# Advances in leishmaniasis

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**Governed by parasite and host factors and immunoinflammatory responses, the clinical spectrum of leishmaniasis encompasses subclinical (inapparent), localised (skin lesions), and disseminated infection (cutaneous, mucosal, or visceral). Symptomatic disease is subacute or chronic and diverse in presentation and outcome. Clinical characteristics vary further by endemic region. Despite T-cell-dependent immune responses, which produce asymptomatic and self-healing infection, or appropriate treatment, intracellular infection is probably life-long since targeted cells (tissue macrophages) allow residual parasites to persist. There is an epidemic of cutaneous leishmaniasis in Afghanistan and Pakistan and of visceral infection in India and Sudan. Diagnosis relies on visualising parasites in tissue or serology; culture and detection of parasite DNA are useful in the laboratory. Pentavalent antimony is the conventional treatment; however, resistance of visceral infection in India has spawned new treatment approaches—amphotericin B and its lipid formulations, injectable paromomycin, and oral miltefosine. Despite tangible advances in diagnosis, treatment, and basic scientific research, leishmaniasis is embedded in poverty and neglected. Current obstacles to realistic prevention and proper management include inadequate vector (sandfly) control, no vaccine, and insufficient access to or impetus for developing affordable new drugs.**

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Leishmaniasis has several diverse clinical manifestations: ulcerative skin lesions, destructive mucosal inflammation, and disseminated visceral infection (kala azar). Epidemiology, immunopathology, and outcome are similarly diverse, since infection occurs in multiple endemic regions, in both children and adults, and is caused by nearly two-dozen distinct Leishmania species.[1–3] Variable disease expression has also been shown in naturally infected animals and, especially, experimentally infected animals.[4,5] Nevertheless, all forms of this protozoal infection share three pathogenetic features: resident tissue macrophages are targeted and support intracellular parasite replication; the host immunoinflammatory response regulates expression and outcome of disease; and persistent tissue infection is characteristic.

## Leishmaniases

Sandflies inoculate the skin with flagellated promastigotes, which invade or are phagocytosed by local and immediately recruited host cells, including neutrophils. Within phagolysosomes of resident macrophages, surviving promastigotes transform and replicate as amastigotes (figure 1), which infect additional macrophages either locally or in distant tissues after dissemination. In susceptible patients, local or systemic inflammation develops but is ineffective, and disease is initiated if specific immune mechanisms are not properly established. With incubation periods of weeks to months, the preceding

### Search strategy and selection criteria

We searched PubMed and MEDLINE with several key words—namely, "leishmaniasis"; "cutaneous", "diffuse cutaneous", "mucosal", and "visceral leishmaniasis"; "kala azar" and "post-kala azar dermal leishmaniasis"—for recent clinical and basic science articles related to leishmaniasis, and we used our own files of published work accumulated over years. We paid particular attention to articles primarily published in English since 1999 when leishmaniasis was last reviewed in *The Lancet*.

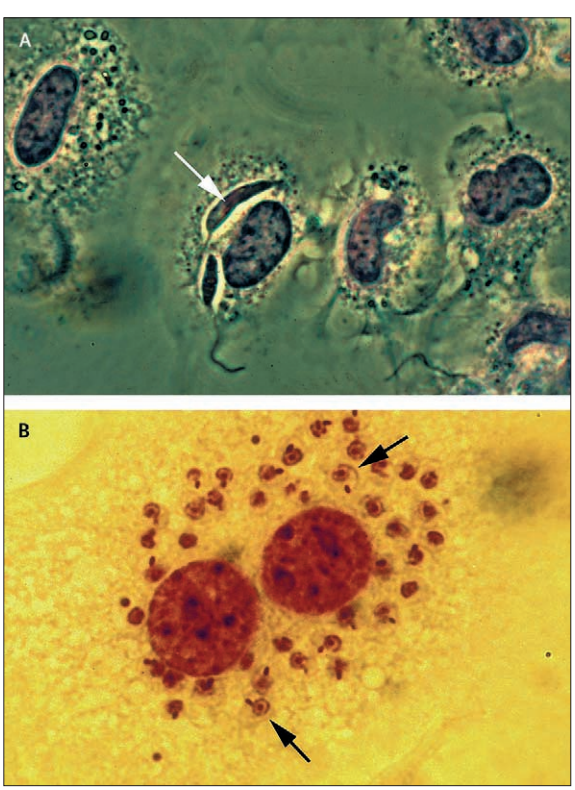


*Figure 1:* **Macrophages infected in vitro with *L donovani***
(A) Ingestion of two flagellated promastigotes (arrow) by human monocyte-derived macrophages. Cells were fixed to show macrophage morphology. (B) Replication of intracellular amastigotes within mouse peritoneal macrophages. Methanol fixation shows characteristic amastigote features (round nucleus, rod-shaped kinetoplast; arrows) seen in clinical specimens. Original magnification, ×500.

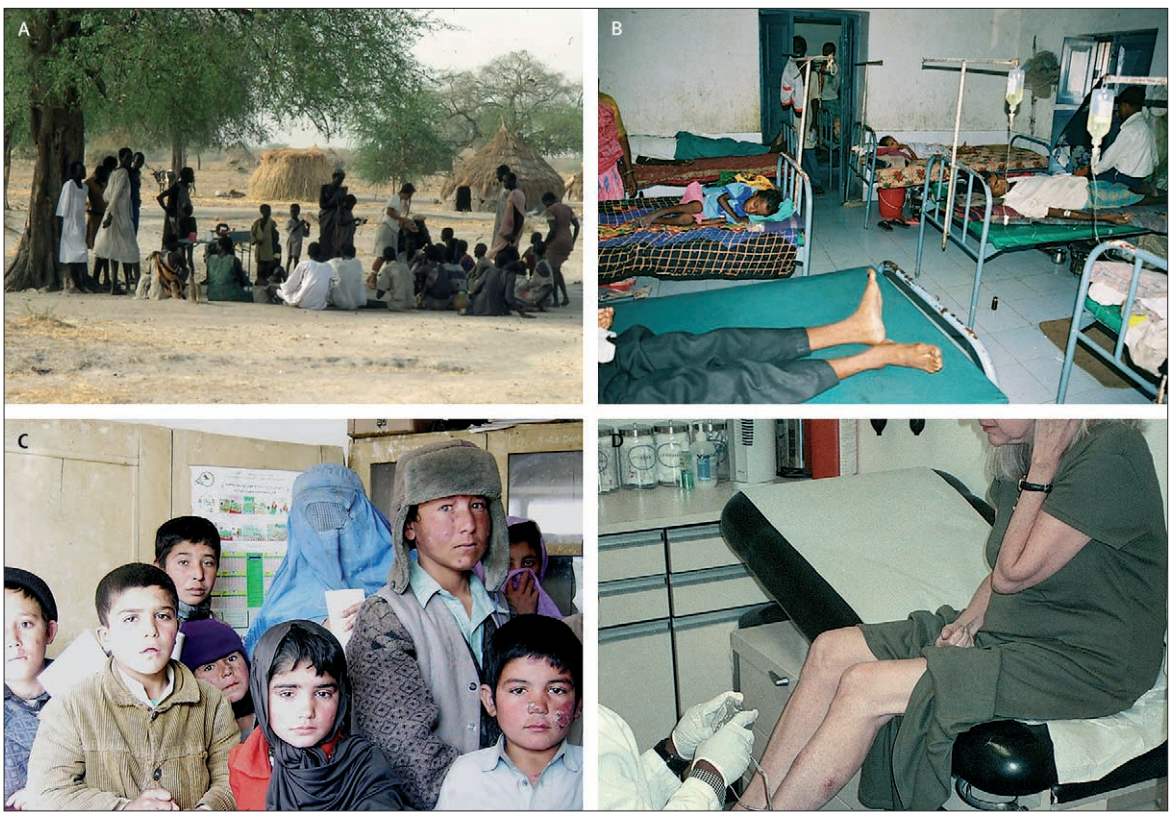


***Figure 2:*** **Leishmaniasis in Sudan, India, Afghanistan, and the USA**
(A) Kala azar clinic in Duar, Sudan, at Médecins Sans Frontières unit (courtesy of R Wilkinson). (B) Ward at Kala azar Medical Research Centre in Muzaffarpur, Bihar State, India (courtesy of S Sundar). (C) Cutaneous infection in Kabul, Afghanistan; school children with facial lesions (courtesy of R Reithinger). (D) Cutaneous infection (both legs) in a contrasting setting (private physician's office in New York City, USA) in a traveller returned from Israel.

