Seminar

The trypanosomiases

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The trypanosomiases consist of a group of important animal and human diseases caused by parasitic protozoa of the genus *Trypanosoma*. In sub-Saharan Africa, the final decade of the 20th century witnessed an alarming resurgence in sleeping sickness (human African trypanosomiasis). In South and Central America, Chagas' disease (American trypanosomiasis) remains one of the most prevalent infectious diseases. Arthropod vectors transmit African and American trypanosomiases, and disease containment through insect control programmes is an achievable goal. Chemotherapy is available for both diseases, but existing drugs are far from ideal. The trypanosomes are some of the earliest diverging members of the Eukaryotae and share several biochemical peculiarities that have stimulated research into new drug targets. However, differences in the ways in which trypanosome species interact with their hosts have frustrated efforts to design drugs effective against both species. Growth in recognition of these neglected diseases might result in progress towards control through increased funding for drug development and vector elimination.

Parasitic protozoa infect hundreds of millions of people every year and are collectively some of the most important causes of human misery. The protozoan order Kinetoplastida includes the genus *Trypanosoma,* species that cause some of the most neglected human diseases.

There are many species of trypanosome, and the group infects most vertebrate genera. Several trypanosome species cause important veterinary diseases, but only two cause significant human diseases (table).[1] In sub-Saharan Africa, *Trypanosoma brucei* causes sleeping sickness or human African trypanosomiasis,[2] and in America, *Trypanosoma cruzi* causes Chagas' disease (figure 1).[3] Both diseases have been considerably neglected, disproportionately affecting poor and marginalised populations. Despite this neglect, the basic biology of trypanosomes has been the subject of intense study. The kinetoplastida also contains species of the genus *Leishmania* that cause a range of diseases in the tropics and subtropics.[4] These evolutionarily ancient eukaryotic organisms display many fascinating and unique molecular and biochemical phenomena. A key challenge facing trypanosome research is to exploit this knowledge in clinical advances. A substantial amount of similarity exists between these organisms: both are transmitted by insect vectors and share many aspects of their basic biochemical physiology. However, profound differences at the level of the host-parasite interface have frustrated most efforts to use information gained about one species to assist in control of the other.

The aim of this article is to compare two closely related parasites of major public health importance by outlining similarities and discrepancies in their biology, the diseases they cause, and approaches to their treatment and control.

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The parasites and their vectors

Superficially, there are many similarities between trypanosome species and the diseases they cause (table). Both are single-celled flagellates (figure 2) that are transmitted by insect vectors (figure 3). They share phases of local multiplication in their human host followed by dissemination and localisation in target organs, in which they cause potentially lethal damage. However, key differences between the organisms exist, which can account for why clinical manifestations and susceptibility to treatment differ.

Evolutionary history

Comparison of 18S rRNA gene sequences from multiple trypanosome species obtained from diverse hosts, combined with other molecular approaches, suggests that the genus *Trypanosoma* is monophyletic.[5] By superimposing estimates with the molecular clock on vicariance biogeography, it is suggested that *T brucei* and *T cruzi* shared a common ancestor around 100 million years ago.[6]

This prehistoric dating indicates that human beings were exposed to African trypanosomes concomitantly with their evolution. However, American trypanosomes evolved independently of *Homo sapiens*, who probably joined the host range of *T cruzi* only within the past 12 000–40 000 years, when man arrived on the American continent. *T cruzi* DNA has been identified in mummified human beings in South America dating back 4000 years.[7]

Search strategy and selection criteria

PubMed Central and ISI Web of Science were searched, with the keywords: 'trypanosomiasis', 'sleeping sickness', 'Chagas disease', '*T brucei*', and '*T cruzi*'. Preference was given to reviews that encompassed much of the primary published work on the findings that have led to the prevailing picture of these diseases. WHO websites for Chagas' disease (http://www.who.int/ctd/chagas/index.html; accessed Aug 20, 2003) and human African trypanosomiasis (http://www.who.int/health-topics/afrtryps.htm; accessed Aug 20, 2003) contain many statistics and are frequently updated. WHO/TDR websites for both diseases are also very informative (http://www.who.int/tdr; accessed Aug 20, 2003).

Feature	Human African trypanosomiasis	Chagas' disease
Number of infected people	0·5 million	11–18 million
Deaths per year	50 000	13 000
Disability adjusted life-years	1 598 000	649 000
Current trends	Rising prevalence	Falling prevalence
Distribution	Sub-Saharan Africa	Central and South America
Causative organisms	*T brucei gambiense*; *T brucei rhodesiense*	*T cruzi*
Vector	Mainly *Glossina fuscipes* group (gambiense); mainly *Glossina morsitans* group tsetse fly (rhodesiense)	Triatomine bugs
Natural habitat	Forested rivers and shores (gambiense); savannah (rhodesiense)	Usually peridomestic in roofs and palm trees
Natural hosts	Predominantly anthroponosis (gambiense); large range of ungulates and other mammals (rhodesiense)	Large range of mammals

Features of human African trypanosomiasis and Chagas' disease[1]

Epidemiology and transmission

T brucei and *T cruzi* are transmitted by biting insects, but a fundamental difference between the means of transmission has been incorporated into the classification of these organisms. *T brucei* are known as salivaria because they are transmitted in tsetse saliva. *T cruzi* belongs to the stercoraria because transmission is via vector faeces.

Transmission of both species can also be via blood transfusion, contaminated needles, or the congenital route. Rarely, transmission of *T cruzi* by breastfeeding or through contaminated food has been reported.[8]

Trypanosoma brucei

Trypanosoma brucei is divided into three subspecies. Only two cause human African trypanosomiasis.[9] In west and central Africa, *T brucei gambiense* causes a chronic form of sleeping sickness. In east and southern Africa, *T brucei rhodesiense* causes an acute form. Natural tsetse-mediated transmission of both subspecies happens only in Uganda. Infection with either subspecies is uniformly fatal if untreated. *T brucei brucei* is not infectious to human beings.

Results of molecular studies are clarifying the picture with respect to the different subspecies.[10–12] *T brucei gambiense* (type 1) is genetically distinct from *T brucei brucei* and *T brucei rhodesiense*, which can be judged host-range variants of local *T brucei brucei* populations. Type 2 *T brucei gambiense* resembles *T brucei brucei*. Human infective parasites resist lysis by a component of the HDL fraction termed trypanosome lytic factor,[13–15] which has been identified as apolipoprotein L1.[16] The gene *SRA* (encoding a serum resistance associated protein)[17] is expressed from a variant surface glycoprotein gene expression site in *T brucei rhodesiense* but not *T brucei brucei*.[18] The expressed gene can identify rhodesiense group trypanosomes infectious to human beings that are present in animal populations.[19] *T brucei gambiense* does not contain the *SRA* gene, suggesting that this organism resists lysis through a different mechanism.

