CHAPTER 6

Understanding Human Leishmaniasis: The Need for an Integrated Approach

M. Hide,[1] B. Bucheton,[1] S. Kamhawi,[2] R. Bras-Gonçalves,[3] S. Sundar,[4] J.-L. Lemesre,[3] and A.-L. Bañuls[1]

[1]*Génétique et Evolution des Maladies Infectieuses (UMR CNRS/IRD 2724), IRD de Montpellier, 911 av Agropolis BP 64501, 34394 Montpellier Cedex 5, France*

[2]*Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, USA*

[3]*Pathogenie des Trypanosomatidés (UR008), IRD de Montpellier, 911 av Agropolis BP 64501, 34394 Montpellier Cedex 5, France*

[4]*Infectious Diseases Research Laboratory, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, 6 SK Gupta, Nagar, Lanka, Varanasi 221005, India*

6.1 GENERALITIES ON LEISHMANIASIS

Leishmaniasis has been known for many hundreds of years, with one of the first clinical descriptions made in 1756 by Alexander Russell and called Aleppo boil. Many names correspond to this group of diseases: kala-azar, Dum-dum fever, white leprosy, espundia, pian bois, and so on. Leishmaniases are parasitic diseases spread by the bite of the infected female phlebotomine sand fly (Fig. 6.1). Leishmaniases are caused by approximately 20 species, pathogenic for humans, belonging to the genus *Leishmania* (kinetoplastids order, Honigberg, 1963) and within 500 known phlebotomine species, of which only some 30 have been positively identified as vectors of these pathogenic species.

6.1.1 Geographic Distribution

Human leishmaniases are found on all continents, except Antarctic and Australia. However, cutaneous leishmaniasis was recently revealed in Australian red kangaroos [296]. Approximately 350 million people live in endemic areas, thereby comprising populations at risk, and annual incidence is estimated at 1–1.5 million cases of cutaneous leishmaniasis plus 500,000 cases of visceral leishmaniasis; overall prevalence is 12 million people. Most of the affected countries are in the tropics and subtropics: more than 90% of the world's cases of visceral leishmaniasis are in India, Bangladesh, Nepal, Sudan, and Brazil (Fig. 6.2), 90% of all cases of mucocutaneous leishmaniasis (Fig. 6.3) occur in Bolivia, Brazil, and Peru, whereas 90% of all cases of cutaneous leishmaniasis (Fig. 6.3) occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia, and Syria (for further detail, see http://www.who.int/leishmaniasis/en/).

6.1.2 The Players in Leishmaniasis

Leishmania parasites are responsible for cutaneous forms as well as visceral forms of the disease. Healing or progression of this infection is related to the genetic and immune status of the *host*, the virulence and pathogenicity of different species and strains of *Leishmania*, and the *vector* involved. The hosts can be humans but also rodents, dogs, and other mammals [16,307], and great diversity of immune response exists depending on the host considered (see Section 6.4 for details). Similarly, within 500 known phlebotomine species, only 31 have been positively identified as vectors of the *Leishmania* pathogenic species and 43 as probable vectors [181]. Among them, some vectors such as *Phlebotomus Phlebotomus papatasi* and *P. Paraphlebotomus sergenti* can only be

Encyclopedia of Infectious Diseases: Modern Methodologies, by M. Tibayrenc

Fig. 6.1. *Phlebotomus argentipes*, the vector of kala-azar in India and neighbouring countries, engorged. (Photo taken by Edgar D. Rowton, all rights reserved.)

infected by one *Leishmania* species, whereas *Lutzomyia longipalpis* is a permissive vector, able to transmit different *Leishmania* species (see Section 6.2 for details). Finally, the 20 species described as pathogenic for humans belong to the *Leishmania* genus (Ross, 1903). They are divided into two subgenera (*Leishmania* in the Old World (Saf'Janova, 1983) and *Viannia* in the New World (Lainson and Shaw, 1987)), the *Leishmania* subgenus is composed of several species or species complexes (*Leishmania donovani* complex, *L. mexicana* complex, *L. major*, *L. tropica*, etc.) and the *Viannia* subgenus contains species of the *L. braziliensis* complex (*L. braziliensis* (Viannia, 1911), *L. peruviana* (Velez, 1913), and the *L. guyanensis* complex (*L. guyanensis* (Floch, 1954), *L. panamensis* (Lainson and Shaw, 1972)), *L. lainsoni*, etc.). These *Leishmania* species are associated with different diseases (see Section 6.3 for details). For example, infections by *Leishmania donovani* complex species are associated with visceral leishmaniasis and *L. braziliensis* infections are responsible for mucocutaneous

Fig. 6.2. Distribution of visceral leishmaniasis (WHO website: http://www.who.int/leishmaniasis/leishmaniasis_maps/en/index.html).

Fig. 6.3. Distribution of cutaneous leishmaniasis (WHO website: http://www.who.int/leishmaniasis/leishmaniasis_maps/en/index.html).

leishmaniasis. However, the first species complex is able to generate benign cutaneous lesions, and *L. braziliensis* has been isolated from simple cutaneous lesions but also from visceral forms. It is clear that *the clinical outcome of infection depends on a multifaceted association of factors among the three main players involved*: *hosts, parasites, and vectors*.

6.1.3 The Life Cycle of the *Leishmania* Parasite

Leishmania parasites are transmitted to their host by the bite of an infected female phlebotomine sand fly (Psychodidae family, Phlebotominae subfamily), which needs a blood meal to produce its eggs (Fig. 6.4). The sand fly vectors are primarily infected when feeding on the blood of an infected individual or a vertebrate reservoir host. Many mammal species could act as a reservoir host, for example, rodents or dogs [16,307].

During feeding, host macrophages, containing *amastigotes* (Fig. 6.5), are ingested by the vector. These parasite forms, round and nonmotile (3–7 μm in diameter), are released into the posterior abdominal midgut of the insect, where they transform into *promastigotes* to begin their extracellular life cycle in the vector. This form is motile, elongated (10–20 μm), and flagellated (Fig. 6.6).

The promastigotes then migrate to the anterior part of the alimentary tract of the sand fly where they multiply by binary fission. Approximately 7 days after feeding, the promastigotes undergo metacyclogenesis and become infectious (metacyclic promastigotes). They are released into the host together with saliva when the sand fly lacerates the skin with its proboscis during feeding. The sand flies usually feed at night while the host is asleep.

These metacyclic promastigotes are taken up by host macrophage, where they metamorphose into the amastigote form. They increase in number by binary fission within the phagolysosome until the cell eventually bursts, then infect other phagocytic cells and continue the cycle. In cases of visceral leishmaniasis, all organs, containing macrophages and phagocytes, can be infected, especially the lymph nodes, spleen, liver, and bone marrow.

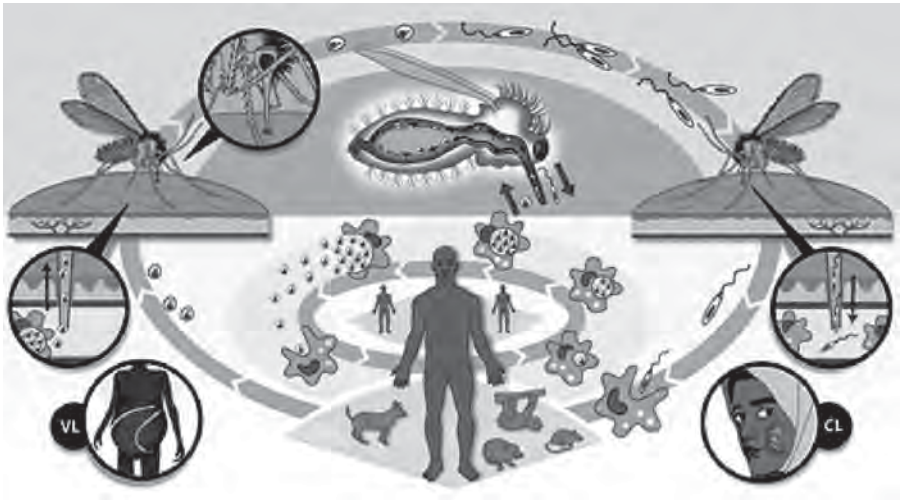

Fig. 6.4. *Leishmania* life cycle (WHO website: http://www.who.int/tdr/diseases/leish/leish.htm).

6.1.4 Symptoms

A high rate of infected people remain asymptomatic, but for others, the infection by *Leishmania* can produce very different clinical symptoms. Indeed, several forms of leishmaniasis exist: cutaneous leishmaniasis and mucocutaneous leishmaniasis, which cause skin sores, and visceral leishmaniasis, which affects some of the internal organs of the body (e.g., spleen, liver, bone marrow). People with cutaneous leishmaniasis usually develop skin sores a few weeks (sometimes as long as months) after being bitten, whereas people with visceral leishmaniasis usually become sick within several weeks or months (rarely as long as years).

The most severe form of the disease is visceral leishmaniasis (VL) (Fig. 6.7), which has a mortality rate of almost 100% if untreated. It is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia. *Leishmania* species responsible for this form mainly belong to the *Leishmania donovani* complex. VL caused by *L. infantum* especially affects children. Other symptoms, called post-kala-azar dermal leishmaniasis (PKDL), can appear several months (or years) after VL treatment. This complication of VL is characterized by a macular, maculopapular, and nodular rash in a patient who has recovered from VL and who is otherwise well [400].

Mucocutaneous leishmaniasis (MCL) (Fig. 6.8.), mainly caused by *L. braziliensis* and more rarely by the *L. guyanensis* complex, produces lesions that can lead to extensive and disfiguring destruction of mucous tissues of the nose, mouth, and body, including the face, arms, and legs, causing serious disability.

The cutaneous leishmaniases (CL) (Fig. 6.9) are the most common and represent 50–75% of all new cases. CL also result in a variety of clinical manifestations, in terms of the number of lesions (up to 200 on the exposed part of the body) and with selfhealing lesions compared with lesions requiring specific anti-*Leishmania* treatment. The lesion is localized at the site of the sand fly bite and satellite lesions in the vicinity of the original lesion can sometimes be observed. CL are mainly attributable to *L. amazonensis*, *L. braziliensis*, *L.*

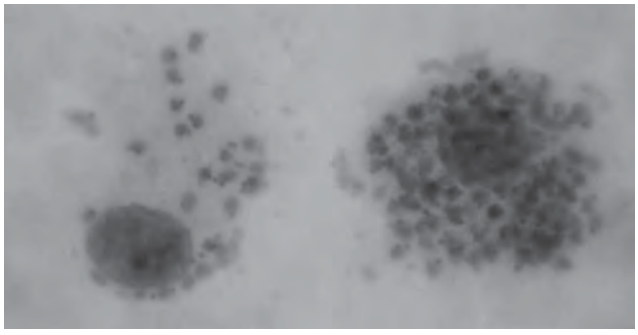

Fig. 6.5. Two human macrophages infected by *L. donovani* amastigotes, all rights reserved.

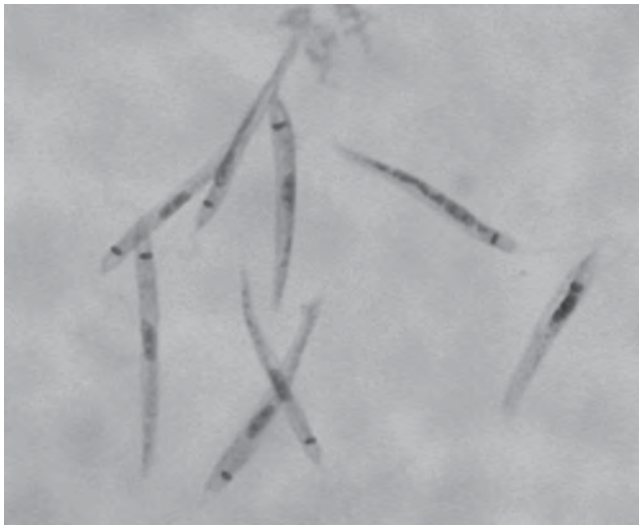

Fig. 6.6. *L. infantum* promastigotes, all rights reserved.

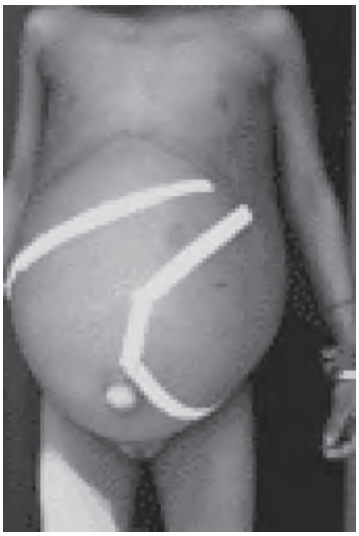

Fig. 6.7. Visceral leishmaniasis. (Photo taken by Philippe Desjeux. WHO website: http://www.who.int/leishmaniasis/disease_epidemiology/en/index.html.)

guyanensis, *L. mexicana* (Biagi, 1953), *L. panamensis*, *L. naiffi*, *L. venezuelensis*, *L. lainsoni*, and *L. shawi* in the New World and *L. major* (Yakimoff and Schockor, 1914), *L. aethiopica* (Ashford and Bray, 1973), *L. tropica* (Wright, 1903), *L. arabica*, and *L. gerbilli* (Wang, Qu, and Guan, 1964) in the Old World, even if other species such as *L. donovani* (Laveran and Mesnil, 1903), *L. infantum* (Nicolle, 1908) have also been isolated from cutaneous lesions. Diffuse CL, mainly caused by *L. amazonensis* and *L. aethiopica*, never heals spontaneously and tends to relapse after treatment. This form is characterized by disseminated nodular lesions that resemble lepromatous leprosy.

Finally, these diseases have not only been found in developing countries since 1985, when the first co-infected patient was detected [93], even if the *Leishmania*–HIV co-infection cases are decreasing in Europe (introduction of Highly Active Antiretroviral Therapy (HAART)). These cases are mostly localized in Europe where intravenous drug users have been identified as the main population at risk. In this case, the immunological status of these people creates a favorable ground for the *Leishmania* parasite.

Fig. 6.8. Mucocutaneous leishmaniasis. (Photo taken by Philippe Desjeux. WHO website: http://www.who.int/leishmaniasis/disease_epidemiology/en/index.html.)

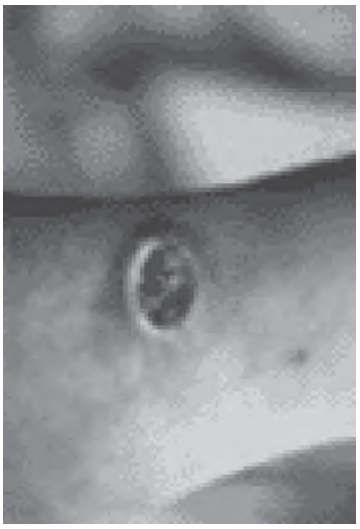

Fig. 6.9. Cutaneous leishmaniasis. (Photo taken by Philippe Desjeux. WHO website: http://www.who.int/leishmaniasis/disease_epidemiology/en/index.html.)

6.1.5 Prevention, Diagnosis, and Treatments

Leishmaniases are a diverse and complex group of disorders. Unfortunately, strict rules cannot be applied for a type of *Leishmania* causing a typical disease, as even subtle changes in host immunity, the environment, and the parasite itself might result in completely different clinical manifestations; therefore, various approaches to disease control are necessary. Hence, prevention, diagnosis, and treatments depend on *Leishmania* species diagnostics and on the disease form; they differ for CL, VL, and MCL.

6.1.5.1 Prevention of leishmaniases

6.1.5.1.1 Zoonotic cutaneous leishmaniasis (ZCL) In the Old World, identification and control of animal reservoirs (small rodents) consist of deep plowing to destroy the burrows (breeding and resting sites) and plant (Chenopodiacae) sources of food for the rodents. Poisoning is no longer used, as it is considered too dangerous for other animals. In New World, especially in Latin America, large mammals living in forests or around houses can help contain the disease. In recent years, there has been an increase in the incidence of ZCL attributable to urbanization and deforestation, leading to domestication of transmission cycles, and the building of dams and new irrigation schemes, which have increased the population of animal reservoirs. Because populations living close to or at the edge of forests are particularly vulnerable, such habitats should be moved away from the forests. Limited clearance of peridomestic forest can reduce the risk of intradomiciliary transmission [101,102].

6.1.5.1.2 Anthroponotic cutaneous leishmaniasis Anthroponotic cutaneous leishmaniasis (ACL) is confined to urban or suburban areas of the Old World. Early diagnosis and treatment of recurring cases are necessary to avoid an increase in transmission risk, as they reduce morbidity, mortality, and transmission (reduction of human reservoir). The best prevention for ACL is the use of long-lasting impregnated bed nets in order to prevent infected sand flies from infecting healthy people and reduce untreated cases that continue infecting sand flies. Residual insecticide house spraying is another important prevention and intervention strategy. Mosquito repellents can be combined with pyrethroid-impregnated clothes (e.g., uniforms for military personnel) for individual protection [78,99]. Mosquito coils and the electrically heated fumigation mats containing pyrethroids are also helpful in protection.

6.1.5.1.3 Zoonotic visceral leishmaniasis In zoonotic visceral leishmaniasis (ZVL) endemic areas, the dog is a major reservoir. Several preventive measures are advocated: insecticide-impregnated dog collars, vaccination of pets against leishmaniasis, and elimination of infected stray dogs can decrease the incidence of infection. Canine and indirectly human leishmaniasis (because dogs are the *Leishmania* reservoir) is prevented by using deltamethrin-treated collars to protect dogs against *L. infantum* infection [127].

6.1.5.1.4 Anthroponotic visceral leishmaniasis (AVL) Elimination of the human reservoir by early diagnosis and treatment of PKDL and VL can reduce the transmission effectively. Furthermore, in anthroponotic foci, vector control through residual insecticide spray and improvement of the environment to control the growth of sand flies are the major tools for prevention.

6.1.5.2 Diagnosis of leishmaniases

6.1.5.2.1 Visceral leishmaniasis Typical clinical features of VL such as fever followed by splenomegaly (*enlargement of the spleen*) and lymphadenopathy (*swelling of the lymph nodes*) in a patient living in the endemic area should arouse suspicion of VL. Presence of antileishmanial antibodies, detected through conventional ELISA, IFAT, or DAT or the popular rapid rK39 strip test, indicates infection [2,12,20,117,149,331,354,385]. This is usually confirmed through demonstration of amastigotes in tissue smears mostly from the spleen, bone marrow, or lymph nodes. Polymerase chain reaction (PCR) is employed for demonstration of parasitic DNA in peripheral blood for diagnosis [249,309].

In India, a rapid strip test based on rK39 antigen has become available and should improve the diagnostic situation [42,136,250,353,354,375,401]. However, there is a need to develop a diagnostic test that has a high degree of specificity for active disease. Detection of antigen in urine (KAtex) is a promising tool, provided its format is improved [17,116,150, 279,312,345]. DNA detection by PCR is another powerful tool that could be established at several nodal centers in endemic areas serving the entire endemic region for diagnosis and evaluation of cure [214,248,249, 265, 308,327,394]. Both KAtex and PCR correlate well with disease activity and thus have a clear edge over tools based on antibody detection.

6.1.5.2.2. Cutaneous leishmaniasis In areas of endemicity without sufficient laboratory infrastructure, CL is often diagnosed on the basis of clinical characteristics of the lesions. However, parasitological confirmation is important, because clinical manifestations may be mimicked by other infections and granulomatous diseases: lupus vulgaris, leprosy, and so on. *Species identification may be important in predicting the course of the disease and selecting therapy*.

Leishmania may be isolated in up to 80% of sores during the first half of their natural course [273]. Parasites seem to be particularly difficult to isolate from sores caused by *L. braziliensis*, responsible for the vast majority of cases in Brazil. Touch preparations from biopsies and histopathology usually have a low sensitivity [81,389]. Slit-skin smears taken from the nodular edge of the lesion, or scrapings from within the ulcer [273] examined microscopically are positive in 32.7–84% [242,389]. Culture of fine needle aspiration material has been reported to be the most sensitive method [242,389]. Mucocutaneous leishmaniasis (MCL) is more difficult to diagnose parasitologically; even hamster inoculation only brings the yield up to 50% [389].

PCR introduced to determine the parasite species is used increasingly for diagnosis, greatly improving the diagnostic rates for CL and MCL [92,203]. For CL in Ecuador, using culture as standard, PCR was 97% sensitive as compared with microscopy (42%) and histology (33%) [18],whereas in Brazil, 71% of MCL cases were detected by PCR compared to 17% detected by conventional method [203]. Clinically, species identification may be important for epidemiological and therapeutic reasons, for example to identify the dominant species in a CL focus in Brazil [91]. Isoenzyme methods [283] and monoclonal antibodies [15,158] have been employed for species typing as well as analysis of amplified minicircle kinetoplast DNA (kDNA), by choosing primers from variable regions of different *Leishmania* species kDNA minicircle [327].

6.1.5.3 Leishmaniasis treatments

Treatment of leishmaniases has centered around *pentavalent antimonials* ($Sb^{V)}$ for six decades except in North Bihar, India, where large-scale antimony resistance is emerging and where Sb^{V}, even with the higher doses, is able to cure only 35–50% of patients [342,352,356,359,360]. In the Old World (*L. major*, *L. tropica*, and *L. donovani* complex) and the New World (*L. mexicana* and *L. braziliensis* complexes), CL and PKDL are commonly treated with Sb^{V}. A species-based approach to treatment has been advocated, especially in countries where several species may cause CL [245,295]. Intralesional Sb^{V} has been used with encouraging results in the Old World selfhealing CL [5,371].

A second-line drug, *pentamidine isethionate*, is expensive and toxic, beacuse it can be responsible for irreversible insulin-dependent diabetes mellitus and death. It was used to treat Sb^{V}-refractory patients with VL, but its efficacy has declined

and its use for VL has been abandoned [160,162,164]. During the late 1980s and the early 1990s, many Indian patients died for want of treatment after failing therapy with Sb^V and pentamidine. Though for some forms of CL, pentamidine is still attractive because very few doses are needed [9,335].

Due to increasing Sb^V-unresponsive VL, especially in India over the last decade, *amphotericin B* has become the drug of choice [230]. However, it is toxic and requires close monitoring. Though the cure rate with amphotericin B is approximately 100% and relapses are rare, the need for hospitalization lasting 5–6 weeks, infusion reactions, occasional serious adverse reactions such as hypokalemia, myocarditis, and death precludes its widespread application in peripheral health posts where monitoring facilities are limited. Thus, a large number of patients have to wait several weeks to months for hospitalization and treatment [132,229,230,361]. In South America, many regard amphotericin as the drug of choice for MCL, because of the low relapse rate [80,291]. The introduction of lipid-associated amphotericin, *i.e., liposomal amphotericin B (AmBisome), amphotericin B lipid complex (ABLC; Abelcet) and amphotericin B colloidal dispersion (Amphocil)*, has been one of the most important developments in the chemotherapy of leishmaniasis. In these formulations, deoxycholate has been replaced by other lipids that mask amphotericin B from susceptible tissues, thus reducing toxicity, and are preferentially taken up by reticuloendothelial cells, thus targeting drug delivery to the parasite and increasing efficacy. Three lipid formulations are commercially available, but their cost is prohibitive [88,89,106,188,227,351]. In India, all three formulations, with comparable efficacy, have been used, with AmBisome being the safest [89,105,188,343,344,346,348,349,351].

Paromomycin, an aminoglycoside, is well tolerated and effective for VL, but less so for CL [74,76,163]. Topical paromomycin ointment has been used for the treatment of CL [187,252,305]. The search for an effective oral antileishmanial drug spans two decades. *Allopurinol*, the azoles, rifampicin, and atovaquone showed activity in experimental systems, but proved disappointing in clinical trials. Oral *miltefosine*, an alkyllysophospholipid, originally developed as an anti-cancer agent, is now approved for the treatment of VL in India [347]. In several clinical trials, miltefosine cured more than 90% of patients with only minor gastrointestinal side effects such as vomiting in about half of the patients and less commonly diarrhea [165,347,350,355]. An asymptomatic transient rise in hepatic transaminases occurs during the second week of treatment, returning back to baseline values on continued treatment. It induces rapid cure, with a majority of patients becoming afebrile within the first week, quick regression of spleen, and recovery of blood counts. However, due to the risk of teratogenicity, Miltefosine should not be given to child-bearing age women except if contraception can be secured during and after treatment. Oral *sitamaquine*, an 8-aminoquinoline derivative, has been shown to have clinically significant antileishmanial activity. This effective oral antileishmanial compound has been tested in Kenya, Brazil, and India [104,161,325,387].

6.1.5.4 Vaccines? *There is no vaccine available* against any form of leishmaniasis for prophylaxis. Control of leishmaniasis remains a source of grave concern worldwide. As most of the available methods for leishmaniasis treatment and control are of limited effectiveness, there is now an urgent need for new low-cost drugs and/or new therapeutic interventions such as a vaccine, which would be the most practical and efficient tool for the control of these parasitic diseases [90].

Although considerable progress has been made over the last decade in understanding the immune mechanisms underlying protective responses, identifying potential candidate antigens, and implementing these principles in animal models, very few candidate vaccines have progressed beyond the experimental stage.

