Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment

M. T. M. Roberts*

Department of Medicine and Infectious Diseases, Worcester Royal Hospital, Charles Hastings Way, Worcester, Worcs WR5 1JG, UK and University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Leishmaniasis is a major tropical disease with a wide clinical spectrum of cutaneous, mucocutaneous and visceral involvement. Presentation is often varied and diagnosis can be challenging. The outcome of infection is determined by the parasite species and the host's immunological response. The CD4⁺ T helper cell is critical with animal models demonstrating that cure is associated with strong IFN-γ, interleukin (IL)-2 and IL-12 responses in the absence of classical Th2 cytokines or IL-10. Prevention has focussed on vector control, control of animal reservoirs and efforts to develop a protective vaccine. Treatment options historically have relied on antimonials though agents with better tolerability and efficacy have been developed including amphotericin and the oral agent miltefosine. Drug resistance, human immunodeficiency virus and changes in vector epidemiology threaten recent advances. Renewed impetus led by the WHO is required to co-ordinate future international effort to develop new drugs and ultimately a vaccine.

Keywords: leishmaniasis, review, vaccines, prevention, treatment

Biology of the parasite

Leishmaniasis is a parasitic infection caused by the obligate, intracellular protozoan of the genus *Leishmania* (family Trypanosomatidae). Over 15 species of *Leishmania* are capable of infecting man classified into two main groups: Old World: *L. major*, *L. tropica*, *L. aethiopica* and the *donovani* complex (*L. donovani*, *L. infantum*) and New World: *L. mexicana*, *L. amazonensis* and *Viannia* complex (e.g. *L. brasiiliensis*, *L. guyanensis*). Leishmaniasis is a zoonosis with important animal reservoirs. The parasite has a digenetic life cycle with an extracellular developmental stage in the insect vector, a female phlebotomine sandfly, and a developmental stage in mammals, which is mostly intracellular. In sandflies, development of the parasite occurs in the alimentary canal with the formation of a motile, flagellated and elongated form termed a...
‘promastigote’. The promastigote matures in the insect midgut into an infective metacyclic promastigote. Inoculation into the mammalian host occurs when sandflies feed on blood, which is a requirement for oviposition.

A typical inoculum contains around 100–1000 metacyclic promastigotes which quickly become engulfed by leucocytes, particularly macrophages, neutrophils and dendritic cells. The parasites undergo a further transformation within these cells to form amastigotes. A morphological change occurs as the parasite takes on an ovoid shape with a short flagellum, hence the term ‘amastigote’, and possibly a metabolic change with a switch to anaerobic metabolism under acidic conditions found chiefly in the phagolysosome compartment. The distribution and manifestations of the disease vary widely according to the parasite species and the underlying immune response of the host. However, a feature common to mammalian infection with all parasite species is that the infection is intracellular with parasites only briefly exposed to an extracellular environment at inoculation. This has important implications for host immunity and in designing potential vaccine candidates.

*Leishmania* parasites possess a variety of virulence mechanisms that enable the amastigote stage to survive in the hostile environment of the phagolysosome (reviewed by Hommel [1]). Sandfly saliva can also act as a virulence factor by enhancing invasion of macrophages by promastigotes, which leads to disease with lower parasite inoculations [2].

### Epidemiology

Leishmaniasis is endemic in 88 countries with an estimated 12–15 million individuals infected and an annual incidence of around 2 million [3]. The incidence of fatal visceral leishmaniasis is rising, largely secondary to urbanization and the human immunodeficiency virus (HIV) pandemic. Epidemics have led to an even greater impact at a local level, such as occurred in southern Sudan in the 1990s [4]. Although most human cases occur as a result of transmission by sandfly bites, contaminated blood products and sharing of needles by intravenous drug users are other reported mechanisms of transmission [5]. Host genetic factors probably play an important part in the disease (reviewed by Blackwell [6]).

### Clinical presentation and diagnosis

Three major clinico-pathological categories are recognized: cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis and visceral leishmaniasis (VL) each caused by distinct species. Following treatment of VL, the skin can become the focus of infection with a condition termed...
Leishmaniasis: immunology, prevention and treatment

‘post-kala-azar dermal leishmaniasis’. The typical lesion of CL is a chronic ulcer with histological features of an intense lymphoid and monocytic infiltrate with granuloma formation. Some species particularly, *L. mexicana* and *L. aethiopica*, may lead to a diffuse cutaneous leishmaniasis characterized by widespread non-ulcerating nodules resembling lepromatous leprosy. VL is characterized by dissemination of parasites throughout the reticuloendothelial system and, after an incubation period ranging from 1 month to 2 years, patients typically develop pyrexia, wasting and hepatosplenomegaly.

