Review

Sleeping sickness and the brain

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Abstract. Recent progress in understanding the neuropathological mechanisms of sleeping sickness reveals a complex relationship between the trypanosome parasite that causes this disease and the host nervous system. The pathology of late-stage sleeping sickness, in which the central nervous system is involved, is complicated and is associated with disturbances in the circadian rhythm of sleep. The blood-brain barrier, which separates circulating blood from the central nervous system, regulates the flow of materials to and from the brain. During the course of disease, the integrity of the blood-brain barrier is compromised. Dysfunction of the nervous system may be exacerbated by factors of trypanosomal origin or by host responses to parasites. Microscopic examination of cerebrospinal fluid remains the best way to confirm late-stage sleeping sickness, but this necessitates a risky lumbar puncture. Most drugs, including many trypanocides, do not cross the blood-brain barrier efficiently. Improved diagnostic and therapeutic approaches are thus urgently required. The latter might benefit from approaches which manipulate the blood-brain barrier to enhance permeability or to limit drug efflux. This review summarizes our current understanding of the neurological aspects of sleeping sickness, and envisages new research into blood-brain barrier models that are necessary to understand the interactions between trypanosomes and drugs active against them within the host nervous system.

Key words. Sleeping sickness; central nervous system; blood brain barrier; trypanosome; chemotherapy.

Introduction

Human African trypanosomiasis (HAT), or sleeping sickness, is caused by infection with haemoflagellates of the *Trypanosoma brucei* subspecies which are introduced to the human bloodstream by the bite of infected tsetse flies of the genus *Glossina*. The ecology of the insect vector is such that the disease is only found in the intertropical regions of Africa. After an insect bite, parasites replicate at the site of infection, producing a local inflammatory reaction, then spread to the regional lymph nodes. From there, they become disseminated throughout the host, eventually becoming established in the central nervous system (CNS).

Although there are many species of trypanosomes, only two, belonging to the *brucei* group, are infectious to humans. *T. b. gambiense,* found in West and Central Africa, leads to a chronic form of the disease. *T. b. rhodesiense,* found in East and Central Africa, leads to a more virulent and acute condition. For each species of trypanosome, there are strains of different virulence contributing to the inter-individual variability in the clinical course.

At the present time, sleeping sickness is a re-emerging infectious disease. The number of infected individuals is estimated at more than 300,000 [1], and about 55 million people are at risk of infection with trypanosomes. Only four million of these are subject to active surveillance or have access to health centres where reliable diagnosis and treatment are available. The disease is invariably fatal unless treated, and lost Disability-Adjusted Life Years and

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social problems associated with the disease, which in its final stages causes profound neurological disturbances, makes its impact far more profound than indicated by the number of deaths associated with the disease. The relationship between the parasite and the central nervous system is the subject of this review.

Clinical symptoms

The clinical signs of HAT are manifold [2, 3]. The early stage of the disease, defined as the period preceding CNS involvement, is dominated by general signs of infection (irregular and moderate fever, intense pruritus, headache, oedema – especially facial oedema, ocular disturbances and frequently amenorrhoea and impotence). The subsequent meningo-encephalitic phase (late stage) of the disease is characterised by a severe and complex neuropsychiatric syndrome, leading to death if untreated.

Most late-stage patients display disturbances of consciousness and of the circadian rhythm of sleep, represented typically by diurnal somnolence and nocturnal insomnia [4–6]. While the total time spent sleeping by patients with this disease differs little from uninfected individuals [4], the disease was named sleeping sickness because of the alterations to sleep patterns and a pronounced lethargy seen in patients at the late stage of the disease. Sensory disturbances, including hyperpathia and extrapyramidal symptoms, are common. Psychological symptoms are also characteristic of the disease, with memory loss, dementia, depression, agitation, mania, irritability and effective indifference all common. Patients can suffer hallucinations and a number of other neurological disorders have been reported [7]. The vast majority of sleeping sickness patients are unaware of their disease prior to the manifestation of symptoms associated with late-stage infection.

The blood-brain barrier

To maintain the physico-chemical composition of the CNS, a unique regulatory and protective structure, the blood-brain barrier, is required. All of the defining symptoms of sleeping sickness relate to the presence of parasites in the brain which is normally protected from invasion by micro-organisms by this blood brain barrier [8]. In fact, two separate barriers enclosing the CNS can be distinguished: the blood-brain barrier and the blood-cerebrospinal fluid barrier. The blood-cerebrospinal fluid barrier is more permeable than the blood-brain barrier and is found at locations in the CNS, such as the choroid plexus, where enhanced solute exchange is necessary.

The blood-brain barrier (fig. 1) is a highly dynamic structure and consists of endothelial cells, which are charac-
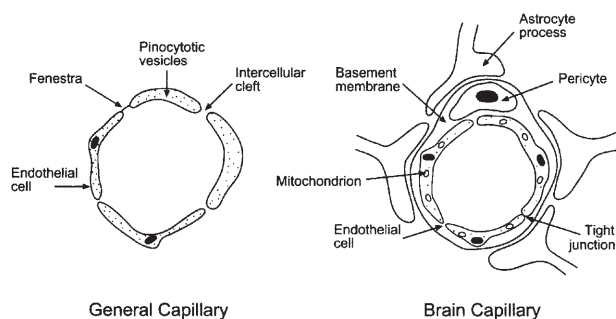

Figure 1. A comparison of the anatomy of a general capillary with a capillary comprising the blood-brain barrier.

terised by the presence of tight junctions and a relative lack of endocytic vesicles [9]. The tight junctions are reinforced by the foot processes of astrocytes. Selective permeability of the blood-brain barrier results from the presence of the tight junctions that restrict paracellular passive diffusion between endothelial cells of the cerebral vessels. Epithelial cells, making up the blood-cerebrospinal fluid barrier of the choroid plexus, are also rich in tight junctions.

In healthy individuals, the blood-brain barrier regulates the entry of compounds into the brain, and it also restricts cellular infiltration. The normal blood-brain barrier endothelial cell layer provides a thromboresistant surface that limits platelet and leukocyte adhesion and activation of any coagulation system and it also plays a key role in rendering the CNS an immunosecluded site [10].

How do trypanosomes penetrate the blood-brain barrier?