events, which culminate in the expression of clinical disease, unfold slowly.[6]

Every year, an estimated 1·5–2 million children and adults develop symptomatic disease (cutaneous 1–1·5 million; visceral 0·5 million), and the incidence of infection is substantial when subclinical infections are included.[1] Leishmaniasis is associated with about 2·4 million disability-adjusted life years and around 70 000 deaths per year.[1] 90% of cutaneous leishmaniasis infections develop in Afghanistan, Pakistan, Syria, Saudi Arabia, Algeria, Iran, Brazil, and Peru; 90% of visceral leishmaniasis occurs in India, Bangladesh, Nepal, Sudan, and Brazil (figure 2 and figure 3).[1] In view of this geography, leishmaniasis remains embedded in poverty as a neglected disease.[7] Except in southern Europe, bare-bones national health services in endemic regions block access to ready diagnosis, affordable treatments, and effective disease control. With little prospect for financial return, antileishmanial drug development remains stalled.[8]

Nevertheless, and despite such obstacles, the past 5 years have produced tangible progress in diagnosis, treatment, and vector control.[9–12] Research has increased understanding of susceptibility, disease expression,[13] and of the host-parasite relationship; antileishmanial immunity has been more precisely clarified; and a vaccine is in development.[14,15] The genome sequence of both *Leishmania major*[16,17] and the sandfly vector *Lutzomyia longipalpis*[18] have also recently been completed. These accomplishments will clearly advance opportunities to address basic, incompletely understood aspects of parasite biology, transmission, and pathogenesis, and could yield new targets for diagnostic assays, drugs, or vaccines.

## Intrinsically diverse spectrum of disease

Parasite properties (infectivity, pathogenicity, virulence)[19] and host factors and host responses regulate heterogeneous disease expression and clinical manifestations; expression and manifestations vary still further by parasite species and endemic region. Four selected examples illustrate some of the basic diversity in leishmaniasis.

### Subclinical infection

Leishmania infections, especially those caused by viscerotropic species, can remain entirely asymptomatic.[1,8,19–22] This observation draws attention to host factors in susceptibility and disease expression—age, nutritional

state, and efficacy of innate and timely acquired T-cell-dependent immune responses.[23–28] The latter can in turn be affected by immunogenetic polymorphisms.[13,29]

### Localised versus disseminated infection

Overall, most clinically apparent leishmanial infections remain localised in the skin or adjacent lymph nodes. Nonetheless, certain species escape to nasal and oropharyngeal mucosa, multiple cutaneous sites, or to liver, spleen, bone marrow, and distant lymph nodes (kala azar). Dissemination points mainly, but not exclusively, to differing parasite properties—temperature sensitivity,[30] tissue tropism,[31] and capacity for immunoevasion and persistence.[32–34] How leishmania parasites enter the bloodstream is not well understood. Amastigotes circulate in active visceral leishmaniasis[35] and parasitaemia is frequent in immunodeficient patients with HIV-associated kala azar.[36] Paradoxically, however, asymptomatic parasitaemia also occurs in immunocompetent individuals with subclinical visceral infection.[21,22]

### Host response to infection

T-cell responses and cytokine-induced macrophage activation, determinants of cell-mediated immunity, disease expression, and parasite burden, are also particularly variable (figure 4). Delayed-type hypersensitivity (positive leishmanin skin test), antigen-specific T-cell reactivity, and activating cytokine secretion are, for example, maintained in asymptomatic infection and localised cutaneous leishmaniasis,[19,27,28,37–40] present but ineffective in visceral leishmaniasis and post kala azar dermal leishmaniasis (PKDL),[39,41–43] and exuberant but apparently pathological in mucosal infection.[44,45]

### Regional variations

Basic clinical features in both cutaneous leishmaniasis and visceral leishmaniasis also vary substantially by, and even within, endemic regions, probably indicating an interaction between local parasite properties, vector biology, and host factors. Regional variations include: population primarily targeted (young children *vs* adolescents and adults), mode of transmission (zoonotic *vs* anthroponotic), particular clinical findings and syndromes, probability and pace of self-healing, and risk of recurrence. Treatment approaches and responses to chemotherapy also vary by region. Thus, diversity in both cutaneous leishmaniasis and visceral leishmaniasis often makes further clinical subcategorisation by endemic region necessary.[1,2]


***Figure 3:*** **Leishmaniasis treatment in Sudan, India, and Afghanistan**
Duar, Sudan, kala azar treatment at Médecins Sans Frontières unit: (A) preparing to treat dozens of patients with pentavalent antimony (courtesy of Barbara Herwaldt), and (B) four clinical trial participants receiving liposomal amphotericin B with infusion bags hung from a tree (courtesy of R Wilkinson). (C) Bihar State, India, wage-earning head of household with kala azar closely attended by dependent mother, wife, and children during 30 days of amphotericin B treatment. (D) Kabul, Afghanistan, intralesional pentavalent antimony treatment for cutaneous infection (courtesy of R Reithinger).

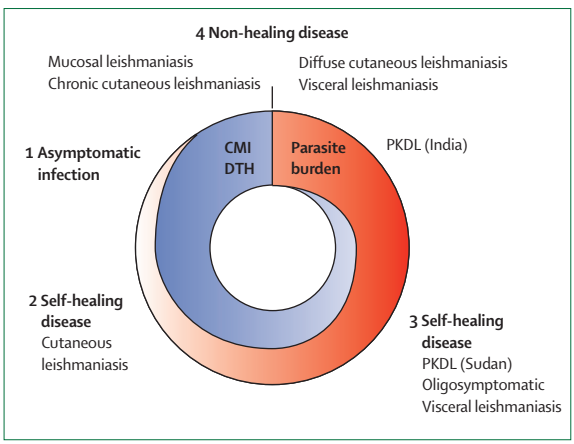


***Figure 4:*** **Dynamic relation in the clinical spectrum of human leishmaniasis between effective cell-mediated immunity (CMI), delayed-type hypersensitivity (DTH), and parasite burden**
Starting with (1) asymptomatic infection (vigorous but not pathologic CMI/DTH; low parasite burden) and moving counterclockwise, CMI/DTH and parasite burden are inversely related. At end of spectrum (4), however, non-healing chronic disease is produced differently, by either: (a) uncontrolled infection because of absent (diffuse cutaneous leishmaniasis) or ineffective CMI/DTH (visceral leishmaniasis, PKDL in India), or (b) unrestrained CMI/DTH and pathologic inflammation despite low parasite burden (mucosal leishmaniasis, chronic cutaneous leishmaniasis). Self-healing disease, associated with cutaneous (2) or visceral infection (3), is shown at separate points since clinical disease is different and initially CMI/DTH is less well-developed and parasite burden higher in (3). In this scheme, reactivation (not shown) of subclinical (asymptomatic), self-healing, or successfully-treated infection would be associated with impaired CMI/DTH and high parasite burden.

## Basic similarities

Fortunately, shared features help in understanding pathogenesis, host defence, and problems of chronic persistence and reactivation, and in devising new approaches to treatment and, hopefully, prevention by vaccination. In the leishmaniases the portal of entry is the same, the tissue macrophage is the target, inflammation and macrophage-activating immunity regulate disease expression and initial outcome, and parasite persistence is probably the rule (eg, sterile immunity is not achieved) despite spontaneous or treatment-induced clinical resolution.[11,21,22,,34,46–49] Intracellular quiescence, including in normal-appearing skin, produces a life-long, double-edged effect—apparent immunity to reinfection in most, but not all,[19] instances and a ready source for recrudescence if protective T-cell mechanisms fail.[11,36,49,50]

## Transmission and epidemiology

Female sandflies (*Phlebotomus* and *Lutzomyia* spp) seek a bloodmeal at or after dusk, becoming infected if they suck the blood of infected human beings (anthroponoses) or terrestrial mammals (zoonoses). Imbibed amastigotes transform in the sandfly gut and replicate as promastigotes; at a subsequent bloodmeal, metacyclic promastigotes are regurgitated[51] and injected into the skin to complete the cycle. About 70 of around 1000 known sandfly species transmit leishmaniasis. Vector competence in most species seems to be controlled by parasite ability to resist proteolytic enzymes during bloodmeal digestion and avoid excretion by binding to midgut epithelium. Binding is mediated by promastigote surface lipophosphoglycan and the phosphoglycan domains differ between species.[52] Sandfly saliva affects local host immune responses, promoting experimental cutaneous infection;[49] conversely, bites of uninfected sandflies or vaccination with salivary protein induce protection against experimental challenge.[52] Although T-cell-dependent responses to salivary antigens probably mediate such protection, the presence of anti-saliva antibodies suggests recent exposure to sandflies and therefore risk of infection. Anti-saliva antibodies are detectable in infected human beings.[53] Occasionally, sandflies are not involved in transmission. Visceral leishmaniasis can be directly initiated by amastigotes via blood (shared needles, transfusion, transplacental spread) or organ transplantation;[54-56] cutaneous infection can develop after inadvertent needlestick if the needle or syringe contains infected material.

