Kinetoplastids: related protozoan pathogens, different diseases

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Kinetoplastids are a group of flagellated protozoans that include the species Trypanosoma and Leishmania, which are human pathogens with devastating health and economic effects. The sequencing of the genomes of some of these species has highlighted their genetic relatedness and underlined differences in the diseases that they cause. As we discuss in this Review, steady progress using a combination of molecular, genetic, immunologic, and clinical approaches has substantially increased understanding of these pathogens and important aspects of the diseases that they cause. Consequently, the paths for developing additional measures to control these “neglected diseases” are becoming increasingly clear, and we believe that the opportunities for developing the drugs, diagnostics, vaccines, and other tools necessary to expand the armamentarium to combat these diseases have never been better.

Introduction

Kinetoplastids are a group of flagellated protozoans that are distinguished by the presence of a DNA-containing region, known as a “kinetoplast,” in their single large mitochondrion. Although the different kinetoplastid pathogens have a similar genomic organization and similar cellular structures and all undergo morphological changes during their life cycles, these flagellated protozoans cause distinct human diseases and are transmitted by different insect vectors (Table 1). The most common human diseases caused by kinetoplastids include human African trypanosomiasis (HAT; also known as African sleeping sickness), which is caused by infection with two of the three subspecies of Trypanosoma brucei; Chagas disease, which is caused by infection with Trypanosoma cruzi; and various forms of leishmaniasis, which are caused by infection with different species of Leishmania (1). Half a billion people, primarily in tropical and subtropical areas of the world, are at risk of contracting these diseases, and it is estimated that more than 20 million individuals are infected with the pathogens that cause them, resulting in extensive suffering and more than 100,000 deaths per year.

Much of the cellular biology of the different kinetoplastids is very similar. For example, they are all motile protozoans with a single flagellum that originates near their large single mitochondrion and emanates from a pocket in the cell membrane, where endocytosis also occurs; their peroxisomes are modified to perform glycolysis and are therefore known as “glycosomes”; their cell membrane is underlain with a sheet of microtubules and is highly decorated with species-specific molecules that are critical for their survival; they typically grow asexually, and although sexual recombination has been shown for T. brucei, inferred for T. cruzi, and might occur in some species of Leishmania, it is not obligate in any; and they divide by binary fission during which their nucleus does not undergo membrane dissolution or chromosome condensation. Despite these similarities, which are not surprising given that of the more than 8,000 genes in the genome of each kinetoplastid there are more than 6,000 orthologs in common, the diseases that they cause are very distinctive. Understanding the differences between these human disease–causing pathogens at the genetic, molecular, and cellular levels might provide new approaches to the development of diagnostics, vaccines, drugs, and other tools needed to combat these diseases. Achieving these goals is more feasible now due to a combination of new knowledge of parasite genomes and biochemistry, new insight into host immune responses, and the injection of substantial philanthropic support. These recent advances together with the knowledge from epidemiological studies could complement surveillance, vector control, and other public health efforts to provide for optimum control, and hopefully the elimination, of these diseases.

The diseases, their pathogenesis, and their diagnosis

HAT. About 50 million people live in HAT transmission areas. Its incidence surged in the 1990s due to civil unrest and a lack of awareness of the disease, with major flare-ups in Angola, Democratic Republic of the Congo, Uganda, and Sudan. Mortality rates are uncertain, but prevalence was estimated to be about 500,000 cases in 1998, of which only 40,000 were reported. These rough estimates reflect the rural and focal nature of the disease as well as under-surveillance. Subsequent decreases in incidence seem to be due to greater political stability and funding of surveillance and control programs as well as pharmaceutical company drug donations, all of which helped reduce the reported new cases of HAT to 17,600 in 2004 (2).

HAT is fatal if left untreated (1). The first stage of the disease (the hemolymphatic stage) is accompanied by fever, headache, adenopathy, joint pain, and pruritus. Rapid parasite growth is countered by host immune responses, but parasite antigenic varia-
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Table 1
The kinetoplastid diseases