In recent years, great interest has been focused on the development of vaccines against localized cutaneous disease. Comparatively, VL has received limited attention. Indeed, only studies to identify the immunological factors of VL patients after chemotherapy and in asymptomatic subjects have been reported so far [231]. In regions where VL is endemic, such as the Mediterranean area, severe disease only occurs in a small population of around 10–33%, whereas the majority of infected individuals show no clinical symptoms and a significant part have self-resolving infection [21]. Furthermore, patients who have recovered from kala-azar are usually immune to reinfection, suggesting that *vaccination against VL should be possible*. The fact that a large proportion of the people living in endemic areas has self-resolving subclinical infections and the immunological mechanisms that control parasite multiplication in asymptomatic subjects are not well defined provides a rationale for designing immunoprophylactic strategies against VL.

Historically, "leishmanization" with live organisms was used to protect against disfiguring CL, because of the knowledge that individuals whose skin lesions had healed were immune. Knowledge of pathogenesis fortified by immunological understanding and genetic sequencing studies have gradually led to rational approaches toward the induction of protective immunity to *Leishmania* in animal models. Thus far, attempts at human vaccination have been unsuccessful, but several promising candidate vaccines are being explored in mouse models and in dogs.

In humans, measurement of cytokines in culture supernatants of *Leishmania* antigen-activated PBMCs and T-cell clone analysis support the view that (i) cell-mediated immunity, regulated by Th1 $CD4^+$ lymphocytes, was required for the destruction of *Leishmania* parasites in macrophage phagolysosomes [179]; (ii) control of infection in asymptomatic subjects was partially associated with the expansion of parasite-specific $CD8^+$ lymphocytes [211]; and (iii) these measurements revealed a coexistence of Th1 and Th2 responses in kala-azar patients as well as in cured individuals [253]. Therefore, even in humans, it is difficult to demarcate the responses leading to either visceral disease ("susceptible") or protective immunity ("resistance") against *Leishmania* parasites. Successful resistance

is probably the result of cooperation between the various arms of the immune system.

Recently, a vaccine against canine VL involving *Leishmania* excreted–secreted antigen has been developed (*Li*ESAp) [226]. It proved efficient in both experimentally and naturally *L. infantum*-exposed dogs in southern France [147,194]. In dogs, the vaccine-induced protection correlates with an early production of IFN-γ by a Th1 subset of $CD4^+$ T cells, which activate macrophages to destroy intracellular amastigotes through NO production. This was demonstrated by anti-*Li*ESAp IgG2 reactivity, *Li*ESAp-specific lymphocyte proliferation assays, and enhanced NO-mediated anti-leishmanial activity of canine monocyte-derived macrophages (CM-DM). In vaccinated dogs, NO-mediated *Leishmania* killing was associated with higher IFN-γ production by T cells when *L. infantum*-infected CM-DMs were co-cultured with autologous lymphocytes [147,194]. The main scientific issues in the design of a *Leishmania* vaccine are no different from those for any other vaccine. On a positive note, there is currently rapid progress in our understanding of the molecular nature of potential vaccine candidates and the mechanisms that determine infection-preventing immune responses. Multidisciplinary approaches integrating studies on parasite and host factors would facilitate our understanding of the disease and help in the design of a vaccine against human VL.

6.1.6 Why an Integrated Approach?

Even if we can generalize the life cycle of *Leishmania* because it always contains one vector, one parasite, and one host, the outcome of transmission, infection, and disease are dependent on the intrinsic characteristics of these three players. Indeed, *the epidemiology of leishmaniasis will be reflective of the particular combination of interactions among all players*: *parasite, vector, reservoir host, and environmental conditions*. In many endemic areas, the exact role of these players and their relations to human infections are unknown and it is difficult to generalize. Integrated analysis of both parasite genetics, parasite virulence factors, host immune responses, vector competence, host genetics, socioeconomic, and environmental risk factors is necessary for a better understanding of the interplay between these different factors and the risk of developing leishmaniasis. This approach could also provide information on the critical biological pathways involved in the host resistance or susceptibility to leishmaniasis and therefore help in orienting new therapeutic or vaccine strategies. Indeed, factors determining the host resistant/susceptible status are complex and largely unknown. Environmental factors acting on the phlebotomine and/or animal reservoir populations could modulate exposure of the human host to infected sand fly bites. Moreover, it has been suggested that the host immune response may also depend on the parasite strain, and different parasitic factors directly or indirectly responsible for the disease outcome have been described. Factors affecting the patient immune competence such as HIV infection or malnutrition have also been described to mediate susceptibility to VL. Immunity in leishmaniasis is considered mainly T-cell mediated, but more and more nonspecific factors acting in the early stage of infection are now considered as important for either the progression or control of the disease. Therefore, we will first expose the advances in the identification of the factors involved, due to the vector (Section 6.2), parasite (Section 6.3), and host (Section 6.4), and in the interactions between these players. The last section will focus on kala-azar in India, and we will demonstrate the necessity of this integrated approach to better understand this complex epidemiologic focus.

6.2 IMPACT OF SAND FLY VECTORS ON LEISHMANIASIS

Phlebotomine sand flies belong to the order Diptera, suborder Nymatocera, and family Psychodidae. They are small, about 3 mm in length, hairy flies characterized by a "hopping" flight and wings that remain erect above the abdomen when at rest. Sand flies are widely distributed and occupy tropical, subtropical, and temperate biotopes [4].

Phlebotomine sand flies are biological vectors of *Leishmania* in which the parasites undergo a complex developmental cycle beginning with ingested amastigotes and terminating with transmission of infective metacyclic promastigotes. Not all sand fly species transmit *Leishmania* parasites, however, with the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) accounting for all incriminated vectors to date. The bite of an infective sand fly vector is the only means by which any *Leishmania* species can be transmitted at a sustained and significant level. Importantly, the impact of sand flies on the establishment and spread of leishmaniasis extends beyond the transmission of *Leishmania* parasites to a direct effect on the host response to infection. In this section, the complexity of sand fly—*Leishmania* and sand fly—mammalian host interactions is outlined.

6.2.1 The Life Cycle of *Leishmania* in a Competent Sand Fly Vector

The life cycle of *Leishmania* parasites is contained within the digestive tract of the sand fly and begins with the ingestion of an infected blood meal containing amastigotes. Around 4 h after blood feeding, a chitinous *peritrophic matrix* (*PM*) is secreted, surrounding the blood meal within 24 h. The PM acts as a barrier that slows the diffusion of digestive enzymes secreted by the sand fly in response to blood ingestion and indirectly protects the parasites from the harmful effects of the enzymes [260]. This provides the opportunity for amastigotes to differentiate into sluggishly dividing procyclics, and by day 2 into large flagellated nectomonads (Fig.6.10). The blood meal is digested around 3–4 days after feeding. At this point, the PM breaks down, permitting escape of nectomonads and their attachment to the midgut epithelium. The degradation of the PM was initially attributed in full to the secretion of chitinases by *Leishmania* parasites [315]. Recently, however, Ramalho-Ortigao

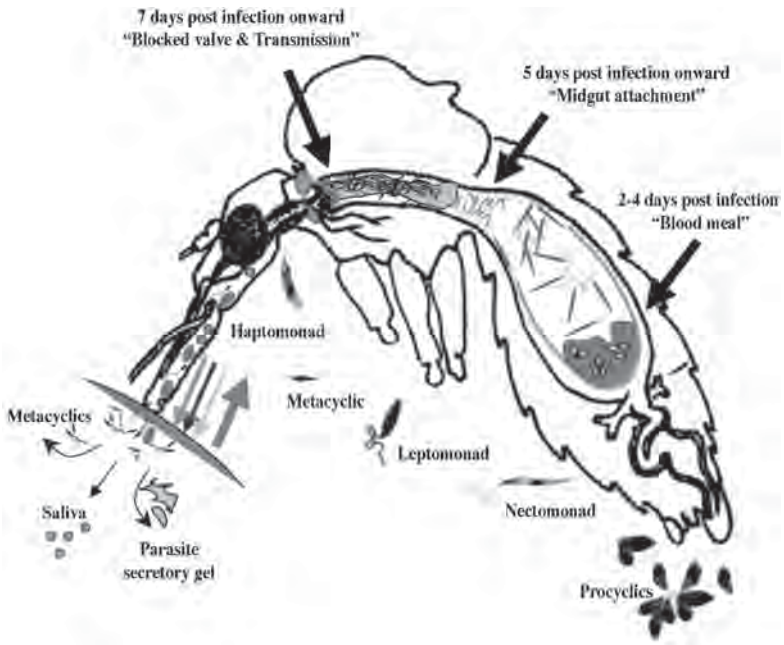

Fig. 6.10. Life cycle of *Leishmania* in a competent sand fly vector. See color plates.

et al. [272] showed that sand flies secrete their own chitinases after induction by the bloodmeal. The activity of sand fly chitinases peaks at about 48 h post blood feeding, coinciding with the time of the escape of nectomonads from the confinement of the PM [269]. Once in the gut lumen, attachment to the epithelium allows the nectomonads to persist in the midgut and prevents their expulsion with remnants of the undigested blood meal. Thereafter, nectomonads differentiate into leptomonads that divide rapidly as they migrate anteriorly to the thoracic part of the midgut [135,293]. Around day 7 after feeding, leptomonads give rise to infective metacyclics that accumulate in the anterior midgut below the stomodeal valve [135,293]. Metacyclics are characterized morphologically by their small cell body and long flagellum, and functionally by their free and rapid motility [311]. Simultaneously, haptomonads, highly specialized forms that adhere to each other and to the stomodeal valve, form a concentric parasite plug that blocks the opening of the valve (Fig. 6.10).

With such a complex life cycle, the parasites have to overcome several adverse conditions before they can successfully complete their development in the fly [180,299]. Such obstacles include digestive enzymes secreted by the sand fly [50,108,270], midgut lectins [381,382, 384], excretion of bloodmeal remnants [182,261], and sand fly innate immune responses [51,271]. As a result, different species of *Leishmania* closely evolved to fit distinct sand fly species, overcoming these obstacles and giving rise to the term "vector competence."

6.2.2 Vector Competence

A major determinant of vector competence is the ability of parasites to attach to the midgut epithelium of the sand fly to avoid expulsion with the blood meal remnants. Numerous studies, some involving mutants specifically deficient in *lipophosphoglycan* (*LPG*), a large and abundant molecule on the surface of *Leishmania* promastigotes, have implicated LPG as the ligand that mediates this attachment [62,262,302,303]. LPG is a tripartite GPI-anchored molecule with a backbone of conserved disaccharide repeats consisting of phosphorylated galactose-mannose sugars $-6Gal\beta 1,4Man\alpha 1\text{-}PO_4-$ capped with a neutral sugar. The LPG of different *Leishmania* species is highly polymorphic where the backbone can be unsubstituted (*L. donovani*, Sudan; and *L. chagasi*), partially substituted (*L. donovani*, India), or completely substituted (*L. major* and *L. tropica*) by side chains varying in the number and nature of their sugar residues [206,216,217,332,368] (Fig. 6.11A). The driving force for the observed LPG side chain substitutions is thought to be dependent on the complexity of the receptor present on the midgut epithelium of the targeted sand fly vector. Experimental infections showed that some sand fly species, such as *Lutzomyia longipalpis* and *Phlebotomus argentipes*, developed mature transmissible infections when infected with several foreign *Leishmania* species [168,261,294,304]. These species were termed *permissive vectors*. Others, including *P. papatasi* and *P. sergenti*, can only support the growth of the *Leishmania* species they are found infected with in nature (*L. major* and *L. tropica*, respectively) [168,261]; as such, they are considered *restricted vectors*. It is important to note that this species-restricted vectorial competence can also be strain specific. Certain natural variants of *L. major*, such as the West African Seidman strain, which lacks galactose side chains, do not maintain infection in *P. papatasi* but do maintain infection in another, closely related species *P. duboscqi* [206]. The strain-specific variability of LPG galactosylation in *L. major* was

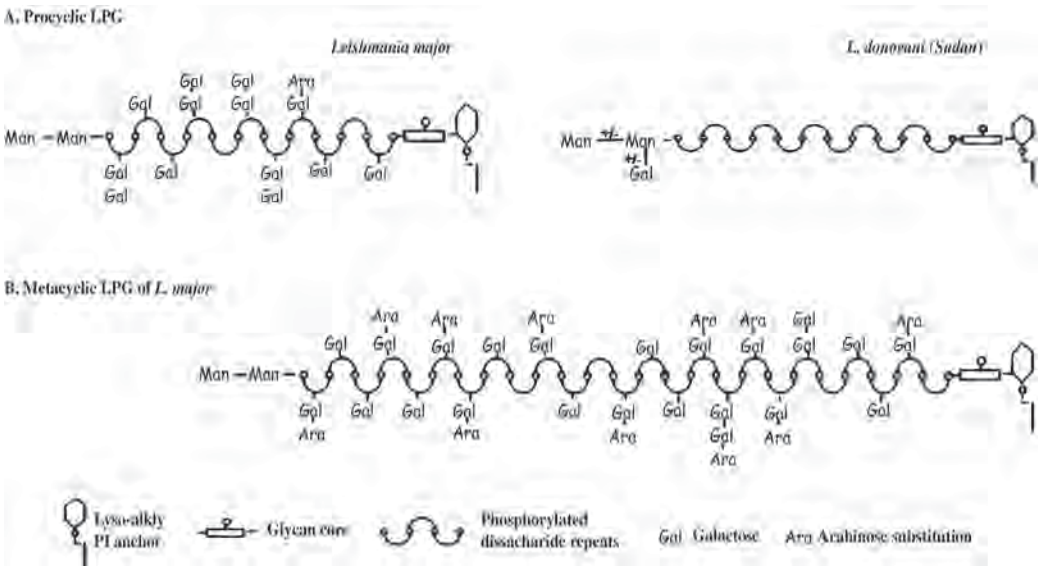

Fig. 6.11. Illustration of (A) *Leishmania major* (completely substituted) and *L. donovani* "Sudan" (unsubstituted) LPGs and (B) the changes during metacyclogenesis of *L. major* LPG.

attributed to the differential expression of a family of six genes encoding *L. major galactosyltransferases* that vary in their expression and activity [110]. Additionally, in the north of Israel, a strain of *L. tropica* whose LPG terminates with galactose instead of glucose residues, known to decorate the LPG of previously characterized *L. tropica*, was isolated from *P. arabicus* and not the classical vector *P. sergenti* [332]. As for sand fly midgut receptors, the first and only identified receptor to date is *PpGalec*, a tandem repeat galectin responsible for the observed specificity of *P. papatasi* for *L. major* [169].

Though appropriate LPG polymorphisms are necessary, vector competence has also been associated with the ability of certain *Leishmania* species to overcome other adverse conditions in the midgut of their respective competent vectors. For example, *Leishmania* species are able to overcome the harmful effects of digestive enzymes in a competent vector, but not in a foreign sand fly species, by specifically inhibiting or retarding the peak activity of these enzymes [50,107,316]. Secreted glycoconjugates, a family of LPG-related molecules characteristic of *Leishmania*, were implicated in this protection [302,317], highlighting the degree of adaptation necessary for parasite survival in competent vectors.

6.2.3 Metacyclogenesis and Transmission

Transmission of the parasites from the sand fly to the mammalian host requires detachment of the parasites from the midgut epithelium. This event is again mediated by *LPG*, which undergoes *stage-specific modifications* involving elongation of the molecule and/or changes to the nature of sugar residues on its side chains or neutral cap [206,217,262,301,303]. For example, during metacyclogenesis, the LPG of *L. major* elongates to approximately twice its procyclic length, and the majority of terminal galactose sugars get capped by arabinose residues (Fig. 6.11B). This modified LPG cannot bind to PpGalec, the midgut receptor for *L. major* procyclic LPG in *P. papatasi* [169]. In *L. chagasi*, metacyclics downregulate the glucose substitutions in their LPG, which, in contrast to procyclic parasites and procyclic LPG, becomes unable to bind to the midgut of its natural vector *L. longipalpis* [333]. This detachment frees the metacyclics and ensures their availability for transmission to the mammalian host. The trigger that initiates metacyclogenesis is not well understood. The only available evidence to date is a negative regulation by tetrahydrobiopterin, a byproduct of pteridine metabolism, whose levels are high following a bloodmeal and decline with time elevating metacyclogenesis [84].

To further enhance their chances for successful transmission, *Leishmania* parasites evolved the haptomonad stage, whose specific function is to block the stomodeal valve separating the midgut from the foregut. These parasites are nonmotile and adhere to the chitinous lining of the valve. The physical blockage of the valve is compounded by the secretion by the parasites of a proteophosphoglycan-rich gel termed the *promastigote secretory gel* (*PSG*) [156,340]. Both act in concert to obstruct the intake of blood during feeding, requiring more bites and a longer period to feed, and promoting *regurgitation of metacyclics* into the skin of the mammalian host [33,183,294]. In addition, parasite chitinases destroy the chitinous lining of the stomodeal valve, further contributing to the defective feeding mechanism in infected flies [314,380]. Another aspect of sand fly feeding that promotes transmission is sand fly probing. Due to their small mouth parts, sand flies need to lacerate multiple skin-surface capillaries to create the pool of blood upon which they feed [277,278]. Beach et al. [32] have shown that infected sand flies can transmit *Leishmania* parasites while probing. Moreover, infected flies

with mature infections and a stomodeal valve destroyed by chitinases and blocked by haptomonads and PSG probe longer in their efforts to feed [33,293,314], thus further promoting transmission. Based on a pool of 50 flies, the number of metacyclics egested into a membrane feeder by an infected sand fly was averaged at 1000 parasites [294]. However, considering the modification of feeding behavior mentioned above, the full potential of transmission in infected sand flies has yet to be accurately defined.

6.2.4 Sand Fly Modulation of the Mammalian Host Immune Response

6.2.4.1 Sand fly saliva During the act of probing and feeding, sand flies salivate into the wound. Consequently, *Leishmania* metacyclics are always egested in the presence of saliva. Sand fly saliva consists of a complex mixture of pharmacologically active compounds such as vasodilators, anticoagulants, and platelet inhibitors [166,278,372], as well as a number of immunogenic proteins of unknown function [72,246,373]. Numerous studies have shown that *sand fly saliva enhances* Leishmania *infections and has the ability to modulate the host immune response* (reviewed in [131,166,299]). Moreover, preexposure to saliva protected mice against infection with *L. major* [37,167]. Therefore, salivary molecules identified as disease enhancing or immunogenic may be targets for vaccine development. *Maxadilan*, a vasodilatory peptide identified from *Lu. longipalpis* [195], and *SP15*, a salivary molecule of unknown function identified from *P. papatasi* [373], both protected mice against infection with *L. major* [235,373]. Currently, salivary proteins of various sand fly vector species are being evaluated for their ability to protect against the *Leishmania* species they transmit in nature. This is made possible by the development of a high-throughput approach to DNA plasmid production combined with an immunization strategy that accelerates the identification of salivary molecules producing a cellular response, an antibody response, or a combination of both [246].

6.2.4.2 Promastigote secretory gel PSG is produced by leptomonad forms of *Leishmania* and accumulates at the anterior midgut region of an infected sand fly where it is egested with metacyclics during transmission by bite [31,293]. *Filamentous proteophosphoglycan* (*fPPG*), a component of PSG, was found to enhance *L. mexicana* infection in mice, causing long-term disease exacerbation [294]. Again, an intimate adaptation of *Leishmania* parasites to their vectors is reinforced, where molecules of parasitic origin and delivered by the fly insure the successful transmission of *Leishmania* and its establishment in its mammalian host.

6.2.4.3 Conclusions The role played by phlebotomine sand fly vectors in the development of *Leishmania* parasites, their successful transmission, and the outcome of disease is substantial. The complexity of the life cycle of *Leishmania* parasites in the digestive tract of the sand fly, from surviving the onslaught of digestive enzymes and immune molecules to attaching to receptors on midgut epithelial cells, exerts a powerful evolutionary pressure that restricts the species of *Leishmania* that can be successfully transmitted by a particular species of sand flies. In some instances, as for *L. major* and *P. papatasi*, the specificity of this vector–parasite association is so reliable as to enable the identification of the vector following characterization of the *Leishmania* species circulating in a focus of disease. Appreciation of the full significance of sand flies as vectors of leishmaniasis came with the demonstration of their influence on the progress and outcome of disease in the mammalian host that extends beyond their delivery of parasites. Sand flies can alter the immune response of the mammalian host through the modulatory effect of molecules they inject into the skin. These include salivary molecules and/or molecules of parasite origin, such as PSG. Some of the most exciting fields of research today pertain to an integrated approach in the search for an effective anti-*Leishmania* vaccine that combines protective salivary molecules with *Leishmania* antigens. Taking all of the above into consideration, further research is needed to identify the key molecules involved in *Leishmania* – sand fly interactions, from those important to the survival of the parasite within the digestive tract of the vector to those influencing their transmission and establishment in the mammalian host.

6.3 BIODIVERSITY AND GENETICS OF PARASITES: IMPLICATIONS IN VIRULENCE AND PATHOGENICITY IN HUMANS

6.3.1 *Leishmania* Species and Epidemiological Diversity

The *Leishmania* (Ross, 1903) parasites are protozoa belonging to the Kinetoplastida order (Honigberg, 1963) and to the *Leishmania* genus. Kinetoplastida have a unique mitochondria-like organelle called the *kinetoplast*, an appendix of their single mitochondrion, located near the basal body of the flagellum. As described above, this genus is characterized by ecological, epidemiological, and clinical complexity. The presence of these organisms throughout the world, except Antarctica, and their capacity to infect a large range of vertebrate hosts and sand fly species shows that *Leishmania* spp. have the ability to adapt and survive in very diverse environments. The hypothesis based on epidemiological data is that almost all *Leishmania* hosts are adapted to these environments, and the infections remain inapparent [189]. On the contrary, within animals that are less well adapted, such as humans, infections can produce a wide range of diversified pathologies, from asymptomatic carriers and benign cutaneous lesions to more serious cases such as the visceral form (see Section 6.1.4. for details). Indeed, when humans are bitten by a sand fly, the parasite inoculation can lead to the development of leishmaniasis but can also have no incidence on humans. The rate of asymptomatic carriers (infected individuals without clinical manifestations) is not accurately known, but different studies have revealed that it

seems to be higher than expected. For example, on the Balearic Islands, *L. infantum* was amplified by PCR in 22% of blood donors [280] and asymptomatic carriers were also revealed in Brazil [77], southern France [192], and India [323].

This great phenotypic variability is also expressed by the high number of *Leishmania* species described in the literature. A large part of these species has been defined on the basis of epidemiological, clinical, geographical, and biological data, for example, *L. guyanensis* (isolated in Guyana), *L. peruviana* (isolated in Peru), *L. infantum* (isolated from a child in Tunisia), *L. gerbilli* (isolated from gerbils), and so on. These extrinsic characteristics were first used to determine the species because morphological characteristics cannot be used for species identification. Even if differences in length have been observed among *Leishmania* spp. [125,174], the different species are indistinguishable in morphology in both the promastigote and amastigote stages. The development of genetic and phenotypic tools has provided means to reconsider the *Leishmania* taxonomy more rigorously. The first problem noted was that *these organisms could not be defined on the basis of the biological concept of species* [215]. Indeed, the studies of population genetics published show a basic clonal population structure in different species [25,23,362,364,365]. However, this model is not as simple as it appears because these organisms have been shown to use different multiplication strategies, with several *hybridization events* between species evidenced in the literature [24,41,112,120,175]. For example, in the New World, hybrids between *L. braziliensis* and *L. peruviana*, and *L. guyanensis* and *L. braziliensis* were described [24,112], and in the Old World, hybrids have been shown between *L. major* and *L. arabica* [120]. However, these recombination events do not seem frequent enough to disturb the *clonal propagation* of clones stable in space and time. Thus, the species definition of these "agamospecies" (*a group of individuals in which reproduction is almost exclusively done by asexual means*) still remains arbitrary and is based on a mix of intrinsic and extrinsic characteristics considered together. In this framework, different analyses clearly showed that the species status of some taxa was not taxonomically valid or questionable [23,26,85,137,212,213,283,396].

It must be kept in mind that there is a need for a rigorous and clear nomenclature for efficient communication between the scientific and medical professions. Indeed, first the various *Leishmania* species require different medical posologies to treat patients (see Section 6.1.5.3 for details) and second, clinical data suggest *a close association between the clinical outcome of the disease in humans and the species responsible for the infection*. Concerning the second point, for examples, (i) the *L. donovani* complex is mainly responsible for visceral forms; (ii) mucosal lesions are generally associated with *L. braziliensis*; (iii) *L. major*, *L. tropica*, *L. mexicana*, *L. guyanensis*, and *L. peruviana* produce a variety of *Leishmania* skin lesions in humans; and (iv) *L. amazonensis* is generally associated with diffuse cutaneous leishmaniasis. But once again, the clinical picture is more complex since at an intraspecific level, we can observe different disease outcomes: for example, *L. amazonensis* was isolated from six patients, three with cutaneous lesions, one with mucosal lesions, and two with diffuse cutaneous forms [205]; *L. infantum* can cause both cutaneous and visceral forms; and *L. braziliensis* produces cutaneous lesions and in around 10% of cases metastasizes.