Risk of acquiring infection is determined by local sandfly behaviour and by the presence of an infected animal or human reservoir. Subclinically infected human beings are less likely to transmit infection.[57] The global burden of leishmaniasis has remained stable, but risk patterns have changed.[1] There is a continuing urbanisation of zoonotic visceral leishmaniasis—*Leishmania (Leishmania) chagasi* identical to *L (L) infantum*—and domestic transmission of zoonotic cutaneous leishmaniasis—*L (Vianna) braziliensis*, *L (L) mexicana*—in Latin America;[2] an epidemic of anthroponotic cutaneous leishmaniasis—*L (L) tropica*—in west Asia[58] and visceral leishmaniasis—*L (L) donovani*—in east Africa (associated with mass movement of non-immune persons);[59,60] and increasing HIV-visceral leishmaniasis co-infection worldwide,[61] except in southern Europe where highly active antiretroviral therapy (HAART) is available and cases of co-infection have declined.[2,62]

## Pathogenesis and host defence

Parasite factors and host mechanisms are inextricably linked in pathogenesis. To initially establish infection, promastigotes enter macrophages silently to evade triggering host responses;[33,34] progressive intracellular (amastigote) infection depends on the maintenance of macrophages in an inert, deactivated state.[5,6,34] At the same time, however, the immunocompetent host is also equipped and responds with interdigitating non-specific (innate) and antigen-specific (acquired) mechanisms (cell-mediated immunity, delayed-type hypersensitivity). These inflammatory responses mediate disease expression and may (asymptomatic infection, self-healing disease) or may not (non-healing disease) produce the desired clinical end-result (figure 4).

Under optimum conditions, macrophages are eventually activated to a leishmanicidal state largely

governed by an intact T-helper cell-type 1 (Th1) response. This complex response revolves around antigen-presenting dendritic cells, responding CD4+ T cells, and secretion of pro-inflammatory cytokines, including interleukin 12, interferon γ, and tumour necrosis factor.[5,11,34,49,63] This same Th1 response also prevents recrudescence of latent, chronic infection. In subclinical infections, host responses are by definition effective and presumably tightly regulated since signs of inflammation are not noticeable. The alternative, that many human infections are caused largely by avirulent species, seems unlikely. By contrast, inflammation is prominent and underlies pathogenesis in nearly all forms of clinically apparent infection; thus, some tissue injury (eg, ulcerative skin lesions) is inevitable at sites of infection.

## Parasite effects and factors

Depending on parasite stage and species, leishmania can evade humoral innate defences, remodel intracellular compartments and pathways, and impair macrophage and dendritic cell mechanisms.[5,33,34] Infection manipulates intracellular kinases and phosphatases, downregulates activating-type signalling pathways, upregulates suppressive-type signalling pathways, and affects transcription factors and gene expression.[5,33,34,64–66] In turn, macrophage responsiveness to and secretion of cytokines, surface molecule expression, and generation of leishmanicidal mechanisms (reactive oxygen and nitrogen intermediates) are compromised.[34,35,66,67] Parasite effects extend to dendritic cells, which are critical to antigen presentation, T-cell co-stimulation, and efficient development of acquired Th1 responses.[49,68] Effects on dendritic cells include inhibition of migration, maturation and activation, and interleukin 12 production.[33,68] Lipophosphoglycan, which interferes with macrophage and dendritic cell function,[5,33,34,69] and surface membrane metalloprotease gp63, represent two promastigote virulence factors. gp63 protects against innate complement-mediated lysis and enables entry into macrophages.[70] In amastigotes, A2 gene locus products promote *L donovani* infectivity and visceralisation,[31] and cysteine proteases in *L mexicana* impede protective Th1 immunity by targeting interleukin 12.[71] Leishmania homologue of activated C kinase receptor (LACK) can induce suppressive (Th2) CD4+ cell responses and is essential in establishing experimental *L major* infection.[72] Amastigotes also survive within and have thus adapted to the acidic, hydrolytic milieu of the macrophage phagolysosome.

## Host responses

Acting in tandem, innate and acquired immune responses dictate overall outcome of infection, including spontaneous healing and prevention of reactivation,[5,11,34,49,73] and determine responses to chemotherapy.[11,41,73–75] At sites of infection, complex innate responses include multiple factors:[76] cells (neutrophils, monocytes, natural killer cells, macrophages, dendritic cells); recognition receptor mechanisms (eg, toll-like receptors);[68] and soluble products (complement, released cytokines including interleukin 1α, interleukin 12, TNF).[33] If provoked, specific humoral antibody responses are not protective. Antileishmanial immunoglobulin G titres are highest in chronic, non-healing disease, especially in visceral leishmaniasis, and do not prevent reactivation.[19,36,50,77,78]

### *Acquired pro-host defence responses*

Innate mechanisms, especially interleukin 12 secretion,[76,79] help to drive the parallel induction of cell-mediated immunity.[5,49] This similarly complex set of mechanisms, largely initiated and orchestrated by migrating parasitised dendritic cells,[68] is expressed by activation of CD4+ and CD8+ cells.[5,49,63] These effector T cells circulate, are recruited to cutaneous or visceral sites via adhesion molecule and chemokine mechanisms, and, along with influxing blood monocytes, direct local inflammatory responses, including granuloma assembly and lesion development.[5,34,49,63,80] Predominant Th1-type CD4+ cell responses are associated with interferon γ-induced macrophage activation, indicating a network of pleiotropic cytokines in which interleukin 12 shapes the basic response and interleukin 2, TNF, and other cytokines participate.[5,34,63,80] Paradoxically, experimental data suggest that this inflammatory response could also foster cutaneous infection under certain conditions. CD8+ T cells also produce interferon γ and promote CD4+ Th1 cell development and disease healing; CD8+ cells and memory CD4+ cells also regulate resistance to reinfection and vaccine-induced immunity.[5,34,80,81]

### *Downregulating responses*

In any inflammatory environment, counterbalancing mechanisms are normally produced to curtail the process. Among several such mechanisms in leishmaniasis,[5,82] generation and effects of suppressive-acting cytokines are especially well recognised. Interleukin 4, interleukin 10, and interleukin 13 (Th2 cell-associated cytokines) and transforming growth factor β (TGFβ) are capable of derailing Th1-type responses and deactivating macrophages,[5,34,49,73,80] thereby moderating tissue injury but promoting intracellular infection. Although polarised Th1 and Th2 responses can be produced in animals and related to resistance and susceptibility, respectively,[5,49,82] Th1 and Th2 independent pathways also modify experimental disease expression.[83] In patients with clinically apparent infections, Th1 and Th2 type responses are not characteristically polarised, as both activating (eg, interferon γ, interleukin 12) and suppressive cytokines (eg, interleukin 10, interleukin 13, interleukin 4, TGFβ) are detected.[27,28,37-44,73,84–87] Thus, perhaps affected in some patients by genetic factors,[13,29]

progressive non-healing infection (eg, visceral leishmaniasis, PKDL, chronic cutaneous leishmaniasis) seems more likely to indicate a net suppressive-type response (eg, Th2>Th1) rather than an inert Th1 response; under either condition, cell-mediated immunity would be ineffective. Nevertheless, such a net suppressive response does not extinguish persistent inflammation, the hallmark of clinically apparent leishmaniasis.