<table>
<thead>
<tr>
<th>Principal disease forms or stages</th>
<th>HAT</th>
<th>Chagas disease</th>
<th>The leishmaniases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causative organisms</td>
<td>T.b. gambiense, T.b. rhodesiense</td>
<td>T. cruzi</td>
<td>–21 Leishmania spp., e.g., L. donovani (VL), L. braziliensis (MCL), L. major (CL)</td>
</tr>
<tr>
<td>Host cell/tissue</td>
<td>Extracellular, in blood, lymph, cerebral spinal fluid, and intercellular spaces</td>
<td>Intracellular, in cytoplasm of heart, smooth muscle, gut, CNS, and adipose tissue cells</td>
<td>Intracellular, in phagolysosomes of macrophages</td>
</tr>
<tr>
<td>Vectors of medical importance</td>
<td>Tsetse flies (–20 Glossina spp.) (palpalis group, T.b. gambiense, morsitans group, T.b. rhodesiense)</td>
<td>Reduvid bugs (–12/–138 Triatominae spp.) (Triatoma, Rhodnius, and Panstrongylus spp.)</td>
<td>Phebotomine sandflies (–70 spp.) (Phlebotomus spp. in Old World, Luizomyia spp. in New World)</td>
</tr>
<tr>
<td>Transmission</td>
<td>Infected fly bite, congenital (rare), blood transfusion (rare)</td>
<td>Contamination by feces of infected bugs (e.g., at bite site, in mucus membranes, in food or drink), blood transfusion, congenital, organ transplantation (rare)</td>
<td>Infected fly bite</td>
</tr>
<tr>
<td>Geographic distribution</td>
<td>Sub-Saharan Africa (–20 countries)</td>
<td>South and Central America (19 countries)</td>
<td>South and Central America, Europe, Africa, Asia (88 endemic countries)</td>
</tr>
<tr>
<td>Population at risk Infected</td>
<td>50 million</td>
<td>100 million</td>
<td>350 million</td>
</tr>
<tr>
<td>Health burden (DALYs)</td>
<td>70,000–80,000^</td>
<td>8–11 million</td>
<td>12 million</td>
</tr>
<tr>
<td>Deaths (per annum)</td>
<td>1.5 million</td>
<td>0.7 million</td>
<td>2.1 million</td>
</tr>
<tr>
<td>Prevention and control measures</td>
<td>–30,000</td>
<td>14,000</td>
<td>51,000 (VL)</td>
</tr>
<tr>
<td>Treatment of homes with residual insecticides, blood donor screening, drug treatment for acute, early indeterminate and congenital cases, better housing (plastered walls and metal roof replacing adobe-walled, thatch-roofed dwellings)</td>
<td>Treatment of homes with residual insecticides; reservoir (dog) control (VL); treatment of homes with residual insecticides; insecticide-treated bed nets, fabrics, and dog collars; live vaccination (“leishmanization,” CL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from refs. 132–138 and from http://www.who.int/neglected_diseases/diseases/en/. ^Calculated based on the reported incidence in 2004 of ~18,000 cases identified from 3.3 million people under active surveillance, assuming 50 million at risk (8). DALY, disability-adjusted life year; MCL, mucocutaneous leishmaniasis.
Chagas disease. Chagas disease is prevalent throughout the Americas. It mostly affects people whose housing provides a habitat for the Triatominae insects, commonly called "kissing bugs," which act as a vector for the disease-causing parasite T. cruzi. There are many domestic and wild mammal reservoirs of T. cruzi. Infection with T. cruzi can also result from blood transfusion (11), organ transplantation (12), vertical transmission (13), and by ingestion of contaminated food or drink (14).

Chagas disease is the result of persistent infection with T. cruzi and complex interactions between the pathogen and host immune response, which, in the absence of immune system dysfunctions (e.g., in individuals with AIDS), results in very low parasite numbers but rarely elimination of the infection in this chronic or indeterminate phase (15) (Figure 2). Nondividing transmissible extracellular trypomastigotes infect and replicate as amastigotes in cells of multiple organs and tissues, including the heart, gut, CNS, smooth muscle, and adipose tissue (16–18). An estimated 30% of individuals infected with T. cruzi develop clinical Chagas disease, which manifests as cardiac disease or pathological gut enlargement, decades after the initial infection. Although an autoimmune etiology was initially hypothesized, the continuous immune assault on persistent parasites is likely to be the primary cause of cumulative tissue damage in chronic Chagas disease.