The blood-brain barrier presents a formidable barrier to microbes. How trypanosomes penetrate the barrier is not clear. However, the protective effect of the barrier is diminished when inflammation, such as that seen when trypanosomes become associated with the brain capillaries, occurs. Details of the events occurring at the blood-brain barrier in response to inflammation are emerging. Within minutes after the release of inflammatory mediators, including cytokines and eicosanoids, neutrophils arrive at the site of inflammation. These are followed by a migration of antigen-specific B and T lymphocytes and monocytes [11]. This migration of mononuclear cells into the CNS is often accompanied by an increased flux of serum proteins that are transferred to the cerebrospinal fluid. This general permeabilisation of the barrier could influence the migration of trypanosomes. Adhesion molecules responsible for the migration of leukocytes into an inflamed site are found on endothelial cells, perivascular cells, and some astrocytes and they include intercellular adhesion molecules-1 and -2 (ICAM-1 and -2) and vascular cell adhesion molecule-1 (VCAM-1).

These molecules recognise their leukocytic ligand and permit adhesion and migration of leukocytes out of the bloodstream [11]. ICAM-1 expression on cultured brain microvascular endothelial cells can be up-regulated in a time- and dose-dependent manner by interferon-gamma (IFN-γ) and interleukin-1 (IL-1) [12]. In vitro studies revealed that administration of tumour necrosis factor-alpha (TNF-α), IL-1 and IL-6 to monolayers of endothelial cells leads to an increase in permeability [13]. Prostaglandin E2 (PGE2) and PGE12 are also involved at the site of the inflammation and both are secreted by inflamed tissue and by vascular endothelium [14]. Pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-1, released during infections by cells of the blood-brain barrier, are also able to induce inducible nitric oxide synthase (iNOS) present in endothelial cells, astrocytes and brain macrophages [15]. Nitric oxide also increases permeability of the barrier.

While the mechanism by which African trypanosomes permeate the blood-brain barrier is unknown, a number of studies have investigated this question. Other microbial pathogens can also escape the blood and lymph to reach the CNS and several routes have been described [16]. These include:

1) Entry via the choroid plexus epithelium leading to the cerebrospinal fluid space.
2) Entry via the cerebral capillary endothelium leading to the brain parenchyma.
3) Penetration through disrupted tight junctions.
4) Entry together with, or inside, leukocytes.

The peri-ventricular regions, the tubero-infundibula and thalamic-hypothalamic regions, appear to be involved in trypanosome invasion of the CNS [17]. In experimental *T. brucei* infections, the parasites localise to regions of the nervous system that are protected by the blood-cerebrospinal fluid barrier rather than the blood-brain barrier. These regions include the choroid plexus, the trigeminal and dorsal root ganglia and the circum-ventricular organs including the pineal gland, median eminence and the area postrema [18].

The choroid plexus is a highly vascularised epithelium found within each of the four cerebral ventricles. It is structurally analogous to the distal and collecting tubules of the kidney, using capillary filtration and epithelial secretory mechanisms to maintain the chemical stability of the cerebrospinal fluid [19]. Secretory capacities of the choroid plexus are bi-directional, accounting for both continuous production of cerebrospinal fluid and active transport of metabolites out of the CNS into the blood. The choroid plexus and peri-ventricular regions may be damaged shortly after trypanosome infection, with parasites penetrating these tissues [20]. Trypanosomes have been speculated to escape from the peripheral circulation in the early stages of infection reaching the CNS before observed cerebrospinal fluid changes. Parasites may en-

ter by direct passage through the cerebral capillaries, or following an early breakdown of the barrier at the choroid plexus [21], followed by seeding into the cerebrospinal fluid and passage through the subependymal or subpial tissues into the parenchyma [22].

Trypanosomes have also been suggested to migrate by transcytosis through the cerebral endothelial cells rather than via disrupted junctions between them, indicating that a breakdown of the blood-brain barrier may not be a prerequisite for invasion of trypanosomes. A recent study [23], in a rat model of chronic disease, showed that trypanosomes cross the blood-brain barrier while tight junction proteins are preserved. Indeed, some trypanosomes were clearly observed in the brain parenchyma, and these parasites were probably derived from cells that had penetrated directly through the cerebral vessels. The conclusion is that trypanosomes are able to cross the blood brain barrier in vivo, without a generalised loss of tight-junction proteins.

An intracellular form of *T. brucei,* that could play a role in entry to the CNS, was surmised several years ago [24], although supporting evidence was hard to come by. Intact trypanosomes were recently identified inside glial cells including astrocytes [25], although whether these intracellular forms of parasite were able to survive and/or grow is not known. In addition, the early detection of parasites in parenchyma and some brain areas may be expected since trypanosomes do not survive well in cerebrospinal fluid [26].

Do trypanosomes proliferate in the cerebrospinal fluid?

For any parasite to establish a pathogenic infection it must proliferate. Trypanosomes do proliferate in the blood and lymph; however, their proliferation in the relatively nutrient-depleted environment of the cerebrospinal fluid is less certain [26] (table 1). Parasite numbers in the brains of mice with late-stage sleeping sickness increased after suramin treatment had cleared parasites from the blood, indicating that trypanosomes may be able to proliferate in

Table 1. Composition of serum and cerebrospinal fluid (CSF).

Component	CSF	Serum
Water content (%)	99	93
Protein (mg/dl)	35	7000
Glucose (mg/dl)	60	90
Osmolarity (mOsm/l)	295	295
Na^+ (meq/l)	138	138
K^+ (meq/l)	2.8	4.5
Ca^{2+} (meq/l)	2.1	4.8
Mg^{2+} (meq/l)	0.3	1.7
Cl^- (meq/l)	119	102
pH	7.33	7.41

the cerebrospinal fluid [B. Enanga, unpublished observation] although definitive evidence is lacking.

Little is known about the biochemical physiology of the parasites within this environment. This could be of importance in contemplating responses to drugs. For example, a number of trypanocidal drugs enter parasites via an unusual nucleoside transporter called P2 [27]. Expression of nucleoside transporters in trypanosomatids can be regulated in response to environmental stimuli. Should expression of a transporter involved in drug uptake be down-regulated in parasites within the cerebrospinal fluid, these cells would not respond to drugs in the same way as bloodstream-form organisms.

Factors of host and parasite that influence infection and pathogenesis

Dysfunction of the nervous system may be induced by molecules released either by parasites or by host cells responding to the infection. The difficulty of accessing trypanosomes in the cerebrospinal fluid and the paucity of in vitro model systems for the CNS have hindered our understanding of these processes. A little is known about parasite factors released into the blood and lymph which influence pathogenesis, and there are indications that host molecules can influence parasite proliferation.