Other points complicate the clinical picture: the existence of hybrids (see above) and mixed infections with different *Leishmania* strains. Concerning hybrids, *L. braziliensis* can produce cutaneous or mucocutaneous lesions in humans requiring care, whereas *L. peruviana* is responsible for dry benign cutaneous lesions that heal spontaneously. The hybrids between these two species found in Peru were isolated from patients either with mucocutaneous lesions or with benign lesions typical of the *L. peruviana* species [112]. These strains are thus capable of producing the different pathologies found in each species. Concerning mixed infections by different *Leishmania* species, few cases have been described in the New and Old World in the literature [13,30,154,210,341]. However, the molecular epidemiology studies evidenced that many foci exist in which several species circulate simultaneously [205]. It is hypothesized that the number of mixed infections is underestimated because of a selection problem during the parasite culture required by molecular techniques. This is confirmed by a study conducted in Bolivia [30] and also presents a problem for *Leishmania* diagnosis, prognosis, and for the understanding of the real role of parasites in pathogenicity in humans.

Moreover, it seems important to note and to consider the cases of co-infection of *Leishmania* with other pathogens. This is relatively frequent according to the literature and various pathogens in association with *Leishmania* such as *Mycobacterium tuberculosis* [94,386], *Trypanosoma cruzi* [30], *Salmonella* and *Schistosoma* [109], and of course HIV (for reviews see [97,103,234,268]) have been studied. Furthermore, in some cases, these co-infections can produce unusual clinical forms of leishmaniasis [66,75].

Another aspect of the incredible environmental adaptation of *Leishmania* parasites is their ability to become drug resistant. Indeed, drug and multidrug resistance has emerged as a major problem in treating both VL and CL. In particular, the appearance of antimonial resistance has changed the pattern of leishmaniasis treatment in the world. Indeed, pentavalent antimony has long been the cornerstone of anti-*Leishmania* chemotherapy, but resistance to this drug class is so high in some parts of the world, particularly in northeast India (see Section 6.1.3 for details), that it is quickly becoming obsolete [251,352]. There are many factors that can influence the efficacy of drugs in the treatment of leishmaniasis. These include both an intrinsic variation in the sensitivity of *Leishmania* species, described for pentavalent antimonials, but also paromomycin, azoles, and other drugs that have reached clinical trials, as well as acquired drug resistance to antimonials [79]. Thus, *the understanding of the molecular mechanisms that the parasite adopts or may adopt in the future is of high clinical relevance*. We know that the parasite is able to adapt itself to become resistant. For example, some results on glibenclamide-resistant

Leishmania parasites suggest that drug resistance involves a metabolic adaptation that promotes a stage-dependent modulation of energy substrate uptake and use as a physiological response to the challenge imposed by drug pressure [370]. Resistance of *Leishmania* species, in many instances, is due to overexpressed efflux pumps belonging to the superfamily of ABC (ATP-binding cassette) transporters [193].

6.3.2 Different Pathogenic Potential of Species and Within Species: Experimental Data

From all these data, it seems clear that the clinical outcome of the disease in humans is multifactorial. However, despite the complex clinical picture, *the parasites play an important role in human pathology and are not a passive organism*.

The animal models are largely used for immunobiology studies to understand and characterize the host–parasite interactions during infections. The fact that different human parasite isolates produce different infection patterns in a given mouse model suggests that parasite-related factors play an important role in the resistant versus susceptibility status and in the type of immune response elicited by the infected host [173]. The studies showed that animal models such as mice, hamsters, or nonhuman primates respond differently depending on the *Leishmania* species used [220,392]. For example, the *Leishmania* (*Viannia*) subgenus (which are predominant in Latin America), fail to reliably infect mice [220]. Moreover, different experimental data also showed that at an intraspecific level (within species), different strains can have different levels of virulence or different pathogenic properties. Indeed, it was demonstrated in BALB/c IL-4-deficient mice that a particular *L. major* strain induced a non-healing infection, whereas a different *L. major* strain induced a healing infection [186,244] and thus different *L. major* strains can induce somewhat different host immunologic responses [151] in mice. Another example was based on the comparisons of infection in both mouse and hamster models using *L. tropica* metacyclics purified from dermotropic and visceral isolates [200]. They found differences in disease progression that may reflect the parasite tissue tropism and pathogenic potentialities displayed by these strains in their human hosts. The authors suggested a role for parasite-related determinants in the clinical spectrum of disease. Thus, it was shown that in addition to the host factors, parasites also influence susceptibility and immune response following infection.

6.3.3 Genetic Markers and Parasitic Factors Involved in Pathogenicity in Humans

Since the development of molecular tools, scientists have attempted (i) to determine whether there is a *Leishmania* phenotypic or genetic association with virulence of strains and/or with pathogenicity observed in humans and (ii) to identify the markers involved directly or indirectly in the clinical outcome of the disease.

Different direct and indirect parasitic factors influencing disease outcome have been described. These factors were classified into three types: (i) *indirect genetic markers of pathogenicity*, (ii) factors called *invasive/evasive determinants* by Chang and McGwire [71], and (iii) factors called *pathoantigenic determinants* [71]. It should be noted that the distinction between the different groups is somewhat unclear and must not be considered inflexible. Indeed, this classification depends on knowledge acquired on each type of marker and thus it could be questioned in the future.

6.3.3.1 Indirect genetic markers *Indirect genetic markers* regroup genes or loci not directly involved in virulence or pathogenicity; they have been and continue to be widely explored. Different molecular tools such as multilocus enzyme electrophoresis (MLEE), which is the gold standard method for species identification [283], random amplified polymorphic DNA (RAPD)[82,157]. Pulse field gel electrophoresis (PFGE) [113,139], restriction fragment length polymorphism (RFLP) on various gene [82,377], and recently microsatellites [60,159,298] and real-time PCR [267,320], were used and the data were compared with clinical and epidemiological data. This kind of comparison is justified because of the clonal model (see Section 6.3.1 for details) of these organisms [363]. Indeed, the frequency of genetic exchanges (absent or rare for clonal species and frequent or obligatory at each generation for sexual species) conditions the interest of these genes or locus as epidemiological or clinical markers. The clonality implied linkage disequilibrium (nonrandom reassortment of genotypes occurring at different loci) and thus, correlation between independent genetic and phenotypic markers, suggesting strongly the possibility to find some genotypes associated with clinical or biological phenotypes [226]. Genetic markers are numerous to distinguish the different species but only a few of them were found to be associated with various clinical phenotypes at the intraspecies level. For example, within *L. peruviana*, we found a link between MLEE data and severity of lesions in patients [23,111,114]; for *L. infantum*, some zymodemes (all the stocks pertaining to a zymodeme have the same MLEE patterns) were associated exclusively with dermotropic strains and others with strains mainly isolated from visceral forms of the disease [14,142,282]. Other investigations studying different genetic markers showed also a correlation between clinical polymorphism and genetic data in *L. infantum* [139] and in *L. braziliensis* [319]. But finally, these correlations remain weak and do not allow us to understand the role of parasites in the outcome of the disease and to use these tools as prognosis markers.

6.3.3.2 Invasive/evasive determinants Chang and McGwire [71] have identified a second group of markers called *invasive/evasive determinants*. They belong to parasitic mechanisms that are necessary to establish leishmaniasis such as (i) *Leishmania*–macrophage attachment; (ii) the entry of *Leishmania* into macrophages; (iii) intramacrophage survival; and (iv) differentiation and intracellular multiplication of *Leishmania* amastigotes, but these *invasive/evasive determinants are not responsible for the symptoms of the disease*. Thus, they refer

to all determinants that help successfully establish *Leishmania* infection in the host such as *glycosylphosphatidylinositol* (*GPI*), *glycosylphospholipid* (*GIPL*), *lipophosphoglycan* (*LPG*), *leishmanolysin* (*GP63*), *cysteine proteases* (*CPs*), among others. These molecules have been widely studied, especially LPG, GP63, and CPs. *LPG* is the dominant surface molecule of promastigotes involved in (i) binding, migration, and release of the parasite in the sand fly midgut but also in (ii) the modulation of resistance to lysis by the host's complement. It is almost completely absent from amastigotes [83,217,247,262]. LPG is not involved in virulence within all *Leishmania* species. For example, it is not required for infection by *L. mexicana* [155], whereas it is needed for *L. donovani* and *L. major* infection [221,337]. Its structure varies between *Leishmania* species and also differs between procyclic and metacyclic promastigotes (see Section 6.2.2 for details). Some analyses showed, in addition to stage-specific and interspecies variability, an intraspecies polymorphism in lipophosphoglycan structure [206]. This diversity may be linked to the *Leishmania* adaptation to the sand fly species rather than related to the clinical diversity observed in humans.

Another important surface molecule, *GP63*, is an ecto-metalloprotease particularly abundant in promastigotes and also released by this stage of *Leishmania* [219]. Like LPG, GP63 is downregulated in the amastigote form [318]. These molecules may be involved in the evasion from humoral lytic factors and in the attachment of parasites to macrophages followed by their intracellular entry into these phagocytes [395]. GP63 protein is encoded by a multigene family repeated in tandem. Genetic and structural diversity was extensively studied and showed a high polymorphism at both inter- and intraspecific levels [119,140,289,339,376]. Like LPG, this protein seems to be subjected to strong host-selection pressure by the vector as well as by the vertebrate host [141]. But no link was found between the genetic or phenotypic diversity of GP63 and the intraspecies clinical polymorphism of strains [139].

Scientists have also shown increased interest in *cysteine proteases* because of the key roles some of them play in infection and expression of the disease, making them potential drug targets or vaccinal antigen. In *L. major*, a total of 65 CPs may exist, many of which are likely to play crucial roles in host-parasite interactions, particularly in facilitating survival and growth of parasites in mammals by destruction of host proteins, nutrition, evasion of the host immune response, and *Leishmania* survival within host macrophages [4,237,240,297]. The functional studies of the most widely studied CPs, CPB, allowed to explore the ways in which these molecules influence the interactions between parasite and mammalian host (see reviews [4,237,240,297] for details). Indeed, the generation of *Leishmania cp*-deficient mutants and inoculation on mice showed the involvement of these proteins in virulence and pathogenicity. For example, the *L. mexicana* strain deficient in the *cpb* array reduced virulence in BALB/c mice [4,238]. As for the *gp63* array, the genetic studies showed a high level of polymorphism, among species as well as within species. Nevertheless, only one publication showed a statistical correlation between gene organization of *cpb* in the *L. infantum* population and the strain tropism (cutaneous versus visceral) [67].

This list is far from exhaustive: other molecules such as *PSA* (GP46), an abundant surface glycoprotein of the promastigote form [35,357], or *A2* protein, shown to have an influence on the outcome of the disease [398], appear to play an important role in the invasive/evasive phases of the *Leishmania* cycle. For example, A2 is an important gene for *L. donovani* virulence but is not expressed in *L. major* [397,398]. Nevertheless, we can note once again the high level of heterogeneity depending on the considered species.

6.3.3.3 Pathoantigenic determinants The third group of factors comprises *Leishmania pathoantigenic determinants* [71]. *This group includes all the molecules described in the literature capable of inducing host immunopathology as the principal cause of clinical symptoms*. Thus, all *Leishmania* antigens eliciting antibodies at high titers compared to antibody titers against the other determinants (invasive/evasive determinants) can be classified in this category. These pathoantigenic determinants are all conserved structural or soluble cytoplasmic proteins, which are often complexed with other molecules to form subcellular particles [71]. Moreover, they have been found to contain immunogenic B-cell epitopes. The list of candidate molecules is based on data obtained from kala-azar patients (the visceral form of the disease as described above) [276]; thus, they clearly differ from those obtained from cutaneous leishmaniasis. For example, the unique 117-basepair repeat, encoding for a 39-amino acid peptide (recombinant products = rK39) in the *Leishmania* kinesin-like gene, is expressed by the amastigotes of visceralizing *Leishmania* (*L. donovani*, *L. chagasi*) and not by dermotropic species (*L. major*, *L. amazonensis*, and *L. braziliensis*) [61]. Indeed, sera from kala-azar patients contains antibodies specific to this 39-amino acid peptide called anti-rK39 at high titers [331]. It is interesting to note that this antigen has been successfully used for serodiagnosis of active kala-azar cases.

To date, the interactions between these molecules and the human immune system as well as activation of specific antibodies production remain unknown. All these molecules are localized in amastigote cytoplasm and are thus beyond the reach of their specific antibodies [71]. However, their potential contributions to immunopathology are apparent. In a study on protective immunity in *Leishmania* [266], Chang and McGwire [71] suggest that some *Leishmania*-specific T-cell epitopes may also exist and cause additional immunopathology.

6.3.3.4 Conclusions In summary, all the experimental and epidemiological data show that the identity of the parasite responsible for infection plays a fundamental role in the clinical diversity observed in humans, as it does when we consider the different species as different parasites of a single species. As described above, *factors or factor groups from the* Leishmania *parasite could clearly be involved in this clinical diversity at both interspecific and intraspecific levels*.

Unfortunately, their true roles and the biological pathways in which they participate remain unknown because of the immunopathological complexity involved. All these studies are based mainly on the comparison of strains responsible for different degrees of pathogenicity. Nevertheless, it seems that the majority of infections remain inapparent in natural populations considering all the vertebrate hosts, but this is also true in humans, as described in the literature [77,209, 255,280]. Although it is known that leishmaniasis is the result of a complex association of host and parasite factors, we do not know what occurs in *asymptomatic carriers*. We do not know whether strains from patients and from asymptomatic carriers are genetically different. To explore the pathogenic potential of strains and identify the parasite factors involved in pathogenicity, it is fundamental to *compare isolates from asymptomatic carriers with parasites from patients*.

As described above, leishmaniasis results from apparently multiple factors of *Leishmania* origin but also host and environmental origin combined (see the other parts of this review for details). Thus, all these data illustrate the value of mechanistic approaches focusing on both parasite and host defense pathways in dissecting the specific biological roles of the different complex virulence factors and pathoantigenic determinants [338].

6.4 THE IMMUNE RESPONSE AND GENETIC FACTORS FROM THE MAMMALIAN HOST

In endemic area populations, it is striking to observe, for a given parasite species, a wide range of interindividual variability in susceptibility/resistance to disease. Furthermore, epidemiological studies have shown that infection by *Leishmania* parasites remains asymptomatic in most cases [21,57,146,399]. These subjects (detected either by a positive serology, the Leishmanin skin test, or detection of parasite by PCR [330]) are either able to clear infection or can remain asymptomatic carriers for years (as evidenced by the development of leishmaniasis in immunosuppressed patients several years after their last stay in endemic areas). Other subjects, however, are unable to control parasite dissemination and/or multiplication and develop clinical symptoms of diverse severity. Malnutrition, immunosuppression (AIDS, malignancy), pregnancy, age, as well as immunological capacities and genetic factors are risk factors associated with the development of leishmaniasis. Malnutrition alters the immune response and leads to increased parasite visceralization during *Leishmania donovani* infection [11,143]. Leishmaniasis in HIV-infected individuals is often the consequence of a reactivation of a latent infection. Accelerate multiplication of parasites and the invasion of multiple visceral sites stems from progressive T-cell immunosuppression [6,393]. *Leishmania*-HIV co-infections appear to be accompanied by changing nonpathogenic into pathogenic strains, and dermotropic strains are seen to induce viscerotropic behavior [7].

Although the general state of health and physiological conditions of the host can and do influence disease progression, genetic predisposition indubitably plays a major role in determining disease outcomes. Thus, the aim of this section is to analyze how the host response to parasite infection mediates susceptibility/resistance to leishmaniasis. First, the different host immunological responses to infection and their relation to susceptibility/resistance to disease will be presented, and then we will focus on how these observed response differences are related to genetic factors from the mammalian host.

6.4.1 The Host Immune Response to *Leishmania*

In their mammalian host, *Leishmania* species are obligate intracellular parasites of hematopoietic cells of the monocyte/macrophage lineage. As such they infect and multiply within cells having a central role in the host immune response, as they are both involved in *innate immunity* (as anti-*Leishmania* effector cells) and in presenting parasite antigens to lymphocytes, and thus in initiating the *acquired immune response* [95,96,300,334] (Fig. 6.12).

6.4.1.1 Early events On infection, *Leishmania* parasites are first confronted with the host's *innate immune response* (see Fig. 6.12). Mechanisms of the innate response leading to the control of infection are mediated by the intrinsic capacity of macrophages [133] to become infected by promastigotes and then by amastigotes and to activate on infection to limit parasite multiplication. The ability of macrophages and dendritic cells [40] to produce interleukin-12 (IL-12) and other pro-inflammatory cytokines (tumor necrosis factor-α [TNF-α], IL-1) early during the course of infection is also a critical step [358,366,367]. IL-12 has a key role in the development of cell-mediated immunity through the induction of naive T cells to differentiate into Th1 cells (see acquired immunity below) and through the activation of NK cells to secrete interferon-γ (IFN-γ) [36,313]. IFN-γ and TNF-α are cytokines involved in the activation of infected macrophages, which is characterized by an increased production of radical oxygen and nitric oxide (NO), which are potent anti-*Leishmania* molecules [49,121,122,148,196,241]. Intramacrophagic radical oxygen (ROS) is produced by the NADPH oxidase complex, whereas NO is produced by the inducible nitric oxide synthase (iNOS).

However, in no way can the parasite be seen as a passive partner in the establishment of the immune response. Indeed, several studies on macrophage gene expression have shown that the pattern of gene expression in infected macrophages is profoundly modified upon infection [54,73,292]: a number of genes encoding molecules involved in the macrophage anti-microbial response are down-modulated [53,68,100, 144,259,275,369,388], whereas fewer genes coding immunosuppressive molecules such as TGF-β, IL-10, IL-10R, are selectively up-regulated [29,48,59,123,138,383].

6.4.1.2 Acquired immunity *Acquired response develops with the surface parasite peptide-presentation by infected macrophages and dendritic cells* (see Fig. 6.12). These peptides are the result of

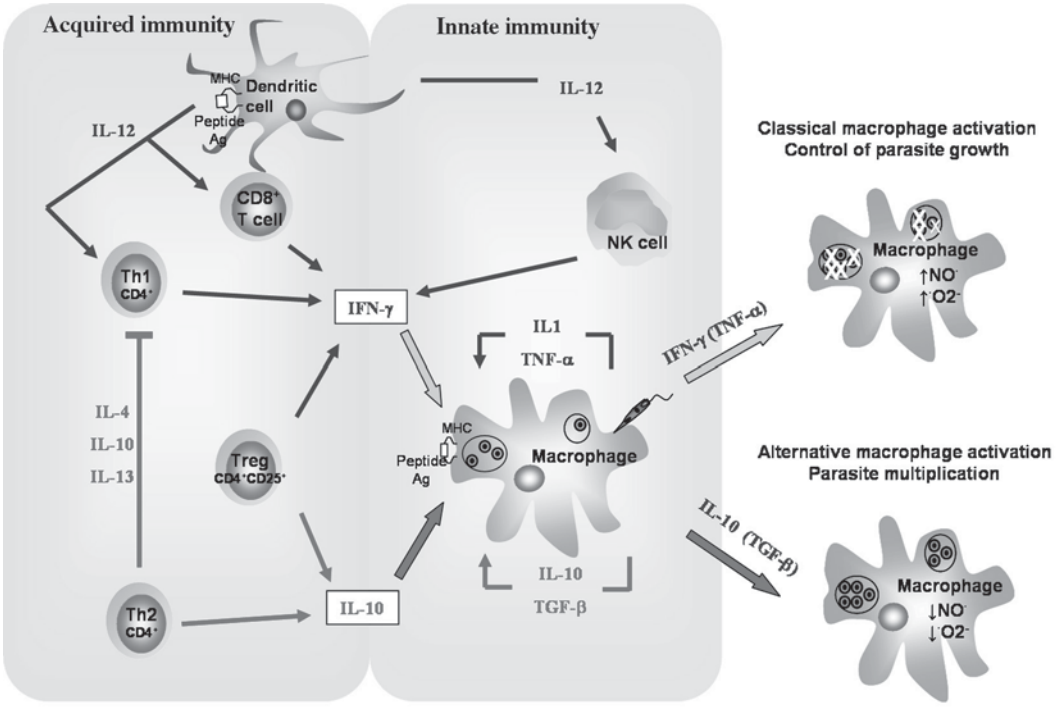

Fig. 6.12. Immunological determinants influencing parasite multiplication. During blood meal infected sand flies transmit metacyclic promastigotes to the vertebrate host, which convert to the amastigote form on entering macrophages and dendritic cells. IL-12 production from infected cells induces NK cells activation, CD4+ T helper cell differentiation, activation of CD8+ cytotoxic T-cells and INF-g production. INF-g stimulates iNOS expression and NO production in the macrophage, which mediates parasite killing. Failure to produce IL-12, to respond to INF-g or alternatively IL-4/IL-13 production results in unregulated parasite replication within the infected cells facilitated by host cell IL-10 production. IL-10 production by CD4+ CD25+ regulatory T-cells can both facilitate disease development as well as maintaining latent infection and concomitant immunity. See color plates.

intracellular processing of *Leishmania* antigens and are presented to T-cell receptors by the major histocompatibility complex (MHC) molecules [185,402]. Depending on the peptide presented and the cytokine context (i.e., presence of IL-12 or IL-4), this will lead or not to the activation and proliferation of $CD8^+$ cytotoxic T cells and to the differentiation of naive $CD4^+$ T helper (Th) cells into Th1 or Th2 subtypes [98,184,236]. *Th1* cells secrete cytokines usually associated with *inflammation* such as IL-2, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and *induce* Leishmania *cell-mediated immune responses* (induction of macrophage microbicide activities and activation of cytotoxic T cells). In contrast, *Th2* cells help in the development of the *humoral* response (production of antibodies by B cells) and produce cytokines (IL-4, IL-5, IL-10, IL-13, etc.) that *inhibit* both *development of Th1 responses and macrophage activation* [274].

Other T-cell populations were shown to be involved in long-term protection. IFN-γ producing $CD8^+$ T cells or $CD8^+$ T cytotoxic cells play a role in immunity to reinfection [39,86,239]. More recently, $CD4^+$ $CD25^+$ regulatory T cells [306, 326] (*Treg* cells) were proved to mediate persistence of *L. major* parasites at a low level in healed cutaneous lesions [38]. Thus, Treg cells seem to suppress the ability of the immune response to completely eliminate parasite infection. This might reflect a *Leishmania* parasite adaptive strategy to maintain its transmission cycle in nature; such persistence can lead to disease reactivation; however, it could also contribute to the maintenance of a lifelong immunity against reinfection [38,225].

6.4.1.3 Anti-*Leishmania *immunity in different* Leishmania *species and hosts The fact that resistant inbreed strains of mice (self-resolution of lesions) develop a Th1 response, whereas susceptible strains (progressive nonhealing lesions) develop a Th2 response upon experimental infection by *L. major* provides an exquisite demonstration that Th1 and Th2 subsets can influence the course of disease toward opposite poles [145,152,191,202,321] (Fig. 6.12). In humans, (i) the observation of a strong humoral response (characterized by high anti-*Leishmania* antibody titers) during the course of disease; (ii) the fact that a delayed-type hypersensitivity (DTH; detected by the leishmanin skin test) response, which is a marker of cellular immunity, develops in cured patients; and (iii) the fact that DTH positivity is also detected in exposed healthy subjects (asymptomatic infection) are also compatible with the Th1/Th2 model of resistance/susceptibility established in mice infected by *L. major*.

However, in mice and other animal models, the importance of the Th1/Th2 dichotomy in determining the course of infection is less clear when animals are experimentally infected with other *Leishmania* species such as the "visceralizing" species of the *L. donovani* complex [28,197,228]. Similar to *L. major* infection, resistant mouse strains such as C57BL/6 develop a Th1 response with $CD4^+$ cells, producing IFN-γ and IL-2 during *L. donovani* or *L. infantum* infections, whereas susceptible strains (BALB/C) exhibit a decrease in IFN-γ production. In contrast to *L. major* infection, the IL-12 effect is delayed for 4 weeks after infection. Furthermore, susceptible strains lack Th2 immune response despite disease progression [172,224,228]. Thus, in "visceralizing" *Leishmania* infection, Th1 response is not suppressed by Th2 response, in contrast to *L. major* infection.

Mice have also been used to evaluate the immune response directed toward other New World *Leishmania* spp. (*L. mexicana*, *L. amazonensis*) causing cutaneous leishmaniasis and it revealed striking differences with the *L. major* model. Overall, although a protective response is quite clearly Th1 mediated in all species studied to date, it has become apparent that the relevant importance of the specific Th2 response in disease progression is clearly *Leishmania* species dependent. Old World species (*L. major* and *L. donovani*) diverged from the New World species some 40–80 million years ago. It is therefore not surprising that these different parasite species have developed different strategies to survive within different tissue sites and/or a different range of mammalian hosts [220].