Both experimental[47,88] and clinical data[39-42,75,84] support a relevant pathogenetic role for interleukin 10, especially in visceral leishmaniasis and PKDL, and cytokine balance (eg, interferon γ to interleukin 10 ratio) can affect both clinical outcome and responses to treatment.[39-42,75,84] Experimentally targeting interleukin 10 for therapeutic inhibition allows activation of Th1 cell responses, promoting parasite killing and synergy with chemotherapy in acute infection.[88] The same experimental approach eliminates persistent tissue infection maintained by interleukin 10-producing natural CD4+ CD25+ or inducible T-regulatory cells.[47,82] The latter can also maintain persistent infection by secreting TGFβ.[82]

### Immunotherapeutic interventions

To harness endogenous host mechanisms, immunointervention with or without chemotherapy has been well tested experimentally and used clinically, but mostly in limited settings—eg, pilot or small clinical trials.[11,73] One exception is immunostimulation by injections of killed promastigotes plus BCG. This treatment, used in cutaneous leishmaniasis in South America, seems to promote healing even in diffuse cutaneous and mucosal leishmaniasis, the immunologically hyporesponsive and hyper-responsive poles of cutaneous leishmaniasis, respectively.[89] In visceral, cutaneous, or mucosal leishmaniasis other approaches have included use of activating cytokines (interferon γ, interleukin 2, granulocyte-macrophage colony-stimulating factor),[11,73,90] inhibition of TNF-induced inflammation,[44,91] and topical immunomodulators.[92]

## Cutaneous and mucosal disease

### Clinical spectrum

Multiple species produce cutaneous leishmaniasis in children and adults, primarily *L major*, *L tropica*, and *L (L) aethiopica* (old world cutaneous leishmaniasis); *L infantum and L chagasi* (Mediterranean and Caspian sea regions); and *L mexicana*, *L (L) amazonenesis*, *L braziliensis*, *L (V) panamensis*, *L (V) peruviana*, and *L (V) guyanensis* (new world cutaneous leishmaniasis). A papule typically begins at the sandfly bite, enlarges to a nodule, and ulcerates over 1–3 months (figure 5).[93–95] Flat plaques or hyperkeratotic or wart-like lesions also develop in old world disease. Patients, including travellers and military personnel,[95–97] first seek attention because of one to two, or sometimes several (up to dozens) non-healing skin lesions on nocturnally exposed skin. In old world cutaneous leishmaniasis, most lesions are papules, nodules, or nodule-ulcers,[93] whereas ulcerative lesions are most common in new world cutaneous leishmaniasis.[98] Disseminated lesions[99] and localised lymphadenopathy preceding skin ulcers[100] occur in Brazil. HIV-associated cutaneous leishmaniasis has been infrequent thus far,[101,102] but prevalence will probably increase in the future.

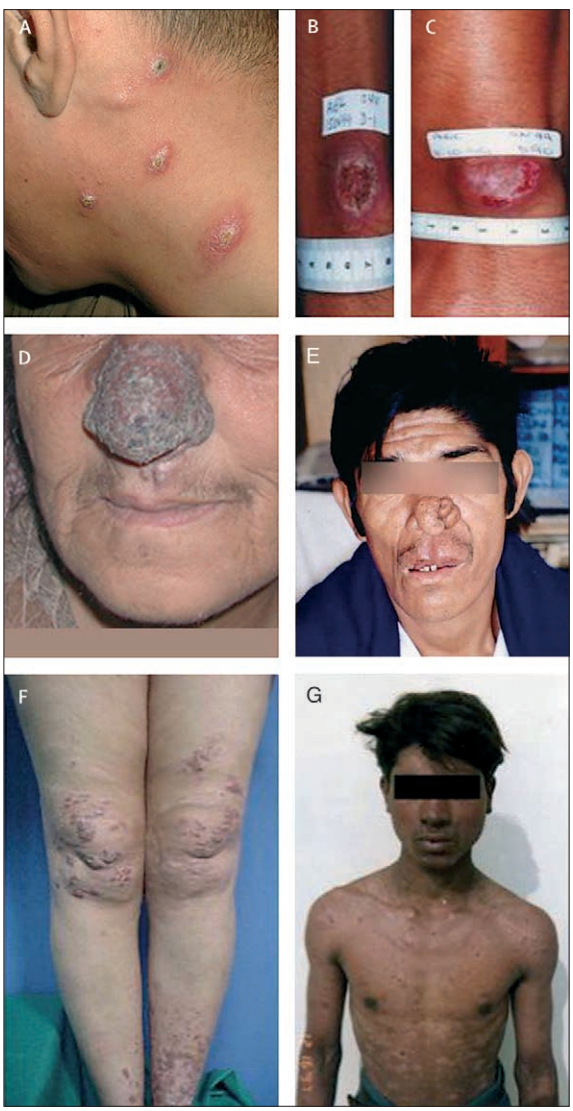


***Figure 5:*** **Cutaneous and mucosal disease**
(A) Old world infection (*L major*) acquired in Iraq; note five papular and nodular lesions on neck (courtesy of P Weina). (B) New world infection (*L panamensis*) in Colombia; purely ulcerative lesion is characteristic of new world disease (courtesy of J Soto). (C) Healed infection in patient shown in (B) 70 days after 20 days of meglumine antimoniate treatment; note paper-thin scar tissue over flat re-epithelialised skin. (D) Old world (*L tropica*) infection in Afghanistan with extensive hyperkeratotic plaque (courtesy of R Reithinger). (E) Destructive mucosal disease (presumed *L braziliensis*) in Peru (courtesy of R Reithinger). (F) Diffuse cutaneous disease (*L panamensis*) in Venezuela with mutliple nodular lesions (courtesy of J Convit). (G) Post kala azar dermal leishmaniasis in India (*L donovani*) with papular-nodular lesions over face, chest, and arms (courtesy of S Bhattacharya).

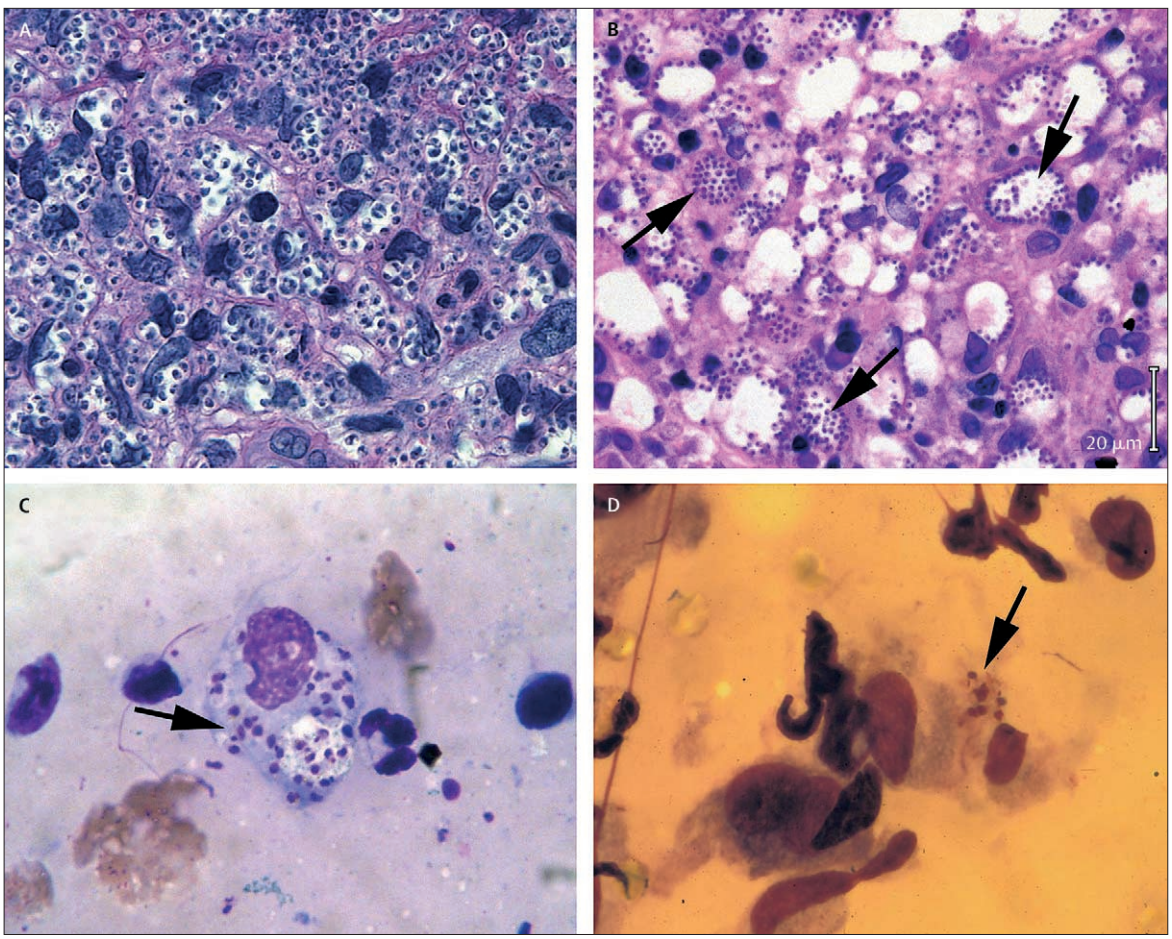


***Figure 6:*** **Histological diagnosis of cutaneous leishmaniasis in haematoxylin-stained and eosin-stained skin lesion biopsies and Giemsa-stained lesion smears**
Arrows indicate amastigotes. Biopsies show sheets of amastigotes in (A) *L major* (courtesy of H Bishop) and (B) *L mexicana* infection (courtesy of S McNutt); in (B), note large, parasite-laden macrophage vacuoles seen in *L mexicana* infection. (C) Smear of lesion scraping in probable *L panamensis* infection (courtesy of CIDIEM image library). (D) Impression smear in *L mexicana* infection obtained by pressing glass slide to open ear lesion. Original magnification, ×500, except in (A), ×1000.