The current diagnostics for Chagas disease are inadequate yet crucial for identifying infected individuals as well as monitoring treatment and other interventions (19, 20). Direct detection of parasites after the acute stage of infection is very difficult due to their low numbers. Multiple serological tests using different platforms (e.g., ELISA, indirect immunofluorescence, and indirect hemagglutination) are routinely used to attempt to obtain a consensus result. However, the infection status of those positive in only one test is inconclusive (21), and multiple tests on individuals with detectable parasites can give negative results (22). Thus, current tests are inadequate for monitoring treatment efficacy and other interventions. New tests need to be developed to inform policy development for treatment and control strategies.

The Leishmaniases. There are more than 21 species of Leishmania that cause various diseases ranging from self-healing cutaneous leishmaniasis (CL) to debilitating and lethal (if untreated) visceral leishmaniasis (VL; also known as kala-azar) (23). Less frequent but devastating forms of the disease include mucosal leishmaniasis (ML), which is disfiguring (24); diffuse CL, which causes numerous nodular lesions (25); and post kala-azar dermal leishmaniasis, which occurs as a sequelae to VL (26). VL is caused by infection with either Leishmania infantum or Leishmania donovani and has an estimated global incidence of 200,000–500,000 cases; CL is caused by infection with any one of several different Leishmania spp. and has a prevalence of more than 5 million cases worldwide (http://who.int/tdr). However, most L. infantum infections are subclinical and develop to disease only if immune responses are depressed (27–29). Leishmania major and Leishmania mexicana lesions usually self cure within 3–12 months, leaving deep scarring, whereas Leishmania tropica and Leishmania braziliensis lesions, which are also cutaneous, take up to 18 months to

levels (9). The low numbers of trypanosomes in blood and cerebrospinal fluid necessitate concentration by centrifugation or anion exchange chromatography, two methods that have been adapted of contaminated food or drink (14).

The parasites pass via the lymphatic system into the bloodstream, which carries them throughout the body. The parasites continue to replicate by binary fission. In late-stage disease the parasites invade the CNS and reside in the cerebrospinal fluid and intercellular spaces. Figure modified with permission from Alexander J. da Silva and Melanie Moser, Centers for Disease Control Public Health Image Library.

Figure 1
Life cycle of T. brucei. HAT is caused by infection with the T.b. rhodesiense or T.b. gambiense subspecies. Over 20 species and subspecies of tsetse flies transmit these parasites, but only approximately 1 per 1,000 flies has the mature salivary gland infection that is necessary to transmit the parasite to humans. Trypanosomes enter the fly when it takes a meal of parasite-containing blood from an infected human or animal and, over a period of four weeks, undergo morphological and physiological transformations in the alimentary tract and in the salivary glands, where they become infective. During a blood meal on the mammalian host, an infected tsetse fly injects parasites into skin tissue. The parasites pass via the lymphatic system into the bloodstream, which carries them throughout the body. The parasites continue to replicate by binary fission. In late-stage disease the parasites invade the CNS and reside in the cerebrospinal fluid and intercellular spaces. Figure modified with permission from Alexander J. da Silva and Melanie Moser, Centers for Disease Control Public Health Image Library.
cure and in some cases can develop into the severely disfiguring ML disease state that is difficult to cure.

*Leishmania* parasites invade mammalian macrophages by receptor-mediated endocytosis, for example, via complement receptors that are cleaved by parasite proteases (Figure 3). They multiply in the low-pH, amino acid–rich endolysosomes, to which their metabolism and nutrition are adapted (30). *L. donovani* and *L. infantum*, which infect cells found in lymphoid tissues, including spleen, lymph nodes, and bone marrow, inhibit an effective immune response to the parasite. Parasite lipophosphoglycans reduce the activity of PKC and protein tyrosine kinases (PTKs) in macrophages that have infected, leading to attenuated IFN-γ–induced immune activation (31). Broader effects on the immune system include reduced levels of IL-12 produced by macrophages and T cells (34). The resulting immunosuppression can lead to secondary respiratory infections that can be fatal in individuals with VL. By contrast, patients with CL, in whom the pathogen is largely restricted to the skin and is not found in lymphoid tissues, do not become immunosuppressed and generally develop a curative immune response.

