CED
Clinical and Experimental Dermatology

Cutaneous leishmaniasis: advances in disease pathogenesis, diagnostics and therapeutics

M. Ameen

Royal Free Hospital, Royal Free Hampstead NHS Trust, London, UK

doi:10.1111/j.1365-2230.2010.03851.x

Summary

Cutaneous leishmaniasis is one of the most common tropical dermatoses worldwide and is of major public health importance. It is caused by numerous *Leishmania* protozoa species, which are responsible for its clinical diversity. With changes in vector (sandfly) habitat and increased travel among human populations, its incidence is rising, and in nonendemic countries, including the UK, it is increasingly diagnosed in migrants, returned travellers, and military personnel. Diagnostic tests have not always been sufficiently sensitive, and despite a wide range of treatments, poor therapeutic responses and adverse effects are common. In the past decade, there have been notable advances in molecular diagnostics, in the understanding of host immune responses to infection, and in new therapeutic interventions and vaccine development.

Introduction

Leishmaniasis is a disease caused by protozoa of the *Leishmania* genus. The protozoa are transmitted through the bite of an infected sandfly, and > 20 *Leishmania* protozoa species are responsible for disease in humans.

Epidemiology and aetiology

Leishmaniasis is endemic in > 80 countries throughout Africa, Asia, southern Europe (Old World; OW) and Latin America (New World; NW). It has an estimated prevalence of 12 million cases worldwide, which is continuing to increase, with 1.5–2 million new cases each year. New foci of leishmaniasis are emerging due to changes to ecology and vector habitats caused by deforestation, urbanization and civil conflict.[1,2] Consequently, there has been spread to previous nonendemic regions such as the state of Texas in the USA.[3] In the UK, there has been a marked increase in cases as a consequence of increased travel, both for tourism and for work (e.g., in military personnel returning from Iraq and Afghanistan, where the incidence is high).[4] The numbers of cases seen at the Hospital of Tropical Diseases in London quadrupled over a 10-year period until 2004.[5]

The clinical outcome of infection is determined by the species of *Leishmania*, vector virulence factors and host immune responses, resulting in cutaneous (CL), mucocutaneous (MCL) or visceral (VL) leishmaniasis. *Leishmania major* and *Leishmania tropica* are the predominant species causing OW CL. *Leishmania aethiopica*, which is restricted to East Africa, can also cause the severe mucocutaneous and diffuse variants. *Leishmania mexicana* complex and *Leishmania braziliensis* complex are responsible for NW CL. The latter (Viannia subgenus) is of particular importance as it has the propensity to cause MCL (Table 1).[1]

Clinical presentation

The time from the sandfly bite to the development of skin lesions varies, with incubation periods ranging from weeks to months. Initially, there may be a patch of nonspecific erythema with induration (Fig. 1a) which

Correspondence: Dr Mahreen Ameen, Royal Free Hampstead NHS Trust, Belsize Park, London, NW3 2QG, UK
E-mail: mahreenameen@hotmail.com

Conflict of interest: none declared.

Accepted for publication 10 January 2010

Table 1 The major *Leishmania* species responsible for human leishmaniasis.

Clinical presentation	Location	Species
CL	OW	• *L. major*
		• L. tropica
		• L. aethiopica (rarely MCL/ DCL)
	NW	***L. mexicana* complex**
		• *L. mexicana* (rarely DCL)
		• L. amazonensis (rarely DCL)
		• L. venezuelensis
CL/MCL	NW	***L. braziliensis* complex**
		• L. braziliensis
		• L. colombiensis
		• L. guyanensis
		• L. panamensis
		• *L. peruviana*
VL/CL	OW	***L. donovani* complex**
		• L. donovani
		• L. infantum
	NW	***L. donovani* complex**
		• L. chagasi

OW, Old World; NW, New World; DCL, diffuse CL.

progresses to papular lesions (Fig. 1b). Nodules may also develop, which can ulcerate (Fig. 1c). OW CL caused by *L. tropica* typically does not ulcerate but produces markedly hyperkeratotic lesions (Fig. 1d). Lymphangiatic spread with satellite lesions often occurs (Fig. 1c). MCL can involve the nasal and buccal mucosae, causing considerable inflammation with risks of necrosis and cartilage destruction (Fig. 1e). A variant of CL endemic in the rainforests of central America is the 'chiclero ulcer', which characteristically affects only the pinna (Fig. 1f). Diffuse CL is rare, and produces widespread, multiple nonulcerative papulonodular or infiltrative cutaneous lesions with features similar to lepromatous leprosy (Fig. 1g).[1]

