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Proteins and Proteomics of *Leishmania* and *Trypanosoma*

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Editorial

The so-called neglected tropical diseases comprise a group of infections that is especially endemic in low-income population in developing countries of Africa, Asia and the Americas. In this group, the American (*Trypanosoma cruzi*) and African (*Trypanosoma brucei* complex) trypanosome species and microorganisms belonging to the *Leishmania* genus are the etiologic agents of major human and animal parasitic diseases worldwide, being responsible for large socio-economic losses, especially in developing countries. The effective treatment of diseases caused by trypanosomatids is still an open issue, nevertheless of paramount importance. Chemotherapy still relies on drugs developed decades ago, showing limited efficacy and possibility of toxic side effects. Nowadays, new approaches have been employed to improve treatment, and researches conducted to discover strategies that are safer, more efficacious and accessible. These strategies include the use of lipid formulations such as amphotericin B or miltefosine to treat the leishmaniasis, and chemotherapeutic combinations for other parasitic diseases. However, this still remains expensive considering the target population. Moreover, the emergence of resistance has been reported. Efforts to tackle these diseases require research on the molecular components that regulate the infection initiation, which is critical for a better understanding of the diseases' pathogeneses. Considering that these infections represent a major health concern worldwide, the development of a new generation of chemotherapeutic agents is of extreme importance. For that, research on the relevant aspects of targeted drug development is a critical priority. Several proteins/glycoproteins are being explored as targets for chemotherapy development, since they play key roles in different phases of the life cycle of trypanosomatids, including: hydrolytic enzymes, surface proteins with adhesive properties and extracellular components capable in helping the parasites to evade the host immune responses. Better biochemical and/or molecular characterisation of these (glyco)proteins can help to decipher their real relevance in the life cycle of human pathogenic trypanosomatids. In this context, without doubt, proteomic techniques are an interesting approach to discover novel potential parasite molecules to be used as target to generate novel anti-trypanosomatid compounds. With regarding to all these issues,

the present book focuses on the description of relevant proteins that participate in key steps of biological events and virulence of parasites belonging to the *Leishmania* and *Trypanosoma* genera.

The authors believe that this book will serve as a basis for consultations by specialists in the field of microbiology, in particular parasitologists, because it contemplates matters of the utmost importance in the proposed area. The chapters are written by experts who actively contribute to international scientific literature, generating knowledge about protein molecules synthesised by pathogens belonging to the genera *Leishmania* and *Trypanosoma*. We also believe that the theme "Proteins and proteomics of *Leishmania* and *Trypanosoma*" is a very attractive proposal and the compilation of interesting data will benefit authors around the world. This fact can be corroborated by a simple inspection in scientific papers' databases, where a large number of papers have been published over the last years on this subject in order to unravel the mechanisms of pathogenicity of human pathogenic trypanosomatids, which still constitute a major public health problem worldwide.

The book *Proteins and proteomics of* Leishmania *and* Trypanosoma contains (1) an integrated view about the biochemistry of parasites belonging to the *Leishmania* and *Trypanosoma* genera; (2) an updated review on the expression of biologically relevant proteins by human pathogenic trypanosomatids and their possible role in the interaction with host cells and (3) several pictures, diagrams and tables that can be used in both undergraduate and postgraduate teaching as well as scientific lectures. Briefly, Chap. 1 by *Juliany Rodrigues* and co-workers opens the book providing an update on the biology of *Leishmania* and *Trypanosoma*, focusing in epidemiology, life cycle and ultrastructural aspects of special structures and organelles found exclusively in trypanosomatid cells that contain unique proteins responsible for crucial metabolic pathways. Chapter 2 presented by *Despina Smirlis* and *Milena Soares* critically discusses the tools for exploiting, predicting and selecting novel and potential protein targets for rational drug design. In Chap. 3, *Ana Paula Fernandes* and co-workers discuss about visceralising proteins from *Leishmania*, with emphasis on the amastigote-specific antigen A2, which participates in pathogenesis and is a promising target for the development of vaccine against visceral leishmaniasis. Chapter 4 by *Maria Fernanda Silva* and *Lucile Maria Floeter-Winter* describes the relevance of arginine uptake and arginase activity in the establishment and maintenance of *Leishmania* infection. In Chap. 5, *Turán Ürményi* and co-workers review the relevance of heat shock proteins in *Trypanosoma cruzi*, since this parasite experiences several kinds of stress during its complex life cycle. *Cristian Cortez* and co-workers (Chap. 6), *Eliciane Mattos* and co-workers (Chap. 7), *Isadora Oliveira* and co-workers (Chap. 8) and *Sergio Rubin* (Chap. 9) elucidate biochemical, molecular, structural and topological aspects of the well-known gp85/sialidase superfamily that are expressed at the surface of clinically relevant trypomastigote forms of *T. cruzi,* such as gp82 (Chap. 6), gp85 (Chap. 7) and *trans-sialidase* (Chaps. 8 and 9), which participate in the attachment and invasion of both extracellular matrix components and host cells. In Chap. 10, *Anita Freitas-Mesquita* and *José Roberto Meyer-Fernandes* provided details about the occurrence and physiological roles of two ecto-enzymes, ecto-nucleotidases and

ecto-phosphatases, in parasites belonging to *Leishmania* and *Trypanosoma* genera. Chapter 11 presented by *Claudia d'Avila-Levy* and co-workers critically analyses the function of gp63 (leishmanolysin) from the perspective of the interaction of trypanosomatids with the invertebrate host. *Carla Polycarpo* (Chap. 12) provides some important clarifications about the aminoacyl-tRNA synthetases from trypanosomatids, exploring their structural diversity as a rational target for the design of novel drugs. The last three chapters discuss trypanosomatids from an entirely proteomic point of view of trypanosomatids. *Rubem Menna-Barreto* and *Jonas Perales* (Chap. 13), *José Batista de Jesus* and co-workers (Chap. 14) and *Fabricio Marchini* and co-workers (Chap. 15) summarise proteomic and phosphoproteomic maps of different morphotypes of both *T. cruzi* and *Leishmania* as well as describe unique metabolic pathways and parasite-specific molecules with potential participation in essential physiological/pathological events from *T. cruzi* and *Leishmania*, identifying alternative candidates for drug interventions.