The most promising diagnostic tests are a direct agglutination test and the rK39 urinary antigen test with sensitivities up to 95 and 87% reported [7]. An indirect fluorescent antibody test has also been developed but serological tests can be expensive and unreliable in the field setting, give high false negative rates in the immunodeficient, may persist after cure and are less helpful in CL. The gold standard is parasitological diagnosis by biopsy of the affected tissue, lymph nodes, splenic or bone marrow aspirate with positivity rates around 80% for the latter [8]. PCR-based techniques are increasingly used [9].

**Mammalian immune responses to leishmaniasis**

Evidence for the critical role for IFN-γ in the control of *Leishmania* infection comes from the demonstration that IFN-γ knockout (KO) mice fail to cure infection [10]. Furthermore, in experimental *L. major* infections genetically resistant mice develop a T-cell response dominated by a CD4+ T helper 1 (Th1) phenotype characterized by IFN-γ secretion, whilst in susceptible mice the dominant response is a CD4+ T helper 2 (Th2) phenotype characterized by interleukin (IL)-4, IL-5 and IL-13 secretion. The correlation between a polarized immune response and outcome to infection led to the concept that the balance of Th1 to Th2 responses determines the outcome [11]. These observations of *L. major* in mice led to the emergence of the Th1/Th2 paradigm as opposing cytokine responses in the control of infections. Hence, the quest to discover how naïve T cells, with the potential for differentiation to either Th1 or Th2, are directed towards one of these opposing extremes.

Studies on the early immune response to high-dose infection with *L. major* in mice on resistant C57BL/6 or C3H backgrounds or a susceptible BALB/c background revealed three distinct patterns. Infection in C3H mice was dominated by an IL-12-driven, CD4+ Th1 response with high IFN-γ levels secreted by natural killer cells and no IL-4 [12]. In contrast, progressive disease in susceptible BALB/c mice was characterized by early IL-4 synthesis in the absence of IL-12 and a bias towards a Th2 response [13]. Evidence that early IL-4 synthesis drives this Th2
response came from experiments in IL-4 KO BALB/c mice and mice treated with anti-IL-4 antibody, demonstrating that they heal infection [14]. The cellular origin of IL-4 in BALB/c mice is confined to an oligoclinal CD4+ T-cell population with a Vβ4Vα8 T-cell receptor, recognizing the Leishmania homologue of the receptor for activated C kinase (LACK) [15].

The critical importance of IL-12 in mediating a Th1 response and resistance is demonstrated by IL-12 depletion experiments leading to susceptibility in naturally resistant mice [16] and the conversion of susceptible BALB/c mice to a resistant phenotype by treatment with IL-12 [17]. Dendritic cells are the source of IL-12 [18]. The antigens responsible for the IL-12 response and the exact mechanisms are not defined.

Despite a wealth of evidence for IL-4 in the development of a non-healing phenotype, virulent strains of L. major can lead to susceptibility in BALB/c IL-4 KO mice [19]. In a search for an explanation to this, it is observed that IL-4/IL-13 KO BALB/c mice exhibit greater resistance than single KO strains and additionally, IL-4Rα mice displayed greater resistance than IL-4 KO mice, indicating that IL-13 can substitute for IL-4 in promoting Th2 differentiation [20]. However, the discovery that IL-4Rα/IL-10 KO BALB/c mice and IL-4Rα mice treated with anti-IL10R antibody became highly resistant identified IL-10 as a key cytokine [21]. There are three potential sources of IL-10: (1) Th2 cells of the lineage that produce IL-4 as described; (2) a discrete subpopulation of CD4+ T cells termed ‘T regulatory cells’ and (3) dendritic cells (DCs) and macrophages.