The diagnosis of CL and ML usually employs microscopic detection of organisms in the lesions and skin tests, which consist of intradermal injection of whole or lysed promastigote forms of the pathogen followed by measuring local induration at the injection site 48–72 hours later (35, 36). Organisms can be seen in splenic and bone marrow aspirates and can be cultured from the blood of patients with VL, but the high levels of serum antibody specific for parasite antigens allow serological diagnosis of VL using ELISA. Advances in antigen discovery and serological diagnosis have resulted in antibody-based tests for VL that can be used in the field, including a direct agglutination test (DAT) and a lateral flow assay (LFA), both of which detect the presence of the K39 repeat antigen (37) that is expressed by both species of *Leishmania* that cause VL and, to a lesser extent, by other species. The DAT (Royal Tropical Institute) is sensitive and specific but requires substantial laboratory incubation, whereas the Kalazar Detect rapid test (InBios International Inc.) produces results in a few minutes. The rK39-LFA is highly sensitive in patients in India, Bangladesh, and Nepal, but it is less sensitive in patients in Africa, perhaps due to heterogeneity in the species of *Leishmania* that cause VL; however, its sensitivity exceeds that of microscopic detection of parasites. The serological sensitivity of defined antigens is useful for diagnosis in individuals with VL who are also infected with HIV and for determining whether individuals with VL, whose titers of antibody specific for these antigens are falling, have eliminated the causative pathogen (38). An accurate test of cure would be useful for evaluating new therapies. Other promising diagnostics are detection of antigen in the urine by latex agglutination (KAtex; Kalon Biological Ltd.) and detection of modified sialic acid residues on erythrocytes (due to the production of immature erythrocytes by infected bone marrow; Zephyr Biomedical) (39). Molecular diagnosis of leishmaniasis includes PCR detection of *Leishmania* spp. DNA (40). Field-adaptable systems include Liat Analyzer (IQum Inc.), a simplified PCR assay that uses a colorimetric detection system and a portable instrument that can extract and amplify DNA as well as detect the PCR product. Measurement of T cell responses to antigens expressed by *Leishmania* spp. is useful for verifying CL and
subclinical VL, in which cellular immune responses are strong. No skin test that discriminates between *Leishmania* spp., or that discriminates *Leishmania* from other kinetoplastids, is available, but a process to prepare a standardized reagent has been developed (41, 42) and a prototype in vitro assay to measure T cell responses to *Leishmania* spp. has been developed by Cellestis (43).

**Interventions: treatment, vector control, and the potential for vaccine design**

*HAT*. Treatment of HAT currently relies on five drugs, all of which have adverse effects and problems of efficacy, administration, and compliance (44, 45). Suramin and pentamidine (Pentacarinat; Sanofi-Aventis) are used to treat the first stage of disease caused by infection with *T. rhodesiense* and *T. gambiense*, respectively. Both drugs have the drawback that they must be administered parenterally (suramin i.v. and pentamidine i.m.), but efficacy is high and adverse effects are moderate. Two drugs, both of which are active against the first stage of HAT, are approved for the treatment of the second stage of disease. Melarsoprol is effective against second-stage HAT caused by both *T. rhodesiense* and *T. gambiense*, and eflornithine (Ornidyl; Sanofi-Aventis) is only active for second-stage HAT caused by *T. gambiense*. The arsenical melarsoprol is administered i.v. and has severe adverse reactions that can be life threatening. Furthermore, the number of treatment failures following its administration is increasing, due to drug resistance or other unknown factors (46). Pharmacokinetic studies on melarsoprol (47) led to the treatment regimen being changed to a 10-day course rather than the 21- to 35-day course previously followed, thus improving patient compliance and reducing hospital costs (48). Eflornithine, a polyamine biosynthesis inhibitor, is registered for cases of HAT caused by infection with *T. gambiense* that are unresponsive to treatment with melarsoprol. It is administered over 14 days as four daily i.v. infusions and has less severe adverse effects than melarsoprol. However, administration of 56 i.v. infusions presents a logistical challenge, especially in resource-poor rural settings. Clinical trials are ongoing using eflornithine in combination with nifurtimox, a drug that has limited efficacy when used alone, to shorten and simplify the course of eflornithine treatment. In addition, a combination of melarsoprol and nifurtimox has proven to be effective in cases of second-stage HAT that are either refractory to treatment with melarsoprol alone or in situations where ornithine is unavailable (49).