*L tropica* infection disseminates in that new papules can appear around a healed lesion (leishmaniasis recidivans). Mucosal dissemination of new world species (*L braziliensis*, *L panamensis*, *L guyanensis*) occurs in 1–10% of infections, developing 1–5 years after cutaneous leishmaniasis has healed, but sometimes coincident with active skin lesions; about 90% of patients have a preceding cutaneous scar.[19,26,96] Mucosal leishmaniasis begins with erythema and ulcerations at the nares, proceeding to nasal septum perforation and destructive inflammatory lesions. The latter can obstruct the pharynx or larynx and produce remarkable disfigurement (figure 5, E).[103] Mucosal disease is occasionally reported outside of Latin America,[78,104] and can be acquired by travellers.[105] In diffuse cutaneous leishmaniasis, seen rarely in Ethiopia and Latin America, parasite-laden nodules are widespread and do not ulcerate (figure 5, F).[106,107] In PKDL, a disease of unclear pathogenesis that occurs in east Africa and the Indian subcontinent (*L donovani*), a maculopapular-nodular eruption is often first localised around the mouth and then becomes generalised (figure 5, G).[43] In Sudan, PKDL manifests in about half of patients within 0–6 months of visceral leishmaniasis diagnosis, but self-cures within 12 months and usually needs no additional treatment.[43] In India, PKDL develops in 5–10% of patients several years after apparently successful treatment for visceral leishmaniasis and needs lengthy retreatment.[43]

## Diagnosis

In cutaneous leishmaniasis, serum antileishmanial antibody can be detected with standardised and sensitive assays;[108] however, in practice,[10] diagnosis is made microscopically by identification of amastigotes in biopsies, scrapings, or impression smears (figure 6).[3] Material from the ulcer base usually has the highest yield.[97,109] Combination of microscopy and culture increases diagnostic sensitivity to more than 85%,[96,109] and culture (or DNA analysis) allows species identification.[10,97] Detection of parasite DNA in lesion material by PCR is

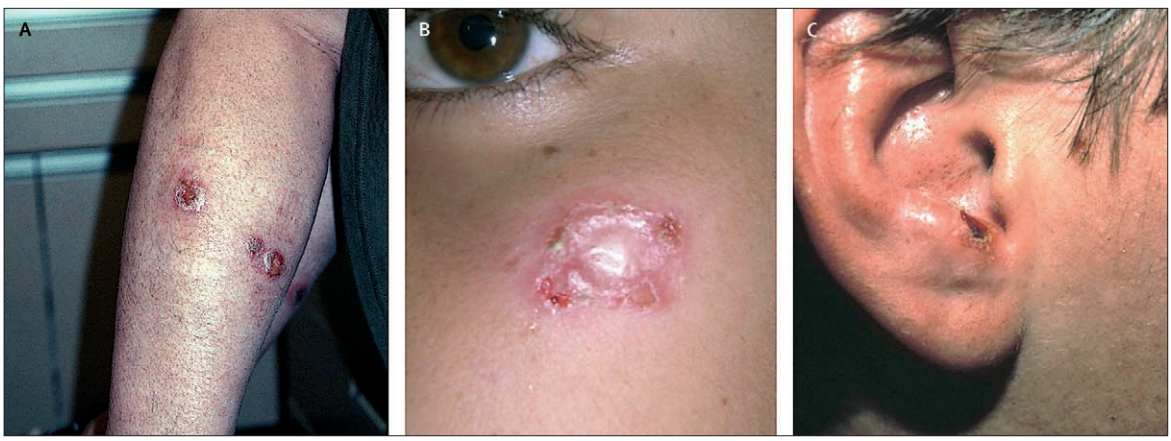


***Figure 7:*** **Cosmetically important cutaneous lesions for which treatment is nearly always requested or given**
(A) Multiple lesions (*L major*, Israel) on both legs of woman shown in figure 2, D (courtesy of P Smith). See biopsy result in figure 6, A. (B) Large lesion (probably *L panamensis*, Colombia) on young girl's face (courtesy of J Villaneuva). See scraping result in figure 6, C. (C) Ear lesion ("chiclero ulcer"; *L mexicana,* Belize, in a travelling US college student. See imprint result in figure 6, D.

usually most sensitive in the diagnosis of both cutaneous and mucosal leishmaniasis,[10,97,110–112] but is seldom the only test producing positive results.[97,112] Culture and PCR testing are technically difficult laboratory techniques that are not currently practical in developing countries.

## Treatment

Cutaneous disease heals by re-epithelialisation with scarring (figure 5, C). Most old world lesions self-cure within 2–4 months (*L major*)[93,113–115] or 6–15 months (*L tropica*).[93,96] In new world cutaneous leishmaniasis, self-healing after 3 months is rapid in *L mexicana* (>75%),[116] but slow in *L braziliensis* (about 10%)[117] and *L panamensis* infections (about 35%).[118–120]

Cutaneous leishmaniasis is treated to accelerate cure, reduce scarring especially at cosmetic sites (figure 7), and to attempt to prevent dissemination (eg, mucosal disease) or relapse. Treatment is especially likely to be given for persistent lesions (≥6 months) or lesions that are located over joints, multiple (5–10 or more), or large (4–5 cm or more).[96,97] Parenteral antimony would probably be successful in cutaneous leishmaniasis in all regions (table 1); however, up to 20 daily injections of a moderately toxic drug (table 2) approximates the

| | Species | Treatment regimen* | Cure rate at 3 months | Reference |
|---|---|---|---|---|
| **Cutaneous disease** | | | | |
| Old world | *L major* | Observation alone | 60–70% | 93,113–115 |
| | | Sb: IL ≥1 mL per lesion; qod×8–15 injections or every 1–2 wk×3–8 injections | ≥75% | 121–123 |
| | | Sb: IM/IV 20 mg per kg per day ×10 days | In evaluation | |
| | | Fluconazole: oral 200 mg once-daily ×6 weeks | 90% | 115 |
| | | Paromomycin: topical, bid×2 weeks | In evaluation | |
| | *L tropica* | Sb: IL (as above) weekly×8–11 (average) | ≥90%† | 124 |
| | | Sb: IM/IV 20 mg per kg per day×10 days | Not well documented | |
| New world | *L mexicana* | Observation alone | >75% | 116 |
| | | Sb: IM/IV 20 mg per kg per day×10 days | 90% | 125 |
| | *L panamensis* | Sb: IM/IV, 20 mg per kg per day×20 days | 80–90% | 118,119,126 |
| | | Miltefosine: oral, 2·5 mg per kg per day×28 days | 80–90% | 120 |
| | | Pentamidine: IM 2 mg per kg qod×7 injections | 60–95% | 119 |
| | *L braziliensis* | Sb: IM/IV 20 mg per kg per day×20 days | 80–90% | 117,127 |
| | | Pentamidine: IM 2 mg per kg qod×7 injections | <50% | 127 |
| | Other species | Sb: IM/IV 20 mg per kg per day×20 days | 80–90%‡ | Authors' estimate |
| **Mucosal disease** | | | | |
| New world | Any species | Sb: IM/IV 20 mg per kg per day×28 days | Mild disease 75% | 107 |
| | | | Severe disease 10–67% | 103 |
| | | Amphotericin B: IV 1 mg per kg qod×20 infusions | >75% | 128, author's estimate |

Sb=pentavalent antimony; IL=intralesional; IM/IV=intramuscular/intravenous; bid=twice-daily; qod=every second day. *Sb available in branded (sodium stibogluconate [Pentostam], meglumine antimoniate [Glucantime], and generic form.[126] Limiting the maximum daily dose to 850 mg is no longer recommended. Miltefosine is available in 10 mg and 50 mg capsules; for bodyweight ≤25 kg given as single daily dose, for >25 kg in two divided doses. In pregnant women, Sb is not recommended and miltefosine is contraindicated (see text). †Efficacy of intralesional Sb in *L tropica* (*vs L major*) infection may reflect longer treatment duration. ‡Cure rates for *L guyanesis* infection may be lower than those reported here.[129]

***Table 1:*** **Selected treatment regimens for cutaneous and mucosal leishmaniasis**

morbidity of self-healing disease itself and antimony is expensive, except in generic form.[121–134] Carefully tested, well tolerated, inexpensive oral agents are clearly needed in self-curing disease. Whereas there is no consensus on optimum treatment in cutaneous leishmaniasis in general, alternatives to systemic antimony are under active investigation (table 1). Except for intralesional antimony, which is used in a highly variable fashion, alternative regimens have mostly been assessed in single studies.