The editors really hope that the reading of each chapter and, of course, the book as a whole, will arouse enthusiasm and scientific curiosity in young students and researchers around the world to learn more about these intriguing microorganisms, which continue to challenge us and excite our curiosity. With this proposal in mind, new perspectives need to be envisioned and employed. In this context, we need to better understand the physiology of these microorganisms in order to find new cellular targets and new drugs for alleviating the discomfort of millions of individuals who, unfortunately, live with the suffering inflicted by infections caused by *Leishmania* and *Trypanosoma*.

Finally, the editors are extremely grateful to all the contributing authors for their enthusiasm and valuable cooperation and to the consulting editors for their expert and exhaustive scientific review.

Contents

Chapter 1
Biology of Human Pathogenic Trypanosomatids: Epidemiology, Lifecycle and Ultrastructure

Juliany Cola Fernandes Rodrigues, Joseane Lima Prado Godinho, and Wanderley de Souza

Abstract *Leishmania* and *Trypanosoma* belong to the Trypanosomatidae family and cause important human infections such as leishmaniasis, Chagas disease, and sleeping sickness. Leishmaniasis, caused by protozoa belonging to *Leishmania*, affects about 12 million people worldwide and can present different clinical manifestations, i.e., visceral leishmaniasis (VL), cutaneous leishmaniasis (CL),

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mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL), and post-kala-azar dermal leishmaniasis (PKDL). Chagas disease, also known as American trypanosomiasis, is caused by *Trypanosoma cruzi* and is mainly prevalent in Latin America but is increasingly occurring in the United States, Canada, and Europe. Sleeping sickness or human African trypanosomiasis (HAT), caused by two sub-species of *Trypanosoma brucei* (i.e., *T. b. rhodesiense* and *T. b. gambiense*), occurs only in sub-Saharan Africa countries. These pathogenic trypanosomatids alternate between invertebrate and vertebrate hosts throughout their lifecycles, and different developmental stages can live inside the host cells and circulate in the bloodstream or in the insect gut. Trypanosomatids have a classical eukaryotic ultrastructural organization with some of the same main organelles found in mammalian host cells, while also containing special structures and organelles that are absent in other eukaryotic organisms. For example, the mitochondrion is ramified and contains a region known as the kinetoplast, which houses the mitochondrial DNA. Also, the glycosomes are specialized peroxisomes containing glycolytic pathway enzymes. Moreover, a layer of subpellicular microtubules confers mechanic rigidity to the cell. Some of these structures have been investigated to determine their function and identify potential enzymes and metabolic pathways that may constitute targets for new chemotherapeutic drugs.

1 Introduction

Leishmania and *Trypanosoma* belong to the Trypanosomatidae family and cause important human infections; e.g., *Leishmania* causes leishmaniasis, and *Trypanosoma* is responsible for Chagas disease and human African trypanosomiasis (HAT), otherwise known as sleeping sickness. Together, these illnesses are among the most important neglected tropical diseases (NTDs), and they affect about 22 million people worldwide (Figs. 1.1 and 1.2). These protozoan parasites alternate between invertebrate and vertebrate hosts throughout their lifecycles, and different developmental stages are capable of living inside the host cells or circulating in the bloodstream or the insect gut. Another important feature of these parasites is the presence of special organelles that are absent in other eukaryotic organisms or have functions exclusively found in trypanosomatids. As an example, mitochondrion is unique and ramified and has a particular region localized close to the basal body and flagellar pocket; this specific region is known as the kinetoplast and houses the mitochondrial DNA. In this chapter, we will briefly review the most relevant data on the epidemiology, lifecycle, and structural organization of *Leishmania*, *Trypanosoma cruzi* (i.e., causative agent of Chagas disease), and *Trypanosoma brucei* (i.e., causative agent of HAT).

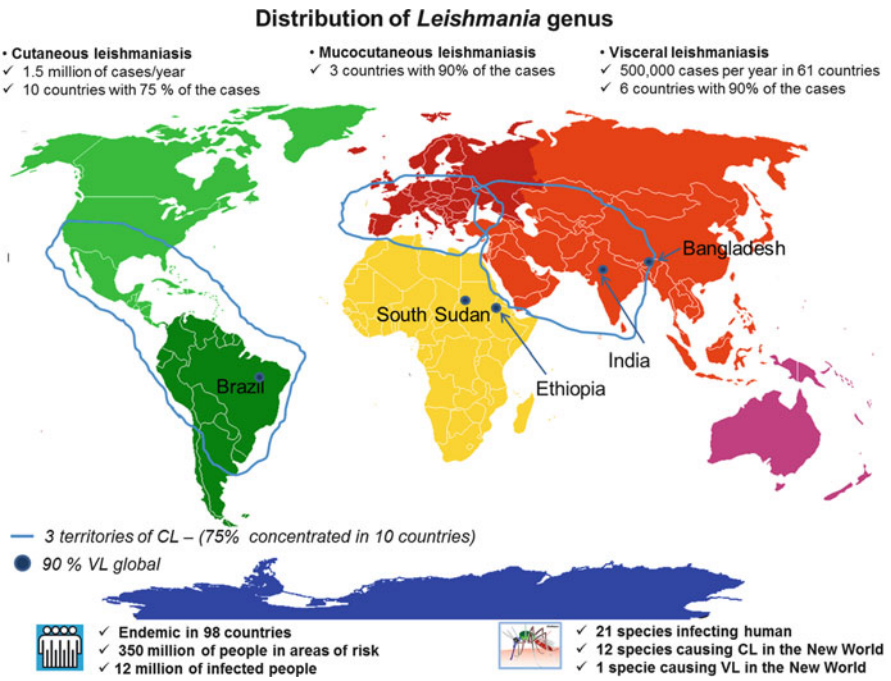

Fig. 1.1 World distribution of the different clinical manifestations of leishmaniasis