Tsetse fly control programs are using insecticides, traps, and sterile insects to eliminate the flies that transmit the HAT-causing *T. brucei* subspecies to thereby reduce pathogen transmission and disease (http://www.africa-union.org/PATTEC/home.htm). Effective and economic tsetse control measures include the use of insecticides on cattle and for ground and aerial spraying (50, 51). The seeking of patients and treatment of infected persons by mobile teams are also key ways to control infection with *T. gambiense*, since humans are the main reservoir of this pathogen. Animal screening is used to control infection with *T. rhodesiense*, since wild and domestic animals are the crucial reservoirs. Chemoprophylaxis is not recommended due to drug toxicity and the relatively low risk of infection. Development of a vaccine for HAT is highly unlikely because these parasites make many thousands of antigenic variants by alternate expression and recombination of a repertoire of approximately 1,000 VSG-encoding genes, enabling them to evade the immune response.
**Chagas disease.** Despite advances in understanding the biology of *T. cruzi*, only the nitrofuran derivative nifurtimox and the nitroimidazole benznidazole are currently available for treatment of Chagas disease. Both are administered orally but have long treatment courses (≥60 days) and side effects (52), and there is variation in the sensitivity of the parasite to the drugs. Benznidazole shows efficacy against the indeterminate phase of infection in children (53). Observational studies have demonstrated the efficacy of benznidazole as a treatment for adults with Chagas disease who have been infected with the parasite for a long time (54–56), but the lower tolerance for drug side effects (52) in adults and the absence of randomized, placebo-controlled studies has adversely affected the frequency of use in adults. Certain antifungal triazoles and protease inhibitors have shown potential in experimental models of infection with *T. cruzi* (57–59), and late preclinical tests, lead optimization, and good manufacturing practice formulation of these drug leads in preparation for phase I clinical trials are in progress. The reduced transmission of Chagas disease due to intensified vector control in some regions has enhanced the focus on evaluation of drugs against indeterminate and early chronic phase disease.

Insecticidal spraying of houses in the Southern Cone of South America (the southernmost areas of South America, below the Tropic of Capricorn) (60) dramatically reduced transmission of *T. cruzi* in these areas. Although highly successful at eliminating domestic *Triatoma infestans*, the spraying had limited impact on *T. infestans* in peridomestic sites and on other vector species (61), and insecticide-resistant strains of *T. infestans* have emerged (62). Advances in the understanding of infection ecology and disease transmission (63) and the use of geographic information systems (GISs) and low-tech transmission control tools (e.g., insecticide-treated bed nets [ref. 64] and dog collars [ref. 65]) are promising for vector control. Effective disease control requires integrated sustainable programs that are customized to local environmental and sociocultural conditions and deployable at the village and regional levels.

Substantial progress has been made in understanding the types of immune responses that are crucial for control of infection with *T. cruzi* and in identifying the targets of those responses, information that is crucial if effective vaccines are to be developed (66). Proteins encoded by the trans-sialidase, mucin, and mucin-associated surface protein (MASP) gene families (each with 1,000 or more members) are prime targets of immune responses, but are problematic vaccine candidates, given their strain-to-strain variation (67). Testing a prophylactic vaccine for an infection that is difficult to document and is rarely detected until years, or even decades, after the initial infection also presents formidable practical and ethical issues. More innovative vaccine approaches, for example, the vaccination of companion animal reservoirs (68) and the development of therapeutic vaccines for individuals chronically infected with *T. cruzi*, deserve exploration.

**Leishmaniasis.** The standard drugs for the treatment of both VL and CL are the pentavalent antimonials, sodium stibogluconate and meglumine antimoniate. The introduction of generic brands has reduced costs. However, they require courses of 28 days of parenteral administration (either i.m. or i.v.), have known toxicities, and are almost obsolete for the treatment of VL in India due to the emergence of drug-resistant parasites (69). The antibiotic amphotericin B, previously a second-line drug, has moved to the forefront of treatment in India due to this resistance to antimonials. Of a range of amphotericin B lipid formulations developed for the treatment of systemic mycoses, the liposomal formulation AmBisome has proved to be the most effective treatment for VL, and a single dose of 5 mg/kg cured 90% of patients in India (70). However, even at a WHO-negotiated cost, high prices are projected to limit the use of this treatment (71). Paromomycin, an aminoglycoside, has efficacy as a topical treatment for CL and as a parenteral drug (administered i.m.) for VL, for which it was registered in India in 2006 following successful clinical trials (72–74). Its efficacy was essentially equivalent to that of amphotericin B but there were more adverse events (6% versus 2%) (72). Miltefosine, a phospholipid derivative and the first oral treatment for VL (75), is effective against CL (76) and was registered for these indications in India and Colombia in 2002 and 2005, respectively. However, its use is limited by potential teratogenicity.