It is worth noting that even susceptible mice experimentally infected, for example, by *L. donovani* complex parasites, are able to finally resolve infection spontaneously, which make them a better model of subclinical infection rather than progressive disease. In contrast, hamsters infected by *L. donovani* develop progressive disease that mimics human visceral leishmaniasis more closely [87,129,130,290,374]. Surprisingly, there are significant amounts of Th1 cytokines expressed in the spleen of hamsters, although little or substantial amount of IL-4 and IL-10 is present. Instead, susceptibility to *L. donovani* in hamster seems more to be mediated by a defect of NO production by iNOS in infected macrophages rather than the development of a Th2 response [224]. In dog also, a natural reservoir of *L. infantum*, studies done so far have not been able to clearly establish the existence of a Th1/Th2 dichotomy in susceptibility to canine leishmaniasis [8,68,243,263]. Although *resistance is associated with a Th1 response* (production of IL-2, TNF-α, and IFN-γ able to stimulate macrophage leishmanicidal activity [254,263,310], *susceptibility has not been shown to be associated with a Th2 response* [70,264].

6.4.1.4 The immune response in human leishmaniasis

In humans as in experimental models, different patterns of immunological response are observed according to the clinical manifestation and exposure to the different *Leishmania* species. Indeed, different T-cell type responses are observed among the different cutaneous forms of leishmaniasis. An absence of a Th1 response (rather than presence of Th2) is seen in diffuse cutaneous leishmaniasis, whereas patients with self-healing lesions develop a Th1 response [1,65,176,177]. High IFN-γ levels are also detected in chronic lesions and mucocutaneous leishmaniasis, which are rather characterized by a mixed Th1/Th2 response [19,69,204,223]. In the case of the immune response directed against visceralizing *Leishmania* spp., it was shown that peripheral blood mononuclear cells (PBMCs) from individuals with asymptomatic or subclinical infection respond to *Leishmania* antigens with proliferation and production of IL-2, IFN-γ, and IL-12. However, visceral leishmaniasis displays a cytokine profile of mixed Th1/Th2 characteristics such as IFN-γ along with IL-10 readily detected [128,170,179]. Furthermore, both Th1 and Th2 clones producing IFN-γ and IL-4 have been isolated from cured patients [178]. Thus, it has not been possible to clearly associate a Th2 polarity with nonhealing, systemic, or reactivation forms of leishmaniasis. Overall, IFN-γ-producing cells or mRNA remain readily detectable in patients with visceral leishmaniasis, PKDL [126], or chronic cutaneous leishmaniasis, and the opposing cytokine most commonly found in these clinical settings is not IL4 as in mice but IL-10 [10]. Interestingly, IL-10 is not a "pure" Th2 cytokine, as it can also be produced by alternatively activated macrophages and, as shown recently in mice, by Treg cells that also produce IFN-γ. In contrast to IL-4, which inhibits Th1 expansion, IL-10 action serves more to down-regulate the activation of macrophage microbicidal activity by IFN-γ producing cells. The role of Treg cells has been proposed to be both to control the severity of inflammation (which occurs within Th1-type responses and can be harmful to the host) and to promote long-term low parasite persistence in order to maintain a memory pool necessary to resist reinfection [38].

6.4.1.5 Conclusions

Although the control of infection is almost always associated with the development of a Th1 response, mechanisms promoting disease susceptibility are not yet fully understood and determinants other than the Th2 cell subset are likely involved depending on the parasite species. Although macrophages are the primary host cell for *Leishmania*, the role of these cells has not been well characterized either in disease prevention or in disease progression. The effector functions of macrophages have always been described in a T-cell-dependent manner and the fate of infected macrophages in the pre-T-cell phase is not well known. It is also obvious that the parasites modulate the macrophage in terms of their antigen-presenting and intracellular signaling capacity. In this regard, intramacrophagic interaction (which needs to be further explored) between host and parasite molecules could regulate the capacity of macrophage to respond to IFN-γ (possibly through the secretion of IL-10 or other mechanisms) and explain the progression of disease in the context of a Th1 response.

6.4.2 Host Genetic Factors in Resistance/Susceptibility to Leishmaniasis

As stated above, immunological studies in experimental models of infection and in endemic area populations have

associated certain clinical manifestations with qualitative or quantitative changes in the host-specific immune response to *Leishmania*. Although these studies identified several immunological components that are markers of disease versus asymptomatic or subclinical infections, these studies did not identify the primary effects causing the observed complex immunological phenotypes. Growing evidence from mouse and human studies suggests that they are in part related to the genetic make-up of the host. This raises the hope that the analysis of host genetic susceptibility will help in identifying these defects and in demonstrating the causal link between immunological phenotypes and clinical diseases.

6.4.2.1 Genetic studies in mouse The existence of genetic host factors involved in resistance/susceptibility was first suggested by the fact that genetically distinct inbreed strains of mice exhibited substantial differences in the infection outcome in experimental infection by a single strain of *Leishmania*. The possibility of intercrossing or backcrossing between resistant and sensitive inbreed strains of mice has made it possible to start unraveling the basis of genetic susceptibility to leishmaniasis in these experimental models of infection. The control of the early stages of *L. donovani* infection (innate immunity) is associated with a mutation in the transmembrane domain of *Nramp1* (Natural resistance-associated macrophage protein 1) [52,378]. This gene, now renamed *Sl11a1*, codes for a macrophage-restricted divalent cation transporter that is recruited to the phagosome upon phagocytosis [322]. Although the mechanisms by which this transporter limits the replication of intracellular pathogens is not yet fully understood, it could be through the alteration of the phagolysosome environment, especially iron concentration, which is critical for the generation of oxygen-free radicals [43,46,390]. Furthermore, late control of *L. donovani* infection in susceptible mouse strains, concomitant with the development of acquired immunity, has been associated with alleles at the MHC locus [44].

It is worth noting that genetic determinants of murine cutaneous leishmaniasis (*L. major*) map to different regions of the mouse genome and appear to be more complex because 10–15 loci were implicated in the control of diverse clinical or immunological phenotypes [34,134,199,284,285,288,379]. Although the causative genes in these regions remain to be identified, they pointed to interesting positional candidate genes: (i) a susceptibility locus encoding a number of Th2 cytokines on mouse chromosome 11 is syntenic with human 5q31-33, which was shown to influence infection levels by *Schistosoma mansoni* [207], *Plasmodium falciparum* [124,281], and susceptibility to asthma [208]; other loci include genes encoding (ii) other cytokines or cytokine receptors (*Ifng*, *Ifngr1*, etc.); (iii) macrophage effectors (*Nos2*); (iv) transcription factors (*Stat6*); and so on.

Analysis of genetic susceptibility (control of lesion growth) of mice to infection by *L. mexicana* (causing human cutaneous leishmaniasis), also revealed important differences with *L. major* because it was mapped to a single locus on mouse chromosome 4 [287]. On the contrary, in *L. mexicana* infections, visceralization seems to be influenced by the *Nramp1* and *H2* loci (MHC) [286], as in *L. donovani*, whereas with *L. major* this phenotype is controlled by different genes (which remain to be identified) located on chromosomes 2 and 11. In mice, susceptibility to disease caused by different *Leishmania* species thus appears to be regulated by multiple, distinct genetic loci; therefore, it is not surprising that the immune regulation of disease and healing to each species also differs.

Infection of BALB/c-susceptible and C57BL/6-resistant mice by *L. major* has been extensively used to study the immunological determinants of a Th2 versus Th1 response. This huge body of work led to the widely admitted idea that *Th1 is protective in leishmaniasis, whereas Th2 is associated with susceptibility*. However, it is not yet clear how the association occurs: *does the host control the parasite because it develops a Th1 response or is it because parasite multiplication is controlled that a Th1 response eventually develops*? Genetic dissection in mice of clinical, immunological, or infection phenotypes [198] will help answer this question. Interestingly, in C57BL/6 and BALB/c mice congenic for three *L. major* susceptibility loci, the cytokine profile (Th1/Th2) correlated with the parental genetic background (C57BL/6 or BALB/c) but not with disease severity [118].

Experimental infection in inbreed strains of mice has made it possible to characterize the host immune response directed against *Leishmania* parasites and to identify genetic determinants responsible for the differences in resistance versus susceptibility. In mice, resistance/susceptibility was found to be controlled by different sets of genes according to the parasite species involved. Moreover, experimental infection by a given parasite species was found to elicit different immune responses according to the animal model under scrutiny. The question now is to validate these experimental observations in humans living in endemic areas, because experimental conditions of infection in mice are very far from natural infection in which only a few promastigotes are delivered in the host derma along with *Phlebotomus* salivary antigens.

6.4.2.2 Human genetic factors involved in resistance/susceptibility Evidence is now emerging that *host genetic factors also influence the outcome of human infection by* Leishmania. Epidemiological studies have shown familial aggregation of clinical phenotypes that are consistent with the existence of inherited factors in susceptibility to CL and VL in humans [58,63,399]. Furthermore, some studies indicated that the distribution of disease phenotypes (CL or VL) in extended pedigrees living in endemic areas are statistically best explained by the segregation of *one or two major susceptibility loci* [3,256,324]. Together with the observation of profound ethnic differences in the ratio of asymptomatic to symptomatic infections [58,153], these epidemiological observations strongly suggest that human susceptibility to cutaneous or visceral leishmaniasis is mediated by host genetic factors.

6.4.2.3 The candidate gene approach Identification of genes or genetic loci in mice accounting for the differences in susceptibility/resistance among the different

inbreed strains of mice opened the way to a mouse-to-man strategy to identify human susceptibility/ resistance genes [45]. Thus, early attempts to identify genetic factors in human leishmaniasis have first focused on polymorphisms in candidate genes tested by means of either case–control studies (association) or family-based studies (transmission disequilibrium test [TdT] or linkage analysis). Associations have been reported between some HLA alleles and the diverse forms of cutaneous leishmaniasis (CL and MCL) [27,190,258]. In contradiction with the mouse model, however, all attempts to demonstrate such an association with visceral leishmaniasis – in Brazil [45,257], India [329], Sudan [57], and the Mediterranean area [222] – have failed, pointing out the existence of important differences between humans and mice. Polymorphisms in the *TNFA* and *TNFB* genes in the MHC class III region, which encode TNF-α and lymphotoxin-α, respectively, have been associated with mucocutaneous leishmaniasis [64] (some of the promoter polymorphisms such as the *TNFA*-308 SNP were shown to be associated with cerebral malaria [218] and/or to drive higher transcription of the gene [391]). Polymorphisms of the *TNFA* promoter were also examined in asymptomatic *L. chagasi* infection and patients with visceral leishmaniasis [171]. Interestingly, in this study, genetic association was observed with the development of *Leishmania*-specific cellular immunity rather than disease itself. This suggests that although *TNFA* polymorphisms may be a risk factor for VL, they cannot alone explain the development of disease.

6.4.2.4 Genetic control of visceral leishmaniasis in two populations of eastern sudan

Two recent studies have started to study the genetic control of visceral leishmaniasis in Eastern Sudan, an area endemic for *L. donovani* and where an upsurge of cases has occurred over the last decade. One of the studies was carried out during an outbreak (1995–2000) that caused infection in almost all inhabitants of a village of the Sudanese–Ethiopian border (Fig. 6.13). Though more than 90% of the villagers showed immunological evidence of infection, 25% developed visceral disease. Substantial differences in disease prevalence were observed between ethnic groups living in sympatry (the Haoussa and Fellata having a decreased risk of developing VL compared to the Aringa), and

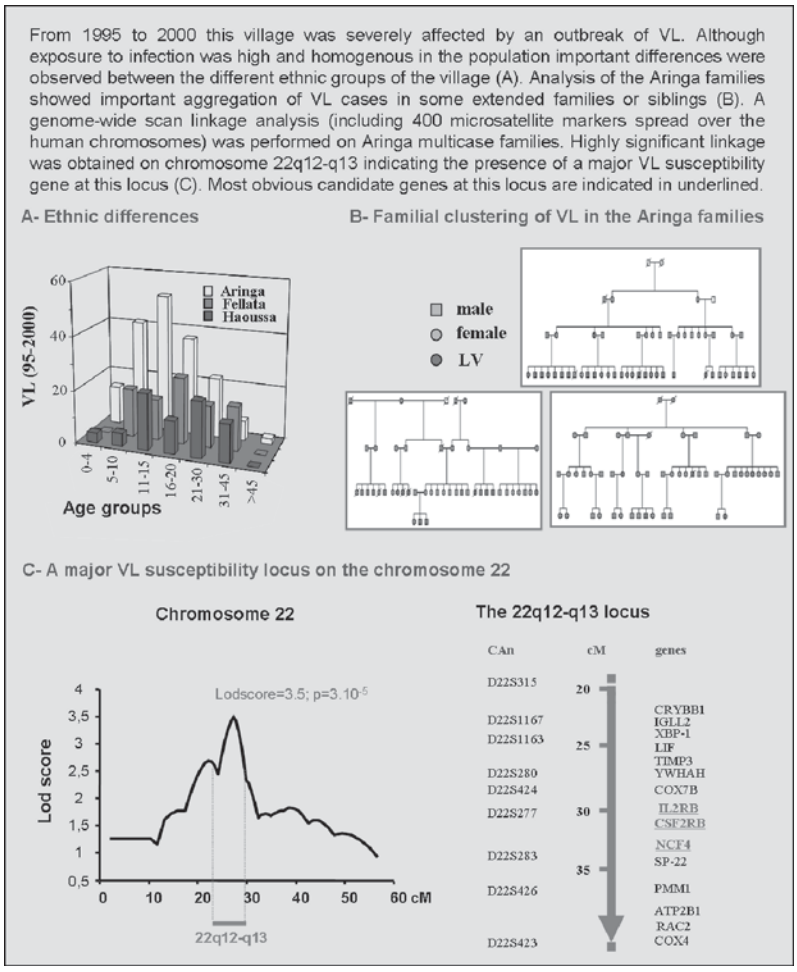

Fig. 6.13. The influence of host factors in an outbreak of VL in the village of Barbar El Fugarra (Eastern Sudan).

certain families among the Aringa ethnic group were more affected than others, suggesting the existence of a familial component in susceptibility to VL [57,58,115]. An attempt to demonstrate that this familial component was attributable to a major gene segregating in the Aringa pedigree was unsuccessful, indicating that the hypothesis of a single major locus is probably not tenable. However, a linkage study carried out on affected sib-pairs, a strategy that accommodates a multigenic control and scanning the entire genome, showed that one locus (chromosome 22q12, $p = 3.10^{-5}$) and possibly another one (chromosome 2q22-q23, $p = 0.0006$) controlled susceptibility to disease in the Aringa population [55]. The two loci identified by this study do not contain classically tested candidate genes in leishmaniasis and therefore allow the formulation of a new hypothesis on susceptibility/resistance genes important in human infection by *L. donovani*. Identification of these genes is now required to characterize the critical steps in the pathological process involved in this lethal disease. Several interesting genes are present at 22q12, such as *CSF2RB*, encoding the β chain of the granulocyte-macrophage stimulating factor (GM-CSF) receptor, and *NCF4*, encoding the soluble P40-PHOX subunit of the NADPH oxidase complex involved in the generation of superoxide in phagocytic cells. Another gene, *IL2RB*, encoding the IL-2 receptor β chain, could also be involved in susceptibility through the modulation the T-cell response by IL-2. However, it is not yet known if a single gene or several genes at the 22q12 locus contribute to the linkage observed in the frame of this study. It is interesting to note that two other genes, *NMI* (N-myc interactor; OMIM 603525) and *STAM2* (signal transducing adapter molecule 2; OMIM 606244), located in the 2q22-q23 region, are also coding proteins, involved in modulating signal transduction downstream of the IL-2 receptor, reinforcing the hypothesis that the IL-2 signaling pathway plays an important role in determining susceptibility to VL. Furthermore, the study carried out in this village population also suggests that host genetics might play an important role in outbreaks determining to a large extent which subjects are at risk of severe disease. The reduction in the number of susceptible subjects, due to patient death and to the induction of protective immunity in cured patients, has probably played a significant role in the termination of the outbreak. Furthermore, the arrival of genetically susceptible immigrants and children born after the outbreak from cured susceptible parents could create the conditions of a new outbreak and account for the periodic occurrence of KA outbreaks in this region of Sudan. In this regard, the Haoussa and Fellata, who first settled in this village in the 1940s, had experienced an outbreak of KA before the one documented in this study and were less affected by the present outbreak than the Aringa who had immigrated to the village more recently.

The other study was carried out in an area located 70 km away from the area covered by the above-described study and involved KA patients from the Massalit ethnic group (a group closely related to the Aringa in that they originate from the same geographic area in Western Sudan and Chad). Only a candidate gene approach was reported from this study [47]. Suggestive linkage (Lodscore = 1.8, $p = 0.002$) and significant association with VL (TdT, $p = 0.008$) were obtained with an intragenic microsatellite of the *IL4* gene in the 5q31-33 region (syntenic with the locus controlling visceralization of *L. major* on mouse chromosome 11). These results are consistent with a functional polymorphism in the close vicinity of the *IL4* gene controlling susceptibility to VL in the Massalit tribe. Another microsatellite marker in the *IFNGR1* locus indicated a possible implication of this gene (Linkage: Lodscore = 0.73, $p = 0.035$; TdT, $p = 0.007$) in the development of PKDL, although a larger sample size is required to confirm this result [232]. Interestingly, the 5q31-33 region and the *IFNGR1* locus showed no linkage in the Aringa study [57]. The reason for this may be that (i) although the Aringa and Massalit are a closely related ethnic group, subtle genetic differences may exist between these two groups; or that (ii) the expression of genetic susceptibility at the population level will depend on the environment, that is, transmission intensity or parasite diversity. In this regard, we can note that the Aringa were submitted to particularly high transmission levels during the Barbar El Fugarra outbreak, which may have driven higher infection rates compared to the Massalit.

Both studies in Sudan tested linkage with *SL11A1* (*NRAMP1*) polymorphisms. Suggestive linkage (Lodscore = 1.29) was observed in the Aringa [57], linkage was confirmed in the Massalit tribe (Lodscore = 1.41), and involvement of the *NRAMP1* gene was further confirmed by transmission disequilibrium test analysis [233]. Thus, the gene encoding NRAMP1, or a closely linked gene, is involved in determining genetic susceptibility to VL. Given the large body of literature on the role of *NRAMP1* in both mouse and human susceptibility to diverse intracellular pathogens [46], these results are consistent with a role of NRAMP1 in susceptibility to VL. However, given the low strength of genetic linkage or association at this locus, its role in determining the infection outcome in humans is minor compared to the strong effect observed in mice.

6.4.2.5 Conclusions Overall studies in mice and in populations living in endemic areas suggest that the risk of clinical leishmaniasis (CL, MCL, and VL) is markedly increased by allelic variants at specific genetic loci. Loci associated or linked with human leishmaniasis are summarized in Table 6.1. Some loci seem specific to particular clinical manifestations such as HLA/cutaneous leishmaniasis (CL, MCL) or *NRAMP1*/VL, whereas others may act across different clinical presentations (cytokines and cytokine receptor genes). Additional work in distinct populations is required to definitively establish the involvement of certain of these genes in susceptibility/resistance to leishmaniasis. It is worth noting that different ethnic groups display different susceptibilities to disease, which seems a common feature and should be carefully taken into account in order to avoid type I errors (false-positive associations) due to population admixture.

TABLE 6.1. Unraveling Human Genetic Susceptibility to Leishmaniasis: a Review of the Literature

Country	Population	Phenotype (*Leishmania* sp.)	Locus	Candidate genes or alleles	Genetic association/ linkage	References
Candidate gene approach						
French Guyana	Hmong refugees	**CL** (*L. guyanensis*)	6p21	HLA	+	[27]
Venezuela		**CL** (*L. braziliensis*)	6p21	HLA	+	[190]
Brazil		**MCL** (*L. braziliensis*)	6p21	HLA	+	[258]
Brazil, India		*VL (L. donovani complex)*	*6p21*	*HLA*	−	*[47,56,222, 257,329]*
Sudan, Mediterranea						
Venezuela		**MCL** (*L. braziliensis*)	6p21	TNFA/TNFB	+	[64]
Brazil		**DTH+** (*L. chagasi*)	6p21	TNFA	+	[171]
Sudan	Aringa, Massalit	**VL** (*L. donovani*)	2q35	NRAMP1	+	[397,398]
	Massalit	**VL** (*L. donovani*)	5q31	IL3, IL4, IL5, IL9, IL12p40, IRF1, CSF2, CSF1R	+	[397,398]
	Aringa	*VL* (*L. donovani*)	*5q31*	*IL3, IL4, IL5, IL9, IL12p40, IRF1, CSF2, CSF1R*	−	*[56]*
	Massalit	**PKDL** (*L. donovani*)	6q23	IFNGR1	+	[232]
Genome-wide approach						
Sudan	Aringa	**VL** (*L. donovani*)	22q12	IL2RB, CSF2RB, NCF4(P40-phox)	++	[55]
			2q23-q24	NMI, STAM2	+	[55]

Results of human genetic studies carried out on diverse populations of the world exposed to diverse *Leishmania* species. Studies are ordered by genetic loci and approaches (candidate gene testing vs. genome-wide analysis). Positive results are given in bold and negative results are in italics.

As evidenced by the genome-wide scan described above, the candidate gene approach, relying heavily on the results obtained in experimental models, is likely to miss important genes specific to natural infection in humans. Other such global approaches on different populations (linkage genome-wide analysis, microarray transcriptom analysis, etc.) in which no hypotheses are made on the genes involved will be required in the future and constitute the first step toward their identification. High-throughput genotyping methods are now becoming available that make it possible to characterize individual genotypes at thousands of polymorphic sites in the human genome in a very short time. Although the related cost of such approaches are today too high to be used extensively (especially for the limited budgets in infectious disease research), we hope that these will become increasingly available in the coming years and help analysis of human susceptibility to leishmaniasis on a global scale. However, the possibility of genotyping hundreds of subjects for thousands of genetic markers should not override the fact that the selection of the population sample to be used in the analysis is the most critical step.

As we have stated earlier in this chapter, not only genetic factors from the host are involved in determining susceptibility/resistance to leishmaniasis. The possibility of integrating other risk factors (from the environment or the parasite) at the individual level should thus help select leishmaniasis patients for whom genetic susceptibility is the most likely and in turn increase the power to detect and identify host genetic effects. The asymptomatic phenotype is also probably quite heterogeneous, with subjects being asymptomatic carriers, those developing unnoticed subclinical disease, and others clearing infection totally. Characterization of the quality of the immune response, demonstration and quantification of the parasite in these "asymptomatic" individuals could help split this complex phenotype into more homogeneous sub-phenotypes for genetic analysis.

In summary, genetics of the host could provide critical information for the discovery of key steps in the pathogenesis of *Leishmania* infections and allow the identification of new targets (targeted on the host response rather than the parasite) for chemotherapy and vaccination. We can also hope that genetic studies will allow the identification of subjects at high risk of severe disease. Such subjects could benefit from targeted prophylactic measures; they will also be evaluated carefully in drug and vaccine trials, as different proportions of susceptible/resistant subjects in the vaccinated or placebo group could be important confounding factors in the analysis.

Fig. 6.14. Transmission of kala-azar in Bihar, India.

6.5 THE NEED FOR AN INTEGRATED APPROACH: THE KALA-AZAR EXAMPLE IN INDIA

In all the previous sections, we have detailed the different known factors involved in leishmaniasis. In this last section, we will focus on the need for an integrated approach considering parasite, vector, and host involvement with the example of Indian VL.

In India, millions are at risk; the state of Bihar accounts for nearly one-fifth of worldwide cases (Fig. 6.14). The current episode of leishmaniasis in India is unique: the disease started in the early 1970s, and for more than 30 years there has been incessant transmission spreading in all directions. There have been efforts to control the disease, mostly knee-jerk reactions, and this has hardly had an impact on transmission. Affected populations (Fig. 6.15) are among the poorest in the world and are not much informed on existing preventive measures. Furthermore, misuse of the first-line drugs in these communities is widespread [342], and the lack of response to pentavalent antimonials has been increasing sharply over the last few years in India, up to more than 50% of the patients in the hyperendemic areas of Bihar [201,328,352].

In these hyperendemic areas, it is now well established that most exposed individuals are asymptomatic [22,336,399]. A multidisciplinary approach, combining parasite, host, and vector studies, could help (i) to understand why in an endemic area in Bihar different clinical outcomes (asymptomatic/paucisymptomatic vs severe visceral disease) result from infection by *L. donovani* and (ii) to identify factors determining resistance or susceptibility to the disease. The integrated analysis of parasite genetics, parasite virulence factors, host immune responses, host genetics, as well as socioeconomic and environmental risk factors will provide a better understanding of the interplay between these different factors and the risk of

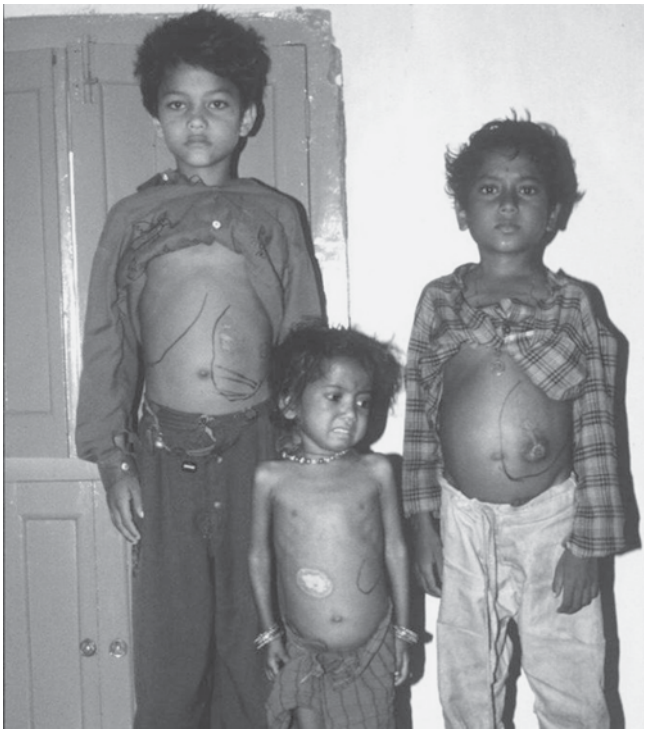

Fig. 6.15. Children with Indian kala-azar with burn marks on the abdomen in an effort to cure by traditional healers (all rights reserved).

developing VL, the critical biological pathways involved in host resistance or susceptibility to VL, and therefore help orient new therapeutic or vaccine strategies.