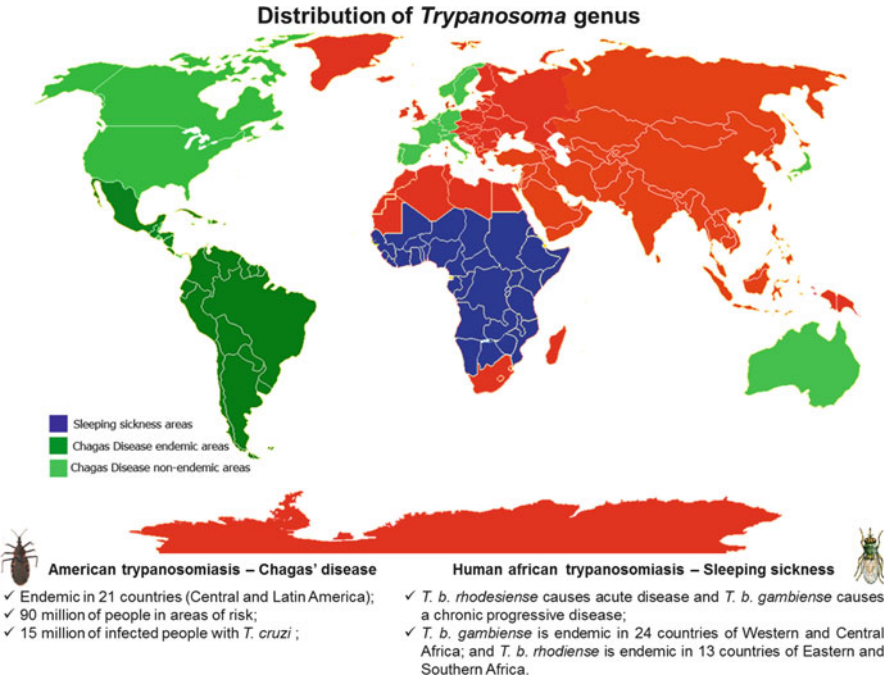

Fig. 1.2 World distribution of Chagas disease and human Africa trypanosomiasis (HAT)

2 Epidemiology, Clinical Manifestations and Chemotherapy

2.1 Leishmania *and Leishmaniasis*

The illness known as leishmaniasis refers to a complex of important NTDs caused by protozoan parasites of the *Leishmania* genus and affects people in both the poorest regions of the world and developing countries, being their distribution quite amazing for the twenty-first century. More specifically, new studies about the epidemiology of this disease revealed that 350 million people are considered at risk for contracting leishmaniasis, and 12 million people were infected worldwide with 2 million new cases yearly (WHO 2010a). Moreover, a recent detailed study based on a comprehensive literature review revealed that a total of 98 countries in five continents have endemic issues related to leishmaniasis transmission (Fig. 1.1) (Alvar et al. 2012). An estimated 0.2–0.4 and 0.7–1.2 million cases of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL), respectively, occur per year. More than 90 % of the global cases of VL are concentrated in the following six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil. However, the distribution of the CL cases is broader; i.e., one-third of these cases are concentrated in the following three epidemiological regions: the Americas, the Mediterranean basin, and Western Asia (i.e., from the Middle East to Central Asia). More specifically, the following ten countries have highest incidence rate of CL: Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru. These countries together are responsible for 70–75 % of the estimated global CL cases (Alvar et al. 2012).

At least 21 species of *Leishmania* can cause disease transmitted to humans by 1 of 30 species of sandflies from the genera *Phlebotomus* or *Lutzomyia*. These species are divided in two subgenera: *Leishmania* and *Viannia*. Together they are responsible for five main clinical manifestations: VL or kala-azar, CL, mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL), and post-kala-azar dermal leishmaniasis (PKDL). Table 1.1 summarizes these clinical manifestations.

In VL, the parasites display a marked tropism for visceral organs, such as the liver, spleen, bone marrow, and lymphatic system. Indeed, VL is considered the most severe form of the disease characterized by undulating fever, weight loss, splenomegaly, hepatomegaly, lymphadenopathy, and anemia. After treatment, the patients may develop a chronic form of cutaneous leishmaniasis that has been called PKDL, which requires long-term treatment. PKDL is a recurrence of VL and can appear up to 20 years after treatment has ended. Sometimes, PKDL appears as a co-infection with human immunodeficiency virus (HIV), which is another important feature of leishmaniasis (Zijlstra et al. 2003). Two specific species are responsible for VL around the world, i.e., *L. donovani* and *L. infantum* (syn *L. chagasi*) in Africa, Asia, Europe and in the Americas (Kuhls et al. 2011).

CL, MCL, and DCL are part of a larger group of diseases also called American tegumentary leishmaniasis, which are widespread with most cases occurring in Brazil. In the case of CL, localized lesions that form can be self-healing; however, when the lesions are multiple and disabling due to the formation of disfiguring

Table 1.1 Summary of the different clinical manifestations of leishmaniasis

Clinical manifestations		Species
Cutaneous leishmaniasis	Red lesions develop at the site of bite that can ulcerate	*L. braziliensis*[a] *L. amazonensis*[a] *L. major*[b]
Mucocutaneous leishmaniasis	The lesions can partially or totally destroy the mucous membranes of the nose, mouth, and throat cavities and surrounding tissues	*L. braziliensis*[a] *L. guyanensis*[a] *L. aethiopica*[b]
Diffuse cutaneous leishmaniasis	Presence of disseminated and chronic skin lesions resembling those of lepromatous leprosy. In this situation, the patients are anergic, and no treatment is available	*L. amazonensis*[a] *L. mexicana*[a] *L. aethiopica*[b]
Visceral leishmaniasis	Also known as kala-azar and characterized by the tropism of the parasites to the visceral tissues and presentation of high fever, substantial weight loss, swelling of the spleen and liver, and anemia	*L. infantum* (syn. *L. chagasi*)[c] *L. donovani*[b]
Post-Kala-azar dermal leishmaniasis	PKDL is a unique eruption that develops after treatment and apparent cure of visceral leishmaniasis	*L. infantum* (syn. *L. chagasi*)[c] *L. donovani*[b] *L. infantum*[b]