In a physiological, low-dose (10^2–10^3) model of infection in C57BL/6 mice, a clear role for CD8+ T cells in primary immunity is defined in the control of L. major infection in resistant mice [22]. However, parasites persist even in resistant mice. Using this low-dose model, it was demonstrated that IL-10 played an essential role in parasite persistence. Only IL-10 KO and IL-4/IL-10 KO mice achieved sterile cure demonstrating the requirement for IL-10 in establishing latency [23]. A role for IL-10 was confirmed when C57/BL/6 mice treated with anti-IL-10R antibody transiently during the chronic phase of infection achieved sterile cure [23]. A key study determined that an endogenous, naturally occurring population of CD4+CD25+ T regulatory cells (Treg), expressing high CTLA-4, are the source of IL-10 controlling L. major persistence and immunity in C57BL/6 mice [24]. Treg constitute 5–10% of CD4+ T cells in normal mice and humans, developing in the thymus where, following high-affinity recognition of self peptides, they up-regulate the transcription factor FoxP3 and the expression of the cell surface marker CD25, essential for their survival and a constitutive marker of Treg in the periphery.
Two distinct sub-populations of Treg have been described: naturally occurring Treg, involved in the maintenance of peripheral tolerance, and antigen-specific T regulatory cells (Tr1) that encounter pathogen-derived foreign antigen in the periphery. In a model of Bordetella pertussis infection, pathogen-specific Tr1 were demonstrated for the first time [25]. A possible mechanism of increased IL-10 production by macrophages involves antibodies via ligation of Fcγ receptors [26]. A study in L. mexicana suggests antibody responses block Th1 development [27]. This highlights the popular view that innate immunity drives adaptive immunity and also indicates that antibody responses may be a further critical component of the immune response against these pathogens.

Another important component of the immune response are natural killer cells acting primarily through their ability to produce IFN-γ, which can optimize the production of IL-12 by DCs and the expression of IL-12R by activated T cells. Finally, production of IFN-γ leads to intracellular death of amastigotes through a common effector mechanism. Macrophage activation is associated with induction of nitric oxide (NO) synthase, which in turn leads to NO-mediated killing. Tumour necrosis factor (TNF)-α is a co-factor with NO. Two other mechanisms of intracellular killing have been proposed involving destruction of infected macrophages by cytotoxic T lymphocytes (CTL) [28] and FasL-mediated macrophage apoptosis [29].

A schematic diagram outlining the basic immunological responses in leishmaniasis is shown in Figure 1.

Human immunology

Cutaneous leishmaniasis usually leads to self-healing disease with lifelong immunity against re-infection. Resolution is characterized by induction of specific IFN-γ releasing CD4+ T cells [30]. Failure to cure is associated with elevated levels of IL-4 with low IFN-γ responses from Leishmania-specific CD4+ T cells [31]. Increased expression of IL-10 in L. major lesions was found to be associated with progressive disease [32]. Studies have also highlighted a dichotomy between Th1 versus Th2 responses in simple versus diffuse CL in humans [33].

Patients with active VL usually demonstrate anergy with a negative skin test to Leishmania antigens. Peripheral blood mononuclear cells from such individuals fail to proliferate or to produce IFN-γ when exposed to specific antigen in vitro [34]. Addition of anti-IL-10R antibody to T cells harvested from these patients restores cytokine responses, indicating a role for IL-10 in suppressing T-cell responses in active disease [34]. Further evidence of a role for IL-10 comes from studies demonstrating increased IL-10 mRNA expression in bone marrow [35],
lymph nodes [34] and spleen [36]. Cure from disease was associated with a fall in IL-10 mRNA levels [34, 35]. Imbalanced IL-10 production may play a role in progression of disease to PKDL. Increased expression of classical Th2 cytokines has been reported in VL with elevated IL-4 particularly associated with treatment failure [37]. Elevated levels of IL-13 have been observed in active disease that returned to normal...
following successful treatment [38]. In these studies IL-10 and not IL-13 was associated with disease relapse. Investigating the potential sources of immunoregulatory cytokines, investigators have found a population of antigen-specific T cells co-producing IL-10 and IFN-γ which expand in response to *L. donovani* infection in humans [39]. However, a role for antigen-specific Tr1 in humans has not been reported. Effector mechanisms are also important in determining the outcome to infection in humans with evidence that host genetic factors play a crucial role [40, 41].

### Prevention

WHO have designated leishmaniasis as a category 1 (emerging and uncontrolled) disease with prevention focussed on vector control, control of animal reservoirs and research into potential vaccines.

#### Vector control

Control of infection through preventing transmission by the sandfly vector is theoretically feasible. Female sandflies usually feed at night though depending on the species some are endophagic, feeding indoors, whilst others are exophagic, feeding outside. Strategies include deterrents, in particular pyrethroids and insecticides, such as DDT. Resistance to DDT has been reported though generally sandflies remain highly sensitive to insecticides. The effectiveness of spraying has been demonstrated at a local level but it is unclear what effect blanket spraying would have on the sandfly population and sustainability of these programmes is problematic.