All *T brucei* group organisms are transmitted by tsetse flies of the genus *Glossina* (order Diptera) (figure 3). Tsetse flies are found exclusively in Africa in a belt that stretches south of the Sahara and north of the Kalahari desert (between 14° North and 29° South of the equator; figure 1). As a result of tsetse occurrence, human African trypanosomiasis has a spatially discrete distribution.[9] Nearly 300 separate active foci are recognised, and some 60 million people are judged at risk in 36 of Africa's 52 countries.[20] Most foci are in rural areas, so substantial under-reporting of the disease takes place. Fewer than 10% of the population in endemic regions are screened, and WHO estimated that in 1998, only 27 000 of an estimated 300 000 cases were reported in the Democratic Republic of Congo alone. Similarly, only 8000 cases were reported in Angola, although observers there expect about 100 000 untreated cases to arise. Presently, WHO estimates that about half a million people carry this potentially fatal infection, although absence of reporting makes estimates difficult, and other observers deem this value to be an overestimate.[9] Irrespective of the precise numbers, the resurgence of human African trypanosomiasis in the latter

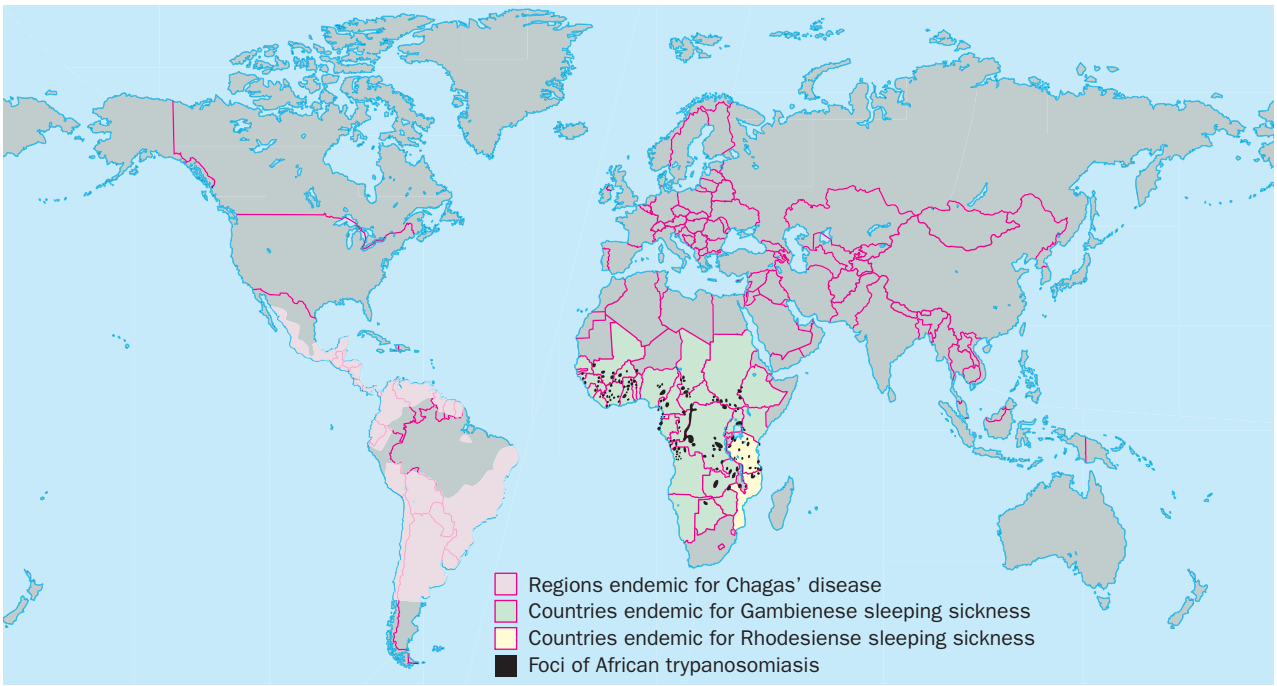


Figure 1: **Geographic distribution of *T brucei* and *T cruzi*, showing endemic countries harbouring these diseases**

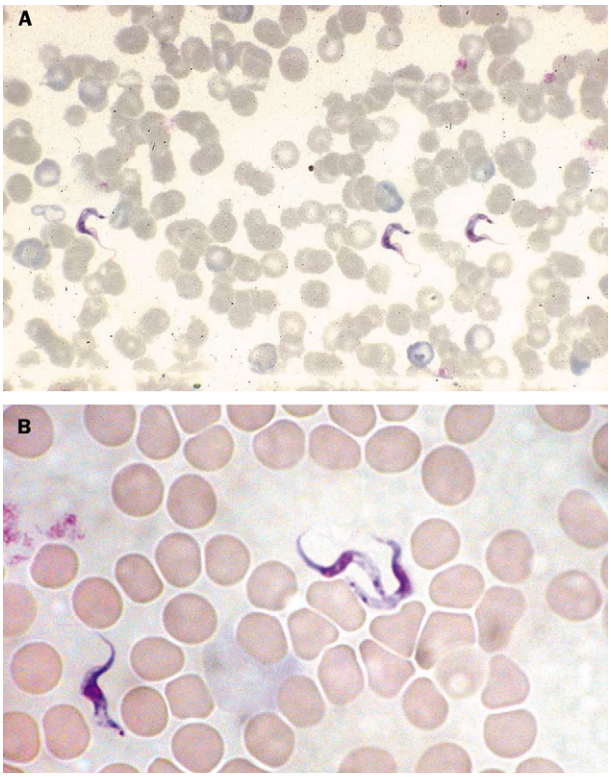


Figure 2: **Blood films with (A) *T cruzi* trypomastigotes and (B) *T brucei* trypomastigotes**

part of the 20th century—after it had been brought largely under control by 1960—has been alarming, and the disease is judged a category 1 resurgent disease by WHO/TDR (WHO special programme for research and training in tropical diseases).[21]

Trypanosoma cruzi

In 1985, WHO estimated that about 100 million people in Latin America were at risk of acquiring Chagas' disease, with a prevalence of human *T cruzi* infection estimated at 18 million cases.[8,22] Since 15–30% of the infected population develops overt clinical manifestations, about 5 million people can be assumed to have clinical changes attributable to Chagas' disease today. Successful programmes in vector control have led to a decline in transmission in recent years (see later).

T cruzi is transmitted by bugs belonging to the subfamily Triatominae in the family Reduviidae (order Hemiptera; figure 3). Synanthropic *Triatoma infestans*, *Rhodnius prolixus*, and *Panstrongylus megistus* are the most important vectors.

Only one lineage of *T cruzi* is recognised to cause Chagas' disease, although molecular typing of the parasite distinguishes at least two lineages, type 1 and type 2.[23] Clonal population structures have been suggested to act as a constraint on genetic divergence,[24] although demonstration that *T cruzi* can engage in genetic exchange within mammalian hosts[25] emphasises the need for caution in interpreting data on the population structures of these organisms. Type 1 *T cruzi* is widespread in mammalian hosts within a sylvatic (wild) cycle, whereas type 2 is more restricted in its host range to only a few mammalian species in a peridomestic habitat. Type 2 parasites are the major cause of Chagas' disease.[3,8]

The diseases

Human African trypanosomiasis and Chagas' disease are both chronic diseases that undergo distinct stages in their natural course. Both are potentially fatal. Sterilising acquired immunity does not exist after natural infection, and there are no vaccines.