Immunological responses to trypanosomes

African trypanosomes are in constant contact with the mammalian host immune system. The appearance of successive parasitic waves correlates with new antigenic variants as the parasites evade the immune response by periodically switching their major variant surface glycoprotein (VSG). Antigenic variation [28] appears to be one of several mechanisms that African trypanosomes use to evade the immune system of their host. Many of the immune events that occur during an infection are likely to be the result of the response to the variant and invariant antigens [29].

A non-specific polyclonal B cell activation results in the production of large quantities of IgM. The greatly elevated levels of IgM and resultant antigen-antibody complexes in turn cause hyperplasia of the reticulo-endothelial system, especially the spleen and lymph nodes.

Other humoral (B cell) and cellular (T cell) immune functions are suppressed during the disease [30]. Many secondary immune events are inhibited, for example the enormous IgM production is not followed by the usual increase in IgG and the other antibody classes. Secondary opportunistic infections are consequently common in sleeping sickness.

The continuous stimulation of the immune system due to variant antigens leads to deregulation of immunoglobulin production [31]. Moreover, trypanosomes have recently been shown to produce a protein that is similar to a surface protease of *Leishmania* which may play a role in protecting against complement-mediated lysis [30].

The level of circulating immune complexes is increased in experimental sleeping sickness [32] and these complexes could play a role in pathogenesis, although clear evidence that this is the case has yet to be presented. Deposits of immunocomplexes in the CNS and the release of trypanosomal antigens, which subsequently bind to brain cells and attract antibodies or T lymphocytes, have also been proposed to play a critical role in treatment-induced encephalopathies [33]. However, a similar incidence of neurological sequelae associated with melarsoprol administration [34] is seen when trypanosomes are not present, indicating that the drug alone is sufficient to induce encephalopathy.

The involvement of the CNS in the advanced stages of infection leads to an irreversible demyelinating process which, without treatment, ends in death [35]. Antibodies against CNS components such as galactocerebrosides [36, 37], neurofilaments [38] and tryptophan-like epitopes [39] have also been described in serum and cerebrospinal fluid of sleeping sickness patients. Immune complexes and antibodies against brain-specific substances have been observed in the serum of infected rodents and humans [36, 40]. Demyelination, therefore, could be due to epitopes that are shared between the host and trypanosome that can lead to a self-propagating autoimmune reaction.

Antibody-independent destruction of trypanosomes

While trypanosomes appear to be able to avoid the onslaught of the host's adaptive immune system through their ability to undergo antigenic variation, they can be susceptible to innate immune mechanisms. Trypanosome lytic factor (TLF) is a primate-specific molecule involved in the innate defense mechanism that restricts the host range of African trypanosomes [41]. *T. b. brucei* is susceptible to TLF and therefore cannot survive in human serum. *T. b rhodesiense*, however, is resistant to this factor. TLF resistance in *T. b. rhodesiense* appears to be related to the selective, high-level expression of a serum-resistance-associated (SRA) gene [42]. Cells expressing SRA are apparently no longer susceptible to the effects of TLF, which is a component of high-density lipoprotein particles in serum, and is probably a haptoglobin-associated protein that is believed to induce oxidant stress within lysosomes/endosomes of trypanosomes that internalise it. The SRA protein is structurally related to the VSGs and is believed to be a cell-surface-associated protein. Expression of the SRA gene, and consequently serum resistance, can be regulated. Therefore, trypanosomes from a cloned line can be either resistant or

sensitive to human serum in an unstable manner. *T. b. gambiense* does not appear to have the SRA gene and thus resistance to human serum in these parasites must involve a separate mechanism.

Trypanosome-derived lymphocyte-triggering factor and IFN-γ

Trypanosomes release several substances that stimulate both the immune system and also the neurons of the host. *T. brucei* has been shown to release a trypanosome-derived lymphocyte-triggering factor (TLTF) that triggers T cells to produce IFN-γ, which in turn may provide a growth stimulus for the parasite [43, 44]. High levels of IFN-γ have been detected in the cerebrospinal fluid of sleeping sickness patients [45] and anti-IFN-γ antibodies have been observed in the serum and cerebrospinal fluid of humans and experimental animals infected with trypanosomes [46].

TLTF has also been reported to cause the release from neurons of a constitutively expressed neuron-derived IFN-γ-like molecule (N-IFN-γ) that may also stimulate *T. brucei* proliferation [47]. TLTF was originally identified as a secreted molecule, but the role eponymous with its original definition has been controversial and it has recently been shown to be associated with the trypanosome flagellum [48], casting doubts on whether it really plays a role in triggering T cells. Moreover, increased IFN-γ has been proposed to correlate with reduced parasitaemia in experimental trypanosome infections, and thus reported results on the relationship between TLTF, IFN-γ and trypanosome growth are difficult to interpret when considered together.

Cytokines

Various cytokines are released in response to *T. brucei* infection, although results obtained from studies in mice can often differ profoundly from the situation in humans. IFN-γ may have a central role in the host response to trypanosomes. Among its immunoregulatory effects, IFN-γ induces the release of TNF-α, interleukins, and prostaglandins, as well as stimulating the release of the gaseous free radical nitric oxide from macrophages [49]. These inflammatory mediators have varied effects on different stages of the disease. Several cytokines are involved in both pathogenic and protective immune responses but the precise role of individual cytokines is currently unclear.

In the early stages of the disease, TNF-α may be involved in the elimination of blood parasites. This cytokine also influences inflammatory reactions, immunosuppression and auto-antibody production. In the late stages of the disease, the number of bloodstream trypanosomes is low but levels of TNF-α are high in serum [50].

Resistance to *T. b. rhodesiense* infections in mice, characterised by low parasitaemia and increased survival, was shown to be dependent on IFN-γ [51]. IL-4, commonly induced in brain tissues during experimental African trypanosomiasis with chronic meningo-encephalitis, has also been reported to be involved in controlling the levels of parasitaemia by its effects on immunoglobulin synthesis in mice [52]. IL-10 and IL-4 have also been linked with resistance to late-stage African trypanosome infections in mice via the induction of the IgG1 antibody response to VSG [53].

Recently, in the early stages of experimental African trypanosomiasis, IFN-γ and TNF-α were reported to limit parasite growth, while IL-10 plays an important role in survival of infected mice, probably by damping-down excessive inflammatory responses [54]. This result suggests that IL-10 may play a regulatory role in the equilibrium between pathology and trypanotolerance in infected mice. The presence of IL-10 in the blood and cerebrospinal fluid may be a marker of the second stage of African trypanosomiasis. This could have profound importance in the development of diagnostic tests that do not involve lumbar puncture for direct identification of parasites in cerebrospinal fluid (see below).