Cutaneous leishmaniasis and human immunodeficiency virus

Leishmaniasis is an emerging opportunistic infection in patients infected with human immunodeficiency virus (HIV). It has been a significant problem with VL co-infection, and these patients have shown poor responses to systemic chemotherapy and high relapse rates, although relapse has been reduced by highly active antiretroviral therapy and secondary prophylaxis.[6] A predictably similar clinical picture is emerging with HIV co-infection with CL, which often presents with atypical lesions. Such cases require aggressive management to ensure cure and prevent relapse.[7]

Immunopathogenesis

The immune response to *Leishmania* infection is cell-mediated, and the clinical outcome is dependent on host-mediated T helper (Th)1 or Th2 responses. A Th1 response mediated by interferon (IFN)-γ, tumour necrosis factor and interleukin (IL)-12 is associated with disease resolution and resistance, and a Th2 IL-4-producing response confers disease susceptibility and progression. These immune responses account for the diverse clinical spectrum of CL. Localized self-healing lesions have a predominantly Th1 cytokine profile, whereas Th2-mediated responses characterize diffuse and nonhealing lesions. MCL demonstrates a mixed Th1 and Th2 response, which may account for both its aggressive inflammatory activity and chronicity.[8] Therefore, host immune responses dictate prognosis, suggesting potential for immunotherapeutic interventions.

Diagnosis

Traditionally, CL has been diagnosed microscopically by the identification of *Leishmania* parasites or amastigotes within macrophages of sample tissue. However, the presence of amastigotes depends on the duration of lesions, being fewer in number in chronic lesions.[9] Therefore, failure to visualize amastigotes on histopathology does not exclude a diagnosis of CL. To maximize sensitivity and specificity, the following four investigations should ideally be performed, facilities permitting: direct microscopy, histopathology, culture and PCR. Tissue samples can be obtained by simple aspiration, scrapings, slit incisions or skin biopsy. Biopsy specimens are preferred as they enable all tests to be performed; they can be used to prepare impression smears for direct microscopy and inoculated for culture. The sampling site within lesions influences the sensitivity of parasitological diagnosis; for maximum sensitivity, incisional biopsies should be taken from the active edge of skin lesions rather than from the centre or the base of an ulcer.[10] A minimum of two biopsies are necessary: one fixed in formalin for histopathology and the other sent as fresh tissue in saline-soaked gauze for all other investigations. Species identification is critical as it determines the clinical course, prognosis and choice of treatment, and until the recent development of molecular methods, it was only possible through isoenzyme analysis of *Leishmania* culture. Culture sensitivity varies widely, ranging from only 40% to 75%.[11] In contrast, the development of PCR technology has enabled the rapid detection of *Leishmania*-specific DNA, with 100% specificity and > 90% sensitivity.[12]