Any pathogen must evade host-cell immunity to establish infection. In the mammalian host, by contrast with *T cruzi* and many other pathogenic protozoa that adopt an intracellular existence, which protects them from humoral immunity, African trypanosomes remain exclusively extracellular. They are fully exposed to the host's immune response. A dense, highly immunogenic, glycoprotein coat protects against complement-mediated lysis.[26] Once specific antibody maturation has taken place, immunoglobulins lyse trypanosomes bearing the same surface coat. However, a small percentage of every new parasite generation switches to a new antigenically distinct glycoprotein.[27] Up to 1000 different genes encoding these so-called variant surface glycoproteins are present in the *T brucei* genome.[28] Sequential expression of genes encoding these glycoproteins produces antigenically distinct parasite populations, allowing survival in the mammalian host. Variant surface glycoprotein gene expression has led to novel ideas for studies on monoallelic gene expression in eukaryotes.[26–28]

Polyclonal B-cell activation and raised IgM production are characteristic of human African trypanosomiasis. These reactions could be associated with responses to variant surface glycoproteins and might relate to general immunosuppression associated with the disease. Polyclonal lymphocyte activation is also associated with *T cruzi* infection; an unusual parasite-derived proline racemase activity has been associated with this effect,[29] although little is known on molecular mechanisms linking these common events in both trypanosome species.

After entry into human beings, *T cruzi* invade host cells. Macrophages have an important early role in reacting to infection and in carrying parasites to other sites within the body. *T cruzi* trypomastigotes differentiate into replicative amastigotes within cells before differentiating back to bloodstream-form trypomastigotes that leave one cell type and invade others. The surface of the parasite is covered by mucin-type glycoproteins that attach to the membrane by glycosylphosphatidylinositol (GPI) anchors similar to those of the variant surface glycoproteins in African trypanosomes.[30,31] Mucins are the major recipients of the sialic acid transferred by surface trans-sialidase.[30] *T cruzi* has several hundred mucin genes, and the exposed N-terminal moiety of the molecules belonging to one of the mucin families is hypervariable.[32] Because the African trypanosome has received lots of attention with respect to antigenic variation, the mucins of *T cruzi* will inevitably be postulated to have a role in diversion of the immune response of the host, in addition to roles in protection of the parasite's surface and in cell adhesion.

T cruzi-derived GPI anchors can activate host macrophages.[33] *T cruzi* is a potent stimulator of cell-mediated immunity, and induction of macrophage proinflammatory cytokines is judged important in control of infection and outcome of Chagas' disease. Variant surface glycoprotein GPI anchors from African trypanosomes have been suggested to have a similar role,[34] although other events could underlie endotoxin-type reactions during *T brucei* infection.[35]

Pathology and diagnosis

Human African trypanosomiasis

This disorder is classed as stage 1 or 2 depending on whether parasites have become manifest in the cerebrospinal fluid. The pathology has been reviewed elsewhere.[2,36–38]

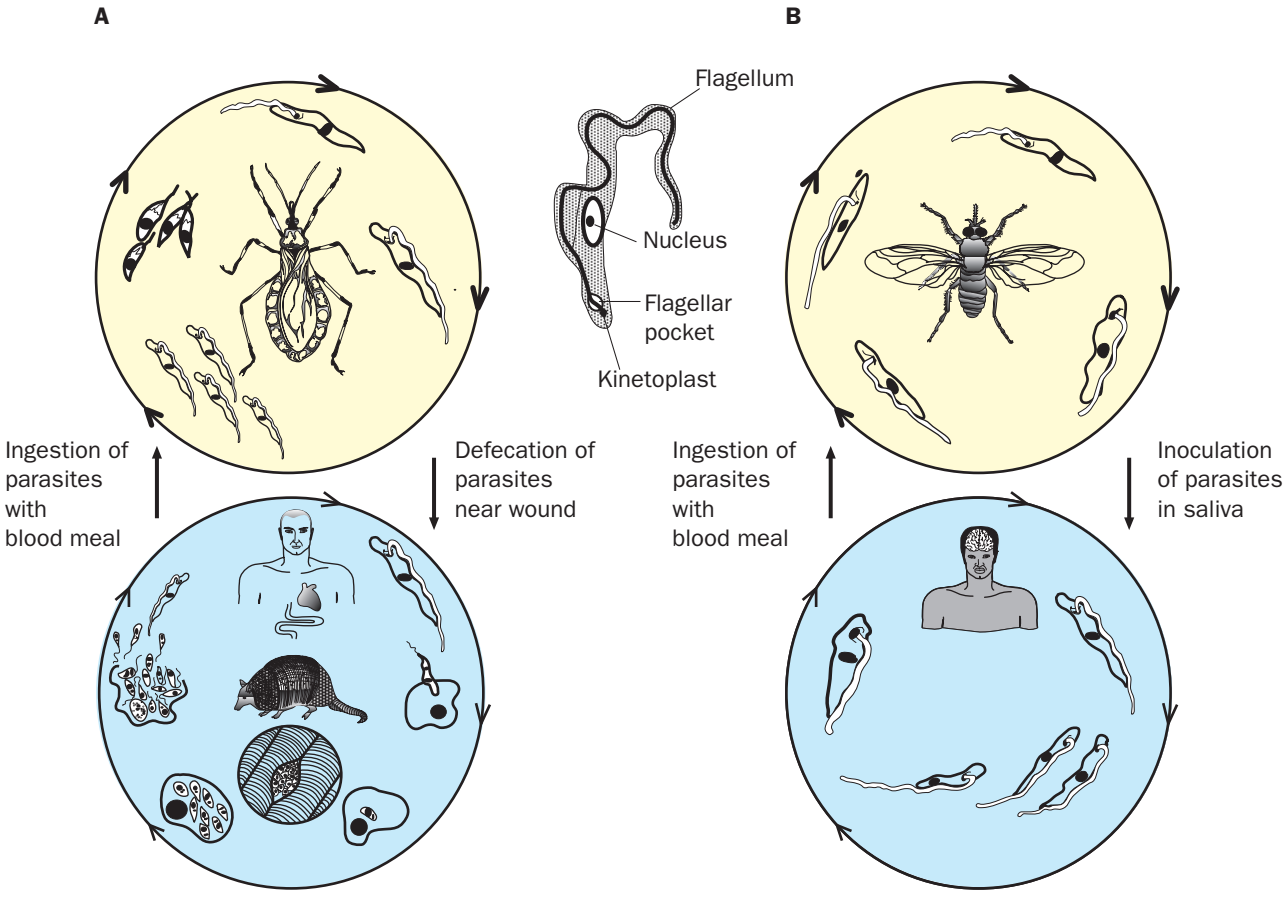


Figure 3: **Life cycles of (A) *T cruzi* and (B) *T brucei***
Upper cycles represent different stages that take place in the insect vectors. Lower cycles represent different stages in man and other mammalian hosts. Cellular invasion and presence of clusters of amastigotes in striated muscle is shown for *T cruzi*. In human beings, the main organs involved in pathogenesis are shown.

Stage 1 disease

After their inoculation, parasites proliferate at the site of infection, leading to an inflammatory nodule or ulcer. This trypanosomal chancre (figure 4) arises in about 50% of all rhodesiense—but rarely in gambiense—infections. After 3–4 weeks, the chancre usually heals with overlying desquamation, sometimes with altered pigmentation. Parasites spread to the draining lymph node and reach the bloodstream, initiating the haemolymphatic stage of the disease. This stage is characterised by general malaise, headache, and fever of an undulating type. In rhodesiense infection, with its more acute course, pancarditis with congestive heart failure, pericardial effusion, and pulmonary oedema can cause fatalities at this early stage, whereas gambiense infection shows a more insidious development that is frequently unrecognised or misdiagnosed. A typical sign of gambiense human African trypanosomiasis is generalised lymphadenopathy that develops after several weeks, frequently in the posterior triangle of the neck. Thomas Masterman Winterbottom noted that slave traders in the late 18th century used neck swellings as an indicator of the sleepy distemper rendering particular slaves undesirable. Winterbottom's sign (figure 5) is still used to describe posterior cervical lymphadenopathy. Other non-specific features of stage 1 human African trypanosomiasis are a circinate rash, pruritus, and generalised oedema.