Bednets provide protection against endophagic species [42] with pyrethroid-impregnated nets providing additional protection reducing biting rates by up to 64–100% [43]. As a long-term control measure effectiveness of bednets depends on regular re-impregnation, replacement of damaged nets and distribution to rural communities. The success of bednets in reducing malaria transmission to children has added impetus to further develop this control strategy including the evaluation of long-lasting insecticide-treated bednets.

#### Animal reservoirs

In many regions of the world, leishmaniasis is zoonotic with major reservoirs of infection in domestic and sylvatic animals. In the Mediterranean basin and Brazil, the dog population has been targeted. The most effective strategy has been the use of deltamethrin-impregnated dog collars with up to 86% protection of dogs in seasons of high transmission [44].
Culling dogs has also been employed with modelling from a Brazilian study indicating that both culling and dog collars should have a higher proportional impact in regions of low endemicity but the relative advantage of dog collars increases with transmission rates [45].

However, reductions in the use of insecticides and increasing prevalence amongst urban populations have reduced the impact of vector and animal reservoir control programmes. Hence, given the variety of epidemiological situations, the multiplicity of factors that influence disease transmission, and continuing uncertainty about the biology of the parasite, its vector and its reservoir hosts, disease control has proven very difficult to achieve. A human or dog vaccine is an attractive option to circumvent these problems.

Vaccines

The observation that spontaneous or drug-induced recovery from CL or VL is accompanied by solid immunity against re-infection provides a rational basis for vaccine development. This fact led to the traditional practice of using live parasites recovered from skin lesions to induce lesions in preferred body sites to prevent disease on re-infection, a process called leishmanization. Such a practice dates back at least 2000 years. Nearly 1.2 million people in Iran between 1982 and 1986 received such a live vaccine [46]. Approximately 50% of those who received this vaccine developed skin lesions and of those, 93% demonstrated a positive leishman-delayed hypersensitivity skin test, a good field marker of population immunity. Furthermore, a significant decrease in disease incidence was observed, falling from 14% in the non-vaccinated group to 2.5% in the vaccinated group. The rationale for using heterologous organisms of lower pathogenicity as vaccines against a more virulent species is based on the high level of immunological cross-reactivity between species at the humoral and cellular levels, though this has not necessarily translated into cross-species protection. However, the risk of localized disease and dissemination in the context of HIV, together with the impracticality of delivering fresh cultures of a live vaccine in the field, has made the practice of leishmanization obsolete.

An alternative strategy using attenuated organisms allows the development of an immune response closest to that of natural infection, with exposure to a much larger range of antigens than achieved by using more refined subunit vaccines. However, despite pursuing such a strategy for human or experimental murine leishmaniasis using naturally avirulent organisms [47], irradiated organisms [48] or genetically manipulated organisms [49], there has been little success. Similarly, killed vaccines have shown limited immunogenicity and efficacy even when combined...
with adjuvants, either BCG or alum [50]. Interestingly, BCG alone led to a positive leishmanin skin test in some individuals, presumably due to antigenic cross-reactivity between *Mycobacteria* and *Leishmania*.

Whilst in dogs single-dose alum-precipitated *L. major* vaccine plus BCG demonstrated efficacy of around 70% [51]. Naturally excreted secreted antigens purified from culture supernatant of *L. infantum* promastigotes delivered subcutaneously to dogs has shown promise as a potential vaccination approach against natural *L. infantum* infection [52]. Three recombinant leishmanial antigens (TSA, LeIF and LmSTI1) have demonstrated immunogenicity in canine VL [53]. Given the importance of dogs as reservoirs of infection, recent advances in the development of a transmission-blocking vaccine are encouraging. Fucose–mannose ligand (FML) antigen of *L. donovani* in combination with saponin (FML vaccine and Leishmune) induced 92–97% protection against zoonotic visceral leishmaniasis with significant protection demonstrated out to 12 months [54].

A contrasting approach has been to investigate the use of individual molecules as human vaccines. A gp63 peptide vaccine was tested successfully in animal models [55]. However, the success of these vaccines in humans has generally been poor due to the failure to elicit adequate cellular immunity, an essential feature for the control of intracellular infections.

**DNA vaccines**

A novel approach to this problem has been the development of DNA vaccines. Wolff *et al.* demonstrated that intramuscular inoculation of plasmid DNA encoding several different reporter genes could induce protein expression in muscle cells [56]. This study provided the strong basis for the concept that purified recombinant nucleic acids can be delivered in vivo to direct protein expression. Subsequent studies showed that DNA vaccines could protect mice against CL [57].

