

## Leishmaniasis

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In 1903, Leishman and Donovan separately described the protozoan now called *Leishmania donovani* in splenic tissue from patients in India with the life-threatening disease now called visceral leishmaniasis. Almost a century later, many features of leishmaniasis and its major syndromes (ie, visceral, cutaneous, and mucosal) have remained the same; but also much has changed. As before, epidemics of this sandfly-borne disease occur periodically in India and elsewhere; but leishmaniasis has also emerged in new regions and settings, for example, as an AIDS-associated opportunistic infection. Diagnosis still typically relies on classic microbiological methods, but molecular-based approaches are being tested. Pentavalent antimony compounds have been the mainstay of antileishmanial therapy for half a century, but lipid formulations of amphotericin B (though expensive and administered parenterally) represent a major advance for treating visceral leishmaniasis. A pressing need is for the technological advances in the understanding of the immune response to leishmania and the pathogenesis of leishmaniasis to be translated into field-applicable and affordable methods for diagnosis, treatment, and prevention of this disease.

Leishmaniasis, a vector-borne disease caused by obligate intramacrophage protozoa, is characterised by diversity and complexity (panel 1). Leishmaniasis is endemic in areas of the tropics, subtropics, and southern Europe, in settings ranging from rain forests in the Americas to deserts in western Asia, and from rural to periurban areas. Several clinical syndromes are subsumed under the term leishmaniasis: most notably visceral, cutaneous, and mucosal leishmaniasis, which result from replication of the parasite in macrophages in the mononuclear phagocyte system, dermis, and naso-oropharyngeal mucosa, respectively. These syndromes are caused by a total of about 21 leishmanial species, which are transmitted by about 30 species of phlebotomine sandflies.<sup>1-3</sup> With some exceptions (eg, visceral leishmaniasis in India, and cutaneous leishmaniasis caused by *Leishmania tropica*), human beings are incidental hosts of infection, and other mammals (such as rodents and canids) are reservoir hosts.<sup>3,6</sup> However, specificity is found among the diversity: particular species of parasite, vector, and host maintain the transmission cycle in a given ecological setting.<sup>6</sup> Both the specificity and the diversity have implications for selection and implementation of control measures.<sup>1,5,6</sup>

If clinically evident but untreated, visceral leishmaniasis (also known as kala-azar, Hindi for black sickness or fever) causes life-threatening systemic infection (figure 1); cutaneous leishmaniasis can cause chronic skin sores (figure 2); and mucosal leishmaniasis (also known as espundia), a dreaded metastatic complication of new-world cutaneous leishmaniasis, causes facial disfigurement (figure 3). Thus, the primary goals for clinical management are straightforward—to prevent death from visceral leishmaniasis and morbidity from cutaneous and mucosal leishmaniasis. However, even tropical medicine clinicians are often baffled by the complexities of leishmaniasis: by the apparently

innumerable possible combinations of different leishmanial syndromes, species, and geographical areas of acquisition of infection, each combination varying by clinical presentation, ease of diagnosis, natural history, and response to therapy.



Figure 1: Cachectic Sudanese woman with visceral leishmaniasis

The increasing interface between leishmaniasis and more-developed countries has prompted growing interest in this disease. Contributing factors include the recognition of cases of leishmaniasis in overseas travellers, US Gulf War veterans<sup>4</sup> (panel 1), and people with HIV infection,<sup>7-10</sup> and the emergence of leishmaniasis as a model system for exploring the immune response to intracellular pathogens.<sup>11,12</sup> Translation of the increased interest in leishmaniasis and the advances in the understanding of the immunoregulation of this disease into field-applicable methods for diagnosing, treating, and preventing infection is challenging. Rapid methods for diagnosis and species identification are needed, as are therapies, prophylactics, and control measures that are effective, safe, affordable, and easily administered.<sup>1,5,6,12,13</sup>

The need for such measures has been highlighted by the occurrence of epidemics on several continents. In northeastern India (particularly the state of Bihar), the latest in the series of epidemics of anthroponotic kala-azar caused by *L donovani* flared up in the 1970s, probably in part because of cessation of insecticide spraying for malaria, and some years still generates an estimated

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## Panel 1: Key facts about human leishmaniasis

### Aetiological agent

Obligate intracellular protozoa of the genus *Leishmania* (order Kinetoplastida); about 21 of 30 species that infect mammals infect human beings.<sup>1-3</sup>

### Stages of parasite

*Promastigote*—flagellated form found in sandflies and culture; 15–20  $\mu\text{m}$   $\times$  1.5–3.5  $\mu\text{m}$  with 15–28  $\mu\text{m}$  flagellum.

*Amastigote*—non-flagellated tissue form (2–4  $\mu\text{m}$  in diameter); replicates in macrophage phagosomes in mammalian hosts.

### Visceral leishmaniasis: causative leishmanial species

*L. donovani* species complex (ie, *L. donovani* and *L. infantum* in old world and *L. chagasi* in new world); also *L. tropica* (old world) and *L. amazonensis* (new world).

### Variations on visceral leishmaniasis

*Post-kala-azar dermal leishmaniasis (PKDL)*—a syndrome that develops at variable times after resolution of visceral leishmaniasis, can be associated with relapse of visceral disease, and is manifested by skin lesions that can be of various types and initially are most prominent on the face; people with chronic PKDL can serve as reservoir hosts of infection.