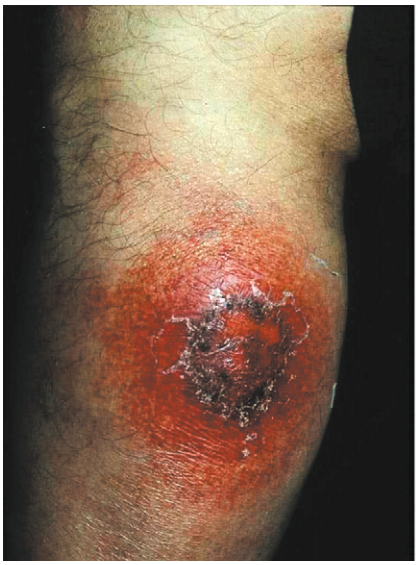
At this stage, parasites can be detected in blood, lymph, or tissue aspirates. However, they are usually below detection levels, especially in gambiense infection, even if concentration methods like microhaematocrit centrifugation, quantitative buffy-coat analysis, or mini-anion exchange columns, which are presently deemed the best way of concentrating trypanosomes[39] to view microscopically, are used. The card agglutination test for trypanosomiasis, which assays for anti-*T brucei gambiense* antibodies in serum, is highly sensitive for gambiense infection,[40] but microscopic visualisation should accompany the technique.

PCR-based approaches are undergoing development[41] and evaluation. However, standardised conditions have yet to be established, and logistical difficulties mean that technical advances will be needed before PCR-based assays can enter routine field use.

Stage 2 disease

In the second stage of the disease, parasites invade internal organs, including the CNS.[42–45] This event happens within a few weeks of infection with *T brucei rhodesiense* but over a period lasting between several months and years with *T brucei gambiense*. Immunosuppression accompanies this stage, with nitric oxide and prostaglandin apparently involved early, and cytokines including interleukin 10—which reaches amplified concentrations even in the cerebrospinal fluid—later. How and why parasites invade the CNS is not clear, although the portal of entry is probably through the choroid plexus.

As stage 2 disease progresses (figure 6), headache becomes severe and sleep disorders—predominantly caused by a disturbance in the circadian rhythms of normal sleep—become manifest.[46] The name sleeping sickness was coined because of early recognition of diurnal somnolence and nocturnal insomnia in some patients, although overall sleep



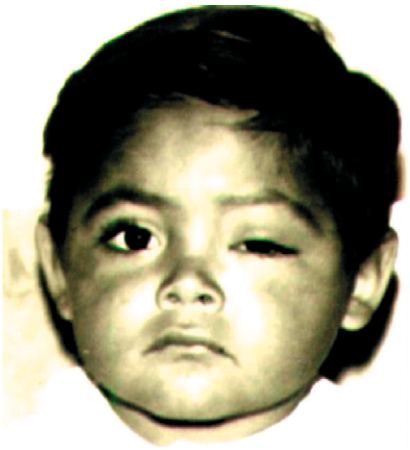


Figure 4: **Trypanosomal chancre (A) and Romaña's sign (B)**
(B) Courtesy of Humberto Lugones, Santiago del Estero, Argentina.

time is unaltered. Personality changes can be striking and mental functions become increasingly impaired. A specific focal neurological pattern has not been described, although an ataxic dyskinesia is present in most patients. Basal ganglia involvement with clinical features that overlap with Parkinson's disease is usual and confirmed in case reports with MRI. Weight loss and endocrine abnormalities, including impotence, are also typical. Progressive CNS involvement culminates in coma and then death in untreated cases.

Histopathological changes include leukoencephalitis with demyelination and accentuation of the periventricular regions. Demyelination might have an autoimmune basis,[47] which can make cure—even once parasites have been pharmacologically removed—impossible once this point has been reached. The main characteristic is infiltration of lymphocytes and plasma cells around cerebral vessels (perivascular cuffing).

Trypanosomes themselves are infrequently seen in the cerebrospinal fluid, even if concentration techniques such as double centrifugation improve diagnostic sensitivity. The host's inflammatory response is a major pathophysiological factor and is also the main diagnostic indicator. IgM concentrations are high.[39,40] Lymphocyte counts are usually high ($>5\times10^6$/L), foamy plasma cells (pathognomonic Mott's morular cells) might be seen, and cerebrospinal fluid protein is increased (>25 mg per 100 mL). Actual cutoffs to define stage 2 disease for cells and protein are still debated.

Chagas' disease

Acute stage

T cruzi is usually transmitted as infected triatomine vectors gorge themselves on blood from sleeping hosts. Faeces, deposited by the insect during feeding, contains metacyclic trypomastigotes. Itching produced by the vector's bite induces the individual to scratch, introducing micro-abrasions that allow the trypomastigotes to breech the skin and invade host cells.[3,8]

Clinical manifestations of the acute stage begin 6–10 days after infection and last 1–2 months.[3] Frequently, inflammation at the site of infection leads to an oedematous swelling known as a chagoma. Romaña's sign (figure 4) entails swelling of the eyelids after infection at this site and allows facile symptomatic diagnosis in up to half of those cases showing overt manifestations of acute disease.

Most acute cases arise before the age of 15 years, mainly between ages 1 and 5 years. Usually, the acute phase passes unnoticed, with many symptoms typical of general malaise noted in other infections. However, death can ensue during this stage in a few cases, usually from myocarditis or meningo-encephalitis, complications that are reminiscent of *T brucei rhodesiense* infection. Hepatosplenomegaly and lymphadenopathy, fever and rash are also frequently seen. Oedema of the face and elsewhere is typical. The electrocardiogram can show abnormalities, including sinus tachycardia, prolongation of the atrioventricular interval, and primary T-wave changes.[3,48] Autoimmune responses to many host antigens have been identified, although the origins and role of these in pathology is not certain and highly controversial.[49–53]

During the acute stage, parasites can be easily detected in peripheral blood.[54] This phase of the disease ends when the immunological balance between the host and the parasite greatly reduces the number of circulating trypomastigotes, rendering direct parasitological diagnosis impossible.

Chronic stage

After the acute phase, patients become asymptomatic, with typical findings on electrocardiography and chest radiography. About 70–85% of infected people continue in this state, known as the indeterminate form of chronic Chagas' disease, for the rest of their lives. However, 15–30% will develop manifestations of organ damage, producing the cardiac, digestive, or nervous forms of chronic Chagas' disease 10–25 years after initial infection. This occurrence is especially typical in men 20–45 years of age. Chest pain, palpitations, dizziness, and peripheral oedema are usual. Arrhythmia, thromboembolism, heart failure, and sudden death are also frequent outcomes.

Chest radiography exposes cardiomegaly associated with differing degrees of four-chamber dilatation and contrasting with an absence of pulmonary congestion due to right ventricular failure (figure 7). Echocardiography also shows changes to the heart, and the electrocardiogram presents typical changes that allow diagnosis. Right bundle branch block, alone or with accompanying left anterior hemiblock, is distinctive. Q waves, pronounced alterations of ventricular repolarisation, and different degrees of atrioventricular conduction defects are also generally seen.