### *Old world cutaneous leishmaniasis*

*L major* infection self-cures rapidly. However, particularly for lesions at exposed sites (figure 3, D), intralesional antimony is frequently used in a variety of seemingly arbitrary regimens.[96,97,121–124] A short, 10-day course of parenteral antimony, currently being tested, could be useful if multiple or large lesions preclude intralesional treatment or in recalcitrant cases. Oral fluconazole was more effective than placebo in a study in Saudia Arabia,[115] but no other oral drug is active in *L major* infection.[135] Topical formulations have intuitive appeal,[96,136] but must penetrate to the dermis where leishmania reside. Topical paromomycin applied for up to 20 days has had variable effects, which can be species dependent or formulation dependent;[97,113,114,136] a penetration-enhancing base is under study. *L tropica* infection does not self-heal rapidly and can be difficult to treat; however, without placebo-controlled trials, efficacy of any of the multiple current intralesional antimony regimens is difficult to judge. Intralesional antimony is given once or twice weekly for up to 4–8 weeks (or longer) or thrice weekly for 2–4 weeks, but practices vary widely depending on the region. 10 days of parenteral antimony or cryotherapy has been used in unresponsive cases.[124] Oral miltefosine is being tested in Afghanistan. Cutaneous species are heat sensitive; local heat therapy via a radio frequency device shows promise.[30]

### *New world cutaneous leishmaniasis*

*L mexicana* infection rapidly self-cures and virtually never affects the mucosa. However, observation alone has not in general been assessed formally; thus, a short, 10-day course of parenteral antimony[125] represents a fair compromise versus observation. Cutaneous leishmaniasis caused by *L panamensis, L braziliensis*, and other species is treated with parenteral antimony for 20 days.[117,119,126,127] In regions where the risk for mucosal disease is very low, topical paromomycin was effective in Guatemala (*L braziliensis, L mexicana*),[137] but not in Colombia (*L panamensis*).[138] Conversely, miltefosine is effective and has been approved (2005) in Colombia (*L panamensis*; >90% cure), but was not effective in Guatemala (*L braziliensis* ).[120] Miltefosine is teratogenic in animals and should not be used in pregnant women; women of child-bearing age also need effective birth control during and for 2 months after treatment.

| | Reactions in patients with cutaneous or visceral leishmaniasis | Comments† |
|---|---|---|
| **Pentavalent antimony** | | |
| Intralesional for CL[124] | Pain, erythema, oedema | Frequent, transient |
| Parenteral[126,127,130] | Raised amylase/lipase or LFTs | Very frequent. Pancreatitis often asymptomatic |
| | Myalgias and arthralgias | Frequent. Mild–moderate |
| | Abdominal pain, nausea | Frequent. Mild–moderate |
| | Thrombocytopenia or leucopenia | Infrequent. Mild decreases |
| | ECG changes or cardiotoxicity | Infrequent. Mild in CL, mild to severe (even fatal) in VL |
| **Paromomycin** | | |
| Topical for CL | Erythema, pain, oedema, blisters | Infrequent, transient. Reactions vary by formulation |
| Parenteral for VL[131] | Ototoxicity‡ | Infrequent. Nephrotoxicity not observed‡ |
| **Miltefosine[120,132]** | | |
| | Nausea, vomiting and/or diarrhoea | Frequent. Usually mild and transient |
| | Raised creatinine | Frequent. Mild increases |
| | Raised LFTs | CL, infrequent; VL, frequent. Mild increases |
| **Pentamidine[127,133]** | | |
| | Nausea, vomiting or diarrhoea | Very frequent |
| | Hyperglycaemia | Infrequent. Can be severe in VL |
| | Cardiotoxicity | Infrequent. Can be severe in VL |
| **Amphotericin B for VL[132,134]** | | |
| | Infusion-related§ | Very frequent |
| | Azotaemia§ | Frequent |
| | Anaemia or hypokalaemia§ | Infrequent |

CL=cutaneous leishmaniasis. VL=visceral leishmaniasis. LFT=liver function test. ECG=electrocardiogram. *For treatment regimens shown in tables 1 and 3. Note that other adverse reactions can also occur with these drugs. †Reactions categorised as infrequent (<25%), frequent (25–50%), or very frequent (>50%). ‡For aminosidine (paromomycin). §For convention amphotericin B. For lipid formulations, infusion-reactions (fever, rigors) are less frequent (especially with liposomal amphotericin B), and azotaemia, anaemia, and hypokalaemia seldom seen since short-course regimens are used (table 3).[11,134]

***Table 2:*** **Common adverse reactions to drugs used to treat leishmaniasis***

Oral ketoconazole had as yet unconfirmed success in Panama (*L panamensis*)[139] and Guatemala (*L mexicana*);[117] itraconazole was ineffective in Colombia (*L panamensis*).[119]

### *Response to treatment*

Physical manifestations take time to improve with any treatment, although lesion size should diminish by at least two-thirds by 6 weeks after drug treatment. If ulcer size has diminished by 33–66%, the clinician can choose another regimen or continued observation; less than 33% reduction in size should prompt different treatment. Patients should be followed up for 6–12 months to show complete healing and no relapse. Parasitological testing after clinical cure is of uncertain importance. Although an active T-cell-dependent immune response accompanies clinical cure, parasites are seldom actually eradicated.[46] Even if evidence of parasites is found in a healed scar, further chemotherapy is not needed.

### *Mucosal disease*

Mucosal leishmaniasis can produce potentially life-threatening inflammatory disease and must be treated. The standard regimen, 28 days of parenteral antimony, induces cure in around 75% of cases of mild disease (nares only), but advanced disease responds less well (table 1).[26,103] Amphotericin B can be used as rescue therapy.[128]

## Visceral Infection

### Clinical findings

Visceral leishmaniasis is caused by *L donovani* in the Indian subcontinent, Asia, and Africa (in adults and children), and by *L infantum* or *L chagasi* in the Mediterranean region, southwest and central Asia, and South America (primarily in young children); other species (eg, *L tropica* in the middle east, *L amazonensis* in South America) are occasionally viscerotropic.[1,2,8] Expression of newly acquired infection varies from none (subclinical), to oligosymptomatic, to fully established (kala azar). Active visceral leishmaniasis may also represent relapse (recurrence 6–12 months after apparently successful treatment) or late reactivation (recrudescence) of subclinical or previously treated infection. Reactivation can be spontaneous, but is often provoked by an intercurrent insult to T (CD4) cell number or function—corticosteroid or cytotoxic therapy, anti-rejection treatment in transplant recipients, or advanced HIV disease.[11,36,50]

In clinically expressed visceral leishmaniasis, fever, weakness, night sweats, anorexia, and weight loss are common and progress over weeks to months.[60,78,140] Children can develop diarrhoea and growth retardation and, in Brazil, can show incomplete oligosymptomatic infection, which usually resolves spontaneously over a variable period but can also progress to kala azar.[141,142] Fever, pallor, wasting, hepatomegaly, and often striking splenomegaly are typical in visceral leishmaniasis (figure 8, A, B). Darkening of the skin (kala azar means black fever in Hindi) is infrequent. Anaemia, leucopenia or thrombocytopenia, and hypergammaglobulinaemia are characteristic. With time, untreated disease in any age-group can produce profound cachexia, multisystem disease, bleeding from thrombocytopenia, susceptibility to secondary infections, and death.[60,78,140]