*Viscerotropic leishmaniasis*—an oligoparasitic syndrome with non-specific manifestations caused by visceral infection with *L. tropica*, which more typically is dermatotropic;<sup>4</sup> 12 parasitologically confirmed cases (*L. tropica* confirmed as aetiological agent in eight) were noted among US servicemen in Persian Gulf conflict in 1990s.

### Cutaneous leishmaniasis: causative leishmanial species

*Old-world cutaneous leishmaniasis*—*L. tropica*, *L. major*, and *L. aethiopica*; also *L. infantum* and *L. donovani*.

*New-world (American) cutaneous leishmaniasis*—*L. mexicana* species complex (especially *L. mexicana*, *L. amazonensis*, and *L. venezuelensis*) and *Viannia* subgenus (most notably *L. [V] braziliensis*, *L. [V] panamensis*, *L. [V] guyanensis*, and *L. [V] peruviana*); also *L. major*-like organisms and *L. chagasi*.

### Variations on cutaneous leishmaniasis

*Mucosal leishmaniasis*—*Viannia* subgenus (typically *L. [V] braziliensis* but also *L. [V] panamensis* and *L. [V] guyanensis*); also *L. amazonensis* (see text).

*Leishmaniasis recidivans*—a chronic, hyperergic, oligoparasitic variant of old-world cutaneous leishmaniasis typically manifested by solitary facial lesion that heals centrally but gradually enlarges over many years and can resemble lupus vulgaris; typically caused by *L. tropica* (sometimes *L. major*) and found in Iran, Iraq, and neighbouring areas.

*Diffuse cutaneous leishmaniasis*—a chronic, progressive, polyparasitic variant that develops in context of leishmanial-specific anergy and is manifested by disseminated non-ulcerative skin lesions, which can resemble lesions of lepromatous leprosy; caused by *L. aethiopica* (old world) and *L. mexicana* species complex (new world).

### Modes of transmission

*Vector-borne*—by bite of infected female phlebotomine sandflies (about 2–3 mm long), which become infected by taking blood meal from infected mammalian host. A total of about 30 species in *Phlebotomus* genus (old world) and *Lutzomyia* genus (new world) have been identified as vectors.<sup>1</sup>

Sandflies are relatively weak, noiseless fliers; they rest in dark, moist places, and are typically most active in evening and night-time hours.

*Other modes*—congenital and parenteral (ie, by blood transfusion, needle sharing, and laboratory accident).

### Geographical distribution and estimates of numbers of cases<sup>1,5</sup>

*Leishmaniasis-endemic countries*—88 countries. Leishmaniasis is endemic from northern Argentina to southern Texas (not in Uruguay, Chile, or Canada), in southern Europe, Asia (not southeast Asia), the middle east, and Africa (particularly east and north Africa, with sporadic cases elsewhere), but not in Australia or Oceania.

*Estimated number of people at risk of infection*—about 350 million.

*Estimated annual number of new cases of visceral leishmaniasis*—about 500 000; over 90% of worldwide cases are in Bangladesh, northeastern India (particularly Bihar State), Nepal, and Sudan (old world), and in northeastern Brazil (new world).

*Estimated annual number of new cases of cutaneous leishmaniasis*—about 1.5 million; over 90% of worldwide cases are in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, and Syria (old world), and in Brazil and Peru (new world). Geographical distribution of cases evaluated in developed world reflects travel and immigration patterns.

200 000 or more cases.<sup>1,14</sup> In southern Sudan, which has been affected by civil war, an epidemic of what is locally called the killing disease has occurred in a remote area not previously considered endemic for *L. donovani*



Figure 2: Skin lesion of a Guatemalan patient with cutaneous leishmaniasis

Note raised outer border of lesion. (Photograph by permission of Dr T Navin)

infection.<sup>15</sup> The epidemic first came to the attention of outsiders in 1988 and continued into the 1990s. Médecins Sans Frontières, Holland, which has treated more than 20 000 patients with limited resources (eg, in makeshift clinics held under shade trees), estimates that the excess mortality has been about 100 000 deaths among about 300 000 people at risk.<sup>15</sup> An epidemic of anthroponotic *L. tropica* infection in another war-affected area, Kabul, Afghanistan, shows that leishmaniasis is not limited to rural areas and that even cutaneous leishmaniasis can occur on a large scale, with hundreds of thousands of cases, and can be personally and socially disruptive (figure 4).<sup>16</sup> Northeastern Brazil is another example of a region where leishmaniasis is encroaching on urban areas. Visceral leishmaniasis caused by *L. chagasi*, with the domestic dog as the primary reservoir host, has emerged in shanty settlements that have sprung up around large cities.<sup>17</sup>

### Immunology

Scientists are studying the immunoregulation of leishmaniasis to improve understanding of the immune response to intracellular pathogens in general and



Figure 3: Mucosal leishmaniasis, with a perforated nasal septum, in a patient who had been in several countries in the Americas

(Photograph by permission of Dr RReves)

leishmania in particular, to find out whether manipulations of the immune system could be therapeutic, and to rationalise vaccine development. Many insights about immunoregulation have been gained through murine models of leishmaniasis,<sup>11</sup> but the complexities of clinical leishmaniasis require further study. The use of cytokine and other types of immunotherapy combined with chemotherapy for leishmaniasis remains experimental, and the results have been mixed.<sup>12,18,19</sup> Various types of vaccines (eg, killed or attenuated whole parasites, synthetic or recombinant peptides, or recombinant live vaccine vectors), with or without cytokines or other adjuvants, are being investigated, but no vaccine is ready for general use to prevent leishmaniasis.<sup>20</sup>