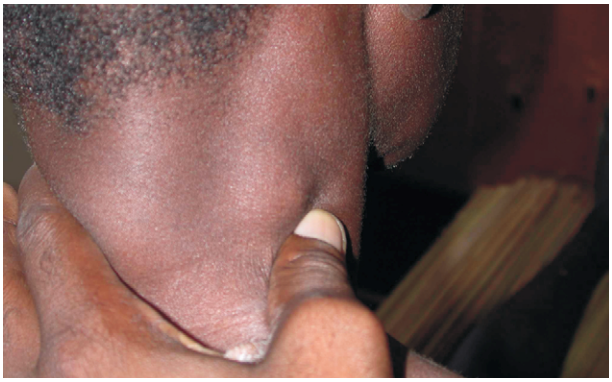


Figure 5: **Winterbottom's sign**

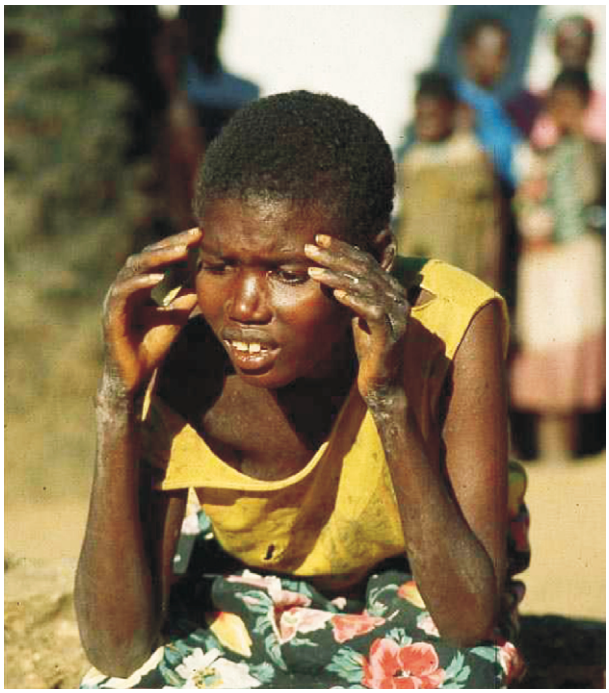


Figure 6: **A patient with stage 2 human African trypanosomiasis**

Chagas' disease presents a unique variety and density of cardiac arrhythmias. Polymorphous ventricular extrasystoles and tachycardia are typical, as well as complete atrioventricular block or sick sinus syndrome, which predisposes to syncope or sudden death.

With pronounced geographic differences, Chagas' disease also produces megaviscera, mainly megaoesophagus and megacolon. Megaoesophagus, in its advanced form, presents as dysphagia. In its initial stages, the disorder is diagnosed by contrasted radiographic studies (figure 7). Megacolon produces different degrees of constipation. When complicated by a faecaloma, the disorder is easily recognised by external examination because of its particular consistency. Barium enema is the best method to detect megacolon. A complication of this disorder is torsion of the dilated part of the intestine causing an emergency situation.

In addition to symptomatic diagnosis, serological diagnosis is routinely undertaken. IgM and IgG antibodies are raised in the acute and chronic phases, respectively, and are routinely exploited in serological diagnosis. Indirect haemagglutination, indirect immunofluorescence, and ELISA techniques are all used. PCR techniques have also been assessed,[55] and they are starting to be available for routine diagnosis. Xenodiagnosis—a technique that entails feeding blood of patients to laboratory reared nymphs of the triatome bugs followed by investigation of insect faeces for parasites over 90 days—and haemoculture are used as classic indirect parasitological methods.

Case management of trypanosomiases

Drugs are available for both human African trypanosomiasis[56–59] and Chagas' disease[60,61] (panel 1). Although available drugs are useful in management of both diseases, new and better drugs are urgently needed.

Treatment of human African trypanosomiasis

Four drugs are licensed for treatment of this disorder.[56] Two of these, pentamidine and suramin, are used before CNS involvement. Against late-stage disease, first-line treatment is melarsoprol. Eflornithine is only useful against *T brucei gambiense* (see later); it has few side-effects, but the requirement for large doses limits its use. Nifurtimox, although only licensed for use against Chagas' disease, has been shown to have limited efficacy against late-stage melarsoprol-refractory human African trypanosomiasis. Diminazene (berenil), a veterinary drug, has also been used occasionally without licence.

All the presently used drugs can induce adverse effects. Melarsoprol, for example, causes a reactive encephalopathy in about a fifth of all patients receiving treatment. A substantial proportion (2–12%) dies as a result of treatment.[62]

Efforts are presently being made to make better use of current drugs. New regimens of melarsoprol[63] and eflornithine[64] have been tested. A shorter treatment regimen of melarsoprol was just as effective as the traditional course but needed reduced admission times and resulted in greater patient adherence.

Use of combinations of known trypanocides might allow reduction in dose of individual drugs, thereby lessening dose-related side-effects and extending the limited supplies of drugs.[65] However, only few clinical trials have been done with combination regimens.[56,59,66,67]

In addition to the correct choice and application of trypanocidal drugs, effective management of patients with human African trypanosomiasis needs skilled nursing staff guided by medical carers who are familiar with the complications of the disease and its treatment. For example, in our experience, standardised treatment protocols that include attention to good calorific intake, antimalarial treatment, and micronutrient supplementation before starting treatment help improve patients' care. However, even these simple measures are hard to achieve in many disease-endemic regions.

Drug resistance

The present epidemics of human African trypanosomiasis are characterised by unusually high rates of relapse after melarsoprol treatment.[68,69] Treatment failure might indicate the emergence of drug-resistance. Cross-resistance between melamine-based arsenicals and diamidines is easy to select in the laboratory when changes to a transporter that carries these drugs become evident.[70] However, the link between resistance and treatment failure has yet to be established unambiguously.[69]

Drug supply

The pharmaceutical industry in general sees little economic incentive to engage in research, development, and production of new compounds against human African trypanosomiasis. In May, 2001, after much pressure from international and non-governmental organisations (NGOs),[71,72] representatives of the pharmaceutical company Aventis signed an agreement with WHO to guarantee a gratis production of pentamidine, melarsoprol and eflornithine for at least 5 years.[73] The agreement also provided unprecedented support for research towards new drugs and other control measures.[74] Médecins Sans Frontières (MSF) took over the logistical part of storage and transport of the drugs. Shortly afterwards, Bayer joined this public-private-partnership, providing suramin and reconsidering a decision to halt production of nifurtimox, for which a licence extension for use against human African trypanosomiasis is presently being pursued through WHO and Bayer. This achievement, for which WHO, NGOs, and the pharmaceutical industry have joined forces to tackle a common goal, is very encouraging, and could serve as an example for successful approaches in the fight against other tropical diseases. However, the infrastructure to distribute