One of the key factors determining the success of a vaccine is the development of long-term memory. DNA encoding leishmanial antigen LACK is more effective than vaccination with leishmanial protein plus IL-12 protein in maintaining antigen-specific Th1 responses that are able to control *L. major* infection [58]. Reasons for the enhanced efficacy of DNA vaccination over protein and adjuvant include antigen persistence and induction of IL-12 through CpG motifs. Parasite persistence has been shown to be crucial for the maintenance of immunity in experimental leishmaniasis in a study demonstrating that anti-IL-10R antibody led to sterile cure but loss of immunity to re-infection [23]. However, long-term immunity can be maintained by DNA vaccines in the absence of
detectable antigen either through persistence of undetectable antigen, perhaps in follicular DCs, or via antigen-independent responses [59].

Studies indicate that memory T cells are heterogeneous with a subset that migrate through lymph nodes (central memory T cells) and those that migrate to tissues and make effector cytokines (effector memory T cells) [60]. Recent evidence demonstrates that central memory T cells can mediate long-term memory in the absence of parasites [61]. Hence, a major challenge for DNA vaccines will be to identify mechanisms that induce central memory T cells. Several approaches have been explored to enhance the immunogenicity of DNA vaccines. Novel adjuvants such as CpG motifs and monophosphoryl lipid A act as ligands for TLRs triggering IL-12 release and promoting a Th1 response. Another strategy to augment human responses to vaccination has been heterologous prime-boost immunization with a number of combinations investigated, though priming with DNA followed by a boost with MVA [62]. This study indicated that IL-10 from regulatory T cells determined vaccine efficacy for these antigens in this BALB/c model reaffirming a key role for IL-10 in the immune response.

Initial studies identified TryP, the most frequently sampled gene in expressed sequence tags from cDNA libraries of *L. major* [63], as a reproducibly protective antigen against infection in susceptible BALB/c mice. Other antigens effective in mice and non-human primates are LACK, *L. major* stress-inducible protein 1 and *Leishmania* elongation factor and in mice HASPB1, a stage-specific hydrophilic acylated surface protein. Use of salivary antigens in plasmid DNA has also been investigated to vaccinate against *L. major* challenge containing saliva [64].

Now that the genomic sequence of *L. major* Friedlin is complete the 8500 identified genes provide a source of potential vaccine candidates [65]. As a consequence of the intracellular location of the parasite, there is no *a priori* requirement that the target antigen should be a surface molecule, which considerably expands the potential number of vaccine candidates. One such large vaccine screen has been undertaken of 100 unique amastigote-expressed *Leishmania* genes in a BALB/c mouse model challenged with *L. major* LV39 substrain using DNA vaccination [66]. A heterologous prime-boost regime for vaccination against experimental visceral leishmaniasis in dogs with DNA/recombinant cysteine proteinases type I and II was protective [67]. Further dog trials and the results of a human trial of a DNA vaccine are eagerly awaited.

## Treatment

Until recently treatment options for leishmaniasis were limited and further hampered by poor diagnostic tools for case finding and the
challenge of delivering complicated and toxic treatment regimens to populations with the highest prevalence. New agents herald renewed optimism for eradication and cure though this comes at a time of increasing drug resistance and co-infection with HIV leading to high recurrence rates. A summary of treatment regimens is shown in Table 1.

### Cutaneous leishmaniasis

Most cases of cutaneous leishmaniasis spontaneously heal with drug therapy targeted to those with disfiguring facial disease, recurrent disease or mucocutaneous leishmaniasis. New World CL Leishmania Viannia subgenus may disseminate leading to MCL if untreated. The most widely used drugs are antimonials. Paromomycin has demonstrated cure rates of up to 97% [68]. Trials with oral miltefosine demonstrate high cure rates and antifungal agents have some activity against the parasite too [69]. The use of immunomodulatory agents, IFN-γ [70] and imiquimod [71] as adjunctive therapy has been reported.

### Visceral leishmaniasis

Historically, the antimonials, sodium stibogluconate and meglumine have been most widely used to treat visceral disease. Antimonials need to be delivered by intramuscular or intravenous routes daily for 28 days.