The fundamental principle of the immunoregulation of leishmaniasis is that the parasite, which replicates in quiescent macrophages, is killed by activated macrophages. Murine models of *L major* disease exemplify the Th1/Th2 paradigm, in which the outcome of disease is determined by the nature and magnitude of the T-cell and cytokine responses early in infection. In infected inbred mice, production of interferon gamma by Th1 and natural killer cells mediates resistance, whereas expansion of interleukin-4-producing Th2 cells confers susceptibility.<sup>11</sup> Interleukin 12, which is an effective adjuvant in experimental vaccination against and treatment of *L major* infection, has a key role in the development of cell-mediated immunity by

inducing naïve T cells to differentiate into Th1 cells and by inducing T cells and natural killer cells to produce interferon gamma.<sup>21,22</sup> Vaccination with the DNA that encodes the LACK antigen (ie, the leishmania homologue of receptors for activated C kinase), an immunodominant *L major* antigen, induces an interleukin-12-mediated, protective Th1 response.<sup>23</sup>

Not surprisingly, the T-cell and cytokine responses in infected human beings are more complex and less polarised than they are in mice, and the immune responses differ among the leishmanial syndromes and species.<sup>12,24</sup> Nonetheless, interferon gamma seems to be important for cure of human disease, making interleukin 12 an attractive potential adjuvant for vaccination and therapy. The presence of interleukin 10 seems to be associated with the disease process in visceral leishmaniasis;<sup>24</sup> interleukin 4 may also contribute to disease progression.<sup>25</sup> Although the genetic mechanisms involved in immunoregulation are also more complex in human beings than in mice, genetic susceptibility to different forms of leishmaniasis may exist.<sup>26,27</sup>

### General principles about diagnosis

Ideally, all cases of leishmaniasis should be confirmed by demonstration of the parasite, which is straightforward (except for needing an invasive procedure) if parasites are plentiful (eg, in kala-azar) but otherwise can be difficult (eg, for viscerotropic leishmaniasis, mucosal disease, and chronic skin lesions [panel 2]). Examination of giemsa-stained slides of the relevant tissue is still the technique

most commonly used to visualise the parasite. The sample should be examined by light microscopy under oil immersion for amastigotes, the tissue form of the parasite (panel 1). To ensure that the visualised structures are amastigotes, rather than other "dot"-like organisms (eg, *Histoplasma* spp), an experienced observer should look for the characteristic size (2–4 µm in diameter), shape (round to oval), and internal organelles (the nucleus and kinetoplast) (figure 5). In particular, the kinetoplast should be visualised; it is a rod-shaped, specialised mitochondrial structure that contains extranuclear DNA in catenated maxicircles and minicircles, a characteristic exploited by some molecular methods.<sup>32–34</sup> With giemsa staining, the cytoplasm typically is pale blue and the nucleus and kinetoplast pinkish red or violet blue.

Other conventional methods for parasitological diagnosis include in-vitro culture of infected tissue or inoculation into animals (eg, golden hamsters). Species



Figure 4: Cutaneous leishmaniasis in Kabul, Afghanistan Despite bitter cold and the devastation of civil war, women and girls crowd around the treatment centre for cutaneous leishmaniasis. Although not life-threatening, their skin lesions constitute much more than a minor irritation. (Photograph taken in February, 1997, and provided by permission of Dr R W Ashford.)

Panel 2: A practical guide for parasitological diagnosis of cutaneous leishmaniasis<sup>28-30</sup>

**General comments**

Preferentially sample active lesions without superinfection. To increase sensitivity, use several techniques with several samples per technique. For new-world disease, even under optimal circumstances, the maximum overall sensitivity of this approach (with conventional parasitological methods) may be 70–75% of clinically compatible cases and is even lower for chronic lesions and mucosal disease.<sup>28-31</sup>

Inject anaesthetic (1% lidocaine with epinephrine 1 to 100 000), particularly if biopsy samples will be obtained, through intact skin cleansed with 70% alcohol, into dermis underlying area to be sampled. High concentrations of anaesthetic could inhibit parasite growth in culture, as could residual iodine if used to cleanse skin. Before obtaining dermal scrapings and biopsy samples, debride relevant portions of lesions and apply pressure with sterile gauze to achieve haemostasis.

**Needle aspirates for leishmanial culture**

Obtain three to five aspirates from different lesions or portions of lesions. Draw up about 0.1 mL preservative-free sterile 0.9% saline into a 1.0–3.0 mL syringe. For ulcerative lesions, insert needle through intact skin into dermis of active border. Use a 23 to 27 gauge needle; small-gauge needles are appropriate for facial lesions. Repeatedly move needle back and forth under skin, tangentially to ulcer, simultaneously rotating syringe and applying suction, until pink-tinged tissue fluid is noted in hub of needle. If none is noted, inject 0.05–0.1 mL saline under skin and resume suction. Discharge each aspirate into separate tube of culture medium (eg, Novy-MacNeal-Nicolle medium). Thin smears of aspirates are typically suboptimal unless a cytospin preparation is used.

**Biopsy samples for cultures and histopathology**

Obtain one to two full-thickness punch-biopsy samples at active border of lesion, including some non-ulcerated tissue.

Divide sample into three portions, or obtain more than one sample:

Use one portion for leishmanial and other cultures (ie, bacterial, mycobacterial, and fungal).

Use one portion for impression smears (ie, touch preparations).