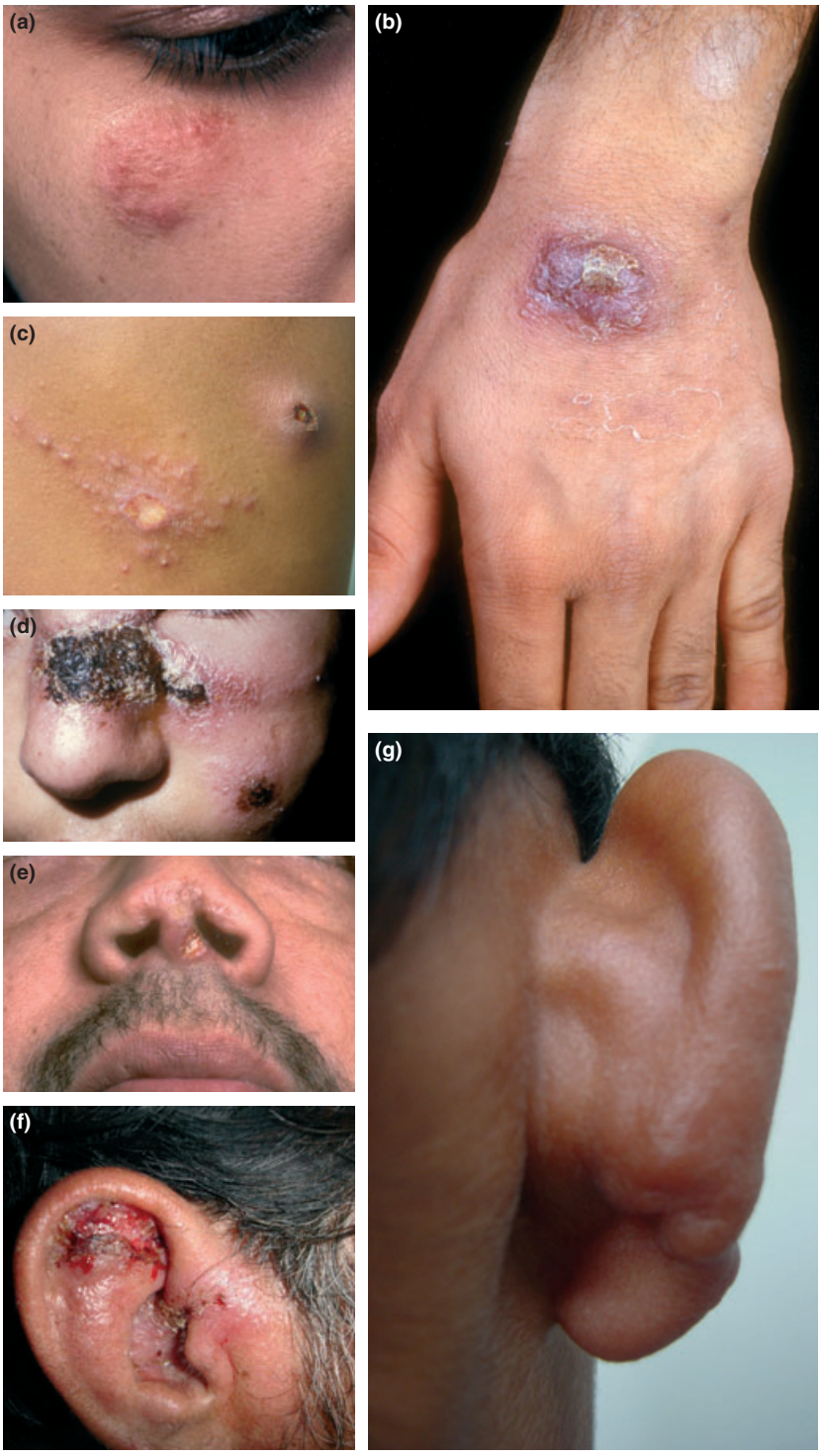

Figure 1 (a) The initial features of cutaneous leishmaniasis (CL) may be non-specifc, with erythema and induration only; (b) a large solitary papulnodular lesion on the dorsum of the hand; (c) the lesions begin as papules or nodules before ulcerating, and lymphangiatic spread is also seen; (d) hyperkeratotic lesion of Old World *Leishmania tropica*; (e) New World CL due to *Leishmania braziliensis* complex with nasal mucosal inflammation and ulcerative lesions; (f) 'chiclero ulcer' due to *Leishmania mexicana* affecting the pinna; (g) diffuse CL due to *Leishmania amazonensis* with cutaneous infiltration of the ear.

Treatment

The goal of treatment is to accelerate healing, reduce the risk of scarring and prevent disease progression. The choice of treatment depends on the size and location of the lesion, the number of lesions and the potential for dissemination. Pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) have been used as standard first-line treatment for CL since 1929. Parenteral treatment is given for severe

Table 2 Treatments for cutaneous leishmaniasis.[14–16]