Development of vaccines to protect against leishmaniasis seems feasible, in part because the mechanisms underlying protective immunity following infection with *Leishmania* spp. are relatively well understood and because antigens are highly conserved between species. Proof-of-concept has been demonstrated with crude vaccine preparations, particularly when applied as a therapy. Traditional vaccination against CL by inoculating exudate in inconspicuous body areas produces self-healing lesions. Vaccination trials with CL promastigote preparations had efficacies in protecting against initial infections ranging from 0% to 75% (77). Therapeutically, killed vaccines when used with chemotherapy had increased efficacy over drug alone. Killed *L. major* together with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) was used to treat post kala-azar dermal leishmaniasis (78, 79), and a killed parasite vaccine is registered in Brazil to treat drug-refractory ML (29, 80).

Acquired immunity directed toward *Leishmania* spp. is mediated by T cells (81–84). Specifically, CD4+ T cells are crucial for host defense, and mouse strains that are resistant to infection with *Leishmania* spp. develop a dominant Th1 response. Interfering with this response increases susceptibility to infection (85–88). In addition, IFN-γ-producing cells are associated with healing. In patients with VL, there is a direct correlation between IL-10 production and disease (89). Experimental animals can be protected against leishmaniasis using defined antigens (90), but few studies have used clinically relevant adjuvants. Indeed, the lack of safe and effective T cell adjuvants has been a major roadblock to the development of a vaccine to protect against infection with *Leishmania* spp. approved adjuvants alum and squalene induce potent antibody responses, which do not seem to mediate protection, and only weak Th1 responses. Protection against experimental leishmaniasis requires induction of IL-12 by APCs, achieved via stimulation of TLR4, TLR9, TLR7, or TLR8, and protection against experimental leishmaniasis requires TLR signaling and/or IL-1/IL-18 signaling (91, 92). A three-protein fusion, Leish-11 If, protects against CL and VL in mouse, hamster, and monkey models of these diseases (93–96), and a vaccine formulated in monophosphoryl lipid A is being evaluated in combined immuno- and chemotherapy trials (97).

Ideal anti-leishmanial vaccines should be safe, affordable, effective against multiple *Leishmania* spp., and effective as either a prophylactic or therapeutic. They should be based on recombinant antigens to provide for manufacturing consistency and have a clear path toward standardization and regulatory approval. Effective vaccines might be developed within the next 5–10 years. Their potential as therapeutics is particularly attractive, given the problems current therapies have of toxicity, expense, and tendency toward drug resistance (98).
Genomes, metabolism, and drug targets

As indicated earlier, each kinetoplastid has more than 8,000 genes with more than 6,000 orthologs in common. L. major has the fewest genes and T. cruzi the most, with the differences primarily due to large gene families. In addition to their common core genome, they have a similar unusual genomic organization (99). Most of their orthologous genes occur in clusters in the same order (i.e., they are syntenic) and on the same DNA strand. This organization mirrors their unusual characteristics of cotranscription and processing of adjacent gene transcripts by coordinated polyadenylation of the upstream transcript and capping by spliced leader addition to the downstream transcript. Their genomic organizations primarily differ in the arrangement of the syntenic clusters, which results in different chromosome numbers and sizes (99). The differences in gene content primarily localize to the syntenic cluster boundaries and to chromosome ends. The key gene content differences reflect critical features of these pathogens. T. brucei subspecies have numerous VSG-encoding genes in subtelomeric clusters and in minichromosomes reflecting their antigenic variation (99). A large proportion of the T. cruzi genome contains clusters of large gene families of surface proteins described above. These trans-sialidase, mucin, and MASP gene families might function in immune evasion and adaptation to an intracellular environment. They are expressed in the mammalian life cycle stages and are among the strongest targets of host immune responses, but their abundance and diversity might present problems as vaccine candidates. Leishmania spp. have a simpler genome but also have the ability to amplify genomic regions. They contain genes for synthesis of complex surface glycoconjugates that are likely to provide for survival in the macrophage phagolysosome.