Use one portion for histological examination of tissue stained with haematoxylin and eosin; giemsa; and special stains to rule out mycobacterial, fungal, and other infectious causes. Although histopathology is generally the least sensitive technique for diagnosing cutaneous leishmaniasis (sensitivity <20% in some studies<sup>29-30</sup>), it helps exclude other diagnoses. Amastigotes are more easily recognisable in touch preparations and in thin smears of tissue scrapings.

Molecular-based and monoclonal-antibody analyses and animal inoculation can also be done.

**Tissue impression smears**

Grasp biopsy sample with forceps. Gently blot cut surface onto paper towel or gauze to remove excess blood. Gently press blotted surface, with rolling or circular motion, onto glass slide. Repeat in parallel row down slide. Air-dry slide, fix in methanol, and stain with giemsa.

**Dermal scrapings for thin smears**

Obtain three to five dermal scrapings from different lesions or portions of lesions (eg, beneath necrotic lip of lesion). If aspirates and biopsy samples for culture are obtained, obtain scrapings last to minimise risk of contaminating the sites. Some practitioners use the slit-skin smear technique and first make an incision before obtaining scrapings. For this technique, pinch skin to exclude blood and use scalpel blade to incise several mm long and deep slit through intact skin into dermis. For ulcerative lesions, start incision in active border and proceed radially out across several mm of intact skin.

Obtain tissue fluid and flecks of tissue by scraping dermis (eg, beneath necrotic lip of lesion or along walls of incision) with sharp instrument (eg, scalpel blade or stainless steel spatula). After obtaining as much tissue as possible, make as thin a smear as possible. Air-dry slide, fix in methanol, and stain with giemsa. Although dermal scrapings can also be cultured, risk of contamination is high.

identification can be accomplished by isoenzyme analysis of cultured promastigotes or with various molecular methods<sup>32,34</sup> or monoclonal antibodies, which also can be used for in-situ diagnosis. PCR, which is currently a research tool, has the potential to increase sensitivity.<sup>32-34</sup> Further assessment of its performance (both sensitivity and specificity) and field applicability are needed,

including its ability to facilitate diagnosis by detecting scarce parasites and by obviating the need for invasive procedures (eg, if testing blood rather than tissue can suffice for visceral leishmaniasis).

Immunodiagnostic methods include serological tests to detect antibody or antigen, and assays to detect leishmania-specific cell-mediated immunity, such as intradermal skin testing and detection of proliferative responses of circulating lymphocytes to leishmanial antigens. The usefulness of such methods depends on the clinical syndrome and the assay. Another issue is that the methods may not reliably differentiate remote from recent or current infection. Advances in molecular methods (eg, production of recombinant and synthetic antigens) have the potential to lead to the development of improved and field-applicable diagnostic techniques.<sup>13,35</sup>

**General principles about treatment**

The good news is that leishmaniasis is treatable. However, antileishmanial therapy is a bewildering subject, largely because of the complexities of the disease and the inadequacies of published information. The plethora of published reports based on anecdotal or otherwise suboptimal data creates the illusion that many good treatment options exist. The harsh realities are that few of the touted agents have been assessed adequately in clinical trials; few of the many combinations of

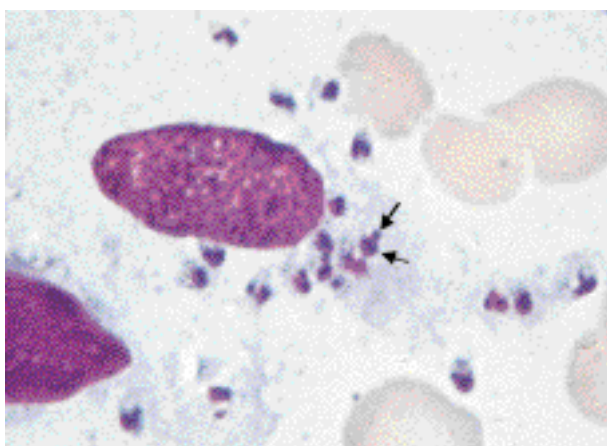


Figure 5: Bone-marrow sample from a patient with visceral leishmaniasis acquired in Spain

Eash amastigote (tissue form of the parasite) has a nucleus (bottom arrow) and kinetoplast (top arrow); the extracellular amastigotes were probably released from mononuclear phagocytes during manipulation of the sample. (Photograph by permission of Dr M Eberhard)