	Treatment regimen	Species	Therapy response (% cure rate)	Adverse effects
Local therapy				
Intralesional antimonial	Lesion infiltrated at base Weekly therapy until cure	*L. major* *L. tropica* *L. mexicana* complex	Up to 95%	Pain at injection site
Topical paromomycin in 10% urea or 12% methylbenzethonium chloride	Twice daily application for up to 4 weeks Can be combined with intralesional antimonials	*L. major* *L. mexicana* complex	Highly variable 30–90%	Local irritation
Cryotherapy	Weekly treatment of two freeze/thaw cycles for 10–25 s until cure More suitable and effective for smaller lesions	*L. major* *L. tropica* *L. mexicana* complex	70–80% Higher when combined with intralesional antimonials or topical paramomycin	Risk of secondary infection and post-inflammatory dispigmentation
Thermotherapy	1–3 applications of radiofrequency waves at 50°C for 30 s Use depends on lesion size and number	*L. tropica* *L. mexicana* complex	70%	Usually well-tolerated
Oral chemotherapy				
Azoles	Fluconazole 200 mg daily for 6 weeks Ketoconazole 600 mg daily for 4–6 weeks	*L. major* *L. mexicana* complex	70–80% Generally better response with fluconazole	High doses of ketoconazole increase risk of hepatoxicity
Miltefosine	2.5 mg/kg/day for 28 days	*L. panamensis*	> 90%	Gastrointestinal disturbance, teratogenic
Parenteral chemotherapy				
Antimonials (Sodium stibogluconate or meglumine antimoniate)	20 mg/kg/day for 20–28 days	*L. braziliensis* complex	> 90%	Cardiotoxic, hepatotoxic, nephrotoxic
Pentamidine isethionate	Three doses on alternate days at 2–4 mg/kg	*L. braziliensis* complex	> 90%	May induce diabetes mellitus
Amphotericin	Second-line agent for severe infection. 0.5–1.0 mg/kg alternate days for up to 8 weeks (total dose <1.5–2.0 g)	*L. braziliensis* complex	Case studies only	Nephrotoxic Liposomal preparation less toxic

cutaneous disease, for the treatment of *Leishmania* species with the potential to disseminate, and for established MCL. However, systemic antimonials are associated with considerable toxicity, and there are reports of emerging leishmanial resistance.[13] There are now viable alternatives to standard antimonial treatment, such as pentamidine, miltefosine and amphotericin, but these too are limited by toxicity, parenteral route of administration, potential for developing drug resistance, and inadequate efficacy (Table 2).[14,15] In addition, therapeutic response seems to be species-specific, and is also associated with the endemic region and vector biology.

For uncomplicated, localized CL there are a range of local and physical therapies. Although such cases are usually self-limiting, treatment is advocated to speed up disease resolution and minimize scarring. Most lesions due to *L. major* or *L. mexicana* spontaneously resolve within 3 months, and therefore could be left untreated if treatments are unavailable. However, *L. tropica* takes considerably longer to resolve (up to 1 year) and should be actively treated. The efficacy rates of topical paromomycin, cryotherapy, thermotherapy and intralesional antimonials vary widely, making it difficult to assess their relative merits. This is largely due to poor clinical trial data, which often fail to define clinical endpoints and follow-up periods and rarely include placebo controls, which are important given the potential of CL in particular to resolve spontaneously. Generally, most studies have found the highest clinical efficacy and

lowest recurrence rates with intralesional antimonials.[16] A comparative study of intralesional antimonials and cryotherapy for OW CL found cure rates of 92% and 78%, respectively.[17]

Photodynamic treatment (PDT) is emerging as an attractive local treatment option with excellent cosmetic results.[18] Weekly treatments for approximately a month are required, and repeat analysis at the end of treatment should be performed to confirm parasitological cure. Its mechanism of action seems to involve tissue destruction accompanied by depopulation of macrophages.[19] PDT may also be effectively combined with other physical treatments.[20]

Immunotherapy

Knowledge of host immune responses to infection has led to studies investigating the use of immunomodulatory agents to enhance disease resolution. Although early studies investigating treatment with IFN in an attempt to induce a Th1 immunological profile found no significant benefit,[21] topical immunomodulators have been more successful. In a study using topical granulocyte–macrophage colony-stimulating factor as an adjunct to antimonial treatment, significantly higher efficacy was seen in the dual treatment group.[22] Topical 5% imiquimod, which induces the production of cytokines stimulating a Th1 response, has demonstrated efficacy in experimental CL.[23] In clinical studies, the addition of 5% imiquimod to parenteral antimonial therapy has been shown to improve clinical outcomes. In a recent randomized double-blind clinical trial of NW *L. braziliensis* complex infection, parenteral antimonial monotherapy vs. parenteral antimonial and topical 5% imiquimod dual therapy demonstrated 58% and 75% cure rates respectively.[24] However, topical imiquimod has not demonstrated any therapeutic advantage over antimonial monotherapy for OW *L. tropica* CL.[25]