Vector control should include not only insecticide spraying but also household vector control measures such as insecticide-impregnated bed nets and curtains, sanitation improvement, and elimination of the sand fly breeding sites. There is an absolute lack of awareness regarding the etiology, transmission, and factors favoring growth of sand flies. Thus, the piecemeal efforts are not likely to succeed. A well-coordinated effort with a combined IEC (information, education, and communication) approach, multipronged vector-control measures, including elimination of breeding sites, personal protection, and insecticide spraying, early diagnosis and effective treatment accessible to all, either free or at a subsidized cost, can only make a favorable impact on transmission.

Nevertheless, to be efficient, these measures require an accurate knowledge of the vector's ecology (geographical distribution, species involved, habitat, transmission rate).

Lack of a vaccine is one of the strongest drawbacks in controlling VL in India and other endemic regions. Intensive efforts toward vaccine development with fast-track clinical development and approval are crucial. Exact immunological aberration in VL has yet to be unraveled, and continued research in human VL can improve the understanding of the disease and provide important clues toward immunotherapy as well as vaccine development. Concerning a VL vaccine, evaluation of the different players' involvement is a critical step in a phase III vaccine evaluation trial. Indeed, inclusion in the vaccinated and placebo groups of different proportions of resistant and susceptible subjects could seriously impair the results of the study. Concerning this last point, a multidisciplinary approach would help in identyfing resistant and susceptible populations in a given endemic area. Moreover, the study of the vaccine antigen-specific antibodies and the cellular immune responses induced *ex vivo* in T-cell stimulation assays in VL patients before and after treatment and in asymptomatic subjects will provide the identification of a number of immunologic parameters in subjects exhibiting patterns of progressive disease or apparent resistance. This is a fundamental prerequisite to identifying the interactions between various cell types that are involved both in processing and effector responses. This would facilitate our understanding of the disease and help in the design of a vaccine against VL.

6.6 CONCLUSION

In this chapter, we attempted to demonstrate the multifactorial aspect of leishmaniasis. The exact involvement of hosts, vectors and parasites, and interactions among them in the outcome of the disease remains unknown. Although we know that all of them have an impact on the manifestation of the disease: (i) there is a strong vector–parasite specificity; (ii) *Leishmania* species are statistically associated with certain clinical forms and some factors have been described as associated with clinical diversity; and (iii) the risk of leishmaniasis is markedly increased by allelic variants at specific host genetic loci – *it is necessary to study all of these factors in a population of a single focus and it is especially important to cross the results*. The integrated analysis on the same subjects (epidemiological, parasitological, immunological, and genetic studies) would therefore provide a clear picture of the interplay between environmental, parasitic, and host factors in the development of the disease. For example, in the case of the Indian focus, an integrated approach could help us to better understand (i) the increasingly worrying problem of drug resistance (Is it due evolution of the host-parasite system? What are the biochemical mechanisms involved?) and (ii) asymptomatic carriers (Are these people infected by a "particular" parasite and/or are they able to contain the infection by themselves? In both cases, what are the molecular processes involved?

ACKNOWLEDGMENTS

We are grateful to the IRD (Institut de Recherche pour le dévepoppement) and the CNRS (Centre National de la Recherche Scientifique) for financial support.

ABBREVIATIONS

ACL:	Anthroponotic cutaneous leishmaniasis
AIDS:	Acquired Immune Deficiency Syndrome
ATP:	Adenosine triphosphate
AVL:	Anthroponotic visceral leishmaniasis
CL:	Cutaneous leishmaniasis
CM-DM:	Canine monocyte-derived macrophages
CP:	Cysteine protease
DAT:	Direct antigenemia test
DNA:	Deoxyribonucleic acid
DTH:	Delayed-type hypersensitivity
ELISA:	Enzyme-linked immunosorbent assay
ESA:	Excreted-secreted antigen
fPPG:	Filamentous proteophosphoglycan
GM-CSF:	Granulocyte-macrophage stimulating factor
GP63:	Glycoprotein 63
GPI:	Glycosylphosphatidylinositol
HAART:	Highly Active Antiretroviral Therapy
HIV:	Human immunodeficiency virus
HLA:	Human leukocyte antigen
IFAT:	Immunofluorescence antibody detection test
IFN:	Interferon
Ig:	Immunoglobulin
IL:	Interleukin
iNOS:	Inducible nitric oxide synthase
KA:	Kala-azar
LPG:	Lipophosphoglycan
MCL:	Mucocutaneous leishmaniasis
MHC:	Major histocompatibility complex

MLEE: Multilocus enzyme electrophoresis
mRNA: Messenger ribonucleic acid
NADPH: Nicotinamide adenine dinucleotide phosphate reduced
NK: Natural killer
NMI: N-myc interactor
NO: Nitric oxyde
Nramp: Natural resistance-associated macrophage protein
PBMC: Peripheral blood mononuclear cell
PCR: Polymerase chain reaction
PFGE: Pulse field gel electrophoresis
PHOX: Phagocyte oxidase
PKDL: Post-kala-azar leishmaniasis
PM: Peritrophic matrix
PSA: Protein surface antigen
PSG: Promastigote secretory gel
RAPD: Random amplified polymorphic DNA
RFLP: Restriction fragment length polymorphism
Sb^V: Stibogluconate (pentavalent antimonials)
SNP: Single nucleotide polymorphism
STAM: Signal transducing adapter molecule
TdT: Transmission disequilibrium test
TGF: Transforming growth factor
Treg: Regulatory T cells
Th: T helper cells
TNF: Tumor necrosis factor
VL: Visceral leishmaniasis
ZCL: Zoonotic cutaneous leishmaniasis
ZVL: Zoonotic visceral leishmaniasis

GLOSSARY

Acquired immune response: Immunity mediated by lymphocytes and characterized by antigen specificity and memory. It is a specific, inducible immune response to pathogens.

Affected sib-pair study: This is the most familiar form of nonparametric linkage analysis. This is observed if affected sibling pairs inherit the same marker allele from their parents more frequently than would be expected by chance.

Allele: An allele is a variant of a single gene, inherited at a particular genetic locus; it is a particular sequence of nucleotides, coding for messenger RNA.

Antibodies: Any of numerous molecules of immunoglobulin superfamily produced by the B cells as a primary immune defense in response to specific proteins (antigens).

Antigen: Any substance recognized by the body as being foreign, that stimulates the production of antibodies. These antigens produce an immune response by the organism in response to their presence.

Biological concept of species: It defines species in terms of interbreeding. For instance, Ernst Mayr defined a species as follows: "species are groups of interbreeding natural populations that are reproductively isolated from other such groups." The biological species concept explains why the members of a species resemble one another, that is, form phenetic clusters, and differ from other species.

Case–control studies: They are based on the comparison of genotypes or allele frequencies between affected cases and unaffected controls groups.

Chemotherapy: The treatment of disease by means of chemicals that have a specific toxic effect on the disease-producing microorganisms (e.g., antibiotics) or that selectively destroy cancerous tissue (anticancer therapy).

Clone, *clonal*, *clonality*: From a genetic point of view, this term refers to all cases in which the daughter cells are genetically identical to the parental cell, whatever the actual mating system.

Cytokines: Small proteins or biological factors (in the range of 8-30 kDa) that are released by cells and have specific effects on cell–cell interaction, communication, and behavior of other cells.

Endemic disease: Present or usually prevalent in a population or geographical area at all times.

Epidemiology: The study of the distribution and determinants of health-related states and events in populations and the control of health problems.

Genetic association: It is related to observing if a particular gene polymorphism is statistically associated with disease, it can be carried out by different methods (case–control studies or family-based association tests).

Genetic locus: The site in a linkage map or on a chromosome where the gene for a particular trait is located. Any one of the alleles of a gene may be present at this site.

Glycoconjugates: Carbohydrates covalently linked to a nonsugar moiety (lipids or proteins). The major glycoconjugates are glycoproteins, glycopeptides, peptidoglycans, glycolipids, and lipopolysaccharides.

Immune response: Alteration in the reactivity of an organism immune system in response to an antigen; in vertebrates, this may involve antibody production, induction of cell-mediated immunity, complement activation or development of immunological tolerance.

Immunization: The act of inducing antibody formation leading to immunity.

Immunogenic: Producing immunity, evoking an immune response.

Immunosuppression: This occurs when T and/or B lymphocytes are depleted in size or suppressed in their reactivity, expansion, or differentiation.

Incidence: The frequency of new infections during a designated time period expressed.

Innate immunity: Early nonspecific immune response to pathogens. Also called natural immunity, it is immediate first line of defense, including the complement system, natural killer cells and phagocytic cells (e.g., macrophages, dendritic cells).

Kinetoplast: Mass of mitochondrial DNA, usually adjacent to the flagellar basal body, in flagellate protozoa (these microorganisms belong to the group of kinetoplastids (Kinetoplastida order).

Leishmanization: An ancient practice of immunization to protect against infection to *Leishmania* by inoculating live parasites.

Linkage analysis: A mean for determining the localization in the genome of an unknown susceptibility gene with respect to positionally known genetic markers. This is based on the tendency for closely positioned sequenced to be inherited together.

Lodscore: It represents the intensity of linkage between two markers on the genome (the logarithm of the likelihood ratio for the odds in favor of linkage over no linkage). It provides a statistical test of the null hypothesis of free recombination (no linkage) over the alternative hypothesis of linkage.

Lutzomyia: A genus of New World sand flies or bloodsucking midges (family Psychodidae) that serve as vectors of leishmaniasis and Oroyo fever; formerly combined with the Old World sand fly genus *Phlebotomus*.

Major histocompatibility complex (*MHC*): The set of gene loci specifying major histocompatibility antigens. The MHC molecules display antigenic peptides to T lymphocyte receptors and initiate the specific immunity.

Metacyclogenesis: Process by which noninfective procyclic promastigotes are transformed into metacyclic promastigotes, the infectious form. This process is characterized by morphological changes of the parasite and also biochemical transformations.

Multilocus Enzyme Electrophoresis (*MLEE*): Protein extracts from given samples, for example various pathogen stocks, are separated by electrophoresis. The gel is then subjected to a histochemical reaction involving the specific substrate of a given enzyme, and the zone of activity of this enzyme is specifically stained. The same enzyme from different samples may migrate at different rates. These different electrophoretic forms of the same enzyme are referred to as isoenzymes or isozymes.

Pathogenicity: The ability of a pathogen to inflict damage on the host.

Pedigree: A multigenerational family health history diagrammed with a set of international symbols to indicate the individuals in the family, their relationships to one another, those with a disease, and so on.

Phagocytosis: Phagocytosis involves the ingestion and digestion by phagocyte cells of microorganisms, insoluble particles, damaged or dead host cells, cell debris or activated clotting factors. The principal phagocytes include the neutrophils and monocytes (types of white blood cells).

Phenotype: The observable characteristics of an organism, the expression of gene alleles (genotype) as an observable physical or biochemical trait. It is the result from interaction between the genotype and the environment.

Phlebotomus: A genus of psychodidae that functions as the vector of a number of pathogenic organisms, including *Leishmania*.

Polymerase chain reaction (*PCR*): A technique to amplify a specific region of double-stranded DNA. An excess of two amplimers, oligonucleotide primers complementary to two sequences that flank the region to be amplified, are annealed to denatured DNA and subsequently elongated, usually by a heat-stable DNA polymerase from Thermus aquaticus (Taq polymerase).

Prevalence: The proportion of individuals in a population having a disease.

Promastigote: Term now generally used instead of "leptomonad" or "leptomonad stage," to avoid confusion with the flagellate genus *Leptomonas*. It denotes the flagellate stage of a trypanosomatid protozoan in which the flagellum arises from a kinetoplast in front of the nucleus and emerges from the anterior end of the organism; usually an extracellular phase, as in the insect intermediate host (or in culture) of *Leishmania* parasites.

Prophylaxis: The administration of chemicals or drugs to members of a community to reduce the number of carriers of a disease and to prevent others contracting the disease.

Reservoir host: A reservoir is the source of an infecting microorganism. It serves as a source from which other individuals can be infected. For example, a zoonosis is a communicable disease that is transmitted from a nonhuman animal (*reservoir*) to a human.

Sib-pairs: See affected sib-pair study.

Taxonomy: The theory and practice of biological classification. The theories and techniques of naming, describing, and classifying organisms, the study of the relationships of taxa, including positional changes that do not involve changes in the names of taxa.

Transmission disequilibrium Test (*TdT*): It is a family-based association test. In this case, only cases and their parents are included in the analysis. The TdT is used to look for bias in transmission of alleles from heterozygous parents to affected offspring (different from 0.5 if there is association).

Vector: An agent, usually an animal or an insect, that transmits a pathogen form one host to another.

Zymodeme: Regroups all the *Leishmania* strains that have the same MLEE patterns for all the loci.

REFERENCES

1. Ajdary S, Alimohammadian MH, Eslami MB, Kemp K, Kharazmi A. Comparison of the immune profile of nonhealing cutaneous Leishmaniasis patients with those with active lesions and those who have recovered from infection. *Infect Immun* 2000;**68**(4):1760–4.
2. Alam MJ, Rahman KM, Asna SM, Muazzam N, Ahmed I, Chowdhury MZ. Comparative studies on IFAT, ELISA & DAT for serodiagnosis of visceral leishmaniasis in Bangladesh. *Bangladesh Med Res Counc Bull* 1996;**22**(1):27–32.
3. Alcais A, Abel L, David C, Torrez ME, Flandre P, Dedet JP. Evidence for a major gene controlling susceptibility to tegumentary leishmaniasis in a recently exposed Bolivian population. *Am J Hum Genet* 1997;**61**(4):968–79.
4. Alexander J, Coombs GH, Mottram JC. *Leishmania mexicana* cysteine proteinase-deficient mutants have attenuated virulence for mice and potentiate a Th1 response. *J Immunol* 1998;**161**(12):6794–801.
5. Alkhawajah AM, Larbi E, al-Gindan Y, Abahussein A, Jain S. Treatment of cutaneous leishmaniasis with antimony: intramuscular versus intralesional administration. *Ann Trop Med Parasitol* 1997;**91**(8):899–905.
6. Alvar J. Leishmaniasis and AIDS co-infection: the Spanish example. *Parasitol Today* 1994;**10**(4):160–3.
7. Alvar J, Canavate C, Gutierrez-Solar B, et al. *Leishmania* and human immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev* 1997;**10**(2):298–319.
8. Alvar J, Canavate C, Molina R, Moreno J, Nieto J. Canine leishmaniasis. *Adv Parasitol* 2004;**57**:1–88.
9. Amato V, Amato J, Nicodemo A, Uip D, Amato-Neto V, Duarte M. Treatment of mucocutaneous leishmaniasis with pentamidine isothionate. *Ann Dermatol Venereol* 1998;**125**(8):492–5.
10. Anderson CF, Mendez S., Sacks DL. Nonhealing infection despite Th1 polarization produced by a strain of *Leishmania major* in C57BL/6 mice. *J Immunol* 2005;**174**(5):2934–41.
11. Anstead GM, Chandrasekar B, Zhao W, Yang J, Perez LE, Melby PC. Malnutrition alters the innate immune response and increases early visceralization following *Leishmania donovani* infection. *Infect Immun* 2001;**69**(8):4709–18.
12. Anthony RL, Christensen HA, Johnson CM. Micro enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of New World leishmaniasis. *Am J Trop Med Hyg* 1980;**29**(2):190–4.
13. Antoniou M, Doulgerakis C, Pratlong F, Dedet JP, Tselentis Y. Short report: treatment failure due to mixed infection by different strains of the parasite *Leishmania infantum*. *Am J Trop Med Hyg* 2004;**71**(1):71–2.
14. Aoun K, Bouratbine A, Harrat Z,et al. Epidemiologic and parasitologic data concerning sporadic cutaneous leishmaniasis in northern Tunisia. *Bull Soc Pathol Exot* 2000;**93**(2):101–3.
15. Ardehali S, Moattari A., Hatam GR, Hosseini SM, Sharifi I. Characterization of *Leishmania* isolated in Iran. 1. Serotyping with species specific monoclonal antibodies. *Acta Trop* 2000;**75**(3):301–7.
16. Ashford RW. The leishmaniases as emerging and reemerging zoonoses. *Int J Parasitol* 2000;**30**(12–13):1269–81.
17. Attar ZJ, Chance ML, el-Safi S, et al. Latex agglutination test for the detection of urinary antigens in visceral leishmaniasis. *Acta Trop* 2001;**78**(1):11–6.
18. Aviles H, Belli A, Armijos R, Monroy FP, Harris E. PCR detection and identification of *Leishmania* parasites in clinical specimens in Ecuador: a comparison with classical diagnostic methods. *J Parasitol* 1999;**85**(2):181–7.
19. Bacellar O, Lessa H, Schriefer A, et al. Up-regulation of Th1-type responses in mucosal leishmaniasis patients. *Infect Immun* 2002;**70**(12):6734–40.
20. Badaro R, Benson D, Eulalio MC, et al. rK39: a cloned antigen of *Leishmania chagasi* that predicts active visceral leishmaniasis. *J Infect Dis* 1996;**173**(3):758–61.
21. Badaro R, Jones TC, Carvalho EM, et al. New perspectives on a subclinical form of visceral leishmaniasis. *J Infect Dis* 1986;**154**(6):1003–11.
22. Badaro R, Jones TC, Lorenco R, et al. A prospective study of visceral leishmaniasis in an endemic area of Brazil. *J Infect Dis* 1986;**154**(4):639–49.
23. Bañuls AL, Dujardin JC, Guerrini F, et al. Is *Leishmania (Viannia) peruviana* a distinct species? A MLEE/RAPD evolutionary genetics answer. *J Eukaryot Microbiol* 2000;**47**(3):197–207.
24. Bañuls AL, Guerrini F, Le Pont F, et al. Evidence for hybridization by multilocus enzyme electrophoresis and random amplified polymorphic DNA between *Leishmania braziliensis* and *Leishmania panamensis/guyanensis* in Ecuador. *J Eukaryot Microbiol* 1997;**44**(5):408–11.
25. Bañuls AL, Hide M, Tibayrenc, M. Molecular epidemiology and evolutionary genetics of *Leishmania* parasites. *Int J Parasitol* 1999;**29**:1137–47.
26. Bañuls AL, Jonquieres R, Guerrini F, et al. Genetic analysis of *Leishmania* parasites in Ecuador: are *Leishmania (Viannia) panamensis* and *Leishmania (V.) guyanensis* distinct taxa? *Am J Trop Med Hyg* 1999;**61**(5):838–45.
27. Barbier D, Demenais F, Lefait JF, David B, Blanc M, Hors J, Feingold N. Susceptibility to human cutaneous leishmaniasis and HLA, Gm, Km markers. *Tissue Antigens* 1987;**30**(2):63–7.
28. Barbosa Junior AA, Andrade ZA, Reed SG. The pathology of experimental visceral leishmaniasis in resistant and susceptible lines of inbred mice. *Braz J Med Biol Res* 1987;**20**(1):63–72.
29. Barral-Netto M, Barral A, Brownell CE, et al. Transforming growth factor-beta in leishmanial infection: a parasite escape mechanism. *Science* 1992;**257**(5069):545–8.
30. Bastrenta B, Mita N, Buitrago R, et al. Human mixed infections of *Leishmania* spp. and *Leishmania-Trypanosoma cruzi* in a sub Andean Bolivian area: identification by polymerase chain reaction/hybridization and isoenzyme. *Mem Inst Oswaldo Cruz* 2003;**98**(2):255–64.
31. Bates PA, Rogers ME. New insights into the developmental biology and transmission mechanisms of *Leishmania*. *Curr Mol Med* 2004;**4**(6):601–9.

32. Beach R, Kiilu G, Hendricks L, Oster C, Leeuwenburg J. Cutaneous leishmaniasis in Kenya: transmission of *Leishmania major* to man by the bite of a naturally infected *Phlebotomus duboscqi*. *Trans R Soc Trop Med Hyg* 1984;**78**(6):747–51.
33. Beach R, Kiilu G, Leeuwenburg J. Modification of sand fly biting behavior by *Leishmania* leads to increased parasite transmission. *Am J Trop Med Hyg* 1985;**34**(2):278–82.
34. Beebe AM, Mauze S, Schork NJ, Coffman RL. Serial backcross mapping of multiple loci associated with resistance to *Leishmania major* in mice. *Immunity* 1997;**6**(5):551–7.
35. Beetham JK, Donelson JE, Dahlin RR. Surface glycoprotein PSA (GP46) expression during short- and long-term culture of *Leishmania chagasi*. *Mol Biochem Parasitol* 2003;**131**(2):109–17.
36. Belkaid Y, Butcher B, Sacks DL. Analysis of cytokine production by inflammatory mouse macrophages at the single-cell level: selective impairment of IL-12 induction in *Leishmania*-infected cells. *Eur J Immunol* 1998;**28**(4):1389–400.
37. Belkaid Y, Kamhawi S, Modi G, et al. Development of a natural model of cutaneous leishmaniasis: powerful effects of vector saliva and saliva preexposure on the long-term outcome of *Leishmania major* infection in the mouse ear dermis. *J Exp Med* 1998;**188**(10):1941–53.
38. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. *Nature* 2002;**420**(6915):502–7.
39. Belkaid Y, Von Stebut E, Mendez S, et al. CD8+ T cells are required for primary immunity in C57BL/6 mice following low-dose, intradermal challenge with *Leishmania major*. *J Immunol* 2002;**168**(8):3992–4000.
40. Bell D, Young JW, Banchereau J. Dendritic cells. *Adv Immunol* 1999;**72**:255–324.
41. Belli AA, Miles MA, Kelly JM. A putative *Leishmania panamensis/Leishmania braziliensis* hybrid is a causative agent of human cutaneous leishmaniasis in Nicaragua. *Parasitology* 1994;**109**: 435–442.
42. Bern C, Jha SN, Joshi AB, Thakur GD, Bista MB. Use of the recombinant K39 dipstick test and the direct agglutination test in a setting endemic for visceral leishmaniasis in Nepal. *Am J Trop Med Hyg* 2000;**63**(3–4):153–7.
43. Biggs TE, Baker ST, Botham MS, Dhital A, Barton CH, Perry VH. Nramp1 modulates iron homoeostasis in vivo and in vitro: evidence for a role in cellular iron release involving de-acidification of intracellular vesicles. *Eur J Immunol* 2001;**31**(7):2060–70.
44. Blackwell J, Freeman J, Bradley D. Influence of H-2 complex on acquired resistance to *Leishmania donovani* infection in mice. *Nature* 1980;**283**(5742):72–4.
45. Blackwell JM. Genetic susceptibility to leishmanial infections: studies in mice and man. *Parasitology* 1996;**112**(Suppl):S67–74.
46. Blackwell JM, Goswami T, Evans CA, et al. SLC11A1 (formerly NRAMP1) and disease resistance. *Cell Microbiol* 2001;**3**(12):773–84.
47. Blackwell JM, Mohamed HS, M. E. Ibrahim ME. Genetics and visceral leishmaniasis in the Sudan: seeking a link. *Trends Parasitol* 2004;**20**(6):268–74.
48. Bogdan C, Gessner A, Rollinghoff M. Cytokines in leishmaniasis: a complex network of stimulatory and inhibitory interactions. *Immunobiology* 1993;**189**(3–4):356–96.
49. Bogdan C, Rollinghoff M, Diefenbach A. The role of nitric oxide in innate immunity. *Immunol Rev* 2000;**173**:17–26.
50. Borovsky D, Schlein Y. Trypsin and chymotrypsin-like enzymes of the sandfly *Phlebotomus papatasi* infected with *Leishmania* and their possible role in vector competence. *Med Vet Entomol* 1987;**1**(3):235–42.
51. Boulanger N, Lowenberger C, Volf P, et al. Characterization of a defensin from the sand fly *Phlebotomus duboscqi* induced by challenge with bacteria or the protozoan parasite *Leishmania major*. *Infect Immun* 2004;**72**(12):7140–6.
52. Bradley DJ, Taylor BA, Blackwell J, Evans EP, Freeman J. Regulation of *Leishmania* populations within the host. III. Mapping of the locus controlling susceptibility to visceral leishmaniasis in the mouse. *Clin Exp Immunol* 1979;**37**(1):7–14.
53. Brittingham A, Mosser DM. Exploitation of the complement system by *Leishmania* promastigotes. *Parasitol Today* 1996;**12**(11):444–7.
54. Buates S, Matlashewski G. General suppression of macrophage gene expression during *Leishmania donovani* infection. *J Immunol* 2001;**166**(5):3416–22.
55. Bucheton B, Abel L, El-Safi S, et al. A major susceptibility locus on chromosome 22q12 plays a critical role in the control of kala-azar. *Am J Hum Genet* 2003;**73**(5):1052–60.
56. Bucheton B, Abel L, Kheir MM, et al. Genetic control of visceral leishmaniasis in a Sudanese population: candidate gene testing indicates a linkage to the NRAMP1 region. *Genes Immun* 2003;**4**(2):104–9.
57. Bucheton B, El-Safi SH, Hammad A, et al. Antileishmanial antibodies in an outbreak of visceral leishmaniasis in eastern Sudan: high antibody responses occur in resistant subjects and are not predictive of disease. *Trans R Soc Trop Med Hyg* 2003;**97**(4):463–8.
58. Bucheton B, Kheir MM, El-Safi SH, et al. The interplay between environmental and host factors during an outbreak of visceral leishmaniasis in eastern Sudan. *Microbes Infect* 2002;**4**(14):1449–57.
59. Buelens C, Willems F, Delvaux A, et al. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells. *Eur J Immunol* 1995;**25**(9):2668–72.
60. Bulle B, Millon L, Bart JM, et al. Practical approach for typing strains of *Leishmania infantum* by microsatellite analysis. *J Clin Microbiol* 2002;**40**(9):3391–7.
61. Burns JM, Jr, Shreffler WG, Benson DR, Ghalib HW, Badaro R, Reed SG. Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis. *Proc Natl Acad Sci USA* 1993;**90**(2):775–9.
62. Butcher BA, Turco SJ, Hilty BA, Pimenta PF, Panunzio M, Sacks DL. Deficiency in beta1,3-galactosyltransferase of a *Leishmania major* lipophosphoglycan mutant adversely influences the *Leishmania*-sand fly interaction. *J Biol Chem* 1996;**271**(34):20573–9.
63. Cabello PH, Lima AM, Azevedo ES, Krieger H. Familial aggregation of *Leishmania chagasi* infection in northeastern Brazil. *Am J Trop Med Hyg* 1995;**52**(4):364–5.
64. Cabrera M, Shaw MA, Sharples C, et al. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *J Exp Med* 1995;**182**(5):1259–64.
65. Caceres-Dittmar G, Tapia FJ, Sanchez MA, et al. Determination of the cytokine profile in American cutaneous