[a]Species found in the Americas
[b]Species found in Africa, Asia, and Europe
[c]Specie found in the Africa, Asia, Europe and Americas

scars, it is very difficult to treat, leading to a significant disfigurement and social stigmatization. In general, ulcerative lesions are most common in the Americas. CL can be caused by different species of *Leishmania* such as *L. mexicana*, *L. amazonensis*, *L. braziliensis*, or *L. panamensis* in the Americas, and *L. tropica* and *L. major* in other countries. DCL is rare even in leishmaniasis-endemic regions and is caused by an infection with *L. amazonensis* in Brazil or *L. aethiopica* in the Mediterranean basin, Middle East, and Africa. In general, DCL occurs when the immune system fails to react against *Leishmania* antigens in individuals with a defective cell-mediated immune response. Multiple non-ulcerative lesions form around the body, sometimes resembling those found in lepromatous leprosy. These lesions never spontaneously heal, and unfortunately no treatment is currently available for it. In the case of MCL, the parasites have a marked tropism for the oral-nasal and pharyngeal cavities, often causing extensive destruction that involves mutilation of the face and great suffering for the infected patients. In the Americas, it is associated with an infection by *L. braziliensis* and *L. guyanensis*. In contrast, MCL is rare in Africa, Asia, and Europe; however, when it occurs, it is frequently associated with an infection by *L. donovani* or *L. major* or in immunosuppressed patients infected with *L. infantum* (Desjeux 2004; WHO 2010a).

Nowadays, the following two pentavalent antimonials are available as the first line of treatment for leishmaniasis: meglumine antimoniate and sodium stibogluconate (i.e., sold as Glucantime and Pentostan, respectively). In general, these drugs can be administered intramuscularly or intravenously; however, they can also be administrated intralesionally for the treatment of CL. Several common side effects

are possible during treatment, including loss of appetite, vomiting, nausea, abdominal pain, myalgia, arthralgia, headache, metallic taste, and lethargy.

Due a high number of cases of resistance, other treatments have also become available, including amphotericin B deoxycholate, its liposomal formulation, and pentamidine. Amphotericin B is a polyene antibiotic that is administrated by intravenous infusion and has several toxic side effects mainly related to nephrotoxicity, hypokalemia, and myocarditis. Some studies have demonstrated the efficacy of a single dose of liposomal amphotericin B in the treatment of VL (Sundar et al. 2008, 2010). Moreover, miltefosine, an oral drug, is an alkylphosphocholine analog that was originally developed as an anti-cancer drug; however, it was shown to be active against *Leishmania* at the end of 1980 and licensed in India as the first oral treatment for VL in 2002. Besides, it has been tested on several continents in many clinical trials in the treatment of various forms of leishmaniasis, including CL and MCL (Dorlo et al. 2012). Their main side effects are related to gastrointestinal systems, including loss of appetite, nausea, vomiting, and diarrhea. In addition, it is teratogenic in high doses; thus, it is not recommended for use by pregnant women or women who may become pregnant (Ganguly 2002; Oliveira et al. 2011). Given the longer term use of this chemotherapeutic agent in this area, cases of resistance to miltefosine have been reported mainly in India.

Another interesting treatment is based on the intramuscular administration of paromomycin, an aminoglycoside antibiotic that can also be used in a topical lipid formulation (Thaku et al. 2000; Alavi-Naini et al. 2012). In addition to all of the monotherapies mentioned above, combination therapies have been used in some studies and could offer new solutions for the treatment of leishmaniasis (WHO 2010a). Based on their efficacy and function as a monotherapy, different combinations between pentavalent antimonials, amphotericin B, paromomycin and miltefosine have been proposed.

2.2 T. cruzi *and Chagas Disease*

T. cruzi is the etiologic agent of Chagas disease, which is otherwise known as American trypanosomiasis, and it was discovered in 1909 by the Brazilian medical doctor Carlos Chagas. Nowadays, Chagas disease is considered endemic in 21 countries mainly across Latin America and parts of North America (i.e., Mexico and Southern United States) (Fig. 1.2). Around 90 million people are exposed to the parasite, and current estimates indicate that 12 million people are infected with *T. cruzi* (WHO 2010b; Rassi Jr. et al. 2010, 2012). Historically, the disease (i.e., both transmission and morbidity) was restricted to this region; however, due the intense migration activity of Latin American immigrants, the number of cases in non-endemic developed countries (e.g., Australia, Canada, Japan, Spain, and the United States) has significantly increased, making Chagas disease an important public health problem of global concern (Fig. 1.2). Its chronic phase results in significant disability, which ultimately creates significant negative social and economic impacts on countries with high incidence of this disease. For example, Brazil has suffered the loss of around US $1.2 billion in salary and untold losses of industrial and rural productivity due to the number of infected workers (WHO 2010c).

The parasite *T. cruzi* is primarily transmitted to humans by the feces of blood-sucking reduviid insects widely known as "the kissing bugs;" however, non-vectorial mechanisms of infection can occur through such means as blood transfusions, organ transplantations, congenital and oral transmission (i.e., ingestion of contaminated food, especially fruit juices). The disease has the following three characteristic clinical phases: *the acute phase*, where around 5 % of children die but can spontaneously resolve itself in 4–6 weeks; *asymptomatic or indeterminate phase*, where the patients do not present any clinical symptoms of the disease, but they can transmit the parasite to other humans; and the *chronic symptomatic phase* occurring in 10–30 % of the infected patients, where the heart or the gastrointestinal tracts are affected. Note that Chagas disease is one of the most important causes of cardiomyopathy worldwide. In general, acute Chagas disease is asymptomatic. Nevertheless, symptoms can develop at around 8–10 days after infection by vector-borne transmission; in the case of symptom development, an edema known as "chagoma" or Romaña signal appears in the palpebral and periocular regions. In addition, most of the deaths at this stage are due to heart failure. The chronic stage begins 2–3 months after initial infection and after resolution of the acute disease. About 60–70 % of the infected patients with the acute symptoms eventually present the indeterminate form of the disease without any further clinical symptoms except the presence of antibodies against *T. cruzi* in the serum. The other 30–40 % of infected patients with acute symptoms develop the chronic symptomatic disease, which can affect the cardiac muscle, the digestive system (i.e., mainly as megaesophagus and megacolon) or the both. These manifestations can occur usually 10–30 years after the initial infection (Rassi Jr. et al. 2012).