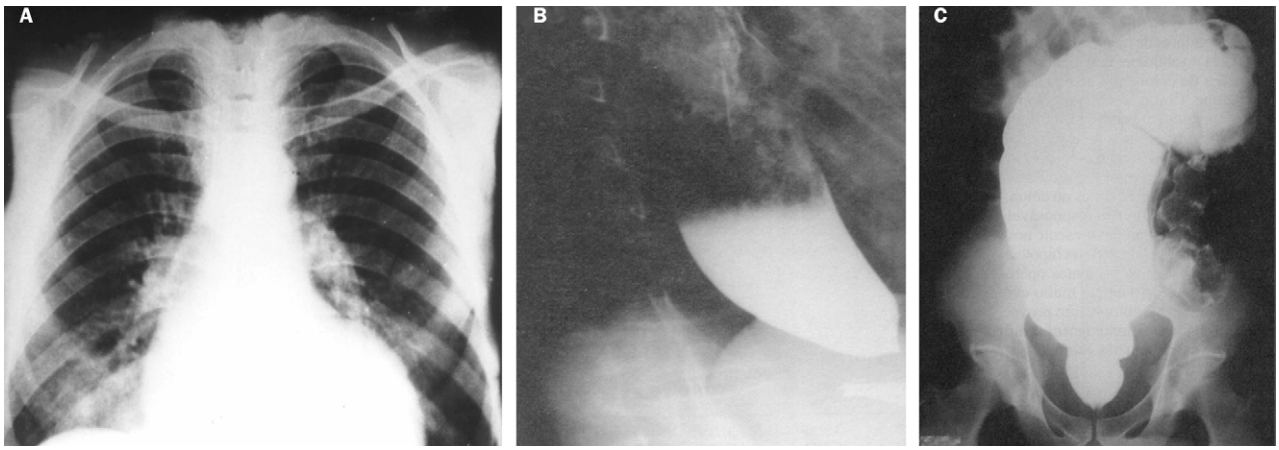


Figure 7: **Radiographs of pathological findings of Chagas' disease**
(A) Enlarged heart, (B) megaoesophagous, and (C) megacolon.

drugs in endemic regions is also poor and needs major improvement. Furthermore, new drugs and treatment ideas are urgently needed to cater for longer term management. The launch by MSF of their Drugs for Neglected Diseases Initiative offers hope that additional support of new drugs might be found.[75]

Treatment of Chagas' disease

Chagas' disease therapy has depended on two nitroheterocyclic drugs: nifurtimox and benznidazole. Nifurtimox, a nitrofuran, was introduced in the 1960s to treat acute Chagas' disease. The drug is trypanocidal, mainly against circulating trypomastigotes, and is better tolerated by young patients. Nifurtimox has not been widely used for Chagas' disease treatment for some years, because its manufacturer halted production in view of an apparent market advantage enjoyed by benznidazole. However, the prospect of a licence extension for nifurtimox for use in human African trypanosomiasis treatment has meant that manufacture will continue, at least temporarily, and the drug could receive a new lease of life for Chagas' disease treatment. Benznidazole, a nitroimidazole, was launched in the 1970s.

Panel 1: **Drugs**

Stage 1 human African trypanosomiasis

Pentamidine
4 mg/kg intramuscularly daily or alternate days for 7–10 injections

Suramin
Day 1: test dose of 4–5 mg/kg
Days 3, 10, 17, 24, and 31: 20 mg/kg, 1 g maximum dose per injection

Stage 2 human African trypanosomiasis

Melarsoprol
Day 1: 1·2 mg/kg
Day 2: 2·4 mg/kg
Days 3 and 4: 3·6 mg/kg repeat course two or three times with 7–10 day interval

Melarsoprol (new short regimen)
Days 1–10: 2·2 mg/kg

Eflornithine
100 mg/kg at 6 h intervals for 14 days (a 7-day schedule of the same dose is recommended for melarsoprol relapses)

Acute Chagas' disease

Benznidazole
5–10 mg/kg per day in two divided doses for 30–60 days

Nifurtimox
8–10 mg/kg in three divided doses after meals for 30–120 days

Both drugs occasionally induce serious side-effects, and neither has high efficacy against chronic Chagas' disease. The need for new drugs is urgent. Results of studies showed that benznidazole depleted anti-*T cruzi* reactivity in specific serological tests[76] and lowered prevalence of heart alterations in up to 62% of schoolchildren in the early chronic stage of the disease.[77] These findings prompted assessment of treatment in patients during the indeterminate and cardiac forms of chronic Chagas' disease, with promising preliminary results. The realisation that parasite persistence is a key feature of chronic disease[53] has reinvigorated research into the benefits of treatment at this stage.

Treatment of the symptomatic complications of Chagas' disease is also effective in improvement of quality of life.[78] Treatment of heart failure based on salt restriction, diuretics, digitalis, angiotensin-converting-enzyme inhibitors and β blockers is also used. Atrial fibrillation needs anticoagulant treatment if available. Sick sinus syndrome, ventricular arrythmias, and severe atrioventricular conduction defects are successfully treated with powerful antiarrythmic drug regimens, pacemaker or automatic cardiodefibrillator implantation. Heart transplantation is a therapeutic option for patients with advanced chagasic cardiomyopathy. Surgical removal of affected areas of oesophagus and colon in the megasyndromes is also practised.

Prospects for new drugs

Biochemical pathways general to trypanosomatids but absent from mammalian hosts have long been regarded as potential drug targets.[79] Differences between *T brucei* and *T cruzi* at the level of the host-parasite interaction, however, lead to huge variation in the pharmacological criteria needed for agents used against the different diseases.[80] Human African trypanosomiasis is seldom diagnosed before the late stage, so drugs must cross the blood-brain barrier to be effective. To target *T cruzi* amastigotes, drugs must also enter many mammalian cell types, which diminishes the quantity of drug available to interact with the parasites and amplifies risks of host-cell toxic effects.

A substantial proportion of research into trypanosomes over the past 20 years has been related to target identification and development of lead compounds that interact with these targets.[74] Action must now be taken to ensure that some of the lead compounds are developed to produce new, clinically relevant, drugs.

Drug targets

The genome sequence of *T brucei* is near completion, and that of *T cruzi* well advanced.[81] Comparative genomics has succeeded comparative biochemistry in the quest for drug

targets in trypanosomes. Genetic manipulation methods to validate targets are now available.[82,83] Gene silencing through RNA interference (RNAi) has greatly advanced this area in *T brucei*.[84] Potential targets, related to biochemical peculiarities of the parasites, are outlined below.

Glucose metabolism and the glycosome

Bloodstream-form African trypanosomes are dependent on glycolysis for energy production. The first seven enzymes of this pathway in these cells reside within unusual peroxisome-like organelles termed glycosomes.[85] *T cruzi* also has glycosomes and, although these parasites are less dependent on glycolysis than *T brucei*, they ferment glucose aerobically.[86] Trypanosomal glycolytic enzymes have structural differences compared with their mammalian counterparts, and are considered good chemotherapeutic targets.[87–89]

Pentose phosphate pathway

In trypanosomes, many enzymes of the pentose phosphate pathway[90] and several other pathways are related to cyanobacterial isoforms rather than to those of eukaryotes.[91] This association has led to a suggestion that the ancestors of trypanosomatids harboured a now lost endosymbiont at some time in their evolutionary past.[91] 6-phosphogluconate dehydrogenase—the pathway's third enzyme—is essential to trypanosomes, and structural differences to its mammalian counterpart have been exploited in design of selective inhibitors.[92]

Thiol metabolism

In trypanosomes, the roles of glutathione in most eukaryotes are undertaken by trypanothione (N^1,N^8-bis-glutathionylspermidine), a unique low molecular weight thiol that consists of two glutathione molecules linked by spermidine.[93] The enzymes involved in trypanothione metabolism, especially the essential enzyme trypanothione reductase, are judged good candidate targets for the rational approach to drug design for both parasites.[94]