leishmaniasis using the polymerase chain reaction. *Clin Exp Immunol* 1993;**91**(3):500–5.
66. Calza L, D'Antuono A, Marinacci G, et al. Disseminated cutaneous leishmaniasis after visceral disease in a patient with AIDS. *J Am Acad Dermatol* 2004;**50**(3):461–5.
67. Campos-Ponce M, Ponce C, Ponce E, Maingon RD. *Leishmania chagasi/infantum*: further investigations on *Leishmania* tropisms in atypical cutaneous and visceral leishmaniasis foci in Central America. *Exp Parasitol* 2005;**109**(4):209–19.
68. Carrera L, Gazzinelli RT, Badolato R, et al. *Leishmania* promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. *J Exp Med* 1996;**183**(2):515–26.
69. Carvalho EM, Johnson WD, Barreto E, et al. Cell mediated immunity in American cutaneous and mucosal leishmaniasis. *J Immunol* 1985;**135**(6):4144–8.
70. Chamizo C, Moreno J, Alvar J. Semi-quantitative analysis of cytokine expression in asymptomatic canine leishmaniasis. *Vet Immunol Immunopathol* 2005;**103**(1–2):67–75.
71. Chang KP, McGwire BS. Molecular determinants and regulation of *Leishmania* virulence. *Kinetoplastid Biol Dis* 2002;**1**(1):1.
72. Charlab R, Valenzuela JG, Rowton ED, Ribeiro JM. Toward an understanding of the biochemical and pharmacological complexity of the saliva of a hematophagous sand fly *Lutzomyia longipalpis*. *Proc Natl Acad Sci USA* 1999;**96**(26):15155–60.
73. Chaussabel D, Semnani RT, McDowell MA, Sacks D, Sher A, Nutman TB. Unique gene expression profiles of human macrophages and dendritic cells to phylogenetically distinct parasites. *Blood* 2003;**102**(2):672–81.
74. Chunge CN, Owate J, Pamba HO, Donno L. Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. *Trans R Soc Trop Med Hyg* 1990;**84**(2):221–5.
75. Colebunders R, Depraetere K, Verstraeten T, et al. Unusual cutaneous lesions in two patients with visceral leishmaniasis and HIV infection. *J Am Acad Dermatol* 1999;**41**(5 Pt 2): 847–50.
76. Correia D, Macedo VO, Carvalho EM, et al. Comparative study of meglumine antimoniate, pentamidine isethionate and aminosidine sulfate in the treatment of primary skin lesions caused by *Leishmania (Viannia) braziliensis*. *Rev Soc Bras Med Trop* 1996;**29**(5):447–53.
77. Costa CH, Stewart JM, Gomes RB, et al. Asymptomatic human carriers of *Leishmania chagasi*. *Am J Trop Med Hyg* 2002;**66**(4):334–7.
78. Croft AM, Baker D, von Bertele MJ. An evidence-based vector control strategy for military deployments: the British Army experience. *Med Trop (Mars)* 2001;**61**(1):91–8.
79. Croft SL. Monitoring drug resistance in leishmaniasis. *Trop Med Int Health* 2001;**6**(11):899–905.
80. Crofts MA. Use of amphotericin B in mucocutaneous leishmaniasis. *J Trop Med Hyg* 1976;**79**(5):111–3.
81. Cuba Cuba CA, Marsden PD, Barreto AC, Rocha R, Sampaio RR, Patzlaff L. Parasitologic and immunologic diagnosis of American (mucocutaneous) leishmaniasis. *Bull Pan Am Health Organ* 1981;**15**(3):249–59.
82. Cuervo P, Cupolillo E, Nehme N, Hernandez V, Saravia N, Fernandes O. *Leishmania* (*Viannia*): genetic analysis of cutaneous and mucosal strains isolated from the same patient. *Exp Parasitol* 2004;**108**(1–2):59–66.
83. Cunningham AC. Parasitic adaptive mechanisms in infection by *Leishmania*. *Exp Mol Pathol* 2002;**72**(2):132–41.
84. Cunningham ML, Titus RG, Turco SJ, Beverley SM. Regulation of differentiation to the infective stage of the protozoan parasite *Leishmania major* by tetrahydrobiopterin. *Science* 2001;**292**(5515):285–7.
85. Cupolillo E, Grimaldi G, Jr, Momen H. A general classification of New World *Leishmania* using numerical zymotaxonomy. *Am J Trop Med Hyg* 1994;**50**(3):296–311.
86. Da Conceicao-Silva F, Perlaza BL, Louis JA, Romero P. *Leishmania major* infection in mice primes for specific major histocompatibility complex class I-restricted CD8+ cytotoxic T cell responses. *Eur J Immunol* 1994;**24**(11):2813–7.
87. Dasgupta S, Mookerjee A, Chowdhury SK, Ghose AC. Immunosuppression in hamsters with progressive visceral leishmaniasis: an evaluation of the role of nitric oxide toward impairment of the lymphoproliferative response. *Parasitol Res* 1999;**85**(7):594–6.
88. Davidson RN, Croft SL, Scott A, Maini M, Moody AH, Bryceson AD. Liposomal amphotericin B in drug-resistant visceral leishmaniasis. *Lancet* 1991;**337**(8749):1061–2.
89. Davidson RN, Di Martino L, Gradoni L, et al. Liposomal amphotericin B (AmBisome) in Mediterranean visceral leishmaniasis: a multi-centre trial. *Q J Med* 1994;**87**(2):75–81.
90. Davies CR, Kaye P, Croft SL, Sundar S. Leishmaniasis: new approaches to disease control. *Br Med J* 2003;**326**(7385): 377–82.
91. De Andrade AS, Gomes RF, Fernandes O, de Melo MN. Use of DNA-based diagnostic methods for human leishmaniasis in Minas Gerais, Brazil. *Acta Trop* 2001;**78**(3):261–7.
92. De Brujin MH, Labrada LA, Smyth AJ, Santrich C, Barker DC. A comparative study of diagnosis by the polymerase chain reaction and by current clinical methods using biopsies from Colombian patients with suspected leishmaniasis. *Trop Med Parasitol* 1993;**44**(3):201–7.
93. De la Loma A, Alvar J, Martinez Galiano E, Blazquez J, Alcala Munoz A, Najera R. Leishmaniasis or AIDS? *Trans R Soc Trop Med Hyg* 1985;**79**(3):421–2.
94. Delobel P, Launois P, Djossou F, Sainte-Marie D, Pradinaud R. American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis coinfection with downregulation of the T-helper 1 cell response. *Clin Infect Dis* 2003; **37**(5):628–33.
95. Delves PJ, Roitt IM. The immune system. First of two parts. *N Engl J Med* 2000;**343**(1):37–49.
96. Delves PJ, Roitt IM. The immune system. Second of two parts. *N Engl J Med* 2000;**343**(2):108–17.
97. Deniau M, Canavate C, Faraut-Gambarelli F, Marty P. The biological diagnosis of leishmaniasis in HIV-infected patients. *Ann Trop Med Parasitol* 2003;**97**(Suppl 1):115–33.
98. Dennert G. Evidence for non-identity of T killer and T helper cells sensitised to allogeneic cell antigens. *Nature* 1974; **249**(455):358–360.
99. Deparis X, Boutin JP, Michel R, et al. Disease vector control strategy in the French army. *Med Trop (Mars)* 2001; **61**(1):87–90.

100. Descoteaux A, Matlashewski G. c-fos and tumor necrosis factor gene expression in *Leishmania donovani*-infected macrophages. *Mol Cell Biol* 1989;**9**(11):5223–7.
101. Desjeux P. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001;**95**(3):239–43.
102. Desjeux P. Worldwide increasing risk factors for leishmaniasis. *Med Microbiol Immunol (Berl)* 2001;**190**(1–2):77–9.
103. Desjeux P, Alvar J. *Leishmania*/HIV co-infections: epidemiology in Europe. *Ann Trop Med Parasitol* 2003;**97**(Suppl 1):3–15.
104. Dietze R, Carvalho SF, Valli LC, et al. Phase 2 trial of WR 6026, an orally administered 8-aminoquinoline, in the treatment of visceral leishmaniasis caused by *Leishmania chagasi*. *Am J Trop Med Hyg* 2001;**65**(6):685–9.
105. Dietze R, Fagundes SM, Brito EF, et al. Treatment of kala-azar in Brazil with Amphocil (amphotericin B cholesterol dispersion) for 5 days. *Trans R Soc Trop Med Hyg* 1995; **89**(3):309–11.
106. Dietze R, Milan EP, Berman JD, et al. Treatment of Brazilian kala-azar with a short course of amphocil (amphotericin B cholesterol dispersion). *Clin Infect Dis* 1993;**17**(6):981–6.
107. Dillon RJ, Lane RP. Bloodmeal digestion in the midgut of *Phlebotomus papatasi* and *Phlebotomus langeroni*. *Med Vet Entomol* 1993;**7**(3):225–32.
108. Dillon RJ, Lane RP. Influence of *Leishmania* infection on blood-meal digestion in the sandflies *Phlebotomus papatasi* and *P. langeroni*. *Parasitol Res* 1993;**79**(6):492–6.
109. Djidingar D, Chippaux JP, Gragnic G, Tchani O, Meynard D, Julvez J. Visceral leishmaniasis in Niger: six new parasitologically confirmed cases. *Bull Soc Pathol Exot* 1997;**90**(1):27–9.
110. Dobson DE, Scholtes LD, Valdez KE, et al. Functional identification of galactosyltransferases (SCGs) required for species-specific modifications of the lipophosphoglycan adhesin controlling *Leishmania major*-sand fly interactions. *J Biol Chem* 2003;**278**(18): 15523–31.
111. Dujardin JC, Bañuls AL, Dujardin JP, Arevalo J, Tibayrenc M, Le Ray D. Comparison of chromosome and isoenzyme polymorphism in geographical populations of *Leishmania (Viannia) peruviana*. *Parasitology* 1998;**117**(Pt 6): 547–54.
112. Dujardin JC, Bañuls AL, Llanos-Cuentas A, et al. Putative *Leishmania* hybrids in the Eastern Andean valley of Huanuco, Peru. *Acta Trop* 1995;**59**(4):293–307.
113. Dujardin JC, Gajendran N, Arevalo J, et al. Karyotype polymorphism and conserved characters in the *Leishmania (Viannia) braziliensis* complex explored with chromosome-derived probes. *Ann Soc Belg Med Trop* 1993;**73**(2):101–18.
114. Dujardin JC, Llanos-Cuentas A, Caceres A, et al. Molecular karyotype variation in *Leishmania (Viannia) peruviana*: indication of geographical populations in Peru distributed along a north-south cline. *Ann Trop Med Parasitol* 1993;**87**(4):335–47.
115. El-Safi S, Bucheton B, Kheir MM, Musa HA, El-Obaid M, Hammad A, Dessein A. Epidemiology of visceral leishmaniasis in Atbara River area, eastern Sudan: the outbreak of Barbar El Fugara village (1996–1997). *Microbes Infect* 2002;**4**(14): 1439–47.
116. El-Safi SH, Abdel-Haleem A, Hammad A, et al. Field evaluation of latex agglutination test for detecting urinary antigens in visceral leishmaniasis in Sudan. *East Mediterr Health J* 2003;**9**(4):844–55.
117. El Harith A, Kolk AH, Leeuwenburg J, et al. Improvement of a direct agglutination test for field studies of visceral leishmaniasis. *J Clin Microbiol* 1988;**26**(7):1321–5.
118. Elso CM, Roberts LJ, Smyth GK, et al. Leishmaniasis host response loci (lmr1-3) modify disease severity through a Th1/Th2-independent pathway. *Genes Immun* 2004;**5**(2): 93–100.
119. Espinoza JR, Skinner AC, Davies CR, et al. Extensive polymorphism at the Gp63 locus in field isolates of *Leishmania peruviana*. *Mol Biochem Parasitol* 1995;**72**(1–2):203–13.
120. Evans DA, Kennedy WPK, Elbihari S, Chapman CJ, Smith V, Peters W. Hybrid formation within the genus *Leishmania*? *Parassitologia* 1987;**29**:165–73.
121. Fonseca SG, Romao PR, Figueiredo F, et al. TNF-alpha mediates the induction of nitric oxide synthase in macrophages but not in neutrophils in experimental cutaneous leishmaniasis. *Eur J Immunol* 2003;**33**(8):2297–306.
122. Gantt KR, Goldman TL, McCormick ML, et al. Oxidative responses of human and murine macrophages during phagocytosis of *Leishmania chagasi*. *J Immunol* 2001;**167**(2): 893–901.
123. Gantt KR, Schultz-Cherry S, Rodriguez N, et al. Activation of TGF-beta by *Leishmania chagasi*: importance for parasite survival in macrophages. *J Immunol* 2003;**170**(5):2613–20.
124. Garcia A, Marquet S, Bucheton B, et al. Linkage analysis of blood *Plasmodium falciparum* levels: interest of the 5q31-q33 chromosome region. *Am J Trop Med Hyg* 1998;**58**(6):705–9.
125. Gardener PJ, Shchory L, Chance ML. Species differentiation in the genus *Leishmania* by morphometric studies with the electron microscope. *Ann Trop Med Parasitol* 1977;**71**(2): 147–55.
126. Gasim S, Elhassan AM, Khalil EA, et al. High levels of plasma IL-10 and expression of IL-10 by keratinocytes during visceral leishmaniasis predict subsequent development of post-kala-azar dermal leishmaniasis. *Clin Exp Immunol* 1998;**111**(1):64–9.
127. Gavgani AS, Hodjati MH, Mohite H, Davies CR. Effect of insecticide-impregnated dog collars on incidence of zoonotic visceral leishmaniasis in Iranian children: a matched-cluster randomised trial. *Lancet* 2002;**360**(9330): 374–9.
128. Ghalib HW, Piuvezam MR, Skeiky YA, et al. Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. *J Clin Invest* 1993;**92**(1):324–9.
129. Ghosh AK, Ghosh DK. Infection pattern of leishmaniasis in hamsters produced by recent isolates from kala-azar patients in India. *Indian J Med Res* 1987;**86**:14–9.
130. Gifawesen C, Farrell JP. Comparison of T-cell responses in self-limiting versus progressive visceral *Leishmania donovani* infections in golden hamsters. *Infect Immun* 1989;**57**(10):3091–6.
131. Gillespie RD, Mbow ML, Titus RG. The immunomodulatory factors of bloodfeeding arthropod saliva. *Parasite Immunol* 2000;**22**(7):319–31.
132. Giri OP, Singh AN. Experience with amphotericin B in sodium stibogluconate – unresponsive cases of visceral Leishmaniasis in north Bihar. *J Assoc Phys India* 1994;**42**(9): 690–1.
133. Gordon S. Macrophages and theimmune response. In *Fundamental Immunology* (ed. W. Paul). Lippincott Raven, Philadelphia, 2003, pp. 481–95.

134. Gorham JD, Guler ML, Steen RG, et al. Genetic mapping of a murine locus controlling development of T helper 1/T helper 2 type responses. *Proc Natl Acad Sci USA* 1996;**93**(22): 12467–72.
135. Gossage SM, Rogers ME, Bates PA. Two separate growth phases during the development of *Leishmania* in sand flies: implications for understanding the life cycle. *Int J Parasitol* 2003;**33**(10):1027–34.
136. Goswami RP, Bairagi B, Kundu PK. K39 strip test-easy, reliable and cost-effective field diagnosis for visceral leishmaniasis in India. *J Assoc Phys India* 2003;**51**:759–61.
137. Grimaldi G, Jr, Tesh RB. Leishmaniases of the New World: current concepts and implications for future research. *Clin Microbiol Rev* 1993;**6**(3):230–50.
138. Groux H, Cottrez F, Rouleau M, et al. A transgenic model to analyze the immunoregulatory role of IL-10 secreted by antigen-presenting cells. *J Immunol* 1999;**162**(3):1723–1729.
139. Guerbouj S, Guizani I, Speybroeck N, Le Ray D, Dujardin JC. Genomic polymorphism of *Leishmania infantum*: a relationship with clinical pleomorphism? *Infect Genet Evol* 2001; **1**(1):49–59.
140. Guerbouj S, Guizani I, Victoir K, Le Ray D, Dujardin JC. Parasite candidate vaccines: a warning from polymorphic *Leishmania* populations. *Parasitol Today* 2000;**16**(6):265.
141. Guerbouj S, Victoir K, Guizani I, et al. Gp63 gene polymorphism and population structure of *Leishmania donovani* complex: influence of the host selection pressure? *Parasitology* 2001;**122**(Pt 1):25–35.
142. Harrat Z, Pratlong F, Belazzoug S, et al. *Leishmania infantum* and *L. major* in Algeria. *Trans R Soc Trop Med Hyg* 1996;**90**(6):625–9.
143. Harrison LH, Naidu TG, Drew JS, de Alencar JE, Pearson RD. Reciprocal relationships between undernutrition and the parasitic disease visceral leishmaniasis. *Rev Infect Dis* 1986;**8**(3): 447–53.
144. Hawn TR, Ozinsky A, Underhill DM, Buckner FS, Akira S, Aderem A. *Leishmania major* activates IL-1 alpha expression in macrophages through a MyD88-dependent pathway. *Microbes Infect* 2002;**4**(8):763–71.
145. Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J Exp Med* 1989;**169**(1):59–72.
146. Ho M, Siongok TK, Lyerly WH, Smith DH. Prevalence and disease spectrum in a new focus of visceral leishmaniasis in Kenya. *Trans R Soc Trop Med Hyg* 1982;**76**(6):741–6.
147. Holzmuller P, Cavaleyra M, Moreaux J, et al. Lymphocytes of dogs immunised with purified excreted-secreted antigens of *Leishmania infantum* co-incubated with *Leishmania* infected macrophages produce IFN gamma resulting in nitric oxide-mediated amastigote apoptosis. *Vet Immunol Immunopathol* 2005;**106**(3–4):247–57.
148. Holzmuller P, Sereno D, Cavaleyra M., et al. Nitric oxide-mediated proteasome-dependent oligonucleosomal DNA fragmentation in *Leishmania amazonensis* amastigotes. *Infect Immun* 2002;**70**(7):3727–35.
149. Hommel M, Peters W, Ranque J, Quilici M, Lanotte G. The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis. *Ann Trop Med Parasitol* 1978;**72**(3):213–18.
150. Hommel M, Sarkari B, Carney J, Chance ML. Katex for the diagnosis of human visceral leishmaniasis. *Med Trop (Mars)* 2001;**61**(6):503–5.
151. Hondowicz B, Scott P. Influence of host and parasite factors on the innate immune response and Th2 stability following infection with *Leishmania major*. *Microbes Infect* 1999; **1**(1):65–71.
152. Howard JG, Hale C, Liew FY. Immunological regulation of experimental cutaneous leishmaniasis. IV. Prophylactic effect of sublethal irradiation as a result of abrogation of suppressor T cell generation in mice genetically susceptible to *Leishmania tropica*. *J Exp Med* 1981;**153**(3):557–68.
153. Ibrahim ME, Lambson B, Yousif AO, et al. Kala-azar in a high transmission focus: an ethnic and geographic dimension. *Am J Trop Med Hyg* 1999;**61**(6):941–4.
154. Ibrahim ME, Smyth AJ, Ali MH, Barker DC, Kharazmi A. The polymerase chain reaction can reveal the occurrence of naturally mixed infections with *Leishmania* parasites. *Acta Trop* 1994;**57**(4):327–32.
155. Ilg T. Lipophosphoglycan is not required for infection of macrophages or mice by *Leishmania mexicana*. *EMBO J* 2000;**19**(9):1953–62.
156. Ilg T, Stierhof YD, Craik D, Simpson R, Handman E, Bacic A. Purification and structural characterization of a filamentous, mucin-like proteophosphoglycan secreted by *Leishmania* parasites. *J Biol Chem* 1996;**271**(35):21583–96.
157. Indiani de Oliveira C, Teixeira MJ, Teixeira CR, et al. *Leishmania braziliensis* isolates differing at the genome level display distinctive features in BALB/c mice. *Microbes Infect* 2004;**6**(11):977–84.
158. Jaffe CL, Bennett E, Grimaldi G, Jr, McMahon-Pratt D. Production and characterization of species-specific monoclonal antibodies against *Leishmania donovani* for immunodiagnosis. *J Immunol* 1984;**133**(1):440–7.
159. Jamjoom MB, Ashford RW, Bates PA, Kemp SJ, Noyes HA. Towards a standard battery of microsatellite markers for the analysis of the *Leishmania donovani* complex. *Ann Trop Med Parasitol* 2002;**96**(3):265–70.
160. Jha SN, Singh NK, Jha TK. Changing response to diamidine compounds in cases of kala-azar unresponsive to antimonial. *J Assoc Phys India* 1991;**39**(4):314–6.
161. Jha T, Sundar S, Thakur CP, Felton J, Sabin A, Horton J. A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India. *Am J Trop Med Hyg* 2005;**73**(6): 1005–11.
162. Jha TK. Evaluation of diamidine compound (pentamidine isethionate) in the treatment resistant cases of kala-azar occurring in North Bihar, India. *Trans R Soc Trop Med Hyg* 1983; **77**(2):167–70.
163. Jha TK, Olliaro P, Thakur CP, et al. Randomised controlled trial of aminosidine (paromomycin) vs sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. *Br Med J* 1998;**316**(7139):1200–5.
164. Jha TK, Sharma VK. Pentamidine-induced diabetes mellitus. *Trans R Soc Trop Med Hyg* 1984;**78**(2):252–3.
165. Jha TK, Sundar S, Thakur CP, et al. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. *N Engl J Med* 1999;**341**(24):1795–800.