The capacity for homologous recombination has been exploited for experimental genetic manipulation of kinetoplastids to reveal gene function (100). All T. brucei subspecies and some Leishmania spp. possess RNAi ability, the function of which is uncertain, especially since it is dispensable within other Leishmania spp. and T. cruzi. Kinetoplastids have distinctive molecular processes that include adding an unusually capped 39nt sequence to the 5’ end of all mRNAs, reprogramming most mitochondrial mRNA sequences by RNA editing (101), and relying heavily on RNA turnover and translational level control rather than transcription initiation to regulate gene expression. T. brucei expresses only one of several VSG expression sites at any time, periodically switches expression to a different site, and employs complex recombinations among its more than 1,000 VSG-encoding genes and pseudogenes to generate a staggering repertoire of antigenic variants (102, 100). T. cruzi might undergo analogous recombination processes. These gene families, distinct molecular processes, as well as other factors yet to be discovered are potential new drug targets.

Analysis of the genomes of L. major, T. cruzi, and T. brucei, the three trypanosomatids (also known as TriTryp) (103), has revealed many common core metabolic functions as well as other pathways that might be specific adaptations to environments associated with life in their respective insect and vertebrate hosts. For example, only L. major seems capable of hydrolyzing disaccharides, possibly because the sandfly vectors of L. major feed on nectar and honeydew. Likewise, only T. cruzi has the potential to convert histidine to glutamate (104), reflecting the abundance of histidine in the excretory and hemolymph of its vector. Similarly, only T. cruzi lacks de novo pathways for polyamine biosynthesis, since polyamines can be obtained from the vector’s excreta or from the host cytoplasm. T. cruzi therefore lacks ornithine decarboxylase, the enzyme that mediates the first step in the polyamine biosynthesis pathway, which is the target of the drug efornithine used to treat second-stage HAT caused by infection with T.b. gambiense (105). Other aspects of these fascinating metabolic adaptations, including how lateral gene transfer from prokaryotes might have shaped metabolism, are reviewed elsewhere (103, 106, 107).

The completion of the TriTryp genomes offers unparalleled opportunities for the identification of much-needed novel drug targets (103). New targets have already emerged from genome analyses as well as phenotypic screens (108). In an ideal world, these would be common to all three disease-causing pathogens, sufficiently different from the mammalian host or unique to the parasite, and certainly essential for growth or survival of the parasite during the mammalian stages of its life cycle (109, 110). Some candidates are to be found in biosynthetic pathways for fatty acids (111), glycosylphosphatidylinositol anchors (112, 113), ergosterol and isopenrenoids (114), as well as in the metabolism and functions of folates, peritins (115, 116), and trypanothione (117).

Other promising areas for intervention include protein farnesyl transferases (118), cysteine proteases (108), N-myristoyltransferase (119), tubulin biosynthesis (120), S-adenosylmethionine and polyamine metabolism (105), purine salvage (121), protein kinases (122), DNA topoisomerases (123), and RNA-editing enzymes (124, 125). To further exploit the TriTryp genomes, the Special Programme for Research and Training in Tropical Diseases (TDR) has recently established the TDR Targets Database, in which the genes from these and other pathogens can be queried and ranked according to different “druggability” criteria (http://tdrtargets.org/).

Identification and characterization of molecular targets is merely a first step. Targets need to be validated as essential for parasite growth or survival using gene knockouts or knockdown technologies and/or using highly specific small molecule inhibitors (110). Current estimates suggest that about 8%–12% of genes in mammalian, insect, helminth, and fungal genomes are druggable, that is, able to bind drug-like small molecules (126). Validating as druggable approximately 800 genes per parasite is a formidable but feasible challenge, even if other targets and pathogenesis determinants are discovered in the approximately 50% of hypothetical proteins in these genomes. However, since drug discovery and development is a risky and expensive undertaking, genetically and/or chemically validated targets also need to be rigorously assessed for chances of success by ranking against additional criteria such as druggability, assay feasibility, toxicity, and potential for the emergence of drug resistance (108, 127, 110).

Perspective and key issues

New tools and efforts are needed to combat diseases caused by infection with a kinetoplastid. The diagnostics are inadequate, and in some cases primitive, the drug arsenal is ancient, blunt, and dangerous, and vaccines are essentially nonexistent. However, the progress in understanding kinetoplastid pathogens and their effects on their hosts, fueled by advances in technologies and increased public awareness, makes this a propitious time to develop the needed tools and interventions. Indeed, the elimination of HAT and Chagas disease as public health problems has been set as a goal by the WHO (128), and the elimination of VL, at least from the Indian subcontinent, is considered technically feasible and operationally achievable (129). Ironically, kinetoplastid diseases of livestock (e.g., trypanosomiasis in cattle) and domestic animals
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