Drug	Syndrome	Dosage regimen <sup>5,12</sup>	Comments
<b>Parenteral</b>			
Pentavalent antimony <sup>36</sup> (intravenous or intramuscular)*	VL	20 mg Sb (V)/kg daily for 28 days	Longer courses of therapy may increase toxic effects. <sup>36</sup>
	CL	20 mg Sb (V)/kg daily for 20 days	Shorter courses may have merit in some situations.
	ML	20 mg Sb (V)/kg daily for 28 days	Longer courses do not necessarily improve effectiveness.
Amphotericin B deoxycholate (intravenous)	VL	0.5–1.0 mg/kg on alternate days or daily (total about 15–20 mg/kg)	Range, total dose about 7–20 mg/kg (varies by region and host status).
	CL	See comments	Infrequently used to treat CL; use if necessary and the toxic effects can be justified.
	ML	1 mg/kg on alternate days or daily (total about 20–40 mg/kg)	..
Lipid formulations of amphotericin B (intravenous)	VL	2–5 mg/kg daily (total about 15–21 mg/kg)	Range, total dose about 5–40 mg/kg (varies by region, drug, and host status). <sup>14,37</sup>
	CL, ML	Not currently recommended	Not clear whether useful for CL or ML: more theoretical basis for use in VL.
Pentamidine isethionate (intravenous or intramuscular)	VL	4 mg/kg on alternate days or three times per week for about 15–30 doses	Considered second-line therapy because of toxicity or suboptimal effectiveness.
	CL	3 mg/kg on alternate days×4 doses or 2 mg/kg on alternate days×7 doses	Based on studies in Colombia (most cases probably caused by <i>Viannia</i> subgenus, particularly <i>L [V] panamensis</i> ).
	ML	2–4 mg/kg on alternate days or three times per week for 15 or more doses	Considered second-line therapy.
Paromomycin sulphate† (intravenous or intramuscular)	VL	15–20 mg/kg daily for about 21 days	Has been used as monotherapy in India and as adjunct to antimony compounds.
	CL	Not currently recommended	Ineffective against <i>L (V) panamensis</i> (Colombia) and <i>L (V) braziliensis</i> (Belize).
Recombinant interferon gamma (subcutaneous or intramuscular)		100 µg/m <sup>2</sup> daily or on alternate days (adult dose)	Sometimes useful as adjunct for difficult cases of VL and other syndromes.
<b>Oral</b>			
Ketoconazole	CL	600 mg daily for 28 days (adult dose)	Consider for <i>L mexicana</i> and <i>L (V) panamensis</i> and possibly for <i>L major</i> .
Itraconazole	CL	200 mg twice daily for 28 days (adult dose)	Failure rate of at least 75% in Colombia (most cases probably caused by <i>Viannia</i> subgenus, particularly <i>L [V] panamensis</i> ).
Dapsone	CL	100 mg twice daily for 6 weeks (adult dose)	Promising results obtained in India but not in Colombia (against mostly <i>L [V] panamensis</i> ).
Allopurinol	CL	See comments	No better than placebo in Colombia (against mostly <i>L [V] panamensis</i> ).
<b>Local/topical</b>			
Paromomycin sulphate ointment‡	CL	Apply twice daily for 10–20 days	Consider especially for <i>L major</i> and <i>L mexicana</i> .
Intralesional Sb (V)	CL	Weekly or alternate-day injections×multiple doses	Infiltrate four opposing sides of lesion until base completely blanched.§

VL=visceral leishmaniasis; CL=cutaneous leishmaniasis; ML=mucosal leishmaniasis; Sb(V)=pentavalent antimony. \*Sodium stibogluconate=100 mg Sb(V)/mL and meglumine antimonate=85 mg Sb(V)/mL; locally made antimony preparations may have different antimony concentrations. Intravenous administration preferable for large volumes.<sup>36</sup> Children, particularly those weighing <20 kg, may benefit from dosing according to body surface area and treating with proportionately >20 mg Sb(V)/kg daily. †500 mg paromomycin sulphate corresponds to 350 mg base. ‡An ointment containing 15% paromomycin and 12% methylbenzethonium chloride in soft white paraffin is modestly effective. Much of the experience has been with *L major* infection. Methylbenzethonium chloride can cause local inflammation (eg, burning sensation, pruritus, vesicles). A product containing 10% urea instead is cheaper and better tolerated but less effective. §Depending on number and characteristics of lesions, intralesional therapy may not be practical. Much of the experience with intralesional therapy has been for old-world disease. Published regimens vary widely in total number of and interval between injections.

#### Drug regimens for treatment of leishmaniasis

syndromes, species, and geographical regions have been studied; and the most effective agents generally have the most potential for toxic effects and are the most difficult to administer. Treatment trials for cutaneous leishmaniasis can be complicated further by rapid self-healing. For many cases of leishmaniasis, decisions about whether and how to treat involve extrapolation from studies done in different settings than the one most relevant to the patient at hand. In the table, the ranges shown for doses and durations of therapy reflect variability both in dosage regimens among clinical trials and in responsiveness in different settings.

Since the 1940s, the pentavalent antimony compounds sodium stibogluconate (Pentostam, Glaxo Wellcome, UK) and meglumine antimonate (Glucantime, Rhône-Poulenc Rorer, France) have been the mainstays of antileishmanial therapy (table).<sup>12,36</sup> Although these drugs are usually highly effective, their disadvantages include: parenteral mode of administration; long duration of therapy (several weeks); suboptimal effectiveness in some settings; bothersome and frequent, albeit almost always reversible, toxic effects (eg, fatigue, body aches, electrocardiographic abnormalities, raised aminotransferase levels, chemical pancreatitis); and the perception that treatment with a heavy-metal compound of uncertain mode of action smacks of alchemy. Amphotericin B and pentamidine, the traditional parenteral alternatives to antimony, were previously relegated to second-line status, partly because they were considered more likely to cause serious or irreversible toxic effects (eg, renal impairment). However, these agents are now being resurrected, with the benefit of new formulations or

dosage regimens, for use in some settings (table).<sup>12,37</sup>

Other new approaches that have merit in some situations (table) include the use of other agents, such as the aminoglycoside paromomycin (the chemical equivalent of aminosidine), adjuncts (eg, interferon gamma), and new modes of treatment (eg, topical therapies). Unfortunately, most of the non-parenteral agents that have been assessed to date have, at best, modest activity (highest cure rates of 70–80%) against a limited range of species and strains.