166. Kamhawi S. The biological and immunomodulatory properties of sand fly saliva and its role in the establishment of *Leishmania* infections. *Microbes Infect* 2000;**2**:1–9.
167. Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science* 2000;**290**(5495):1351–4.
168. Kamhawi S, Modi GB, Pimenta PF, Rowton E, Sacks DL. The vectorial competence of *Phlebotomus sergenti* is specific for *Leishmania tropica* and is controlled by species-specific, lipophosphoglycan-mediated midgut attachment. *Parasitology* 2000;**121**(Pt 1):25–33.
169. Kamhawi S, Ramalho-Ortigao M, Pham VM, et al. A role for insect galectins in parasite survival. *Cell* 2004;**119**(3):329–41.
170. Karp SE, Farber A, Salo JC, et al. Cytokine secretion by genetically modified nonimmunogenic murine fibrosarcoma. Tumor inhibition by IL-2 but not tumor necrosis factor. *J Immunol* 1993;**150**(3):896–908.
171. Karplus TM, Jeronimo SM, Chang H, et al. Association between the tumor necrosis factor locus and the clinical outcome of *Leishmania chagasi* infection. *Infect Immun* 2002; **70**(12):6919–25.
172. Kaye PM, Curry AJ, Blackwell JM. Differential production of Th1- and Th2-derived cytokines does not determine the genetically controlled or vaccine-induced rate of cure in murine visceral leishmaniasis. *J Immunol* 1991;**146**(8): 2763–70.
173. Kebaier C, Louzir H, Chenik M, Ben Salah A, Dellagi K. Heterogeneity of wild *Leishmania major* isolates in experimental murine pathogenicity and specific immune response. *Infect Immun* 2001;**69**(8):4906–15.
174. Kellina OI. On the dimensions of the leishmanial forms of *Leishmania tropica major* and *Leishmania tropica minor*. *Med Parazitol (Mosk)* 1962;**31**:716–8.
175. Kelly JM, Law JM, Chapman CJ, Van Eys GJ, Evans DA. Evidence of genetic recombination in *Leishmania*. *Mol Biochem Parasitol* 1991;**46**(2):253–63.
176. Kemp K. Cytokine-producing T cell subsets in human leishmaniasis. *Arch Immunol Ther Exp (Warsz)* 2000;**48**(3):173–6.
177. Kemp M, Hey AS, Kurtzhals JA, et al. Dichotomy of the human T cell response to *Leishmania* antigens. I. Th1-like response to *Leishmania major* promastigote antigens in individ. uals recovered from cutaneous leishmaniasis. *Clin Exp Immunol* 1994;**96**(3):410–5.
178 Kemp M, Kurtzhals JA, Bendtzen K, et al. *Leishmania donovani*-reactive Th1- and Th2-like T-cell clones from individuals who have recovered from visceral leishmaniasis. *Infect Immun* 1993;**61**(3):1069–73.
179 Kenney RT, Sacks DL, Gam AA, Murray HW, Sundar S. Splenic cytokine responses in Indian kala-azar before and after treatment. *J Infect Dis* 1998;**177**(3):815–8.
180 Killick-Kendrick R. Some epidemiological consequences of the evolutionary fit between *Leishmaniae* and their phlebotomine vectors. *Bull Soc Pathol Exot Filiales* 1985;**78**(5):747–55.
181. Killick-Kendrick R. The biology and control of phlebotomine sand flies. *Clin Dermatol* 1999;**17**(3):279–89.
182. Killick-Kendrick R, Killick-Kendrick M, Tang Y. Anthroponotic cutaneous leishmaniasis in Kabul, Afghanistan: the low susceptibility of *Phlebotomus papatasi* to *Leishmania tropica*. *Trans R Soc Trop Med Hyg* 1994;**88**(2):252–3.
183. Killick-Kendrick R, Molyneux DH. Transmission of leishmaniasis by the bite of phlebotomine sandflies: possible mechanisms. *Trans R Soc Trop Med Hyg* 1981;**75**(1):152–4.
184. Kisielow P, Hirst JA, Shiku H, et al. Ly antigens as markers for functionally distinct subpopulations of thymus-derived lymphocytes of the mouse. *Nature* 1975;**253**(5488):219–20.
185. Klein J, Sato A. The HLA system. First of two parts. *N Engl J Med* 2000;**343**(10):702–9.
186. Kopf M, Brombacher F, Kohler G, et al. IL-4-deficient Balb/c mice resist infection with *Leishmania* major. *J Exp Med* 1996. **184**(3):1127–36.
187. Krause G, Kroeger A. Topical paromomycin/methylbenzethonium chloride plus parenteral meglumine antimonate as treatment of American cutaneous leishmaniasis: controlled study. *Clin Infect Dis* 1999;**29**(2):466–7.
188. Laguna F, Videla S, Jimenez-Mejias ME, et al. Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV: a randomized pilot study. *J Antimicrob Chemother* 2003;**52**(3):464–8.
189. Lainson R, Shaw JJ. Evolution, classification and geographical distribution. In *The Leishmaniases in Biology and Medicine* (eds W. Peters and R. Killick-Kendrick). Academic Press, London, 1987, pp. 1–120.
190. Lara ML, Layrisse Z, Scorza JV, et al. Immunogenetics of human American cutaneous leishmaniasis. Study of HLA haplotypes in 24 families from Venezuela. *Hum Immunol* 1991;**30**(2):129–35.
191. Launois P, Himmelrich H, Tacchini-Cottier F, Milon G, Louis JA. New insight into the mechanisms underlying Th2 cell development and susceptibility to *Leishmania major* in BALB/c mice. *Microbes Infect* 1999;**1**(1):59–64.
192. Le Fichoux Y, Quaranta JF, Aufeuvre JP, et al. Occurrence of *Leishmania infantum* parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. *J Clin Microbiol* 1999;**37**(6):1953–7.
193. Leandro C, Campino L. Leishmaniasis: efflux pumps and chemoresistance. *Int J Antimicrob Agents* 2003;**22**(3):352–7.
194. Lemesre JL, Holzmuller P, Cavaleyra M, Goncalves RB, Hottin G, Papierok G. Protection against experimental visceral leishmaniasis infection in dogs immunized with purified excreted secreted antigens of *Leishmania infantum* promastigotes. *Vaccine* 2005;**23**(22):2825–40.
195. Lerner EA, Ribeiro JM, Nelson RJ, Lerner MR. Isolation of maxadilan, a potent vasodilatory peptide from the salivary glands of the sand fly *Lutzomyia longipalpis*. *J Biol Chem* 1991; **266**(17):11234–6.
196. Liew FY, Millott S, Parkinson C, Palmer RM, Moncada S. Macrophage killing of *Leishmania* parasite in vivo is mediated by nitric oxide from L-arginine. *J Immunol* 1990;**144**(12): 4794–4797.
197. Liew FY, O'Donnell CA. Immunology of leishmaniasis. *Adv Parasitol* 1993;**32**:161–259.
198. Lipoldova M, Svobodova M, Havelkova H, et al. Mouse genetic model for clinical and immunological heterogeneity of leishmaniasis. *Immunogenetics* 2002;**54**(3):174–83.
199. Lipoldova M, Svobodova M, Krulova M, et al. Susceptibility to *Leishmania major* infection in mice: multiple loci and heterogeneity of immunopathological phenotypes. *Genes Immun* 2000;**1**(3):200–6.

200. Lira R, Mendez S, Carrera L, Jaffe C, Neva F, Sacks D. *Leishmania tropica*: the identification and purification of metacyclic promastigotes and use in establishing mouse and hamster models of cutaneous and visceral disease. *Exp Parasitol* 1998;**89**(3):331–42.
201. Lira R, Sundar S, Makharia A, et al. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. *J Infect Dis* 1999;**180**(2):564–7.
202. Locksley RM, Pingel S, Lacy D, Wakil AE, Bix M, Fowell DJ. Susceptibility to infectious diseases: *Leishmania* as a paradigm. *J Infect Dis* 1999;**179**(Suppl 2):S305–8.
203. Lopez M, Inga R, Cangalaya M, et al. Diagnosis of *Leishmania* using the polymerase chain reaction: a simplified procedure for field work. *Am J Trop Med Hyg* 1993;**49**(3):348–56.
204. Louzir H, Melby PC, Ben Salah A, et al. Immunologic determinants of disease evolution in localized cutaneous leishmaniasis due to *Leishmania major*. *J Infect Dis* 1998;**177**(6):1687–95.
205. Lucas CM, Franke ED, Cachay MI, et al. Geographic distribution and clinical description of leishmaniasis cases in Peru. *Am J Trop Med Hyg* 1998;**59**(2):312–7.
206. Mahoney AB, Sacks DL, Saraiva E, Modi G, Turco SJ. Intraspecies and stage-specific polymorphisms in lipophosphoglycan structure control *Leishmania donovani*-sand fly interactions. *Biochemistry* 1999;**38**(31):9813–23.
207. Marquet S, Abel L, Hillaire D, et al. Genetic localization of a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31-q33. *Nat Genet* 1996;**14**(2):181–4.
208. Marsh DG, Neely JD, Breazeale DR, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;**264**(5162): 1152–6.
209. Martin-Sanchez J, Pineda JA, Morillas-Marquez F, Garcia-Garcia JA, Acedo C, Macias J. Detection of *Leishmania infantum* kinetoplast DNA in peripheral blood from asymptomatic individuals at risk for parenterally transmitted infections: relationship between polymerase chain reaction results and other *Leishmania* infection markers. *Am J Trop Med Hyg* 2004;**70**(5): 545–8.
210. Martinez E, Mollinedo S, Torrez M, Munoz M, Banuls AL, Le Pont F. Co-infection by *Leishmania amazonensis* and *L. infantum*/*L. chagasi* in a case of diffuse cutaneous leishmaniasis in Bolivia. *Trans R Soc Trop Med Hyg* 2002;**96**(5):529–32.
211. Mary C, Auriault V, Faugere B, Dessein AJ. Control of *Leishmania infantum* infection is associated with CD8(+) and gamma interferon- and interleukin-5-producing CD4(+) antigen-specific T cells. *Infect Immun* 1999;**67**(11):5559–66.
212. Mauricio IL, Gaunt MW, Stothard JR, Miles MA. Genetic typing and phylogeny of the *Leishmania donovani* complex by restriction analysis of PCR amplified gp63 intergenic regions. *Parasitology* 2001;**122**(Pt 4):393–403.
213. Mauricio IL, Stothard JR, Miles MA. *Leishmania donovani* complex: genotyping with the ribosomal internal transcribed spacer and the mini-exon. *Parasitology* 2004;**128**(Pt 3): 263–7.
214. Maurya R, Singh R, Kumar B, Salotra P, Rai M, Sundar S. Evaluation of PCR in the diagnosis and assessment of cure in Indian kala-azar. *J Clin Microbiol* 2005;**43**(7):3038–41.
215. Mayr. *Animal Species and Evolution*. Belknap Press of Harvard University Press, Cambridge, 1963.
216. McConville MJ, Schnur LF, Jaffe C, Schneider P. Structure of *Leishmania* lipophosphoglycan: inter- and intra-specific polymorphism in Old World species. *Biochem J* 1995;**310**(Pt 3): 807–18.
217. McConville MJ, Turco SJ, Ferguson MA, Sacks DL. Developmental modification of lipophosphoglycan during the differentiation of *Leishmania major* promastigotes to an infectious stage. *EMBO J* 1992;**11**(10):3593–600.
218. McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; **371**(6497):508–10.
219. McGwire BS, O'Connell WA, Chang KP, Engman DM. Extracellular release of the glycosylphosphatidylinositol (GPI)-linked *Leishmania* surface metalloprotease, gp63, is independent of GPI phospholipolysis: implications for parasite virulence. *J Biol Chem* 2002;**277**(11):8802–9.
220. McMahon-Pratt D, Alexander J. Does the *Leishmania major* paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? *Immunol Rev* 2004;**201**:206–24.
221. McNeely TB, Turco SJ. Requirement of lipophosphoglycan for intracellular survival of *Leishmania donovani* within human monocytes. *J Immunol* 1990;**144**(7):2745–50.
222. Meddeb-Garnaoui A, Gritli S, Garbouj S, et al. Association analysis of HLA-class II and class III gene polymorphisms in the susceptibility to mediterranean visceral leishmaniasis. *Hum Immunol* 2001;**62**(5):509–17.
223. Melby PC, Andrade-Narvaez FJ, Darnell BJ, Valencia-Pacheco G, Tryon VV, Palomo-Cetina A. Increased expression of proinflammatory cytokines in chronic lesions of human cutaneous leishmaniasis. *Infect Immun* 1994;**62**(3):837–42.
224. Melby PC, Chandrasekar B, Zhao W, Coe JE. The hamster as a model of human visceral leishmaniasis: progressive disease and impaired generation of nitric oxide in the face of a prominent Th1-like cytokine response. *J Immunol* 2001; **166**(3): 1912–20.
225. Mendez S, Reckling SK, Piccirillo CA, Sacks D, Belkaid Y. Role for CD4(+) CD25(+) regulatory T cells in reactivation of persistent leishmaniasis and control of concomitant immunity. *J Exp Med* 2004;**200**(2):201–10.
226. Merlen T, Sereno D, Brajon N, Rostand F, Lemesre JL. *Leishmania* spp.: completely defined medium without serum and macromolecules (CDM/LP) for the continuous in vitro cultivation of infective promastigote forms. *Am J Trop Med Hyg* 1999;**60**(1):41–50.
227. Minodier P, Retornaz K, Horelt A, Garnier JM. Liposomal amphotericin B in the treatment of visceral leishmaniasis in immunocompetent patients. *Fundam Clin Pharmacol* 2003; **17**(2):183–8.
228. Miralles GD, Stoeckle MY, McDermott DF, Finkelman FD, Murray HW. Th1 and Th2 cell-associated cytokines in experimental visceral leishmaniasis. *Infect Immun* 1994;**62**(3): 1058–63.
229. Mishra M, Biswas UK, Jha DN, Khan AB. Amphotericin versus pentamidine in antimony-unresponsive kala-azar. *Lancet* 1992;**340**(8830):1256–7.

230. Mishra M, Singh MP, Choudhury D, Singh VP, Khan AB. Amphotericin B for second-line treatment of Indian kala-azar. *Lancet* 1991;**337**(8746):926.
231. Modabber F. Vaccines against leishmaniasis. *Ann Trop Med Parasitol* 1995;**89**(Suppl 1):83–8.
232. Mohamed HS, Ibrahim ME, Miller EN, et al. Genetic susceptibility to visceral leishmaniasis in The Sudan: linkage and association with IL4 and IFNGR1. *Genes Immun* 2003;**4**(5):351–5.
233. Mohamed HS, Ibrahim ME, Miller EN,et al. SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in The Sudan. *Eur J Hum Genet* 2004;**12**(1):66–74.
234. Molina R, Gradoni L, Alvar J. HIV and the transmission of *Leishmania*. *Ann Trop Med Parasitol* 2003;**97**(Suppl 1):29–45.
235. Morris RV, Shoemaker CB, David JR, Lanzaro GC, Titus RG. Sandfly maxadilan exacerbates infection with *Leishmania major* and vaccinating against it protects against *L. major* infection. *J Immunol* 2001;**167**(9):5226–30.
236. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;**136**(7):2348–57.
237. Mottram JC, Coombs GH, Alexander J. Cysteine peptidases as virulence factors of *Leishmania*. *Curr Opin Microbiol* 2004;**7**(4): 375–81.
238. Mottram JC, Souza AE, Hutchison JE, Carter R, Frame MJ, Coombs GH. Evidence from disruption of the *lmcpb* gene array of *Leishmania mexicana* that cysteine proteinases are virulence factors. *Proc Natl Acad Sci USA* 1996;**93**(12):6008–13.
239. Muller I, Kropf P, Louis JA, Milon G. Expansion of gamma interferon-producing CD8+ T cells following secondary infection of mice immune to *Leishmania major*. *Infect Immun* 1994;**62**(6):2575–81.
240. Mundodi V, Somanna A, Farrell PJ, Gedamu L. Genomic organization and functional expression of differentially regulated cysteine protease genes of *Leishmania donovani* complex. *Gene* 2002;**282**(1–2):257–65.
241. Murray HW, Nathan CF. Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral *Leishmania donovani*. *J Exp Med* 1999;**189**(4):741–6.
242. Navin TR, Arana FE, de Merida AM, Arana BA, Castillo AL, Silvers DN. Cutaneous leishmaniasis in Guatemala: comparison of diagnostic methods. *Am J Trop Med Hyg* 1990;**42**(1): 36–42.
243. Nieto CG, Garcia-Alonso M, Requena JM, et al. Analysis of the humoral immune response against total and recombinant antigens of *Leishmania infantum*: correlation with disease progression in canine experimental leishmaniasis. *Vet Immunol Immunopathol* 1999;**67**(2):117–30.
244. Noben-Trauth N, Kropf P, Muller I. Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. *Science* 1996;**271**(5251):987–90.
245. Oliveira-Neto MP, Schubach A, Mattos M, Goncalves-Costa SC, Pirmez C. A low-dose antimony treatment in 159 patients with American cutaneous leishmaniasis: extensive follow-up studies (up to 10 years). *Am J Trop Med Hyg* 1997;**57**(6):651–5.
246. Oliveira F, Kamhawi S, Seitz AE, et al. From transcriptome to immunome: identification of DTH inducing proteins from a *Phlebotomus ariasi* salivary gland cDNA library. *Vaccine* 2006;**24**:374–90.
247. Olivier M, Gregory DJ, Forget G. Subversion mechanisms by which *Leishmania* parasites can escape the host immune response: a signaling point of view. *Clin Microbiol Rev* 2005; **18**(2):293–305.
248. Osman OF, Oskam L, Zijlstra EE, el-Hassan AM, el-Naeim DA, Kager PA. Use of the polymerase chain reaction to assess the success of visceral leishmaniasis treatment. *Trans R Soc Trop Med Hyg* 1998;**92**(4):397–400.
249. Osman OF, Oskam L, Zijlstra EE, et al. Evaluation of PCR for diagnosis of visceral leishmaniasis. *J Clin Microbiol* 1997; **35**(10):2454–7.
250. Otranto D, Paradies P, Sasanelli M, et al. Recombinant K39 dipstick immunochromatographic test: a new tool for the serodiagnosis of canine leishmaniasis. *J Vet Diagn Invest* 2005; **17**(1):32–7.
251. Ouellette M, Drummelsmith J, Papadopoulou B. Leishmaniasis: drugs in the clinic, resistance and new developments. *Drug Resist Updat* 2004;**7**(4–5):257–66.
252. Ozgoztasi O, Baydar I. A randomized clinical trial of topical paromomycin versus oral ketoconazole for treating cutaneous leishmaniasis in Turkey. *Int J Dermatol* 1997;**36**(1): 61–3.
253. Palatnik de Sousa CB, Gomes EM, de Souza EP, dos Santos WR, de Macedo SR, de Medeiros LV, Luz K. The FML (Fucose Mannose Ligand) of *Leishmania donovani*. A new tool in diagnosis, prognosis, transfusional control and vaccination against human kala-azar. *Rev Soc Bras Med Trop* 1996; **29**(2): 153–63.
254. Panaro MA, Brandonisio O, Sisto M, et al. Nitric oxide production by *Leishmania*-infected macrophages and modulation by prostaglandin E2. *Clin Exp Med* 2001;**1**(3):137–43.
255. Papadopoulou C, Kostoula A, Dimitriou D, Panagiou A, Bobojianni C, Antoniades G. Human and canine leishmaniasis in asymptomatic and symptomatic population in Northwestern Greece. *J Infect* 2005;**50**(1):53–60.
256. Peacock CS, Collins A, Shaw MA, et al. Genetic epidemiology of visceral leishmaniasis in northeastern Brazil. *Genet Epidemiol* 2001;**20**(3):383–96.
257. Peacock CS, Sanjeevi CB, Shaw MA, et al. Genetic analysis of multicase families of visceral leishmaniasis in northeastern Brazil: no major role for class II or class III regions of HLA. *Genes Immun* 2002;**3**(6):350–8.
258. Petzl-Erler ML, Belich MP, Queiroz-Telles F. Association of mucosal leishmaniasis with HLA. *Hum Immunol* 1991; **32**(4):254–60.
259. Piedrafita D, Proudfoot L, Nikolaev AV, et al. Regulation of macrophage IL-12 synthesis by *Leishmania* phosphoglycans. *Eur J Immunol* 1999;**29**(1):235–44.
260. Pimenta PF, Modi GB, Pereira ST, Shahabuddin M, Sacks DL. A novel role for the peritrophic matrix in protecting *Leishmania* from the hydrolytic activities of the sand fly midgut. *Parasitology* 1997;**115**(Pt 4):359–69.
261. Pimenta PF, Saraiva EM, Rowton E, et al. Evidence that the vectorial competence of phlebotomine sand flies for different species of *Leishmania* is controlled by structural polymorphisms in the surface lipophosphoglycan. *Proc Natl Acad Sci USA* 1994;**91**(19):9155–6.

262. Pimenta PF, Turco SJ, McConville MJ, Lawyer PG, Perkins PV, Sacks DL. Stage-specific adhesion of *Leishmania* promastigotes to the sandfly midgut. *Science* 1992;**256**(5065):1812–5.
263. Pinelli E, Killick-Kendrick R, Wagenaar J, Bernadina W, del Real G, Ruitenberg J. Cellular and humoral immune responses in dogs experimentally and naturally infected with *Leishmania infantum*. *Infect Immun* 1994;**62**(1):229–35.
264. Pinelli E, van der Kaaij SY, Slappendel R, et al. Detection of canine cytokine gene expression by reverse transcription-polymerase chain reaction. *Vet Immunol Immunopathol* 1999; **69**(2–4):121–6.
265. Pizzuto M, Piazza M, Senese D, et al. Role of PCR in diagnosis and prognosis of visceral leishmaniasis in patients coinfected with human immunodeficiency virus type 1. *J Clin Microbiol* 2001;**39**(1):357–61.
266. Probst P, Stromberg E, Ghalib HW, et al. Identification and characterization of T cell-stimulating antigens from *Leishmania* by CD4 T cell expression cloning. *J Immunol* 2001;**166**(1): 498–505.
267. Quispe Tintaya KW, Laurent T, Decuypere S, et al. Fluorogenic assay for molecular typing of the *Leishmania donovani* complex: taxonomic and clinical apllications. *J Infect Dis* 2005; **192**(4):685–92.
268. Rabello A, Orsini M, Disch J. *Leishmania*/HIV co-infection in Brazil: an appraisal. *Ann Trop Med Parasitol* 2003;**97**(Suppl 1): 17–28.
269. Ramalho-Ortigao JM, Kamhawi S, Joshi MB, et al. Characterization of a blood activated chitinolytic system in the midgut of the sand fly vectors *L. longipalpis* and *P. papatasi*. *Insect Mol Biol* 2005;**14**(6):703–12.
270. Ramalho-Ortigao JM, Kamhawi S, Rowton ED, Ribeiro JM, Valenzuela JG. Cloning and characterization of trypsin- and chymotrypsin-like proteases from the midgut of the sand fly vector *Phlebotomus papatasi*. *Insect Biochem Mol Biol* 2003;**33**(2):163–71.
271. Ramalho-Ortigao JM, Temporal P, de Oliveira SM, et al. Characterization of constitutive and putative differentially expressed mRNAs by means of expressed sequence tags, differential display reverse transcriptase-PCR and randomly amplified polymorphic DNA-PCR from the sand fly vector *Lutzomyia longipalpis*. *Mem Inst Oswaldo Cruz* 2001;**96**(1): 105–11.
272. Ramalho-Ortigao JM, Traub-Cseko YM. Molecular characterization of Llchit1, a midgut chitinase cDNA from the leishmaniasis vector *Lutzomyia longipalpis*. *Insect Biochem Mol Biol* 2003;**33**(3):279–87.
273. Ramirez 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**(10):3768–73.
274. Reed SG, Scott P. T-cell and cytokine responses in leishmaniasis. *Curr Opin Immunol* 1993;**5**(4):524–31.
275. Reiner SL, Zheng S, Wang ZE, Stowring L, Locksley RM. *Leishmania* promastigotes evade interleukin 12 (IL-12) induction by macrophages and stimulate a broad range of cytokines from CD4+ T cells during initiation of infection. *J Exp Med* 1994;**179**(2):447–56.
276. Requena JM, Alonso C, Soto M. Evolutionarily conserved proteins as prominent immunogens during *Leishmania* infections. *Parasitol Today* 2000;**16**(6):246–50.
277. Ribeiro JM. Role of saliva in blood-feeding by arthropods. *Annu Rev Entomol* 1987;**32**:463–78.
278. Ribeiro JM. Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infect Agents Dis* 1995;**4**(3): 143–52.
279. Riera C, Fisa R, Lopez P, et al. Evaluation of a latex agglutination test (KAtex) for detection of *Leishmania* antigen in urine of patients with HIV-*Leishmania* coinfection: value in diagnosis and post-treatment follow-up. *Eur J Clin Microbiol Infect Dis* 2004;**23**(12):899–904.
280. Riera C, Fisa R, Udina M, Gallego M, Portus M. Detection of *Leishmania infantum* cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. *Trans R Soc Trop Med Hyg* 2004;**98**(2):102–10.
281. Rihet P, Traore Y, Abel L, Aucan C, Traore-Leroux T, Fumoux F. Malaria in humans: *Plasmodium falciparum* blood infection levels are linked to chromosome 5q31-q33. *Am J Hum Genet* 1998;**63**(2):498–505.
282. Rioux JA, Lanotte G, Maazoun R, Pasteur N. Electrophoresis of enzymes of the genus *Leishmania* Ross 1903. *Parassitologia* 1985;**27**(1–2):141–56.
283. Rioux JA, Lanotte G, Serres E, Pratlong F, Bastien P, Perieres J. Taxonomy of *Leishmania*. Use of isoenzymes. Suggestions for a new classification. *Ann Parasitol Hum Comp* 1990;**65**(3): 111–25.
284. Roberts LJ, Baldwin TM, Curtis JM, Handman E, Foote SJ. Resistance to *Leishmania major* is linked to the H2 region on chromosome 17 and to chromosome 9. *J Exp Med* 1997; **185**(9):1705–10.
285. Roberts LJ, Baldwin TM, Speed TP, Handman E, Foote SJ. Chromosomes X, 9, and the H2 locus interact epistatically to control *Leishmania major* infection. *Eur J Immunol* 1999;**29**(9): 3047–50.
286. Roberts M. Alexander J, Blackwell JM. Influence of Lsh, H-2, and an H-11-linked gene on visceralization and metastasis associated with *Leishmania mexicana* infection in mice. *Infect Immun* 1989;**57**(3):875–81.
287. Roberts M, Alexander J, Blackwell JM. Genetic analysis of *Leishmania mexicana* infection in mice: single gene (*Scl-2*) controlled predisposition to cutaneous lesion development. *J Immunogenet* 1990;**17**(1–2):89–100.
288. Roberts M, Mock BA, Blackwell JM. Mapping of genes controlling *Leishmania major* infection in CXS recombinant inbred mice. *Eur J Immunogenet* 1993;**20**(5):349–62.
289. Roberts SC, Swihart KG, Agey MW, Ramamoorthy R, Wilson ME, Donelson JE. Sequence diversity and organization of the *msp* gene family encoding gp63 of *Leishmania chagasi*. *Mol Biochem Parasitol* 1993;**62**(2):157–71.
290. Rodrigues V, Jr, Santana da Silva J, Campos-Neto A. Transforming growth factor beta and immunosuppression in experimental visceral leishmaniasis. *Infect Immun* 1998;**66**(3):1233–6.
291. Rodriguez LV, Dedet JP, Paredes V, Mendoza C, Cardenas F. A randomized trial of amphotericin B alone or in combination with itraconazole in the treatment of mucocutaneous leishmaniasis. *Mem Inst Oswaldo Cruz* 1995;**90**(4): 525–8.
292. Rodriguez NE, Chang HK, Wilson ME. Novel program of macrophage gene expression induced by phagocytosis of *Leishmania chagasi*. *Infect Immun* 2004;**72**(4):2111–22.