Vaccine development

The rationale for vaccine development came from the observation that recovery from leishmaniasis infection is accompanied by solid immunity against re-infection. The ancient practice of leishmanization in endemic areas involved live vaccination and was done to produce natural resistance, which would minimize the effect of scarring in any subsequent infection. This has largely been discontinued because of instances of unacceptable scarring. However, there is a need for an effective, long-lasting and safe vaccine for CL, and studies in animal models and humans are presently evaluating the potential of genetically modified live attenuated vaccines, recombinant antigens and sandlfy saliva proteins as candidate vaccines.[26,27] The complete genome sequence of *L. major* should provide a further source of vaccine candidates.[28] Vaccines have also been used as a form of immunotherapy and alternative to chemotherapy. An experimental vaccine treated severe mucocutaneous and early diffuse CL by injecting killed promastigotes of *L. braziliensis* together with viable bacillus Calmette–Guérin. This induced a Th1 response, which resulted in complete healing of lesions.[29]

Conclusion

CL is becoming increasingly prevalent in endemic and in nonendemic countries, and an increasing numbers of cases are seen in returning travellers. It is a highly complex and heterogenous disease. Treatment is tailored according to clinical severity, the species of *Leishmania*, and the immune competence of the host. Systemic therapies are often associated with considerable toxicity, necessitating new drug development, but progress has been slow. However, there have been improvements in diagnostics and new therapeutic options, including immunotherapy and ongoing progress in vaccine development.

Learning points

- The incidence of CL is increasing worldwide. The number of cases in nonendemic countries is also rising because of infection in tourists and in returning military personnel.
- Numerous *Leishmania* protozoa species are responsible for CL, which is acquired through the bite of an infected sandfly. *L. tropica* and *L. major* are the main species responsible for OW CL. *L. mexicana* complex and *L. braziliensis* complex cause NW CL or MCL.
- The clinical presentation, treatment choice and prognosis is dependent on *Leishmania* species, host immune responses and vector biology.
- Diagnostic tests should include direct microscopy, histopathology, culture and PCR to ensure maximum sensitivity and specificity.
- CL is often self-limiting, but treatment is given to accelerate healing, reduce scarring, and prevent the risk of progression to mucocutaneous and visceral disease.

- Local and physical therapies (intralesional antimonials, topical paromomycin, cryotherapy and thermotherapy) can be used for limited CL without the risk of dissemination.
- Systemic treatment is required in cases of *Leishmania* species with the potential to disseminate, host immunocompromise, mucocutaneous or diffuse disease, and severe cutaneous disease. Parenteral antinomials are usually first-line agents but are associated with considerable toxicity.

Acknowledgement

I am grateful to Stuart Robertson at St John's Institute of Dermatology, Guys & St Thomas' NHS Trust for Permission to use images 1b, 1d and 1e.