Only two drugs are officially recommended for the treatment of Chagas disease, i.e., benznidazole and nifurtimox. Several clinical studies have demonstrated that benznidazole, a nitroimidazole derivative, is more safe and efficacious than nifurtimox, thereby making it the first-line treatment. Interestingly, both drugs act against the acute phase of Chagas disease, reducing the severity of the symptoms and shortening the clinical course and duration of the detectable parasitemia (Le Loup et al. 2011). However, neither is effective against the chronic phase of the disease. Both benznidazole and nifurtimox present several toxic side effects, such as the formation of a localized allergic dermatitis and gastrointestinal symptoms, respectively (Rassi Jr. et al. 2012). At this moment, clinical trials are taking place in Spain and Argentina using a new agent in the treatment of Chagas disease, i.e., posaconazole, alone or in combination with benznidazole (Loup et al. 2011; Urbina 2010; www.clinicaltrials.gov, accessed on January 10th, 2013).

2.3 T. brucei *and Sleeping Sickness*

T. brucei is the etiologic agent of HAT, which is otherwise known as sleeping sickness; this illness is transmitted by tsetse flies of the *Glossina* genus. Historically, HAT has occurred in the poorest rural areas of Africa, where weak health systems and political instability make disease surveillance and management difficult. Two subspecies are responsible for the transmission of HAT in rural parts of sub-Saharan

Africa. The majority of cases (i.e., >90 %) are caused by *T. b. gambiense*, which is endemic in 24 countries of Western and Central Africa (i.e., mainly Angola, Congo, Guinea, Southern Sudan, and Northwestern Uganda), whereas *T. b. rhodesiense* is endemic in 13 countries of Eastern and Southern Africa (i.e., mainly Malawi, Tanzania, and Southeastern and Central Uganda) (Fig. 1.2). However, the Democratic Republic of the Congo accounts for two thirds of the reported cases. More than 205 discrete, active HAT foci are recognized, most of which are in poor and remote rural areas where health systems are often weak as previously indicated. However, sleeping sickness has also been reported in peri-urban areas (Malvy and Chappuis 2011). As previously mentioned, HAT, leishmaniasis, and Chagas disease represent the most important NTDs and affect mainly the poorest, rural regions. Nevertheless, thanks to important work by the Medicins Sans Frontieres (MSF) and several control and intervention programs from the World Health Organization (WHO), the number of reported cases of HAT have declined from 37,385 in 1998 to 9,589 in 2009 (WHO 2006; Simarro et al. 2012; Blum et al. 2012).

The clinical manifestations that characterize sleeping sickness (i.e., HAT) are generally the same for both causative sub-species. However, *T. b. rhodesiense* causes a more acute disease with overt clinical manifestations developing within days following infection that can lead to death if untreated, while *T. b. gambiense* is characterized by a chronic progressive course of the disease (Malvy and Chappuis 2011). Signs and symptoms of HAT are classified according to the clinical progression of the disease and can be divided into two distinct stages. The first is *Stage 1*, i.e., the hemolymphatic phase, where the trypanosomes are restricted to the blood and the lymphatic systems. In general, this stage involves non-specific symptoms, like headaches, fever, and joint pain, which are difficult to diagnose correctly due the failure of the surveillance systems. *Stage 2*, i.e., the meningoencephalitic phase, is characterized by the active invasion of the central nervous system by the parasite. In this case, the trypanosomes cross the blood-brain barrier and can lead to serious sleep cycle disruptions, paralysis, and progressive mental deterioration, all of which can result in the death of the infected patients in the absence of an effective treatment. Clearly, the sleep disorder inspired the common name of the disease, i.e., *sleeping sickness*. The two stages involved in HAT caused by *T. b. gambiense* have an average duration of around 3 years, which is very different from that of HAT caused by *T. b. rhodesiense*, which is classically described as an acute disease progressing to stage 2 within a few weeks and death within 6 months (Odiit et al. 1997). One important difference between the two infections is the presence of the trypanosomal chancres that can appear after the tsetse bite. In infections of *T. b. gambiense*, the chancre is rarely seen; however, it occurs and can be numerous in approximately 26 % of patients infected with *T. b. rhodesiense* (Boatin et al. 1986; Blum et al. 2012; MacLean et al. 2010). The chancre is defined as the initial lesion at the bite site and is characterized by local erythema, edema, heat, tenderness, and a lack of any suppuration. In the cases where the chancre is present, it can be used as a clinically diagnostic indicator.

Five drugs are available for the treatment of HAT, and they are prescribed according to the stage of the disease and the infecting protozoan sub-species; these five drugs are pentamidine, eflornithine, nifurtimox, melarsoprol, and suramin. For cases of

HAT caused by *T. b. gambiense*, the treatment for stage 1 involves the intravenous administration of pentamidine by slow infusion for 7 days, which is repeated over the course of several decades. The most frequent adverse side effects are pain at the injection site, hypoglycemia, and hypotension. For stage 2 of HAT caused by *T. b. gambiense*, melarsoprol has been the only available effective drug for over 50 years. This drug is poorly tolerated due to a wide range of side effects, including encephalopathic syndrome, peripheral neuropathy, hepatic toxicity, skin rash, acute phlebitis, and vein sclerosis. In addition, a large number of cases of resistance to melarsoprol have been detected in endemic areas. More recently, eflornithine monotherapy has gradually replaced melarsoprol as the first-line treatment in these cases; however, its universal use is complicated due the associated difficulties in logistics and requirements for nursing care. In order to be efficacious, 56 intravenous infusions, each lasting 30 min, must be administered over at least 14 days. Several efforts have been made to attempt to shorten and simplify the eflornithine therapy. As a result, a large clinical trial demonstrated that nifurtimox-eflornithine combination therapy (NECT) has the same efficacy rate as the eflornithine monotherapy (Priotto et al. 2009; Yun et al. 2010). After this study, WHO and Drugs for Neglected Diseases initiative (DNDi) conducted a phase IIIb trial in May of 2009 involving 600 patients from the Democratic Republic of the Congo. Since 2010, MSF has adopted NECT as the first-line treatment in several endemic countries (Simarro et al. 2012). In the case of HAT caused by *T. b. rhodesiense*, the only treatment available is suramin in a complex dose regimen of more than 30 days for stage 1 and melarsoprol for stage 2. Unfortunately, *T. b. rhodesiense* is resistant to eflornithine (Malvy and Chappuis 2011). Table 1.2 summarizes all the treatments presently available for leishmaniasis, Chagas disease, and HAT (i.e., sleeping sickness).