The inflammatory mediators discussed above may also enhance immunosuppression and can alter the blood-brain barrier by acting on cerebral endothelial cells. These are possibly involved in the transmission of inflammatory signals to other cell types in the brain, such as astrocytes, microglia, pericytes and perivascular macrophages [55].

Nitric oxide and prostaglandins

Nitric oxide is known to mediate cytostatic activity against African trypanosomes [56] and immunosuppression in experimental trypanosome infections [57]. In vitro, nitric oxide leads to a rapid blood-brain barrier breakdown, resulting in a reduction in endothelial cell ATP content [58]. Nitric oxide derivatives such as peroxynitrites have been proposed to induce lipid peroxidation [59] and are reactive against protein thiol groups [60]. This may explain the rapid detrimental effects of nitric oxide on blood-brain barrier integrity [61].

A murine experimental model of chronic trypanosomiasis, which mirrors the pathological evolution of human African trypanosomiasis [21], has been used to investigate the origin and role of cerebral nitric oxide production and its toxic derivatives (peroxynitrites) in the CNS pathology of human African trypanosomiasis. Nitric oxide and peroxynitrites are known to induce neuron and astrocyte death by apoptosis. Nitric oxide-linked apoptosis might regulate the number of inflammatory cells in the CNS during a trypanosome infection and this could prolong parasite survival [62].

Loss of neurons and parenchymal cells has been observed in necrotic spaces in murine models of chronic try-

panosomiasis. Peroxynitrite levels in the brain parenchyma were increased in mice that showed neurological disorders, suggesting that these nitric oxide derivatives might be involved not only in the pathogenesis of brain lesions, but also in the appearance of neurological disorders in this infection [63].

In addition to their direct cytotoxic effects against neural cells [64], peroxynitrites can activate prostaglandin biosynthesis [65]. Patients suffering from HAT exhibit an increase in cerebrospinal fluid prostaglandin concentration [66] which may be related to the sleep-wake cycle alterations [67, 68]. The nitric oxide-peroxynitrite pathway may thus be involved in, and explain part of, the circadian disturbances of HAT [5, 69], which can be reversed after trypanocide treatment [70].

By increasing blood-brain barrier permeability in the early neurological stage of trypanosomiasis [71], nitric oxide and particular cytokines might favour the migration of cells and the synthesis of auto-antibodies directed to CNS components [72, 73]. The blood-brain barrier itself may also play an active role in the mediation of the neuro-immune response either by production of inflammatory mediators or by the expression of adhesion molecules. Trypanosomes and inflammatory cells invade the CNS, leading to a progressive meningo-encephalitis with typical perivascular cuffing that may contribute to neurological disorders and neuro-endocrine alterations [44]. Inflammatory cells do infiltrate the leptomeninges and vessels in the peri-ventricular brain parenchyma. Moreover, the few neurological studies conducted on human tissue reveal a preponderance of Mott cells (immunoglobin-containing plasma cells) in trypanosome-infected brains [74].

Diagnosis of CNS involvement

Since different drugs are used before or after CNS involvement, making a correct diagnosis of the stage to which the disease has progressed is crucial. Positive diagnosis depends upon microscopic identification of parasites in blood smears or tissue fluid [1].

Beyond the initial stage, diagnosing the phase of the disease to which the patient has progressed is difficult, particularly in the early neurological phase, due to the absence of neurological signs and noticeable changes in cerebrospinal fluid. Stage determination of HAT is based on the detection of parasites and measurements of biological changes in the cerebrospinal fluid (concentration of white blood cells >5 cells/mm^3; increased total protein levels >40 mg/100 ml) [1]. These diagnoses depend upon the acquisition of cerebrospinal fluid by lumbar puncture, a procedure that is potentially dangerous and that can induce patient non-compliance. Demonstration of the absence or presence of trypanosomes by microscopy fol-

lowing the double centrifugation technique is still the only test available to clinicians for assessing treatment success [75].

Serological tests have been developed to diagnose the presence of a trypanosome infection of blood [76] although these cannot determine whether disease has progressed to the late stage. Studies to evaluate the polymerase chain reaction (PCR) as a tool for assessing the disease stage of trypanosomiasis and for determining treatment success have been carried out [77], although logistical problems of establishing PCR diagnosis in the field remain a hindrance to the practical development of this technology. Another drawback with PCR is that the technique can identify residual parasite DNA retained in the body even when viable parasites are no longer present and thus risks failing to identify cases of successful treatment [78].

Recently, a single centrifugation technique of cerebrospinal fluid in a sealed Pasteur pipette has been developed [79]. Future evaluation of the PCR methods and single centrifugation technique is required to determine whether they could become useful for identifying early treatment failures or stage determination of trypanosomiasis in poorly equipped laboratories in the field. However, these latter techniques still require lumbar puncture with the risks associated with this procedure. The development of techniques to ascertain whether disease has progressed to the late stage would be a major asset in choosing the relevant treatment for patients, and remains an important challenge in trypanosome research.

Chemotherapy of late-stage HAT

Once diagnosis of HAT has been made, the main drugs for treatment of the first stage of disease are suramin and pentamidine, both introduced in the first half of the 20th century. An organo-arsenical drug, melarsoprol (Arsobal), is the drug of choice for use in the second stage of infection. Eflornithine is also effective against late-stage disease caused by *T. b. gambiense*.

Melarsoprol

Melarsoprol is administered by intravenous injection. The standard regimen of 3.6 mg/kg body weight, three to four series of four injections separated by at least 1 week may be superceded by a shorter course that involves less drug (2.2 mg/kg per day for 10 days). This reduces the total amount of drug administered per patient as well as the hospitalisation time, which may increase patient compliance without reducing the effectiveness of the therapy [80].

Adverse effects such as cutaneous reactions, polyneuropathy, diarrhoea and fever are quite common [81]. Encephalopathic syndromes are the most serious complica-

tions of melarsoprol treatment and occur at a rate of 5–10%, being fatal in up to half of these [1]. Immune reactions have been suggested as the trigger for the encephalopathy [82, 83], but this remains controversial. Convulsions similar to the encephalopathic syndromes were reported in some patients treated with melarsoprol against advanced leukaemia in the absence of trypanosomes [34]. These data indicate that trypanosomes are not necessarily involved in the reactive encephalopathies.

Despite its long use, only recently have substantial quantitative studies on the chemical, pharmacological and pharmacokinetic properties of melarsoprol been made [84–86]. Melarsoprol is rapidly metabolised in the host [87], melarsen oxide representing the principal metabolite [88]. Melarsen oxide is toxic to trypanosomes but safety issues led to the preference of melarsoprol over its metabolite as a clinically used drug [89].

