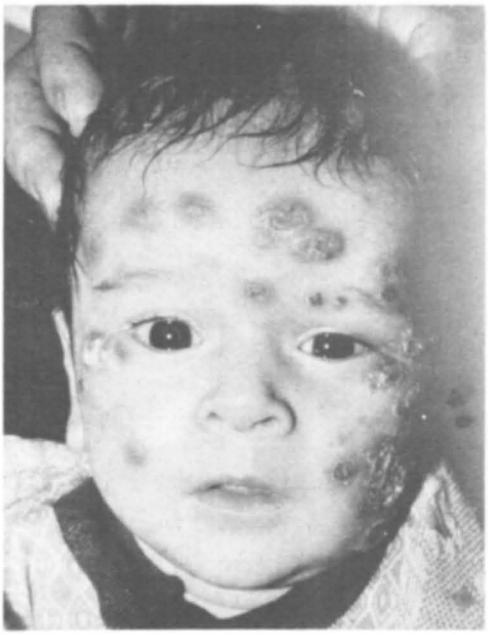

Fig. 6a. A case of fulminant cutaneous leishmaniasis in a Saudi infant. Note multiple crusted and ulcerated nodules showing clustering and skin crease orientation.

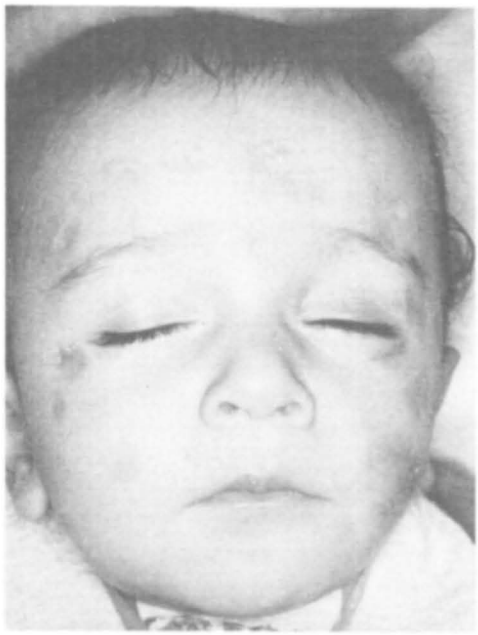

Fig. 6b. The same patient after two courses of sodium stibogluconate. Pentavalent antimonials are the treatment of first choice especially in patients with multiple lesions.

subjects vaccinated with *L. major, L. donovani* antigen yielded better antibody response than *L. major.*[38]

Treatment: The treatment of cutaneous leishmaniasis, unfortunately, remains a bit of a guess work in spite of many new therapeutic studies and voluminous new literature. Pentavalent antimonials i.e. sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), are the best known and most widely used antileishmanial drugs. Pentostam leads the market in the english-speaking world and is recommended as a course of 10-14 days in a dose of 10 mg/kg body weight I.M. or I.V. In resistant cases the course may be repeated after a rest period of 14 days. The mechanism of action of antimonials, until recently, was unknown. It is now postulated that antimonials decrease the viability of *Leishmania* by decreasing the availability of ATP and GTP from ADP and GDP respectively, through inhibition of glycolysis and the citric acid cycle.[39] Antimonials are generally regarded as very toxic. This notion was challenged by a study in which cutaneous leishmaniasis due to *L. aetheopica* was treated with Pentostam 20 mg/kg, twice daily, I.V., for periods of 30 days with mild toxicity.[40] Antimonials are rapidly cleared from the body. After a single I.M. injection, peak levels of 10 mg/litre are reached at 1-2 hours, falling to 1 mg/litre at 8 hours and 0.05 mg/litre at 24 hours.[41] Based on these pharmacokinetics it is being recommended that antimonials be given as two or three divided doses per day.[42]

Ketoconazole, the imidazole antifungal drug, has been shown to have antileishmanial effect in several studies.[43-46] The dosage used ranged from 400 mg to 800 mg in single or divided doses for periods of 4 weeks to 3 months. The mechanism of action is through interference of cell wall biosynthesis; ketoconazole inhibits the 14-alpha demethylation of lanosterol to ergosterol; the ergosterol poor

Leishmania are unable to maintain membrane permeability and succumb.[47] Ketoconazole is well tolerated by children and adults alike, and the idiosyncratic hepatitis associated with it has so far not been encountered in leishmaniasis patients. The efficacy of ketoconazole in leishmaniasis cannot be fully evaluated from published studies. A paediatric preparation (syrup) and an ointment have also become available. Itraconazole, a newer imidazole, has also been evaluated with moderate success in a small study in Venezuela.[48]