**Table 1** Treatments for cutaneous, mucocutaneous and visceral leishmaniasis

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Drug Regimen</th>
<th>Comments/side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous</td>
<td><strong>Antimonials (stibogluconate)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Observation alone</td>
<td>60–70% cure rate</td>
</tr>
<tr>
<td></td>
<td><strong>Antimonials (stibogluconate)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM/IV 20 mg/kg daily × 10 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Paromomycin</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL 1 ml/lesion weekly × 8 weeks</td>
<td>Pain/erythema</td>
</tr>
<tr>
<td></td>
<td><strong>Miltefosine</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Topical bd daily × 2 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Flucnazole</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PO 2.5 mg/kg daily × 28 days</td>
<td>Pain/erythema</td>
</tr>
<tr>
<td></td>
<td><strong>Pentamidine</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PO 200 mg daily × 6 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Imiquimod</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM 8 mg/kg daily × 7 days</td>
<td></td>
</tr>
<tr>
<td>Muco-cutaneous</td>
<td><strong>Antimonials (stibogluconate)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM/IV 20 mg/kg daily × 20 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Liposomal amphotericin</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV 3 mg/kg daily × 20 days</td>
<td>Renal failure/low K*</td>
</tr>
<tr>
<td></td>
<td><strong>Immunotherapy (anti-TNF-α agents)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case reports</td>
<td></td>
</tr>
<tr>
<td>Visceral</td>
<td><strong>Antimonials (stibogluconate)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM/IV 20 mg/kg daily × 20 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Miltefosine</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PO 2.5 mg/kg daily × 28 days</td>
<td>Renal failure/low K*</td>
</tr>
<tr>
<td></td>
<td><strong>Amphotericin B</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV 1 mg/kg daily × 15 days</td>
<td>Renal failure/low K*</td>
</tr>
<tr>
<td></td>
<td><strong>Liposomal amphotericin</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV 3 mg/kg daily × 5 days</td>
<td>Renal failure/low K*</td>
</tr>
<tr>
<td></td>
<td><strong>Paromomycin</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM 20 mg/kg daily × 21 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Combination therapy?</strong></td>
<td>Awaiting trial results</td>
</tr>
</tbody>
</table>

*Species-specific therapy is beyond the scope of this review. IL denotes intraleisional delivery.*
Adverse effects include prolongation of the QT interval sometimes leading to serious arrhythmias [72], pancreatitis [73] and hepatic dysfunction [74]. In Bihar state, India, cure rates with antimonials have dropped to 35% owing to resistance. In Europe and developed countries liposomal amphotericin has become the treatment of choice with high cure rates (90–100%), reduced side effects and shorter hospital stay. However, cost restricts more widespread use of this agent, whilst alternatives including conventional amphotericin, pentamidine and paromomycin though efficacious also require parenteral administration and have significant side effects.

Miltefosine, an alkylphosphocholine analogue, is an oral agent with few side effects (teratogenic in animals) and high cure rates [75]. It is licensed in India, Germany and Colombia for use in adults and children. Concerns about the development of resistance have led to the suggestion of combination drug therapy with results of a trial using sitamaquine awaited [76].

Co-infection with HIV accelerates the clinical course of both infections [77] and highlights the importance of CD4+ T cells in the control of intracellular infections. Severely immunosuppressed patients demonstrate greater organ dissemination of the parasite, reduced response to therapy and higher relapse rates [78]. Immune reconstitution with highly active antiretroviral therapy in combination with anti-parasitic agents improves outcome [79]. There is no consensus regarding secondary prophylactic agents or their regimen.

What is the future?

The international recognition of the importance of this disease coordinated by WHO programmes brings renewed optimism that control is feasible, especially in India where 70% of the global burden of VL is found. New tools for early identification of cases should facilitate surveillance and enable better co-ordinated control programmes. More widespread control in the poorer regions of the world will only be achievable as the local infrastructure develops to enable delivery of healthcare. The emergence of HIV, drug-resistant strains and changes in the epidemiology of the vector challenge this effort. Long-term control will depend on sustained international effort combined with the necessary resources to develop new tools and ultimately a protective vaccine.

Acknowledgements

I am grateful to Professor Jennie Blackwell for the opportunity to study this subject and for reading this manuscript.
References

29 Huang FP, Xu D, Esfandiari EO, Sands W, Wei XQ, Liew FY. Mice defective in Fas are highly susceptible to Leishmania major infection despite elevated IL-12 synthesis, strong Th1 responses, and enhanced nitric oxide production. J Immunol 1998;160:4143–7.


78 Davidson RN, Russo R. Relapse of visceral leishmaniasis in patients who were coinfected with human immunodeficiency virus and who received treatment with liposomal amphotericin B. *Clin Infect Dis* 1994;19:560.