Interestingly, melarsoprol/melarsen oxide accumulates in the cerebrospinal fluid to levels only 2–5% of those found in blood. This is only just sufficient to kill trypanosomes in this compartment, as minimal inhibitory concentrations against most wild-type trypanosomes are close to levels obtainable in the cerebrospinal fluid. The failure of melarsoprol to cure 10% of late-stage sleeping sickness patients possibly relates to the fact that these individuals accumulate sub-curative levels of drug in the brain. However, one study [90] has indicated that levels of drug are similar in the cerebrospinal fluid of patients relapsing or not, so parasites resident at other extravascular sites may be the key to treatment failure [91]. Recent observations indicate that in some regions, treatment failure rates have reached 30% [92]. Parasites retrieved from these treatment failures appear to be somewhat less responsive to melarsoprol than parasites isolated from other foci [93]. Therefore, treatment failure possibly arises from a complex relationship between the drug, parasite susceptibility to the drug and the ability of infected individuals to exclude drug from extravascular compartments that parasites might colonise during the course of infection.

Eflornithine

When melarsoprol fails to cure an individual with a secondary-stage infection, eflornithine (DFMO), the only drug to have been developed in the latter part of the 20th century, is used against *T. b. gambiense* infections [94]. Eflornithine inhibits the enzyme ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis. Selective activity against the parasite appears to result from the fact that the mammalian version of ornithine decarboxylase is very quickly turned over and thus constantly replenished, while the trypanosome one is not. Unfortunately, eflornithine is of limited use against *T. b.*

rhodesiense which appears to have a relatively quickly turned over ornithine decarboxylase [95].

The drug is also less efficient in children than in adults [96]. To avoid treatment failures, a higher eflornithine dosage, based on body surface area rather than on weight, is recommended for children.

Eflornithine is currently very expensive due to the standard 14-day regimen (100 mg/kg, given intravenously every 6 h for 14 days) and the length of hospitalisation. Unfortunately, a shorter and cheaper 7-day course, despite its relatively low toxicity, led to higher relapse rates with late-stage *T. b. gambiense* sleeping sickness [97]. Production difficulties also contribute to the expense and problems of availability of this drug. Recently, however, eflornithine has found a role as an agent used to hinder hirsutism (growth of facial hair) [98], and on the back of its sustained synthesis for this role, the drug will remain available for use against sleeping sickness for at least several more years, although its long-term future remains in jeopardy.

In summary, the number of drugs available for the treatment of late-stage HAT is limited. All are toxic and drug resistance is a growing problem [92]. Up to 30% of patients with secondary-stage trypanosomiasis show resistance to melarsoprol. This is a particularly serious problem since melarsoprol and eflornithine are the only two drugs available for the treatment of late-stage infections, and are expensive, especially for African communities. New and improved chemotherapeutic agents are urgently needed.

The future of HAT chemotherapy

The World Health Organisation and Aventis Pharma AG have recently announced a major initiative to step up efforts against sleeping sickness, with support for the World Health Organisation's activities in the field of African trypanosomiasis over a 5-year period [Press release WHO/23, 3 May 2001]. The project involves three related efforts: drug donation (pentamidine, melarsoprol and eflornithine), disease management and research. The arsenal of agents available to treat late-stage disease is very limited and relatively few new formulations are currently being considered. A few novel products are under consideration for late-stage disease and these are discussed here.

Diamidines and their prodrugs

Pentamidine

Pentamidine is an aromatic diamidine that has been successfully used for more than 60 years for the treatment of infection with *T. b. gambiense* before CNS involvement

[99]. The recommended regimen of pentamidine in trypanosomiasis treatment is seven to ten injections of 4 mg base/kg body weight given daily or on alternate days. Some cases of treatment failure and adverse effects such as nephrotoxicity and diabetes mellitus have been observed. The drug is not usually considered to be effective after trypanosomes have entered the CNS, probably due to poor penetration of pentamidine through the blood-brain barrier. However, pharmacokinetic studies aimed at improving dose schedules for pentamidine measured concentrations in plasma, whole blood and cerebrospinal fluid [100]. Results showed small amounts of drug in the cerebrospinal fluid and inter-individual differences in its pharmacokinetics prompting suggestions that the drug may be useful at the early part of the late stage [101].

The slow elimination of pentamidine from the body [102] may mean that seven to ten successive daily injections are unnecessary and patients given pentamidine on alternate days seem to tolerate the drug well, so modified courses that reduce toxicity may be effective [100].

DB289

Recently a pro-drug analogue of pentamidine, 2,5-bis-(4-amidinophenyl)furan bis-D-methyl amidoximine, or DB289, which can be administered orally and accumulates in the blood across the gastro-intestinal epithelium has entered clinical trials for use against first-stage sleeping sickness [103]. Once systemic, the drug is converted to a dicationic compound that is effective against trypanosomes.

The pro-drug could, in principle, also penetrate the blood-brain barrier due to its amphipathicity, although whether the pro-drug will be converted to its active form in the CNS is not yet certain. A chlorodiazirine analogue of pentamidine, which also acts as a pro-drug in its non-ionic form, can readily cross the blood-brain barrier and has been found to have anti-trypanosomal activity [104]. The prospect of orally available drugs, even if only active against early-stage disease, would be a major advance.

Nitroheterocyclic compounds

Nifurtimox

Nifurtimox is a nitroheterocyclic compound originally licensed for use against Chagas' disease caused by *T. cruzi.* The drug has also been tested against melarsoprol-refractory late-stage sleeping sickness [105].

High-dose nifurtimox (30 mg/kg for 30 days) caused significant host toxicity and was associated with high rates of relapse (36%). This has led to diminished optimism that the drug will be effective against arsenical-resistant sleeping sickness. Recently, used in combination with

melarsoprol (1.2 mg/kg per day during 8 days), nifurtimox (15 mg/kg per day over 8 days) was successful against late-stage sleeping sickness [106]. Pharmacokinetic studies of the drug are required to determine an appropriate dose schedule for the treatment of patients with late-stage sleeping sickness with nifurtimox in combinations with other drugs.

Megazol

One of the most promising experimental trypanocides is another nitroheterocyclic compound, megazol. This nitroimidazole derivative is active following a single oral dose in both rodent and primate models [107, 108]. Since megazol enters parasites predominantly via passive diffusion [109], parasites which have developed resistance to melaminophenyl arsenicals and diamidines through loss of the P2 amino-purine transporter [27, 110] are not cross-resistant to megazol. Interestingly, one line of P2-deficient parasites was actually two- to three-fold more sensitive to megazol than wild-type parasites in mice [109].