There has been renewed interest in applying physical modalities in cutaneous leishmaniasis. Cryosurgery with a carbon dioxide cryomachine using a single freeze technique in zoonotic cutaneous leishmaniasis in Saudi Arabia yielded near perfect results;[49] all patients healed with good cosmetic results in 4-5 weeks, and no complications or relapses were noted. Another study from Israel in which liquid nitrogen was used also gave excellent results (100% success).[50] The efficacy of cryosurgery was questioned in a larger study[51] in which only 24 (27%) out of 88 patients with zoonotic cutaneous leishmaniasis due to *L. major* treated with nitrous oxide cryomachine were cured. In this study,[51] a number of complications were noted, namely, excessive cryo-reaction, patterned depigmentation, and dissemination manifesting as satellite papules and lymphatic subcutaneous nodules. Cryosurgery in Brazil, as an adjunct to chemotherapy in cutaneous leishmaniasis, was also found to be disappointing.[52] Bryceson believes that cryosurgery is biologically unsound.[42] Leishmania are susceptible to heat as well.[8] Local application of heat to achieve a skin temperature of 40-41°C is known to accelerate healing.[41] In a study in Iraq, infra-red heat was used to raise the temperature of cutaneous leishmaniasis lesions to 55°C for 5 minutes; all lesions healed in 5-6 weeks.[53]

Several topical treatments have also been evaluated. A 2% chlorpromazine ointment containing a keratolytic produced parasitological cure within one month in three cases of diffuse cutaneous leishmaniasis in Ethiopia.[54] Although so far not confirmed, there is experimental evidence that phenothiazine neuroleptics and tricyclic antidepressants have leishmanicidal effect.[55, 56] Paromomycin, an aminoglycoside antibiotic, has also figured prominently in the recent literature.[57-60] An ointment containing 15% paromomycin and 12% methylbenzethonium chloride in white soft paraffin (p-ointment) was applied twice daily for 10 days on selected lesions in patients with acute cutaneous leishmaniasis in Isreal.[60] Untreated lesions served as controls. Eighty seven per cent of the patients were clinically cured 10-30 days after completion of the treatment; of these, the majority were parasitologically free at the end of the 10-day treatment period. Treatment induced pigmentation and inflammation in some patients were the only side effects reported.[60] P-ointment was also reported to be effective in two cases of recurrent cutaneous leishmaniasis.[58] The efficacy of paromomycin ointment also awaits confirmation from other centres. Bryceson, in a small personal series, found paromomycin ointment to be unacceptably irritant.[42]

Two diseases with which leishmaniasis is frequently compared are leprosy and tuberculosis and both of these diseases are treated with combination chemotherapy. A burgeoning trend to use drug combinations in leishmaniasis reflects an extension of this comparison. Peters et al[61] reported the potentiating action of rifampicin and isoniazid against *L. mexicana amazonensis* in a patient with diffuse cutaneous leishmaniasis and, subsequently, in experimental infection in mice. A limited evaluation of this combination in cutaneous leishmaniasis due to *L. major* in Israel was considered encouraging and worthy of further evaluation.[62] Rifampicin in combination with intralesional sodium stibogluconate gave good results in cutaneous leishmaniasis in Saudi Arabia.[63] A combination of sodium stibogluconate (600 mg I.M. daily X 14) and ketoconazole (200 mg t.i.d. X

14 days) gave superior results compared to either of the drug alone and without added toxicity.[14] One combination that does not work is ketoconazole with rifampicin or isoniazid because the latter two drugs induce liver microsomal enzymes and thereby lower the serum levels of ketoconazole.[64]

Levamisole,[65] dapsone,[66] intralesional sodium stibogluconate,[67] curettage,[68] and surgery with or without plastic repair,[69] have their advocates.

The dissociation between parasitological cure and clinical cure requires greater awareness. The clinical cure usually takes additional 2-4 weeks. However, the optimal duration of antileishmanial treatment is best linked to the parasitological cure. Rarely, clinical cure has been known to precede parasitological cure.[70,71]

It is now possible, at least in specialised laboratories, to determine the sensitivity of Leishmania to chemotherapeutic agents, using human monocyte cultures. This procedure is beginning to help recognise resistance patterns and explain treatment failures. For a treatment to be successful, not only the parasite has to be susceptible to the chemotherapeutic agent, the host must also cooperate by producing an appropriate immune response.[42]

Vaccination: Protection of the non-immune visitors to the endemic areas and prevention of the disease in residents of such areas should be possible by means of a vaccination. This, unfortunately, is not the situation in leishmaniasis. A safe, commercial vaccine is as yet not available. The experience so far, mainly with cutaneous leishmaniasis in USSR and Israel, has shown that vaccination is possible with vaccines containing live promastigotes.[72] The success rate depends on the size of the inoculum and the virulence of the parasite strain. The protective immunity takes several months to develop. A major problem with currently available vaccines is their short shelf life, and perishability during transportation. In response to this, a frozen vaccine has been developed (promastigotes of *L. major* frozen in 10% glycerine in saline to a temperature of -196 degree C) that remains potent for at least 3 months.[73] It appears that the interest is currently focussed on developing newer vaccines with amastigotes or leishmanial fractions.[72]

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