3 Lifecycle

Leishmania, *T. cruzi*, and *T. brucei* are heteroxenic protozoan parasites; i.e., their lifecycles involve two hosts – the insect vector and the mammalian hosts, including sylvatic and domestic reservoirs and humans. During their lifecycles, different developmental stages essential for progression of the infection occur in both hosts. Figure 1.3 summarizes all the main developmental stages for all species as they occur in the insect and mammalian hosts. Each species has unique aspects in its lifecycle, which are described in the subsequent sections of this text.

3.1 Leishmania *spp.*

During the complex digenetic or heteroxenic lifecycle of *Leishmania* sp., the parasites alternate between elongated promastigotes that have adapted to live in the extracellular space of intestinal cavities of the insect vector and ovoid amastigotes that have adapted to survive in an intracellular habitat inside the mammalian macrophages

Table 1.2 Summary of the currently available treatments and their regimens adopted by the World Health Organization in different countries

Disease	Drugs available	Therapeutic regimen
HAT by *T. gambiense* – stage 1	Pentamidine isethionate	4 mg/kg/day IM or IV (diluted in saline and given in 2 h infusions) × 7 days
HAT by *T. gambiense* – stage 2	NECT (Eflornithine + Nifurtimox)	400 mg/kg/day eflornithine IV in 2 infusions (1 h each) × 7 days + 15 mg/kg/day nifurtimox PO in 3 doses × 10 days
	Eflornithine alone	400 mg/kg/day eflornithine IV in 4 infusions × 14 days
	Melarsoprol	2.2 mg/kg/day melasorprol IV × 10 days (second line treatment)
HAT by *T. rhodesiense* – stage 1	Suramin	Test dose of 4–5 mg/kg suramin (day 1), then 20 mg/kg IV weekly × 5 weeks (maximal dose injection: 1 g)
HAT by *T. rhodesiense* – stage 2	Melarsoprol	2.2 mg/kg/day melarsoprol IV × 10 days or three series of 3.6 mg/kg/day IV × 3 days spaced by intervals of 7 days
Chagas disease – acute phase	Benznidazole	5–7 mg/kg/day orally in two divided doses daily for 60 days for adults; 5–10 mg/kg/day in two divided doses daily for 60 days for infants and children up to 12 years old
	Nifurtimox	8–10 mg/kg/day orally in three divided daily doses ranging from 60 to 90 days for adults; 15–20 mg/kg/day orally in four divided daily doses for 90 days for children
Chagas disease – chronic phase		Specific treatment of the signals and symptoms form the organism in the affected systems
Cutaneous leishmaniasis	Paromomycin	*Worldwide*: Local therapy – 15 % paromomycin/12 % methylbenzethonium chloride ointment twice daily for 20 days; intralesional antimonials, 1–5 ml per session plus cryotherapy (liquid nitrogen: – 195 °C), both every 3–7 days (1–5 sessions); thermotherapy, 1–2 sessions with localized heat (50 °C for 30 s); intralesional antimonials or cryotherapy independently
	Pentavalent antimonials	System therapy: pentavalent antimonials – 20 mg Sb^{5+}/kg/day IM or IV for 20 days; pentamidine isethionate,– IM injections or brief infusions of 4 mg salt/kg/day for 3 doses; amphotericin B deoxycholate – 0.7 mg/kg per day, by infusion, for 25–30 doses; miltefosine – 2.5 mg/kg per day orally for 28 days
	Cryotherapy	
	Thermotherapy	
	Pentamidine isethionate	
	Amphotericin B deoxycholate	
	Miltefosine	

(continued)

Table 1.2 (continued)

Disease	Drugs available	Therapeutic regimen
Mucocutaneous leishmaniasis	Pentavalent antimonials Amphotericin B deoxycholate Liposomal amphotericin B Miltefosine	*The Americas*: pentavalent antimonials – 20 mg/kg/day IM or IV for 30 days; amphotericin B deoxycholate – 0.7–1 mg/kg by infusion every other day up to 25–45 doses; liposomal amphotericin B – 2–3 mg/kg daily by infusion up to a total dose of 40–60 mg/kg; in Bolivia, miltefosine – 2.5–3.3 mg/kg per day orally for 28 days
Diffuse cutaneous leishmaniasis	Pentavalent antimonials	*Africa*, *Asia*, *and Europe*: pentavalent antimonials – 20 mg Sb5+/kg/day IM or IV plus paromomycin, 15 mg (11 mg base)/kg/day intramuscularly for 60 days
	Paromomycin Ketoconazole Miltefosine	*The Americas*: pentavalent antimonials – 20 mg Sb^{5+}/kg/day IM or IV for 20 days; ketoconazole – adult dose, 600 mg oral daily for 28 days; miltefosine – 2.5 mg/kg per day orally for 28 days (B)
Visceral leishmaniasis	Pentavalent antimonials	*Africa*, *Asia*, *and Europe*: Pentavalent antimonials – 20 mg Sb^{5+}/kg/day IM or IV for 30 days; amphotericin B deoxycholate – 0.75–1 mg/kg/day by infusion, daily, or on alternate days, for 15–20 doses; liposomal amphotericin B – 3–5 mg/kg/day by infusion given over 6–10 days up to a total dose of 30 mg/kg
	Amphotericin B deoxycholate	Miltefosine – for children aged 2–11 years, 2.5 mg/kg/day; for people aged ≥12 years and < 25 kg, 50 mg/day; for people 25–50 kg, 100 mg/day; for people > 50 kg, 150 mg/day; orally for 28 days
	Liposomal amphotericin B	Combination: pentavalent antimonials (20 mg Sb^{5+}/kg/day IM or IV) plus paromomycin (15 mg [11 mg base]/kg/day IM) for 17 days
	Miltefosine Pentavalent antimonials + paromomycin	*Worldwide*: Pentavalent antimonials – 20 mg Sb^{5+}/kg/day IM or IV for 30 days; amphotericin B deoxycholate – 1 mg/kg/day by infusion on alternate days for 15–20 doses; liposomal amphotericin B – 1–1.5 mg/kg/day by infusion given over 21 days or 3 mg/kg/day during 10 days
PKDL	Pentavalent antimonials	*Africa*, *Asia*, *and Europe*: East Africa: pentavalent antimonials – 20 mg Sb^{5+}/kg/day IM or IV for 30–60 days; liposomal amphotericin B – 2.5 mg/kg/day by infusion for 20 days
	Liposomal amphotericin B Amphotericin B deoxycholate Miltefosine	*Bangladesh, India, Nepal*: amphotericin B deoxycholate – 1 mg/kg/day by infusion, up to 60–80 doses over 4 months; miltefosine – orally for 12 weeks at dosage