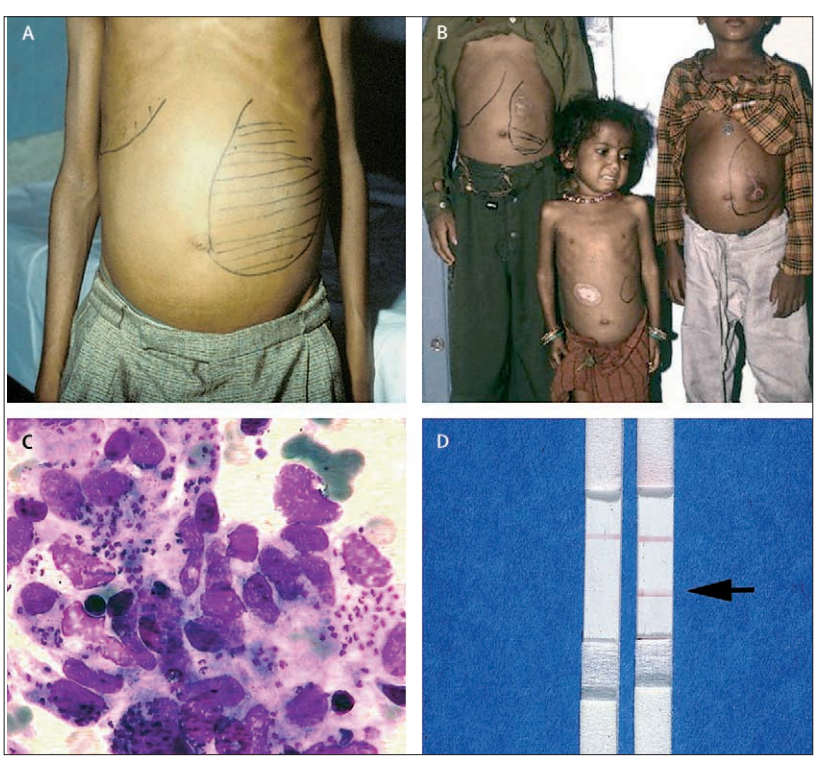


***Figure 8*: Visceral leishmaniasis (*L donovani*) in Bihar State, India**
(A) Hepatosplenomegaly and wasting in a young man (courtesy of D Sacks). (B) Children with burn marks over enlarged spleen or liver—local shaman's unsuccessful remedy (courtesy of R Kenney). (C) Giemsa-stained splenic aspirate smear, showing clumped mononuclear cells and numerous amastigotes (courtesy of S Sundar). Original magnification, ×500. (D) Serodiagnosis of kala azar by anti-K39 antibody detection in immunochromatographic strip test. 5 min after application of one drop of fingerprick blood and buffer, right-hand strip shows second pink band (arrow) indicating presence of anti-K39. Left-hand strip shows a negative result (no second band).

### Diagnosis

Direct visualisation of amastigotes in clinical specimens (figure 8, C) is the diagnostic gold standard in regions where tissue aspiration is feasible and microscopy and technical skill are available. Diagnostic sensitivity for splenic, bone marrow, and lymph node aspirate smears is >95%, 55–97%, and 60%, respectively.[8,9,140,143–145] Elsewhere, including epidemic settings,[8,59,60,140] serum antileishmanial immunoglobulin G in high titre is the diagnostic standard, primarily with direct agglutination tests or other laboratory-based serological assays.[1,3,8,39,140,143,146] Freeze-dried antigen (refrigeration not needed)[146] and rapid detection of anti-K39 antibody with fingerstick blood in an immunochromatographic strip test[9] (figure 8, D) have advanced field serodiagnosis. In symptomatic patients, anti-K39 strip-test sensitivity is high (90–100%),[9,147,148] and while specificity might vary by region[9,12,97,148] this test can safely substitute for invasive diagnostic procedures in Indian visceral leishmaniasis and is useful in PKDL.[9,143] Testing urine for leishmanial antigen or antibody is a new approach.[149,150] In a central laboratory, clinical samples can be cultured for parasite isolation[3,78,148] and leishmanial DNA is readily detectable by PCR testing, including in peripheral blood and serum.[8,78,151,152]

### Treatment

Antimony remains the therapeutic cornerstone in all regions (table 3)[8,11,60,131,132,134,140,144,153–161] except two: Bihar State, India (houses around 90% of India's and about 45% of the world's cases) where the current approximate 35% cure response has ended the usefulness of antimony;[162] and southern Europe. Although the cure rate with antimony (about 90%) has not changed in Europe, many patients now receive liposomal amphotericin B treatment, which produces high-level efficacy in short-course regimens, reducing long hospital stays.[11] Resulting savings in Europe (but in no other endemic region) offset the high cost of liposomal amphotericin B.[11,154,155,161]

In regions other than Bihar, India, and adjacent southeastern Nepal,[162] antimony is effective but has well recognised drawbacks—cost (for branded forms), long duration (table 3), and adverse reactions (table 2). Evidence to show that one particular generic antimony formulation produced in India is as safe and active as branded drug at

| | Treatment and regimen* | Cure rate† | Reference |
|---|---|---|---|
| All except Bihar State, India | Sb: IM/IV 20 mg per kg per day×28–30 days | 90–95% | 8,11,60,140,144,153–155 |
| Bihar, India | Amphotericin B: IV 1 mg per kg qod×15 doses or daily×20 days | 96–100% | 132,134,156 |
| | Liposomal amphotericin B: IV 1–3 mg per kg per day×5 days | 93–97% | 134,157,158 |
| | 5–7·5 mg per kg×1 dose | 90–91% | 157,159 |
| | Amphotericin B lipid complex: IV 2–3 mg per kg per day×5 days | 90–100% | 11,134 |
| | Paromomycin: IM 16–20 mg per kg per day×21 days | 93–97%‡ | 131 |
| | Miltefosine: oral 2·5 mg per kg per day×28 days | 94–96% | 132,160 |
| Mediterranean | Liposomal amphotericin B: IV 3 mg per kg per day×5 days+on day 10 | 95–98% | 155,161 |
| | 10 mg per kg per day×2 days | 98% | 154 |

Sb=pentavalent antimony; IM/IV=intramuscular/intravenous; qod=every second day. *See table 1 legend for Sb preparations, miltefosine dosing, and Sb and miltefosine precautions in pregnant women. In small groups of patients in Brazil and Italy, amphotericin B cholesteryl sulfate (2 mg per kg per day IV×7 days) induced cure rates of 90–100%.[11] †Healthy with no signs or symptoms of relapse ⩾6 months after treatment. ‡Data are for aminosidine[131] (paromomycin). Phase III trial results for paromomycin (15 mg per kg per day×21 days) are pending.

***Table 3:*** **Selected treatment regimens in visceral leishmaniasis in immunocompetent patients by region**

around an eighth of the cost represents a practical advance in treatment.[153]

Pentamidine proved to be an unsatisfactory substitute for antimony in India.[133] However, conventional amphotericin B deoxycholate is highly effective, albeit an arduous treatment because of infusions (figure 2, B), lengthy administration (20–30 days), and adverse reactions.[132,134,156] Lipid formulations of amphotericin B, representing macrophage-targeted treatment, induce side-effects much less frequently than the free drug and are very active in 5–10 day regimens (table 3).[11] Indian kala azar is especially responsive—low total doses and even a single infusion of liposomal amphotericin B cures 90% or more of patients.[134,157–159] Despite short courses, the cost of these highly efficient agents restricts their use in developing countries.[11,134,159] Paromomycin, an aminoglycoside identical to aminosidine,[131] has completed phase III testing in India and is being tested in east Africa. Once commercially available, paramomycin's anticipated high-level efficacy, minimum toxicity, and low cost for the 21-day course[8] should provide an injectable alternative to amphotericin B in India and a potential substitute for antimony worldwide.

Miltefosine, the first effective oral treatment for visceral leishmaniasis, including for antimony-resistant infection (table 3),[8,11,132,160] opened the door to self-administered outpatient therapy; its rapid development in India heads the preceding list of tangible treatment advances. This alkylphospholipid, approved in India (2002), Germany (2004), and Colombia (2005) in a 28-day course, is active in adults and children, and common adverse gastrointestinal reactions are usually transient (table 2).[132,160] Miltefosine has been tested in Nepal and in outpatients in India (results pending), and testing is in progress in east Africa. Concerns have been expressed about miltefosine's cost as well as how to protect the high-level efficacy of this valuable agent from the effects of poor outpatient compliance and the potential development of resistance. Some researchers have suggested combining miltefosine with a second agent[8] in part to maintain its effect but also reflecting a growing interest in combination treatments for visceral leishmaniasis. Results of a phase II study in India of an additional oral agent, sitamaquine,[8,11] have not been reported.