Megazol alone does not cure late-stage disease at doses tested so far in mouse models. However, co-administration of suramin and megazol does cure infected mice with CNS involvement [107, 111].

Megazol levels have been measured in biological fluids of mouse, rat [112] and primate [113]. Using a simple high-performance liquid chromatography method [114], megazol concentrations were measured in plasma, urine and cerebrospinal fluid after oral administration of a single dose of 80–100 mg/kg. Results indicated that megazol was well absorbed from the gut. The maximum levels were detected 2–4 h after megazol administration with an elimination half-life of 2 h. Twenty-four hours after administration, megazol plasma concentrations were still measurable and at trypanocidal levels (100–500 ng/ml). Suramin administered 24 h before megazol affects distribution of the drug. An increased apparent volume of distribution in the blood and a greatly decreased urinary excretion of megazol were observed [112]. In this study, megazol was metabolised only to a minor degree, with 80% of ingested drug recovered in animal urine in an unchanged form. Only three metabolites, found in urine but not in plasma, were identified and their characterisation is in progress.

Megazol concentrations in cerebrospinal fluid were measured at 1.3 µg/ml and 2.4 µg/ml at 8 h for two primates infected with *T. b. gambiense*. One primate infected with *T. b. gambiense* in the late stage was pre-treated with a single dose of suramin 24 h before megazol administration. At 12 h, 1 µg/ml of megazol was measured in the cerebrospinal fluid of this animal. These data suggest that megazol is capable of reaching trypanocidal concentrations in the cerebrospinal fluid whether or not suramin is

administered. The question is therefore raised as to why megazol alone given at this concentration does not cure late-stage disease in mice while the suramin-megazol combination does [111]. More detailed pharmacokinetic analyses in different mammalian models are required to address this question.

The effectiveness of a suramin-megazol combination may be explained by the effects of suramin on the distribution and excretion of megazol in vivo. Amphipathic compounds can cross the blood-brain barrier by simple diffusion but these products are then frequently removed by P-glycoprotein pumps, which are abundant in the cells lining the barrier. Suramin is a substrate and inhibitor of ATP-dependent P-glycoproteins [115], and so may modify megazol extrusion from the cerebrospinal fluid. This may explain why this drug, administered alongside megazol, cures experimental animals of cerebrospinal-fluid-involved disease. However, in the absence of a detailed longitudinal pharmacokinetic study to clarify levels present in the cerebrospinal fluid over a range of time intervals, this remains speculative.

An additional disincentive to the use of megazol arises from the fact that it is positive in Ames tests [116]. However, since Ames tests employ *Salmonella* species with unusual nitroreductase activities [117], results from the classical test should be treated with caution in assessing a nitro-containing drug and further genotoxicity studies using mammalian cell lines are required.

The doses of megazol usually used to cure experimental early-stage infections (80–100 mg/kg) are significantly lower than those used in acute toxicity studies [118, 119] which failed to reveal any significant toxicity even when given at 200 mg/kg over 5 days. Preliminary observations therefore indicate that megazol could be a very useful addition to the trypanocidal drug arsenal; however, a full evaluation of megazol toxicity is needed prior to deciding whether it should proceed to trials in humans.

How to breach the blood-brain barrier?

The blood-brain barrier restricts the passage into the brain of 95% of all drug candidates intended for the CNS [120]. The diffusion of compounds across the plasma membrane of the endothelial cells of the blood-brain barrier is dependent on the physico-chemical properties of these compounds including their lipid solubility, molecular weight, electrical charge and extent of ionisation. Understanding how to deliver drugs to the cerebrospinal fluid is critical to the development of new trypanocides. Entry into the cerebrospinal fluid is achieved in three main ways [121]: (i) by passive diffusion of lipid-soluble substances, (ii) by trans-cytosis, and (iii) by transport of specific water-soluble substances using specific transporters or channels.

Some drugs that are taken up at the endothelial layer of the blood-brain barrier are rapidly removed by several systems including enzymatic barriers and efflux pumps [122]. For example, an abundant endothelial enzyme of the blood-brain barrier, γ-glutamyl transpeptidase, detoxifies glutathione-bound compounds. The multi-drug resistance transporter (mdr1a), a P-glycoprotein originally implicated in the resistance of tumour cells to chemotherapeutic agents, is located on the apical surface of capillary endothelium in the brain [123]. This pump carries many substrates out of cells back to the blood and limits brain penetration of many drugs [124].

Several approaches can be employed to bypass the permeability limitations set by the blood-brain barrier. One approach is to target endogenous blood-brain barrier transport systems as in the case of the anti-retroviral reverse transcriptase inhibitor azidothymidine. Other CNS drug delivery strategies [125] include barrier disruption, novel ways of packaging drugs, inhibition of drug efflux from the brain or manipulation of the brain capillary endothelium permeability to therapeutic agents.

Development of inhibitors of efflux pumps, also used to reverse P-glycoprotein-mediated multi-drug resistance in cancer cells [115], may greatly improve the retention in the brain of valuable drugs that are otherwise removed [126]. P-glycoprotein substrates such as colchicine have been found to accumulate in the brains of *mdr1a* P-glycoprotein gene knockout mice more than in wild-type mice using an in situ mouse brain perfusion model [127]. Whether a given compound is a substrate for P-glycoprotein can be predicted by comparing uptake into the brain or brain capillary endothelial cells when the drug is administered in the presence of P-glycoprotein inhibitors. For example, when rats were pre-treated with GF120918, a potent P-glycoprotein inhibitor, the half-life of morphine in the brain was increased threefold [128].

During the course of inflammatory disease in the brain, decreased P-glycoprotein activity [129] may limit its ability to exclude xenobiotics, thus leading to an accumulation in the diseased state that differs from that measured in healthy animals. Interestingly, the presence of trypanosomes in the brain does lead to inflammation that could have the effect of increasing accessibility to the cerebrospinal fluid of a number of therapeutic agents.

Suramin, usually used only against early-stage African trypanosomiasis, may be used in combination with other trypanocidal reagents to improve activity against late-stage disease [130, 131]. In addition to its ability to modulate P-glycoprotein activity, suramin is a calcium-mobilising P2 purinergic antagonist. Since endothelial cytoplasmic calcium is an important factor in the regulation of blood-brain barrier permeability and transport [132], this too could possibly contribute to the ability of suramin to synergise with other drugs in curing late-stage disease. Pharmacological disruption of blood-brain barrier func-

tion with other agents is another approach that could increase permeability of this barrier. Cereport (also known as labradimil or RMP-7) is a nine-amino-acid peptide that binds bradykinin B2 receptors, agonising bradykinin action and leading to transient relaxation of tight junctions in the blood-brain barrier [133].