HAT human African trypanosomiasis, *PKDL* post-kala-azar dermal leishmaniasis, *IM* intramuscular, *IV* intravenous, *NECT* Nifurtimox-eflornithine therapy, *PO* per os, *Sb^{5+}* pentavalent antimonial

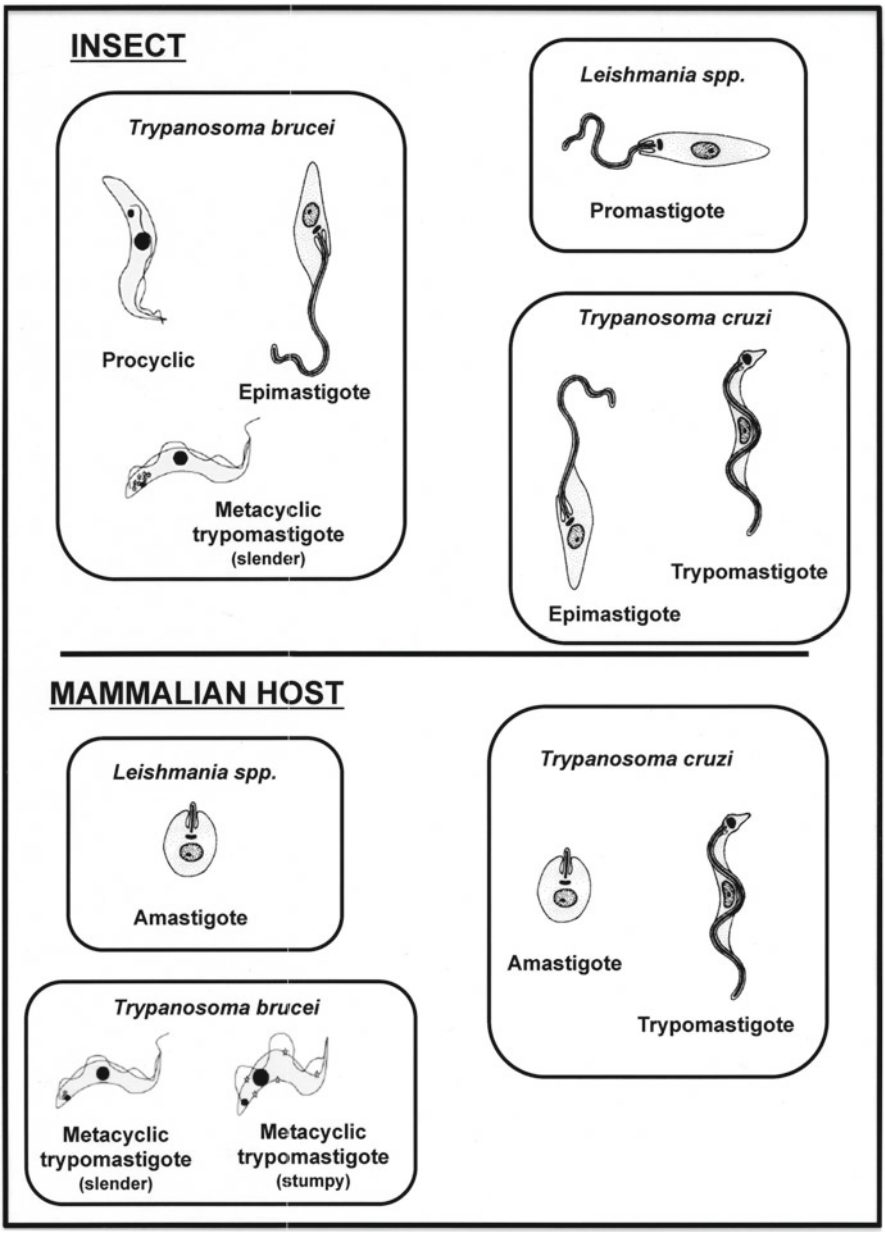

Fig. 1.3 Summary of all developmental stages found in the insect vector and mammalian hosts for the protozoan parasites *Leishmania* sp., *Trypanosoma cruzi*, and *Trypanosoma brucei*