293. Rogers ME, Chance ML, Bates PA. The role of promastigote secretory gel in the origin and transmission of the infective stage of *Leishmania mexicana* by the sandfly *Lutzomyia longipalpis*. *Parasitology* 2002;**124**(Pt 5):495–507.
294. Rogers ME, Ilg T, Nikolaev AV, Ferguson MA, Bates PA. Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. *Nature* 2004;**430**(6998):463–7.
295. Romero GA, Guerra MV, Paes MG, Macedo VO. Comparison of cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis* and *L. (V.) guyanensis* in Brazil: therapeutic response to meglumine antimoniate. *Am J Trop Med Hyg* 2001;**65**(5):456–65.
296. Rose K, Curtis J, Baldwin T, et al. Cutaneous leishmaniasis in red kangaroos: isolation and characterisation of the causative organisms. *Int J Parasitol* 2004;**34**(6):655–64.
297. Rosenthal PJ. Proteases of protozoan parasites. *Adv Parasitol* 1999;**43**:105–59.
298. Russell R, Iribar MP, Lambson B, et al. Intra and inter-specific microsatellite variation in the *Leishmania* subgenus *Viannia*. *Mol Biochem Parasitol* 1999;**103**(1):71–7.
299. Sacks D, Kamhawi S. Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. *Annu Rev Microbiol* 2001;**55**:453–83.
300. Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat Rev Immunol* 2002;**2**(11):845–58.
301. Sacks DL, Brodin TN, Turco SJ. Developmental modification of the lipophosphoglycan from *Leishmania major* promastigotes during metacyclogenesis. *Mol Biochem Parasitol* 1990;**42**(2):225–33.
302. Sacks DL, Modi G, Rowton E, et al. The role of phosphoglycans in *Leishmania*-sand fly interactions. *Proc Natl Acad Sci USA* 2000;**97**(1):406–11.
303. Sacks DL, Pimenta PF, McConville MJ, Schneider P, Turco SJ. Stage-specific binding of *Leishmania donovani* to the sand fly vector midgut is regulated by conformational changes in the abundant surface lipophosphoglycan. *J Exp Med* 1995;**181**(2):685–97.
304. Sadlova J, Hajmova M, Volf P. *Phlebotomus (Adlerius) halepensis* vector competence for *Leishmania major* and *L. tropica*. *Med Vet Entomol* 2003;**17**(3):244–50.
305. Sahibzada NJ, Tareen AK, Khursheed T, Darr N. Evaluation of paromomycin sulphate topical ointment as effective therapeutic agent in cutaneous leishmaniasis *J Pak Med Assoc* 1996;**46**(3):53–5.
306. Sakaguchi S. Regulatory T cells: mediating compromises between host and parasite. *Nat Immunol* 2003;**4**(1):10–1.
307. Saliba EK, Oumeish OY. Reservoir hosts of cutaneous leishmaniasis. *Clin Dermatol* 1999;**17**(3):275–7.
308. Salotra P, Sreenivas G, Beena KR, Mukherjee A, Ramesh V. Parasite detection in patients with post kala-azar dermal leishmaniasis in India: a comparison between molecular and immunological methods. *J Clin Pathol* 2003;**56**(11):840–3.
309. Salotra P, Sreenivas G, Pogue GP, et al. Development of a species-specific PCR assay for detection of *Leishmania donovani* in clinical samples from patients with kala-azar and post-kala-azar dermal leishmaniasis. *J Clin Microbiol* 2001;**39**(3):849–54.
310. Santos-Gomes GM, Rosa R, Leandro C, Cortes S, Romao P, Silveira H. Cytokine expression during the outcome of canine experimental infection by *Leishmania infantum*. *Vet Immunol Immunopathol* 2002;**88**(1–2):21–30.
311. Saraiva EM, Pimenta PF, Brodin TN, Rowton E, Modi GB, Sacks DL. Changes in lipophosphoglycan and gene expression associated with the development of *Leishmania major* in *Phlebotomus papatasi*. *Parasitology* 1995;**111**(Pt 3):275–87.
312. Sarkari B, Chance M, Hommel M. Antigenuria in visceral leishmaniasis: detection and partial characterisation of a carbohydrate antigen. *Acta Trop* 2002;**82**(3):339–48.
313. Scharton-Kersten T, Afonso LC, Wysocka M, Trinchieri G, Scott P. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. *J Immunol* 1995;**154**(10):5320–30.
314. Schlein Y, Jacobson RL, Messer G. *Leishmania* infections damage the feeding mechanism of the sandfly vector and implement parasite transmission by bite. *Proc Natl Acad Sci USA* 1992;**89**(20):9944–8.
315. Schlein Y, Jacobson RL, Shlomai J. Chitinase secreted by *Leishmania* functions in the sandfly vector. *Proc R Soc Lond Ser B Biol Sci* 1991;**245**(1313):121–6.
316. Schlein Y, Romano H. *Leishmania major* and *L. donovani*: effects on proteolytic enzymes of *Phlebotomus papatasi* (Diptera, Psychodidae). *Exp Parasitol* 1986;**62**(3):376–80.
317. Schlein Y, Schnur LF, Jacobson RL. Released glycoconjugate of indigenous *Leishmania major* enhances survival of a foreign *L. major* in *Phlebotomus papatasi*. *Trans R Soc Trop Med Hyg* 1990; **84**(3):353–5.
318. Schneider P, Rosat JP, Bouvier J, Louis J, Bordier C. *Leishmania major*: differential regulation of the surface metalloprotease in amastigote and promastigote stages. *Exp Parasitol* 1992;**75**(2):196–206.
319. Schriefer A, Schriefer AL, Goes-Neto A, et al. Multiclonal *Leishmania braziliensis* population structure and its clinical implication in a region of endemicity for American tegumentary leishmaniasis. *Infect Immun* 2004;**72**(1):508–14.
320. Schulz A, Mellenthin K, Schonian G, Fleischer B, Drosten C. Detection, differentiation, and quantitation of pathogenic *Leishmania* organisms by a fluorescence resonance energy transfer-based real-time PCR assay. *J Clin Microbiol* 2003;**41**(4):1529–35.
321. Scott P, Natovitz P, Coffman RL, Pearce E, Sher A. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. *J Exp Med* 1988;**168**(5):1675–84.
322. Searle S, Bright NA, Roach TI, et al. Localisation of *Nramp1* in macrophages: modulation with activation and infection. *J Cell Sci* 1998;**111**(Pt 19):2855–66.
323. Sharma MC, Gupta AK, Das VN, et al. *Leishmania donovani* in blood smears of asymptomatic persons. *Acta Trop* 2000;**76**(2):195–6.
324. Shaw MA, Davies CR, Llanos-Cuentas EA, Collins A. Human genetic susceptibility and infection with *Leishmania peruviana*. *Am J Hum Genet* 1995;**57**(5):1159–68.
325. Sherwood JA, Gachihi GS, Muigai RK, et al. Phase 2 efficacy trial of an oral 8-aminoquinoline (WR6026) for treatment of visceral leishmaniasis. *Clin Infect Dis* 1994;**19**(6):1034–9.
326. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;**2**(6):389–400.
327. Singh N, Curran MD, Rastogil AK, Middleton D, Sundar S. Diagnostic PCR with *Leishmania donovani* specificity using

sequences from the variable region of kinetoplast minicircle DNA. *Trop Med Int Health* 1999;**4**(6):448–53.
328. Singh N, Singh RT, Sundar S. Novel mechanism of drug resistance in kala azar field isolates. *J Infect Dis* 2003;**188**(4):600–7.
329. Singh N, Sundar S, Williams F, et al. Molecular typing of HLA class I and class II antigens in Indian kala-azar patients. *Trop Med Int Health* 1997;**2**(5):468–71.
330. Singh S, Dey A, Sivakumar R. Applications of molecular methods for *Leishmania* control. *Expert Rev Mol Diagn* 2005; **5**(2):251–65.
331. Singh S, Gilman-Sachs A, Chang KP, Reed SG. Diagnostic and prognostic value of K39 recombinant antigen in Indian leishmaniasis. *J Parasitol* 1995;**81**(6):1000–3.
332. Soares RP, Barron T, McCoy-Simandle K, Svobodova M, Warburg A, Turco SJ. *Leishmania tropica*: intraspecific polymorphisms in lipophosphoglycan correlate with transmission by different *Phlebotomus* species. *Exp Parasitol* 2004;**107**(1–2):105–14.
333. Soares RP, Macedo ME, Ropert C, et al. *Leishmania chagasi*: lipophosphoglycan characterization and binding to the midgut of the sand fly vector *Lutzomyia longipalpis*. *Mol Biochem Parasitol* 2002;**121**(2):213–24.
334. Solbach W, Laskay T. The host response to *Leishmania* infection. *Adv Immunol* 2000;**74**:275–317.
335. Soto J, Buffet P, Grogl M, Berman J. Successful treatment of Colombian cutaneous leishmaniasis with four injections of pentamidine. *Am J Trop Med Hyg* 1994;**50**(1):107–11.
336. Southgate BA, Manson-Bahr PE. Studies in the epidemiology of East African leishmaniasis. 4. The significance of the positive leishmanin test. *J Trop Med Hyg* 1967;**70**(2):29–33.
337. Spath GF, Epstein L, Leader B, et al. Lipophosphoglycan is a virulence factor distinct from related glycoconjugates in the protozoan parasite *Leishmania major*. *Proc Natl Acad Sci USA* 2000;**97**(16):9258–63.
338. Spath GF, Garraway LA, Turco SJ, Beverley SM. The role(s) of lipophosphoglycan (LPG) in the establishment of *Leishmania major* infections in mammalian hosts. *Proc Natl Acad Sci USA* 2003;**100**(16):9536–41.
339. Steinkraus HB, Greer JM, Stephenson DC, Langer PJ. Sequence heterogeneity and polymorphic gene arrangements of the *Leishmania guyanensis gp63* genes. *Mol Biochem Parasitol* 1993;**62**(2):173–85.
340. Stierhof YD, Bates PA, Jacobson RL, et al. Filamentous proteophosphoglycan secreted by *Leishmania* promastigotes forms gel-like three-dimensional networks that obstruct the digestive tract of infected sandfly vectors. *Eur J Cell Biol* 1999; **78**(10):675–89.
341. Strelkova MV, Eliseev LN, Ponirovsky EN, et al. Mixed leishmanial infections in Rhombomys opimus: a key to the persistence of *Leishmania major* from one transmission season to the next. *Ann Trop Med Parasitol* 2001;**95**(8):811–9.
342. Sundar S. Drug resistance in Indian visceral leishmaniasis. *Trop Med Int Health* 2001;**6**(11):849–54.
343. Sundar S, Agrawal G, Rai M, Makharia MK, Murray HW. Treatment of Indian visceral leishmaniasis with single or daily infusions of low dose liposomal amphotericin B: randomised trial. *Br Med J* 2001;**323**(7310):419–22.
344. Sundar S, Agrawal NK, Sinha PR, Horwith GS, Murray HW. Short-course, low-dose amphotericin B lipid complex therapy for visceral leishmaniasis unresponsive to antimony. *Ann Intern Med* 1997;**127**(2):133–7.
345. Sundar S, Agrawal S, Pai K, Chance M, Hommel M. Detection of leishmanial antigen in the urine of patients with visceral leishmaniasis by latex agglutination test (KAtex). *Am J Trop Med Hyg* 2006;**73**(2):269–71.
346. Sundar S, Goyal AK, Mandal AK, et al. Amphotericin B lipid complex in the management of antimony unresponsive Indian visceral leishmaniasis. *J Assoc Phys India* 1999;**47**(2):186–8.
347. Sundar S, Jha TK, Thakur CP, et al. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med* 2002;**347**(22): 1739–46.
348. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin Infect Dis* 2003;**37**(6):800–4.
349. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VR, Buffels R. Low-dose liposomal amphotericin B in refractory Indian visceral leishmaniasis: a multicenter study. *Am J Trop Med Hyg* 2002;**66**(2):143–6.
350. Sundar S, Makharia A, More DK, et al. Short-course of oral miltefosine for treatment of visceral leishmaniasis. *Clin Infect Dis* 2000;**31**(4):1110–3.
351. Sundar S, Mehta H, Suresh AV, Singh SP, Rai M, Murray HW. Amphotericin B treatment for Indian visceral leishmaniasis: conventional versus lipid formulations. *Clin Infect Dis* 2004; **38**(3):377–83.
352. Sundar S, More DK, Singh MK, et al. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis* 2000;**31**(4): 1104–7.
353. Sundar S, Pai K, Sahu M, Kumar V, Murray HW. Immunochromatographic strip-test detection of anti-K39 antibody in Indian visceral leishmaniasis. *Ann Trop Med Parasitol* 2002;**96**(1):19–23.
354. Sundar S, Reed SG, Singh VP, Kumar PC, Murray HW. Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet* 1998;**351**(9102):563–5.
355. Sundar S, Rosenkaimer F, Makharia MK, et al. Trial of oral miltefosine for visceral leishmaniasis. *Lancet* 1998;**32**(9143):1821–3.
356. Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. *J Infect Dis* 1997;**176**(4):1117–9.
357. Symons FM, Murray PJ, Ji H, et al. Characterization of a polymorphic family of integral membrane proteins in promastigotes of different *Leishmania* species. *Mol Biochem Parasitol* 1994;**67**(1):103–13.
358. Sypek JP, Chung CL, Mayor SE, et al. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J Exp Med* 1993;**177**(6):1797–802.
359. Thakur CP, Narayan SA comparative evaluation of amphotericin B and sodium antimony gluconate, as first-line drugs in the treatment of Indian visceral leishmaniasis. *Ann Trop Med Parasitol* 2004;**98**(2):129–38.
360. Thakur CP, Sinha GP, Pandey AK, et al. Do the diminishing efficacy and increasing toxicity of sodium stibogluconate in the treatment of visceral leishmaniasis in Bihar, India, justify its continued use as a first-line drug? An observational study of 80 cases. *Ann Trop Med Parasitol* 1998;**92**(5):561–9.

361. Thakur CP, Sinha GP, Sharma V, Pandey AK, Kumar M, Verma BB. Evaluation of amphotericin B as a first line drug in comparison to sodium stibogluconate in the treatment of fresh cases of kala-azar. *Indian J Med Res* 1993;**97**:170–5.
362. Tibayrenc M. Clonality in *Leishmania*. *Parasitol Today* 1993; **9**(2):58.
363. Tibayrenc M. Towards a unified evolutionary genetics of microorganisms. *Annu Rev Microbiol* 1996;**50**:401–29.
364. Tibayrenc M, Ayala FJ. Evolutionary genetics of *Trypanosoma* and *Leishmania*. *Microbes Infect* 1999;**1**(6):465–72.
365. Tibayrenc M, Kjellberg F, Ayala FJ. A clonal theory of parasitic protozoa: the population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas*, and *Trypanosoma* and their medical and taxonomical consequences. *Proc Natl Acad Sci USA* 1990;**87**(7):2414–8.
366. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 1995;**13**:251–76.
367. Trinchieri G, Kubin M, Bellone G, Cassatella MA. Cytokine cross-talk between phagocytic cells and lymphocytes: relevance for differentiation/activation of phagocytic cells and regulation of adaptive immunity. *J Cell Biochem* 1993;**53**(4):301–8.
368. Turco SJ, Hull SR, Orlandi PA, Jr, et al. Structure of the major carbohydrate fragment of the *Leishmania donovani* lipophosphoglycan. *Biochemistry* 1987;**26**(19):6233–8.
369. Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 2002;**20**:825–52.
370. Uzcategui NL, Figarella K, Camacho N, Ponte-Sucre A. Substrate preferences and glucose uptake in glibenclamide-resistant *Leishmania* parasites. *Comp Biochem Physiol C Toxicol Pharmacol* 2005;**140**(3–4):395–402.
371. Uzun S, Durdu M, Culha G, Allahverdiyev AM, Memisoglu HR. Clinical features, epidemiology, and efficacy and safety of intralesional antimony treatment of cutaneous leishmaniasis: recent experience in Turkey. *J Parasitol* 2004;**90**(4):853–9.
372. Valenzuela JG. High-throughput approaches to study salivary proteins and genes from vectors of disease. *Insect Biochem Mol Biol* 2002;**32**(10):1199–209.
373. Valenzuela JG, Belkaid Y, Garfield MK, et al. Toward a defined anti-*Leishmania* vaccine targeting vector antigens. Characterization of a protective salivary protein. *J Exp Med* 2001;**194**(3):331–42.
374. Vasconcellos RC, Urago KP, Bunn-Moreno MM, Madeira ED. Suppressor activity in *Leishmania donovani*-infected hamster serum: reversion by delipidated bovine serum albumin and role in cell cycle events. *Braz J Med Biol Res* 1996;**29**(5): 615–22.
375. Veeken H, Ritmeijer K, Seaman J, Davidson R. Comparison of an rK39 dipstick rapid test with direct agglutination test and splenic aspiration for the diagnosis of kala-azar in Sudan. *Trop Med Int Health* 2003;**8**(2):164–7.
376. Victoir K, Banuls AL, Arevalo J, et al. The gp63 gene locus, a target for genetic characterization of *Leishmania* belonging to subgenus *Viannia*. *Parasitology* 1998;**117**(Pt 1):1–13.
377. Victoir K, Dujardin JC, de Doncker S, et al. Plasticity of gp63 gene organization in *Leishmania (Viannia) braziliensis* and *Leishmania (Viannia) peruviana*. *Parasitology* 1995;**111**(Pt 3): 265–73.
378. Vidal S, Tremblay ML, Govoni G, et al. The *Ity/Lsh/Bcg* locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the *Nramp1* gene. *J Exp Med* 1995; **182**(3):655–66.
379. Vladimirov V, Badalova J, Svobodova M, et al. Different genetic control of cutaneous and visceral disease after *Leishmania major* infection in mice. *Infect Immun* 2003;**71**(4):2041–6.
380. Volf P, Hajmova M, Sadlova J, Votypka J. Blocked stomodeal valve of the insect vector: similar mechanism of transmission in two trypanosomatid models. *Int J Parasitol* 2004; **34**(11): 1221–7.
381. Volf P, Killick-Kendrick R, Bates PA, Molyneux DH. Comparison of the haemagglutination activities in gut and head extracts of various species and geographical populations of phlebotomine sandflies. *Ann Trop Med Parasitol* 1994;**88**(3): 337–40.
382. Volf P, Skarupova S, Man P. Characterization of the lectin from females of *Phlebotomus duboscqi* sand flies. *Eur J Biochem* 2002; **269**(24):6294–301.
383. Vouldoukis I, Becherel PA, Riveros-Moreno V, et al. Interleukin-10 and interleukin-4 inhibit intracellular killing of *Leishmania infantum* and *Leishmania major* by human macrophages by decreasing nitric oxide generation. *Eur J Immunol* 1997;**27**(4):860–5.
384. Wallbanks KR, Ingram GA, Molyneux DH. The agglutination of erythrocytes and *Leishmania* parasites by sandfly gut extracts: evidence for lectin activity. *Trop Med Parasitol* 1986;**37**(4): 409–13.
385. Walton BC, Brooks WH, Arjona I. Serodiagnosis of American leishmaniasis by indirect fluorescent antibody test. *Am J Trop Med Hyg* 1972;**21**(3):296–9.
386. Wang J, Vanley C, Miyamoto E, Turner JA, Peng SK. Coinfection of visceral leishmaniasis and Mycobacterium in a patient with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 1999;**123**(9):835–7.
387. Wassuna M, Felton J, Sabin A, Horton J. A phase II dose-rising study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. *J Trop Med Hyg* 2005;**73**:871–6.
388. Wei XQ, Charles IG, Smith A, et al. Altered immune responses in mice lacking inducible nitric oxide synthase. *Nature* 1995;**375**(6530):408–411.
389. Weigle KA, de Davalos M, Heredia P, Molineros R, Saravia NG, D'Alessandro A. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. *Am J Trop Med Hyg* 1987;**36**(3):489–96.
390. White JK, Mastroeni P, Popoff JF, Evans CA, Blackwell JM. Slc11a1-mediated resistance to *Salmonella enterica serovar Typhimurium* and *Leishmania donovani* infections does not require functional inducible nitric oxide synthase or phagocyte oxidase activity. *J Leukoc Biol* 2005;**77**(3):311–20.
391. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997;**94**(7):3195–9.
392. Wilson ME, Jeronimo SM, Pearson RD. Immunopathogenesis of infection with the visceralizing *Leishmania* species. *Microb Pathog* 2005;**38**(4):147–60.

393. Wolday D, Akuffo H, Fessahaye G, Valantine A, Britton S. Live and killed human immunodeficiency virus type-1 increases the intracellular growth of *Leishmania donovani* in monocyte-derived cells. *Scand J Infect Dis* 1998;**30**(1):29–34.
394. Wu Z, Bao Y, Ding Y, Yu M, Lu L, Zhang Y. An experimental study on application of PCR in detection of kala-azar. *Southeast Asian J Trop Med Public Health* 1997;**28**(1):169–72.
395. Yao C, Donelson JE, Wilson ME. The major surface protease (MSP or GP63) of *Leishmania* sp. Biosynthesis, regulation of expression, and function. *Mol Biochem Parasitol* 2003;**132**(1): 1–16.
396. Zemanova E, Jirku M, Mauricio IL, Miles MA, Lukes J. Genetic polymorphism within the *Leishmania donovani* complex: correlation with geographic origin. *Am J Trop Med Hyg* 2004;**70**(6):613–7.
397. Zhang WW, Matlashewski G. Characterization of the A2-A2rel gene cluster in *Leishmania donovani*: involvement of A2 in visceralization during infection. *Mol Microbiol* 2001;**39**(4): 935–48.
398. Zhang WW, Mendez S, Ghosh A, et al. Comparison of the A2 gene locus in *Leishmania donovani* and *Leishmania major* and its control over cutaneous infection. *J Biol Chem* 2003;**278**(37): 35508–15.
399. Zijlstra EE, el-Hassan AM, Ismael A, Ghalib HW. Endemic kala-azar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical infection and post-kala-azar dermal leishmaniasis. *Am J Trop Med Hyg* 1994;**51**(6):826–36.
400. Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM. Post-kala-azar dermal leishmaniasis. *Lancet Infect Dis* 2003;**3**(2):87–98.
401. Zijlstra EE, Nur Y, Desjeux P, Khalil EA, El-Hassan AM, Groen J. Diagnosing visceral leishmaniasis with the recombinant K39 strip test: experience from the Sudan. *Trop Med Int Health* 2001;**6**(2):108–13.
402. Zinkernagel RM, Hengartner H. Regulation of the immune response by antigen. *Science* 2001;**293**(5528):251–3.

Hide Mallorie, Bucheton B., Kamhawi S., Bras Gonçalves Rachel, Sundar S., Lemesre Jean-Loup, Banuls Anne-Laure. (2007)

Understanding human leishmaniasis : the need for an integrated approach

In : Tibayrenc Michel (ed.). Encyclopedia of infectious diseases : modern methodologies

Hoboken : J. Wiley, 87-123

ISBN 978-0-471-65732-3