Management of each patient's case should be individualised. Certain questions should be considered when selecting treatment:

- Is treatment indicated? What is the worst that could happen with no or suboptimal treatment (eg, death, substantial morbidity)?
- Does the patient have underlying medical disorders that could affect the course of the infection or increase the risk of toxic effects of certain drugs?
- Which therapeutic agents are available locally? What is known about their efficacy and toxicity profiles both in general, and for treating infection caused by the syndrome and species of interest in the region of interest? Is clinical resistance likely to be encountered?
- Should a drug regimen that is usually highly and rapidly effective be used, or could a potentially less effective but also less toxic and more easily administrable therapy be tried first (eg, an oral or topical agent instead of a parenteral agent for treating non-metastasising cutaneous leishmaniasis)?

Options for persistent or relapsing infection include: monitoring clinically mild disease (eg, cosmetically unimportant skin lesions caused by *L major*); retreatment with the same drug (if some response was noted during the first course) for the same or a longer period, perhaps with addition of an adjunct; or treatment with a different drug.

## Visceral leishmaniasis

### *Clinical manifestations*

Visceral leishmaniasis encompasses a broad range of manifestations of infection. Infection remains asymptomatic or subclinical in many cases, or can follow an acute, subacute, or chronic course. The classic kala-azar syndrome is exemplified by patients such as those in Sudan<sup>15</sup> who are heavily infected throughout the mononuclear phagocyte system; develop life-threatening disease after an incubation period of weeks to months; and have fever, severe cachexia (figure 1), hepatosplenomegaly (splenomegaly usually predominates), pancytopenia (anaemia, thrombocytopenia, and leucopenia, with neutropenia, marked eosinopenia, and a relative lymphocytosis and monocytosis), and hypergammaglobulinaemia (mainly IgG from polyclonal B-cell activation) with hypoalbuminaemia.

### *Differential diagnosis and diagnosis*

The differential diagnosis includes malaria, tropical splenomegaly syndrome, schistosomiasis or cirrhosis with portal hypertension, African trypanosomiasis, miliary tuberculosis, brucellosis, typhoid fever, bacterial endocarditis, histoplasmosis, malnutrition, lymphoma, and leukaemia. Leishmanial parasites can be seen on stained slides or in cultures of a biopsy sample or tissue aspirate (eg, of spleen, bone marrow, lymph nodes). The sensitivity is highest for splenic aspiration (as high as 98% compared with <90% for other organs), but so is the risk (rarely haemorrhage). Whereas in most cases leishmanial-specific cell-mediated immunity becomes detectable only after recovery, high titres of non-protective antileishmanial antibody can typically be detected (eg, with the direct agglutination test) during the illness and can persist for years afterwards. A serological assay for IgG antibody to K39 (a recombinant leishmanial polypeptide), which uses antigen-impregnated nitrocellulose paper strips,<sup>35</sup> looks promising for diagnosis of visceral leishmaniasis under field conditions; but additional field testing is needed.

### *Treatment*

Because death can be imminent for patients with clinical manifestations of visceral infection, use of highly effective, rapidly active therapy is important (table), as is monitoring for bleeding and intercurrent infections, such as pneumonia, tuberculosis, and dysentery. Pentavalent antimony is still commonly used outside of India, with response rates averaging 90%.<sup>12,36</sup> However, good alternatives are available not only for rescue therapy, if antimony therapy fails, but also for primary therapy if non-antimony therapy is likely to be more effective or otherwise advantageous. Treatment of cases in India is particularly challenging because of their sheer abundance and their refractoriness to antimony (and pentamidine<sup>12</sup>) therapy. Currently, up to 50% or more of previously untreated cases in the state of Bihar are unresponsive to,

or relapse after, conventional antimony therapy,<sup>19</sup> perhaps largely because of inappropriate use of antimony by local practitioners.

In Bihar, many practitioners have turned to conventional amphotericin B for first-line therapy, which remains almost 100% effective. The newly available lipid formulations of amphotericin B, in which various lipids have replaced the component deoxycholate, are also highly effective and offer added benefits: passive targeting of drug to macrophage-rich organs decreases nephrotoxic effects and allows higher daily doses of the drug and shorter courses of therapy (table).<sup>12,14,37</sup> Lipid formulations of amphotericin B include liposomal amphotericin B, amphotericin B lipid complex, and amphotericin B cholesteryl sulphate. Dose optimisation studies are in progress. The US Food and Drug Administration recently licensed liposomal amphotericin B for treatment of visceral leishmaniasis and recommended treating immunocompetent patients with 3 mg/kg daily on days 1–5, 14, and 21 (total 21 mg/kg) and immunosuppressed patients with 4 mg/kg daily on days 1–5, 10, 17, 24, 31, and 38 (total 40 mg/kg).<sup>37</sup> An alternative recommendation for immunocompetent patients is treatment on days 1–5 and 10 with 3–4 mg/kg daily for cases in Europe or Brazil, 3 mg/kg daily for Africa, and 2–3 mg/kg daily for India.<sup>37</sup> Unfortunately, the lipid formulations of amphotericin B, even with lower total doses than those recommended by the US Food and Drug Administration, are too expensive for use in the countries where they are needed most.<sup>14</sup> A much cheaper alternative, which may offer similar benefits, is short-course therapy with conventional amphotericin B diluted in a commercially available lipid emulsion. In a study in Bihar, 65 (93%) of 70 patients treated with a total dose of 10 mg/kg (2 mg/kg on alternate days for five doses) were cured (S Sundar and H Murray, personal communication).