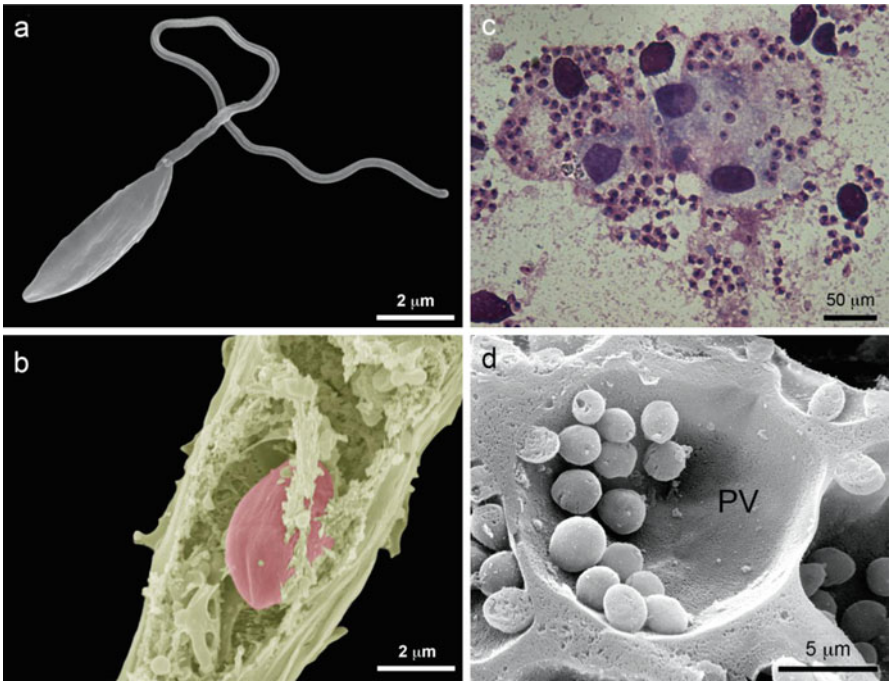

Fig. 1.4 (**a**–**d**) Micrographs of the developmental stages of *L. amazonensis*. (**a**) Extracellular promastigotes; (**b**) murine macrophage infected with intracellular amastigote by scanning electron microscopy; (**c**) light microscopy analysis of skin smears of BALB/c infected tissue stained with Giemsa showing several amastigotes; (**d**) scanning electron microscopy analysis of infected tissue after cleavage in liquid nitrogen to expose the intracellular environment. Note the interaction of amastigotes with the membrane of the parasitophorous vacuole (PV)

(Figs. 1.3 and 1.4). The metacyclic promastigotes, i.e., the infective stage, are inoculated into the host by the sandflies from their proboscis during their blood meals. Due the presence of several glycoconjugates on their surface, these promastigotes adhere to the plasma membrane of macrophages and trigger a phagocytic process (Lodge and Descoteaux 2008). The promastigotes are phagocytized and remain inside the parasitophorous vacuole, a vacuole found in the host cells where most of the protozoan parasites reside and develop during its life cycle. Then, the promastigotes differentiate into amastigotes that live in an acidic pH habitat where they divide by binary fission. After multiple divisions, a large number of amastigotes (Fig. 1.4) leads to the lyses of the macrophages, releasing parasites that can infect new macrophages or be ingested by sandflies during new blood meals. In sandflies, amastigotes transform into procyclic promastigotes in the gut that divide quickly to enter into a differentiation process known as metacyclogenesis. In this process, non-infective forms differentiate into infective metacyclic promastigotes that migrate to the proboscis, thereby starting a new round of infection again (Bates 1994). Note that in the *Viannia* subgenus, the promastigotes develop in the hindgut, while in the *Leishmania* subgenus the metacyclogenesis occurs in the midgut.

3.2 T. cruzi

Several developmental stages make up the digenetic lifecycle of *T. cruzi*; moreover, these stages alternate between blood-sucking triatome insect and hosts (Figs. 1.3 and 1.5). In the peripheral blood of the mammalian host, *T. cruzi* exists as trypomastigotes that exhibit two basic morphologies that are generally described as slender or stumpy. During its blood meal, the insect vector ingests the trypomastigotes, which go to the stomach and undergo differentiation to the spheromastigote form. These non-motile forms are around 3–5 μm in diameter, and, in this habitat, they transform into epimastigotes (Fig. 1.5) that migrate to the midgut; these epimastigotes are the replicative form. Elongated epimastigotes attach to the epithelial intestinal cells as well as to the wax cuticle of the rectum through their long flagella prior to differentiating into the metacyclic trypomastigotes. This differentiation is also called metacyclogenesis. Once differentiated, metacyclic trypomastigotes detach from the intestinal epithelia and migrate to insect rectum to be excreted with feces. This developmental stage is highly infective to a wide range of nucleated mammalian cells. Once in the vertebrate host, the metacyclic trypomastigotes can invade different cells, such as fibroblasts, macrophages, and epithelial cells, through an interaction between the surface glycoconjugates on the parasite and several receptors present on the plasma membrane of the host cell (Tyler and Engman 2001). This stage of mammalian host infection triggers the internalization process by the host cells, starting with the intracellular cycle of *T. cruzi*. Several steps occur in this cycle, including the following: (1) formation of an endocytic compartment known as the parasitophorous vacuole; (2) differentiation of the long and thin trypomastigotes into amastigotes, which have an ovoid shape and a short flagellum (Fig. 1.5); (3) lysis of the parasitophorous vacuole membrane by parasite-secreted enzymes, thus allowing the amastigotes to be released into the cytoplasm that remains in contact with host cells organelles; and (4) transformation of the amastigotes into trypomastigotes that are released into the extracellular space and may thus infect other cells or reach the bloodstream (de Souza et al. 2010).

3.3 T. brucei

The lifecycle of *T. brucei* has four main developmental stages that occur in the tsetse fly of the *Glossina* genus and in the mammalian host, i.e., epimastigotes, procyclic forms, slender metacyclic trypomastigotes, and stumpy metacyclic trypomastigotes (Fig. 1.3). In both *T. b. rhodesiense* and *T. b. gambiense* infections, the lifecycle can start with an infected tsetse fly injecting stumpy metacyclic trypomastigotes in a mammalian host during the blood meal. These parasites enter the lymphatic system and pass to the bloodstream. Inside the host, they transform into proliferative, slender, bloodstream trypomastigotes that go to other parts of the body, thereby reaching other fluids, such as lymph and cerebrospinal fluid, where they multiply