Polyamine metabolism

The only drug for which a mode of action has been identified against human African trypanosomiasis is eflornithine, a suicide inhibitor of ornithine decarboxylase, the first enzyme of the polyamine biosynthetic pathway.[95,96] Turnover for *T brucei gambiense* ornithine decarboxylase is far less rapid than for its mammalian counterpart, so the parasite remains deficient in polyamine biosynthesis for prolonged periods after exposure to eflornithine. The drug acts against the form of the disease caused by *T brucei gambiense* but not that caused by *T brucei rhodesiense*. *T cruzi* is auxotrophic for polyamine biosynthesis, since it does not have ornithine decarboxylase, and is thus naturally refractory to the effects of eflornithine. However, epimastigotes need an exogenous source of polyamines, which they scavenge effectively, and polyamine transporters thus represent potentially important targets in *T cruzi*.[97]

Lipid and sterol metabolism, and cell signalling and differentiation

Lipids have a central role in the structure of biological membranes. They also participate directly and indirectly in cell signalling. Sterol biosynthesis has been targeted in fungi, and—since pathogenic trypanosomatids have similar pathways to fungi for sterol biosynthesis that differ from those in mammalian cells—drugs developed against fungi could be used against parasites. Inhibitors of cytochrome P450-dependent C14 α-methylase—including ketoconazole, D0870, posaconazole, and SCH56592—delay development of *T cruzi*.[98,99] Inhibitors of squalene oxidase, another enzyme of the same pathway, such as terbinafine, might act in synergy with C14 α-methylase inhibitors against the parasite and improve effectiveness.[98]

African trypanosomes have fatty-acid biosynthetic capacity that can produce myristate for use in GPI anchor remodelling. Inhibition of myristate biosynthesis has attracted attention,[100] and thiolactomycin—an inhibitor of fatty-acid biosynthesis—has been shown to kill trypanosomes. Protein farnesyl transferases implicated in protein prenylation have also been shown to be good targets.[101,102]

Protein degradation

Cysteine protease inhibitors have in-vitro and in-vivo activity against *T brucei*[103] and *T cruzi*.[104,105] Turnover of proteins associated with the proteasome—the crystal structure of which has been resolved for *T brucei*,[106]—also takes place in trypanosomes, and proteasome inhibitors such as lactacystin are toxic.

Membrane architecture, transporters, and drug entry

The parasite plasma membrane represents the interface between the parasite and its host. Since, by definition, parasites take nutrients from their host, they have transmembrane transporters to import host metabolites. Blocking of vital transporters would kill the parasites and nutrient transporters could represent good drug targets.[107]

Specific plasma membrane transporters can also assist in selectively targeting drugs to parasites. Melamine-based arsenicals and diamidines, including diminazene and pentamidine, enter *T brucei* via an unusual adenosine transporter termed P2. Pentamidine also enters via two other transporters. Melamine-based arsenicals can also enter via other routes, because removal of the gene that encodes the P2 transporter (*TbAT1*) led to only modest reductions in sensitivity of *T brucei* to melamine-based arsenicals. A recognition motif common to 6-amino-purines and the melamine ring has been attached to various putative toxins for delivery to trypanosomes via this route.[92]

Many major structural surface proteins of parasitic protozoa are attached via GPI anchors.[26] The GPI anchor of variant surface glycoproteins and other proteins, including the surface mucins of *T cruzi*, is constructed by enzymes that differ in some respects from those in mammals. The GPI anchor biosynthetic pathway has been shown to be essential, and specific inhibitors against the trypanosome enzymes have been produced.[108]

Kinetoplast and RNA editing

An unusual network of mitochondrial DNA, consisting of a series of intercatenated circular DNA molecules, is present in trypanosomatids and termed the kinetoplast.[109] Gene expression of some of the kinetoplast genes involves the extraordinary process of RNA editing, whereby U residues are added to (or, less often, removed from) primary transcripts to yield mature translatable transcripts.[110] This mitochondrial RNA editing process is unique to trypanosomatids; hence components of the process could be good targets.

The structure of the kinetoplast itself also offers potential for attack.[109] For example, the complex replication pattern needed to reproduce the catenated network depends on topoisomerase enzymes, and inhibitors of DNA topoisomerases have trypanocidal activity and the ability to disrupt the kinetoplast.[109]

Despite the fact that mitochondrial respiration is suppressed in bloodstream-form organisms, mitochondria are still functional and need a membrane potential rendering the mitochondrion itself a target.[111]

Regulation of nuclear gene expression

Trypanosomatids regulate gene expression in the nucleus in a way that distinguishes them from mammals. Large polycistronic primary transcripts are transcribed by RNA polymerase II and then processed into gene-size units via addition of a 3′ poly-A tail and a common 39 nucleotide 5′ cap structure, termed the spliced leader (or mini-exon).[112] Interference with this trans-splicing process has attracted attention as a possible drug target. Protein expression is essentially regulated at a post-transcriptional level by proteins that amplify or reduce the stability of mature mRNA.

Acidocalcisome

Acidocalcisomes[113] are membrane-bound acidic compartments rich in calcium and pyrophosphate. Short-chain and long-chain polyphosphates, magnesium, sodium, and zinc are also present. Acidocalcisomes have been proposed as possible targets for bisphosphonates.[113]

Trans-sialidase

T cruzi trypomastigotes contain a trans-sialidase, which transfers sialic acid from host glycoconjugates to parasite membrane acceptors, especially the mucins.[30] This enzyme is essential for host-cell invasion and is not present in mammals. It could be judged, therefore, a good potential target for chemotherapy. *T brucei* insect stages also contain a similar enzyme, although its function is so far unknown.

Trypanocides under consideration for clinical development

The orally available prodrug DB289 (2,5-bis [4-amidinophenyl] furan bis-D-methyl amidoximine)[114] is at present the only compound with substantial financial backing for clinical trials against human African trypanosomiasis.[57,59] The compound is absorbed across the intestinal epithelia and is converted into a trypanocidal dicationic form systemically. Whether the pharmacokinetic properties of the prodrug and active metabolite will allow the drug to be used against late-stage disease is not yet known. However, an orally available drug that is effective even against early-stage disease would be very useful in the armoury available against this disorder.

No other new drugs are in clinical trials for human African trypanosomiasis or Chagas' disease. Even optimism that drugs developed as antifungals could be used in treatment of *T cruzi* infections was stifled when D0870 failed to progress through to full clinical development as an antifungal.[61] Posaconazole is a promising triazine that has been studied as an agent active against Chagas' disease.[115] Bisphosphonates, which have been proposed to possibly target the trypanosomal acidocalcisome,[113] show considerable promise. These drugs, such as pamidronate, are already in clinical use for bone diseases, including osteoporosis and Paget's disease, are well tolerated, and achieve parasitological cure in rodents.