Models for the blood-brain barrier

Understanding how trypanosomes, and drugs, interact with the blood-brain barrier has been hindered by a lack of amenable models to study this system. Recent developments in the design of in vitro blood-brain barrier models might help redress this.

Several models of the blood-brain barrier comprising a coculture of brain capillary endothelial cells and astrocytes have recently been developed (fig. 2) [134]. Different cell types have been co-cultured, for example a model using rodent astrocytes with primary brain capillary endothelial cells has been characterised for a number of parameters including morphology, electrical resistance and expression of a number of typical blood-brain barrier markers such as mdr1a [135]. In silico approaches [136] to predict blood-brain barrier permeability based on solvation free energies and other biophysical criteria of chemical structures have also been shown to have good predictive abilities. Using blood-brain barrier models will impact on research into trypanosomiasis in two ways. First, it will enable investigators to gain insight into those compounds that will be able to reach parasites beyond the blood-brain barrier. Models will also facilitate the understanding of the mechanisms by which trypanosomes themselves can penetrate the barrier and establish within the brain.

Conclusions

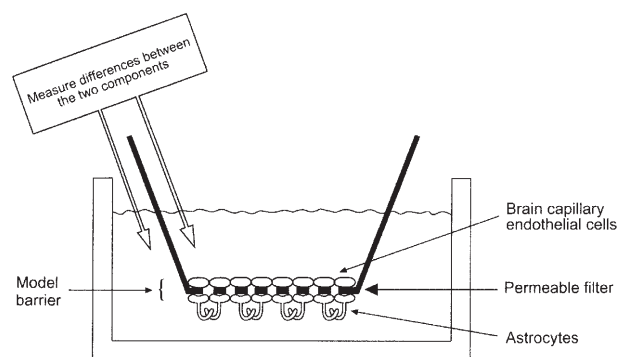HAT, or sleeping sickness, is in resurgence. The disease is defined by the symptoms associated with parasites in the CNS. In the last few years, significant progress has been made in understanding the relationship between the parasite and host in the context of inflammatory mediators which are released from parasites and host cells to influence infection. Many questions, however, remain to be answered including: (i) when and how do parasites cross the blood-brain barrier to reach the CNS? (ii) what inflammatory factors are released both in cerebrospinal fluid and blood when the CNS is involved? (iii) does the blood-brain barrier breakdown that occurs in late stages of disease affect trypanocide accessibility to the cerebrospinal fluid? Future research using blood-brain barrier models will hopefully yield more information about specific markers of CNS involvement and may reveal potential routes for delivering trypanocidal compounds to the CNS.

1 WHO (1998) Control and surveillance of African trypanosomiasis. Report of WHO expert committee. Technical Report Series 881, I–VI, p. 1114
2 Dumas M., Girard P. L. and N'Diaye I. P. (1976) Traitement de la trypanosomose humaine Africaine en milieu hospitalier. Med. Afr. Noire **23:** 39–41
3 Bertrand E., Serie F., Kone I., Rive J., Compaore, Sentilhes L. et al. (1973) Symptomatologie generale de la THA au moment du depistage. Med. Afr. Noire **20:** 303–314
4 Buguet A. (1997) Circadian disorders in sleeping sickness. Neurol. Infect. Epidemiol. **2:** 39–47
5 Buguet A., Bert J., Tapie P., Tabaraud F., Doua F., Lonsdorfer J. et al. (1993) Sleep-wake cycle in human African trypanosomiasis. J. Clin. Neurophysiol. **10:** 190–196
6 Kristensson K., Grassi-Zucconi G. and Bentivoglio M. (1995) Nervous system dysfunctions in African trypanosomiasis. In: Recent Advances in Tropical Neurology, pp. 165–174, Rose F. C. (ed.), Elsevier, Amsterdam
7 Dumas M. (1995) Human African trypanosomiasis. In: Tropical Neurology, vol 1, pp. 339–344, Shakir R. A., Newman P. K. and Poser C. M. (eds), Bailliere Tindal, London
8 Carpenter M. B. and Sutin J. (1983) Human Neuroanatomy, 8th edn, William and Wilkins, Baltimore
9 Chaudhuri J. D. (2000) Blood-brain barrier and infections. Med. Sci. Monit**. 6:** 1213–1222
10 Wekerle H., Livingstone N., Lassmann H. and Meyermann R. (1986) Cellular immune reactivity within the CNS. Trends Neurosci. **9:** 271–277
11 Osborn L. (1990) Leukocyte adhesion to endothelium in inflammation. Cell **62:** 3–6
12 Fabry Z., Waldschmidt M. M., Hendrickson D., Keiner J., Love-Homan L., Takei F. et al. (1992) Adhesion molecules on murine brain microvascular endothelial cells: expression of ICAM-1 and LGP 55. J. Neuroimmunol. **36:** 1–11
13 De Vries H. E., Bloom-Rosemalen M. C. M., Van Oosten M., De Boer A. G., Van Berkel T. J. C., Breimer D. D. et al. (1996) The influence of cytokines on the integrity of the blood-brain barrier in vitro. J Neuroimmunol. **64:** 37–43
14 Moncada S., Flower R. J. and Vane J. R. (1985) Prostaglandins, prostacyclin and thromboxane A2. In: The Pharmacological Basis of Therapeutics, pp. 660–673, Gilman A. G.,

Figure 2. Schematic representation of an in vitro model of the blood-brain barrier.