Other parenteral alternatives (table) that have merit in some settings include pentamidine (limitations include suboptimal effectiveness in India and toxic effects, especially with long courses of treatment), and paromomycin (currently not commercially available). Adjunctive interferon-gamma therapy may accelerate or improve the response to antimony therapy in some difficult cases.<sup>12,18,19</sup> However, this approach may offer, at best, marginal benefit in settings with high-level resistance to antimony compounds<sup>19</sup> and has been little used since the advent of lipid formulations of amphotericin B. An effective oral agent would be a major advance. Therapy with the oral agent miltefosine (100–150 mg daily for 28 days) has been virtually 100% effective and acceptably tolerated in phase I/II studies of adult patients in Bihar<sup>38</sup> (S Sundar and H Murray, personal communication).

Most patients feel better and become afebrile during the first week of treatment.<sup>36</sup> Splenomegaly and biochemical abnormalities do not resolve for weeks to months in some cases. Freedom from clinical relapse for at least 6 months is the best indicator of cure. If the patient's status is in doubt, repeat tissue sampling is indicated. The presence of some residual parasites does not necessarily portend a poor outcome, whereas the apparent absence of parasites does not preclude relapse.

### *Coinfection with HIV*

Visceral leishmaniasis (occasionally other syndromes) is emerging as an important opportunistic infection among

people with HIV-1 infection.<sup>7-10</sup> In fact, the parasite may be a cofactor in the pathogenesis of HIV infection; a major surface molecule, the lipophosphoglycan, of *L donovani* induces transcription of HIV in CD4 cells.<sup>39</sup> To date, leishmania and HIV coinfection has been reported from 31 countries, with most of the cases from southern Europe, where 25-70% of adult patients with visceral leishmaniasis are coinfecting with HIV, and 1.5-9.0% of patients with AIDS develop leishmaniasis.<sup>7</sup> The surveillance data for southern Europe are as follows: 1461 cases of coinfection reported for January, 1990, through June, 1998 (835 [57.2%] from Spain); and 717 cases for January, 1996, through June, 1998 (412 [57.5%] from Spain)<sup>8</sup> (P Desjeux, personal communication). The dual trends of the encroachment of leishmaniasis on urban areas, and of HIV infection on rural areas, are likely to lead to more coinfecting patients elsewhere.

The stereotypical coinfecting patient in southern Europe is a young, male, injecting drug user who is infected with *L infantum* (of the *L donovani* species complex), with a common strain (eg, MON-1) or a strain thought to be relatively avirulent or that is more often associated with cutaneous than visceral leishmaniasis. The leishmanial infection may have been newly acquired via vector-borne transmission or perhaps a contaminated syringe, or have reactivated after years of latency. The CD4-cell count is below 200/ $\mu$ L in up to 90% of patients.<sup>9</sup> If the patient is ill, the clinical manifestations may be typical of visceral leishmaniasis or non-specific (eg, diarrhoea); paradoxically, the illness may become milder and more atypical as the CD4-cell count decreases.<sup>10</sup> Antibody to leishmania may not be detectable, particularly if the HIV infection preceded the leishmanial infection. However, parasites may be abundant, even in atypical sites and cells, which facilitates parasitological diagnosis, both by conventional methods (eg, culture of a bone-marrow aspirate) and unconventional means, such as examination of gastrointestinal tissue or a peripheral blood smear (sensitivity of about 50% for the latter), or culture of a buffy-coat preparation (sensitivity of about 70%).<sup>9</sup>

Management of each patient's case should be individualised, based on assessment of the clinical importance and evolution of the leishmanial infection, with the goal of optimising the patient's quality of life for as long as possible. However, assessment of which clinical manifestations are attributable to leishmaniasis (as opposed to other infections, the host response, or medications) can be difficult. When treatment is indicated, most patients are given one of the standard regimens for visceral leishmaniasis (table), in some cases modified to decrease toxic effects or increase effectiveness. An open, multicentre, randomised trial, in which HIV-infected patients in Spain were enrolled from 1994-96, compared 28 days of either meglumine antimonate (20 mg antimony per kg daily) or conventional amphotericin B (0.7 mg/kg daily).<sup>40</sup> With respect to initial cure (negative giemsa-stained slide and culture of bone marrow aspirate 1 month after the end of therapy), the trial found no difference between the two drugs in an intention-to-treat analysis (cure rates of 66% [29/44] vs 62% [28/45]) or an on-treatment analysis (85% [29/34] vs 93% [28/30];  $p=0.4$ ). The groups also were similar with respect to the probability of not having a relapse by 12 months after therapy (30% vs 44%) and in survival after the diagnosis of leishmaniasis (overall

median of 56 weeks); none of the patients received highly active antiretroviral therapy. Additional randomised, comparative clinical trials of candidate treatment regimens are needed, as are trials of candidate maintenance regimens. Examples of potential maintenance regimens include monthly or fortnightly doses of an antimony compound, pentamidine, or a lipid formulation of amphotericin B, or daily use of an oral agent.