Approaches to control

Prospects for disease management through insect control

The epidemiological features of sleeping sickness and Chagas' disease are defined largely by the insect vectors that carry the parasites. Tsetse flies and triatominae differ ecologically. Tsetse flies are highly mobile winged dipterans needing special conditions of temperature, humidity, and vegetation. Triatominae usually crawl within peridomestic environments. Generally speaking, people are bitten by tsetse flies while active outside and by triatomines while asleep indoors. However, both vector groups share an important ecological feature: they are both K-strategists, having a low reproductive rate complemented by a high survival rate, adapted for the efficient exploitation of their habitat.[116] R-strategists (such as mosquitoes), on the other hand, produce many offspring, with low probability of survival but with high population-genetic variability allowing for rapid adaptation to new environments. As K-strategists, tsetse fly and triatomine populations are vulnerable to interventions aimed at altering their environment and lowering their reproductive rate (panel 2).

American vectors

Triatomine bugs reside within the thatched roofs and cracked walls of rural huts. Exchanging these for corrugated iron, wooden, or stone roofs and smoothed walls creates an adverse milieu for the insects, and improved housing has long been practised in control.

DDT (bis[4-chlorophenyl]-1,1,1-trichloroethane) is not effective against triatominae, although other organochlorides like dieldrin and gamma-hexachlorocyclohexane are. Synthetic pyrethroids show still better effectiveness, and insecticides are widely used in efforts to control triatomine bugs.[116,117] Techniques of insecticide application consist of spraying, use of slow-release insecticide-containing paints, and smoke generating formulations. Control programmes are complemented by continuous surveillance of triatomine reinfestation and by housing improvement and health education.

Successful insecticide control campaigns in Argentina, Brazil, Chile, and Venezuela before the 1970s[117] encouraged other endemic countries to adopt similar methods. In 1991, Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay established the Southern Cone Initiative, aimed at elimination of *Triatoma infestans* and interruption of Chagas' disease transmission through blood transfusion.[118] After the success of these coordinated actions, in 1997, Colombia, Ecuador, Peru, and Venezuela established the Andean Countries Initiative to fight against vectors—not all of them strictly domiciliated, including *Rhodnius prolixus*—implementing control strategies adapted to the local entomological conditions. A year later, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, and Nicaragua adhered to form the Initiative of the Central American Countries. These latter two programmes have also implemented universal blood screening to avoid blood transfusion from infected donors.[116–118]

Today, acute Chagas' disease has been eliminated from many rural areas, and the cost-benefits of the initiatives are highly positive.[116–118] Chagas' disease vectorial transmission has been eliminated in Uruguay and Chile and in large parts of Brazil and Argentina. By 1999, reports of estimates of numbers of infected people suggested disease prevalence was in decline.[117] The chronicity of the disease and absence of mass treatments will, however, jeopardise control if vigilance wanes once transmission rates hit their targets.

Panel 2: Principles of control of trypanosomiases

Human African trypanosomiasis

- Treatment of infected individuals
- Active case finding with mobile teams
- Vector control (use of insecticides, trapping, sterile male release, environmental changes)

Chagas' disease

- Vector control (residual spraying, improvement of housing)
- Personal prophylaxis (bed net)
- Serological control of blood banks
- Treatment of infected individuals

Figure 8: **Tsetse trap**

There is some concern that eradication of the domestic species will open a niche for sylvatic triatominae to inhabit. Notwithstanding, unprecedented political cooperation seems to have been the driving force behind the success of the campaign against Chagas' disease in Latin America

African vectors

Vector control has long been thought to offer great hope for control of human and veterinary African trypanosomiases. During the colonial era, draconian efforts were used to break the tsetse transmission cycle. Large tracts of land were cleared of vegetation, and wild mammals that could act as a reservoir for the rhodesiense form of the disease were destroyed.[116,119]

In the case of *T brucei gambiense* disease, mass treatment campaigns to diminish the reservoir of human hosts was profoundly effective in diminishing disease transmission in west Africa in the mid 20th century.[120]

More subtle approaches to tsetse control are now preferred. Insecticide spraying—generally and selectively targeting the lower part of trees favoured by resting flies in infested areas—has had considerable success, although ecological considerations now limit indiscriminate use of insecticides.[121]

Tsetse traps and targets (figure 8), especially when odour-baited and impregnated with insecticides, have been very useful. Trap efficiency depends on ecology and behavioural pattern of the different species of tsetse. The flies are attracted to visual cues provided by large expanses of blue or black cloth and chemical clues such as acetone, a tsetse-attracting component of cow's breath. Insecticide-coated cattle and oxen live-baits too have been successfully used to destroy tsetse.[122]

Panel 3: **Priorities for research into the trypanosomiases**

- Development of sustainable, multicomponent approaches to control of insect vectors
- Identification of drug targets with genome databases and their validation by genetic approaches
- Development of new trypanocidal drugs with pharmacological properties enabling them to cross the blood-brain barrier in the case of human African trypanosomiasis and, in the case of *T cruzi*, to target amastigotes resident in mammalian cells
- Improvement in practical diagnostic techniques capable of detecting infection and staging the diseases
- Improvement in understanding of the parts played by the parasites and of host responses to them in development of pathophysiology

A much publicised approach to tsetse control involves release of sterile male flies encouraging females to mate unproductively.[123] This strategy contributed to the eradication of *Glossina austeni* from the small island of Unguja (Zanzibar) off the Tanzanian coast. Simpler tsetse eradication procedures (catching flies by hand) successfully eradicated the fly from the island of Principe in 1914.[124] Buoyed by the Zanzibar success, on Feb 19, 2002, the International Atomic Energy Agency—responding to a declaration calling for the eradication of trypanosomiasis from the Organisation of African Unity in July, 2000—stated an intention to release sterile males in 37 countries. Many scientific and technical obstacles limit the probability of successful eradication;[125] however, it is heartening that, at least in principle, politicians in Africa recognise the extent of the trypanosomiasis problem and that international agencies are prepared to contribute where possible. Moreover, the successes of insect-control campaigns for Chagas' disease have served as a stimulus to the human African trypanosomiasis community to reinitiate vector control in Africa.

Outlook

Optimism that similarities in the biochemistry and ecological features of the trypanosome parasites may point to general control strategies has yet to bear fruit because of differences in relations between host, parasite, and the vector arthropods. In human African trypanosomiasis and Chagas' disease, control of transmission through efforts aimed at reducing the prevalence of insect vectors or mammalian reservoirs clearly works. It is essential to establish workable guidelines by which sustainable control through these routes can be achieved, in combination with national and international programmes aimed at lessening the burden of the trypanosomiases through chemotherapy (panel 3).

Human African trypanosomiasis and Chagas' disease will probably not be defeated in the near future. They remain a challenge for the research community, medical personnel in endemic areas, and health planners. More knowledge and a better insight into the diseases and their pathophysiology is necessary. New drugs and better treatment protocols are urgently needed, and the pharmaceutical industry must be encouraged to engage in this process despite the absence of obvious economic incentives. Donors have to be encouraged to commit themselves to long-term programmes. Political decision makers have to be convinced that the trypanosomiases are of vast public-health importance, and that every investment in their control will render a considerable benefit for populations in the affected zones. Above all, major breakthroughs will depend as much on socioeconomic will as on medical know how. Epidemics of human African trypanosomiasis parallel war and civil conflict. Stability, growth, and development in Africa will be the main factors to control this plague. Chagas' disease mainly affects the underprivileged in rural areas in Latin America. Economic progress and prosperity will lead to control of the trypanosomiases.

Conflict of interest statement

MPB is part of a Bill and Melinda Gates Foundation funded consortium, led by the University of North Carolina, working on DB289 development for use against human African trypanosomiasis. No other conflicts declared.

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