### *Response to treatment and outcome*

Most patients respond with clinical improvement after 7–10 days. At or within 2 weeks of treatment end, 90% or more of properly treated individuals show apparent cure responses (afebrile, decreased spleen size, no visible amastigotes if repeat aspiration is feasible) and 5–10% do not respond to or die[60] during treatment (far-advanced disease, intercurrent illness, or drug toxicity; table 2). 5–10% of apparently cured patients relapse, most often within 6 months after treatment; thus, assuming follow-up is possible, complete response is not applied until 6 months or more have passed uneventfully. Relapse warrants a new treatment regimen. Haematological abnormalities improve by the end of treatment and splenomegaly disappears within 6 months; most responders convert their leishmanin skin tests to reactive within a year,[78,140] presumably signalling resistance to reinfection.

### *HIV-visceral leishmaniasis co-infection*

Information primarily from intravenous drug users in southern Europe[36,62,74] has amply illustrated the effects in visceral leishmaniasis of CD4 cell depletion induced by HIV disease. Whereas new leishmania infections can be acquired via shared needles and syringes,[54,55] more than 90% of HIV-associated visceral leishmaniasis represents reactivation of prior subclinical infection.[163] HIV co-infected patients have similar clinical syndromes, including asymptomatic visceral infection.[36,50] However, CD4 cell-depleted patients also show widespread atypical organ involvement, frequent parasitaemia, suboptimal specific immunoglobulin G production, reduced responsiveness to chemotherapy, and predictably high relapse rates once any treatment is discontinued.[36,50,61,62,163] There is still no consensus about long-term maintenance antileishmanial treatment if remission of visceral leishmaniasis is induced or about when such treatment can be safely discontinued in patients who respond to HAART.[164]

## Prevention

Demand for new prevention strategies and improved health education[12,165] continues to grow in leishmaniasis. Case finding and treatment (case management) is difficult to maintain and inefficient even where feasible. Lack of access to affordable, active drugs,[166] incorrect prescribing, and poor compliance undermine case management and perpetuate anthroponotic infection (and simultaneously foster drug resistance).[162,167,168] Although overall attention is rightly focused on prevention of visceral leishmaniasis, the main source of death in leishmaniasis,[1] cutaneous leishmaniasis is also a major burden in certain foci, with serious psychosocial effects (figure 5, D, and figure 7, B).[169,170]

### Vector control

Sandfly control is now mostly dependent on pyrethroids, although the only insecticide resistance in sandflies is for the organochlorine DDT (bis[4-chlorophenyl]-1,1,1-trichloroethane) in India.[12] House spraying to control indoor-resting sandflies can reduce the risk of cutaneous leishmaniasis;[171,172] however, spraying programmes are often unsustainable. Where sandflies are endophagic and most active when people are asleep, bednets provide substantial protection (eg, against visceral leishmaniasis in Bangladesh and Nepal).[173,174] Sandflies are small (wings <3 mm long) and untreated bed nets are only fully effective with narrow mesh (<156 holes per square inch), which are often unacceptable in warmer climates. Protection by wide-mesh nets is enhanced by pyrethroid treatment,[12] which reduces sandfly biting rates by 64–100%.[23,175] However, insecticide-treated bednets require periodic re-impregnation, and long-lasting insecticide-treated bednets in which insecticide is combined with the material during manufacture are currently being assessed for protection against sandflies in Afganistan, Iran, and the Indian subcontinent. Several long-lasting insecticide-treated bednets are under development, and two have been approved for mosquito control by WHO Pesticide Evaluation Scheme (WHOPES):[176] Olyset, polyethylene nets made by Sumitomo Chemical, Tokyo, Japan, and PermaNet, polyester nets made by Vestergaard-Frandsen, Kolding, Denmark. Long-lasting insecticide-treated bednets are more costly than locally made nets, and distribution to rural populations in endemic countries is a challenge.[177] A potentially important development[177] is a polymer resin for mixing with a standard aqueous solution of an insecticide (eg, K-O Tab) to provide long-lasting protection to conventional nets already in use in many leishmaniasis-endemic regions. For example, more than 91% of households in the most visceral leishmaniasis-endemic subdistrict of Bangladesh reportedly own at least one locally produced net.[174] Where transmission is not exclusively associated with sleeping indoors, insecticide-treated bedsheets[172] or window curtains[178] provide protection against cutaneous leishmaniasis. However, away from the domestic environment, treated clothing produced inconsistent results.[179]

Where visceral leishmaniasis is primarily zoonotic (Latin America, Mediterranean basin, central and southwest Asia),[2] reducing transmission to human beings by targeting the animal reservoir—eg, vaccinating dogs to prevent canine visceral leishmaniasis—should be feasible.[12,180,181] However, culling infected domestic dogs in Brazil to reduce human visceral leishmaniasis has apparently not been effective because of incomplete and infrequent coverage; delays between taking blood samples, diagnosis, and culling; and the high dog population turnover rate.[182,183] Dipping in insecticide and applying topical lotions[12,184] protects dogs from infection, but regular re-treatment is needed. By contrast, deltamethrin-impregnated dog collars enable protective effects to persist for more than 8 months. Treated collars reduced sandfly bloodfeeding by up to 90% and decreased *L infantum* incidence rates in domestic dogs in Italy.[185] In Iran, treated collars reduced the infection incidence in dogs by 54% and, importantly, by around 40% in children in intervention areas.[186] Trials testing the epidemiological effect of both treated collars and direct insecticide application are ongoing in Brazil.

### Vaccine

Abundant clinical and experimental evidence indicates that leishmaniasis should be preventable by vaccination.[12,14,15,23,187] The only proven vaccine agent in human beings is live *L major* (leishmanisation), now discontinued because of unacceptable lesions in some recipients.[187] Killed parasites as vaccines produced encouraging results in Brazil in the 1970s.[14] Trials have tested autoclaved *L major* plus BCG versus adjuvant (BCG) alone, assessing the 2-year cumulative incidence of cutaneous leishmaniasis caused by *L tropica*[188] or *L major*[189] (single vaccine dose) or of visceral leishmaniasis caused by *L donovani* (two doses).[190] Although no trial showed a significant effect on disease incidence, vaccination induced skin test conversion and perhaps some protection.[189,190] In Ecuador, two doses of a killed multi-leishmania species cocktail plus BCG reduced cutaneous leishmaniasis incidence by 73% during year 1 (efficacy disappeared after year 2),[191] whereas autoclaved *L amazonensis* plus BCG had no significant effect.[192] In Colombian soldiers, three doses of a killed *L amazonensis* preparation alone provided no protection against cutaneous leishmaniasis.[193] Current laboratory efforts are focused on novel antigens and adjuvants, live-attenuated vaccine, recombinant purified and subunit proteins, naked DNA, bacteria expressing leishmanial antigens, and targeting dendritic cells.[14,15] A great deal is known about the experimental antileishmanial immune response; however, less is known about the human response and precise correlates of immunity have not yet been fully defined.[194] Resistance to live challenge with

*L major* could be an efficient test for future candidate vaccines.[195]

Despite progress in prevention,[12] current leishmaniasis control programmes have largely failed, primarily because of inadequate regional health delivery systems and resources. WHO has identified leishmaniasis as a category 1 disease (emerging and uncontrolled). Sustainability of infection control needs more efficient targeting of activities to areas of highest risk. Such a plan is now feasible, despite generally poor rates of case notification. Clustering of cases within localities is characteristic and measurable,[196] distribution of both cutaneous leishmaniasis[197] and visceral leishmaniasis[198] incidence can be predicted from environmental features with geographic information systems, and early warning systems could be generated for areas of unstable incidence. In the two regions with the greatest burden of disease—the Indian subcontinent and east Africa—long-term sustainability of any control programme, however well targeted, is the major challenge. Nevertheless, given that the Indian subcontinent contains around 70% of the world's cases of kala azar, and that infection is anthroponotic and largely restricted to defined zones, the possibility of local eradication in this one critical region can be envisioned.[199]

#### Conflict of interest statement

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