## Cutaneous leishmaniasis

### *Clinical manifestations*

Like visceral leishmaniasis, both old-world and new-world cutaneous leishmaniasis encompass a broad range of severity and manifestations of infection. Travellers can become infected even after short stays in leishmaniasis-endemic areas. Cutaneous infection can remain subclinical or become clinically apparent after a variable incubation period that averages several weeks. Stereotypically, lesions evolve from papules, to nodules, to ulcerative lesions, with a central depression and raised, indurated border (figure 2), and ultimately, over months to years, to atrophic scars. Some lesions do not ulcerate but persist as nodules or plaques. Some patients have more than one primary lesion, satellite lesions, sporotrichoid-like nodular lymphangitis (common with *L [V] panamensis* and *L [V] guyanensis* infection), regional adenopathy (sometimes bubonic, with *L [V] braziliensis*), lesion pruritis or pain, and secondary bacterial infection. Determinants of the natural history and pathogenicity of cutaneous leishmaniasis, including the propensity for latency, rapid self-cure, persistence, dissemination, reactivation, and reinfection, are poorly understood but include factors related to the behaviour of the vector, the virulence of the parasite, and the host's behaviour and innate and acquired resistance.<sup>31,41</sup>

### *Differential diagnosis and diagnosis*

Disorders that can mimic cutaneous leishmaniasis include tropical and traumatic ulcerative lesions, foreign-body reactions, superinfected insect bites, myiasis, impetigo, fungal and mycobacterial infections, sarcoidosis, and neoplasms. Techniques for obtaining tissue for parasitological diagnosis of cutaneous leishmaniasis are described in panel 2. Serological testing is not helpful in most cases because antibody is undetectable or at low levels, whereas manifestations of cell-mediated immunity (eg, skin-test reactivity) usually develop during active infection. Delays in diagnosis can lead to diagnostic difficulties (because of the scarcity of the parasite),<sup>31</sup> bigger lesions and scars, and more opportunity for development of bacterial superinfection and mucosal leishmaniasis.

### *Treatment*

Decisions about whether and how to treat cutaneous leishmaniasis should first take into account whether the patient is at risk of mucosal leishmaniasis; the desire to prevent mucosal disease is a prime motivator for adequate treatment of new-world cutaneous leishmaniasis. Another important factor is the degree to which the skin lesions are bothersome because of their location (eg, on the face), number, size, evolution, persistence, or other features (eg, nodular lymphangitis).<sup>36</sup>

Unfortunately, no ideal therapy for cutaneous leishmaniasis has been identified. Intravenous or intramuscular antimony therapy is probably still the best option if optimal effectiveness is important (table).<sup>36</sup> Studies in Colombia (predominantly with the *Viannia* subgenus) have shown that relatively short-course pentamidine therapy is effective (96% cure rate) and acceptably tolerated (table).<sup>12</sup> The first sign of a therapeutic response to antileishmanial therapy typically is flattening of the lesion; re-epithelialisation of large, ulcerative lesions may continue after treatment. The need for additional therapy should be assessed 4–6 weeks after therapy, with consideration of the extent and course of the response.<sup>36</sup> Clinical reactivation typically begins at the margins of old lesions.

Second-line agents (or no treatment at all) can be considered for treatment of some relatively benign, cosmetically unimportant lesions, particularly if caused by *L major* (old world) or *L mexicana* (new world).<sup>12,36</sup> These species cause lesions that can persist for months but, other factors being equal, tend to self-heal more rapidly than lesions caused by *L tropica* (old world) or *L (V) braziliensis* (new world);<sup>31</sup> within the *Viannia* subgenus, infection caused by *L (V) panamensis* may be somewhat less virulent and more responsive to second-line agents than *L (V) braziliensis*. The oral agents that have been assessed (table) are at best modestly and slowly active, against only some species and strains. Ketoconazole has modest activity against *L mexicana* and *L (V) panamensis* infection; its usefulness against *L major* infection is unclear. Itraconazole is better tolerated than ketoconazole but may be less effective, at least against the *Viannia* subgenus. The oral agent miltefosine is being investigated for treatment of new-world cutaneous leishmaniasis (J Berman, personal communication). Possible local therapies for some patients with non-metastasising, self-limiting cutaneous leishmaniasis include a paromomycin ointment (commercially available in Israel) and intralesional antimony therapy (table), as well as heat treatment and cryotherapy. Mechanical excision can pose a substantial risk of relapse. Immunotherapy remains experimental.

### Mucosal leishmaniasis

Mucosal leishmaniasis, a dreaded sequela of new-world cutaneous leishmaniasis, is caused by parasites of the *Viannia* subgenus (panel 1) in most cases and results from haematogenous or lymphatic dissemination of amastigotes from the skin to the naso-oropharyngeal mucosa. In most cases it becomes evident within several years of resolution of the original cutaneous lesions, but it can ensue while the lesions are present or decades after they heal. Adequate systemic treatment of cutaneous leishmaniasis is assumed (but not proven) to decrease the already low risk (probably <5%) of mucosal disease. Although the risk factors for mucosal leishmaniasis are poorly understood, they may include having particular alleles for the genes encoding tumour necrosis factor  $\alpha$  and  $\beta$ .<sup>26</sup>

Typically, mucosal disease becomes evident because of chronic nasal symptoms; progressive naso-oropharyngeal destruction may follow (figure 3), in the context of a hyperactive immune response. The differential diagnosis includes paracoccidiodomycosis, histoplasmosis, syphilis, tertiary yaws, leprosy, rhinoscleroma, midline granuloma, sarcoidosis, and neoplasms. Mucosal

leishmaniasis is difficult to diagnose, even when clinically active, because amastigotes are scarce (panel 2). Samples of the lesion should be cultured.<sup>29</sup> Serological tests are more likely to be positive than they are for cutaneous leishmaniasis.

In general, mucosal leishmaniasis is harder to treat than cutaneous leishmaniasis and becomes increasingly so as it progresses. Currently, the best treatment options are pentavalent antimony drugs (cure rates of about 75% for mild disease and 10–63% for more advanced disease) or conventional amphotericin B (table).<sup>12,36</sup> Concomitant corticosteroid therapy is indicated if respiratory compromise develops.

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