References

1 World Health Organization. *Leishmaniasis*. Available at: http://www.who.int/leishmaniasis/en/ (accessed 16 January 2010).
2 Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004; **27**: 305–18.
3 Wright NA, Davis LE, Aftergut KS *et al.* Cutaneous leishmaniasis in Texas : a northern spread of endemic areas. *J Am Acad Dermatol* 2008; **58**: 650–2.
4 Bailey MS, Green AD, Ellis CJ *et al.* Clinical guidelines for the management of cutaneous leishmaniasis in British military personnel. *J R Army Med Corps* 2005; **151**: 73–80.
5 Lawn SD, Whetham J, Chiodini PL *et al.* New world mucosal and cutaneous leishmaniasis: an emerging health problem among British travellers. *QJM* 2004; **97**: 781–8.
6 Pasquau F, Ena J, Sanchez R *et al.* Leishmaniasis as an opportunistic infection in HIV-infected patients: determinants of relapse and mortality in a collaborative study of 228 episodes in a Mediterranean region. *Eur J Clin Micrbiol Infect Dis* 2005; **24**: 411–18.
7 Couppie P, Clyti E, Sobesky M *et al.* Comparative study of cutaneous leishmaniasis in human immunodeficiency virus (HIV)-infected patients and non-HIV-infected patients in French Guiana. *Br J Dermatol* 2004; **151**: 1165–71.
8 Reed SG, Scott P. T-cell and cytokine responses in leishmaniasis. *Curr Opin Immunol* 1993; **5**: 524–31.
9 Andrade-Narvaez FJ, Medina-Peralta S, Vargas-Gonzalez A *et al.* The histopathology of cutaneous leishmaniasis due to Leishmania (Leishmania) mexicana in the Yucatan peninsula, Mexico. *Rev Inst Med Trop Sao Paulo* 2005; **47**: 191–4.
10 Ramírez JR, Agudelo S, Muskus C *et al.* Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. *J Clin Microbiol* 2000; **38**: 3768–73.
11 Boggild AK, Miranda-Verastegui C, Espinosa D *et al.* Optimization of microculture and evaluation of miniculture for the isolation of Leishmania parasites from cutaneous lesions in Peru. *Am J Trop Med Hyg* 2008; **79**: 847–52.
12 Reithinger R, Dujardin JC. Molecular diagnosis of leishmaniasis: current status and future applications. *J Clin Microbiol* 2007; **45**: 21–5.
13 Carter KC, Hutchison S, Henriquez FL *et al.* Resistance of Leishmania donovani to sodium stibogluconate is related to the expression of host and parasite gamma-glutamylcysteine synthetase. *Antimicrob Agents Chemother* 2006; **50**: 88–95.
14 Gonzalez U, Pinart M, Reveiz L, Alvar J. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst Rev* 2008: CD005067.
15 Gonzalez U, Pinart M, Rengifo-Pardo M *et al.* Interventions for American cutaneous and mucocutaneous leishmaniasis). *Cochrane Database Syst Rev* 2009: CD004834.
16 Ameen M. Cutaneous leishmaniasis. therapeutic strategies and future directions. *Expert Opin Pharmacother* 2007; **8**: 2689–99.
17 Gurei MS, Tatli N, Ozbilge H *et al.* Efficacy of cryotherapy and intralesional pentostam in treatment of cutaneous leishmaniasis. *J Egypt Soc Parasitol* 2000; **30**: 169–76.
18 van der Snoek E, Robinson D, van Hellemond J, Neumann H. A review of photodynamic therapy in cutaneous leishmaniasis. *J Eur Acad Dermatol Venereol* 2008; **22**: 918–22.
19 Akilov OE, Kosaka S, O'Riordan K, Hasan T. Parasiticidal effect of delta-aminolevulinic acid-based photodynamic therapy for cutaneous leishmaniasis is indirect and mediated through the killing of the host cells. *Exp Dermatol* 2007; **16**: 651–60.
20 Pizinger K, Cetkovska P, Kacerovska D, Kumpova M. Successful treatment of cutaneous leishmaniasis by photodynamic therapy and cryotherapy. *Eur J Dermatol* 2009; **19**: 172–3.
21 Harms G, Chehade AK, Douba M *et al.* A randomized trial comparing a pentavalent antimonial drug and recombinant interferon-gamma in the local treatment of cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 1991; **85**: 214–16.
22 Santos JB, De Jesus AR, Machado PR *et al.* Antimony plus recombinant human granulocyte-macrophage colony-stimulating factor applied topically in low doses enhances healing of cutaneous leishmaniasis ulcers: a randomized, double-blind placebo-controlled study. *J Infect Dis* 2004; **190**: 1793–6.
23 Buates S, Matlashewski G. Treatment of experimental leishmaniasis with the immunomodulators imiquimod and

S-28463: efficacy and mode of action. *J Infect Dis* 1999; **179**: 1485–94.
24 Miranda-Verastegui C, Tulliano G, Gyorkos TW *et al.* First-Line Therapy for Human Cutaneous Leishman in Peru using the TLR7 Agonist Imiquimod in Combination with Pentavalent Antimony. *PLoS Negl Trop Dis* 2009; **3**: e491.
25 Firooz A, Khamesipour A, Ghoorchi MH *et al.* Imiquimod in combination with meglumine antimoniate for cutaneous leishmaniasis. *Arch Dermatol* 2006; **142**: 1575–80.
26 Kubar J, Fragaki K. Recombinant DNA-derived *Leishmania* proteins: from the laboratory to the field. *Lancet Infect Dis* 2005; **5**: 107–14.
27 Kamhawi S, Belkaid Y, Modi G *et al.* Protection against cutaneous leishmaniasis resulting from bites of uninfected sandflies. *Science* 2000; **290**: 1351–4.
28 Stober CB, Lange UG, Roberts MT *et al.* From genome to vaccines for leishmaniasis: screening 100 novel vaccine candidates against murine Leishmania major infection. *Vaccine* 2006; **24**: 2602–16.
29 Convit J, Ulrich M, Polegre MA *et al.* Therapy of Venezuelan patients with severe mucocutaneous or early lesions of diffuse cutaneous leishmaniasis with a vaccine containing pasteurized Leishmania promastigotes and bacillus Calmette-Guerin – preliminary report. *Mem Inst Oswaldo Cruz* 2004; **91**: 57–62.