Goodman L. S., Rall T. W. and Murad F. (eds), Macmillan, New York

15 Feinstein D. L., Galea E., Roberts S., Berquist Hwang H. and Reis D. J. (1994) Induction of nitric oxide synthetase in rat C6 glioma cells. J. Neurochem. **62:** 315–321

16 Tuomanen E. (1996) Entry of pathogens into the central nervous system. FEMS Microbiol. Rev. **18:** 289–299

17 Dumas M. and Boa F. Y. (1988) Human African trypanosomiasis. In: Handbook of Clinical Neurology 8, pp. 339–344, Vinken P. J., Bryun G. W. and Klawens H. L. (eds), Elsevier, Amsterdam

18 Schultzberg M., Ambatsis M., Samuelsson E. B., Kristensson K. and Van Meirvenne N. (1988) Spread of *Trypanosoma brucei* to the nervous system: early attack on the circumventricular organs and sensory ganglia. J. Neurosci. Res. **21:** 56–61

19 Levine S. (1987) Choroid plexus: target for systemic disease and pathway to the brain. Lab. Invest. **56:** 231–233

20 Bentivoglio M., Grassi-Zucconi G., Olsson T. and Kristensson K. (1994) *Trypanosoma brucei* and the nervous system. Trends Neurosci. **17:** 325–329

21 Keita M., Bouteille B., Enanga B., Vallat J. M. and Dumas M. (1997) *Trypanosoma brucei brucei*: a long-term model of human African trypanosomiasis in mice, meningo-encephalitis, astrocytosis, and neurological disorders. Exp. Parasitol. **85:** 183–192

22 Zang J. R and Tuomanen E. (1999) Molecular and cellular mechanisms for microbial entry into the CNS. J. Neurovirol. **5:** 591–603

23 Mulenga C., Mhlanga J. D. M., Kristensson K. and Robertson B. (2001) *Trypanosoma brucei brucei* crosses the blood-brain barrier while tight junction proteins are preserved in a chronic disease model. Neuropathol. Appl. Neurobiol. **27:** 77–85

24 Abolarin M. O., Evans D. A., Tovey D. G. and Ormerod W. E. (1982) Cryptic stage of sleeping-sickness trypanosome developing in choroid plexus epithelial cells. Br. Med. J. **285:** 1380–1382

25 Stoppini L., Buchs P. A., Brun R., Muller D., Duport S., Parisi L. et al. (2000) Infection of organotypic slice cultures from rat central nervous tissue with *Trypanosoma brucei brucei*. Int. J. Med. Microbiol. **290:** 105–113

26 Pentreath V.W., Owolabi O. A., and Doua F. (1992) Survival of *Trypanosoma brucei brucei* in cerebrospinal fluid. Ann. Trop. Med. Parasitol. **86:** 29–34

27 Carter N.S. and Fairlamb, A.H. (1993) Arsenical-resistant trypanosomes lack an unusual adenosine transporter. Nature. **361:** 173–176

28 Vickerman K. (1985) Developmental cycles and biology of pathogenic trypanosomes. Br. Med. Bull. **41:** 105–114

29 Sileghem M., Flynn J. N., Darji A., De Baetselier P. and Naessens J. (1994) African trypanosomiasis. In: Parasite Infections and the Immune System, pp. 1–51, Kierszenbaum F. (ed.), Academic Press, San Diego

30 Donelson J. E , Hill K. L. and El-Sayed N. M. A. (1998) Multiple mechanisms of immune evasion by African trypanosomes. Mol. Biochem. Parasitol. **91:** 51–66

31 Vincendeau P., Okomo-Assoumou M. C., Semballa S., Fouquet N. and Daulouede S. (1996) Immunology and immunopathology of African trypanosomiasis. Med. Trop. **56:** 73–78

32 Poltera A. A., Hochmann A. and Lambert P. H. (1982) *Trypanosoma brucei gambiense*: cerebral immunopathology in mice. Acta Trop. **39:** 205–218

33 Pepin J. and Milord F. (1991) African trypanosomiasis and drug-induced encephalopathy: risk factors and pathogenesis. Trans. R. Soc. Trop. Med. Hyg. **85:** 222–224

34 Soignet S. L., Tong W. P., Hirschfeld S. and Warrell R. P. (1999) Clinical study of an organic arsenical, melarsoprol, in patients with advanced leukemia. Cancer Chemother. Pharmacol. **44:** 417–421

35 Dumas M. and Bouteille B. (2000) Treatment of human African trypanosomiasis. Bull. WHO **78:** 1474

36 Amevigbe M. D. D., Jauberteau-Marchan M. O., Bouteille B., Doua F., Breton J. C., Nicholas J. A. et al. (1992) Human African trypanosomiasis: presence of antibodies to galactocerebrosides. Am. J. Trop. Med. Hyg. **47:** 652–662

37 Bisser S., Ayed Z., Bouteille B., Stanghellini A., Breton J. C., Dumas M. et al. (2000). Central nervous system involvement in African trypanosomiasis: presence of anti-galactocerebroside antibodies in patients' cerebrospinal fluid. Trans. R. Soc. Trop. Med. Hyg. **94:** 225–226

38 Ayed Z., Brindel I., Bouteille B., Van Meirvenne N., Doua F., Houinato D. et al. (1997) Detection and characterization of antibodies directed against neurofilament proteins during human African trypanosomaisis. Am. J. Trop. Med. Hyg. **57:** 1–6

39 Okomo-Assoumou M. C., Geffard M., Daulouede S., Chaugier C., Lemesre J. L. and Vincendeau P. (1995) Circulating antibodies directed against tryptophan-like epitopes in sera of patients with human African trypanosomiasis. Am. J. Trop. Med. Hyg. **52:** 461–467

40 Lambert P. H., Berney M. and Kazyumba G. (1981) Immune complexes in serum and cerebrospinal fluid in African trypanosomiasis: correlation with polyclonal B cell activation and with intracerebral immunoglobulin synthesis. J. Clin. Invest. **67:** 77–85

41 Milner J. D. and Hajduk S. L. (1999) Expression and localization of serum resistance associated protein in *Trypanosoma brucei rhodesiense*. Mol. Biochem. Parasitol. **104:** 271–283

42 Xong H.V., Vanhamme L., Chamekh M., Chimfwembe C.E., Van Den Abbeele J., Pays A. et al. (1998) A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. Cell. **95:** 839–846

43 Olsson T., Bakhiet M., Edlund C., Hojeberg B., Van Der Meide P. and Kristensson K. (1991) Bidirectionnal activating signals between *Trypanosoma brucei* and CD8+ T-cells: a trypanosome-released factor triggers interferon-gamma production that stimulates parasite growth. Eur. J. Immunol. **21:** 2447–2454

44 Pentreath V. W. (1995) Typanosomiasis and the nervous system: pathology and immunology. Trans. R. Soc. Trop. ed. Hyg. **89:** 9–15

45 Radomski M. W., Buguet A., Bogui P., Doua F., Lonsdorfer A., Tapie P. et al. (1994) Disruption in the secretion of cortisol, prolactin, and some cytokines in human African trypanosomiasis. Bull. Soc. Pathol. Exot. **87:** 376–379

46 Bonfanti C., Caruso A., Bakhiet M., Olsson T., Turano A. and Kristensson K. (1995) Increased levels of antibodies to IFN-gamma in human and experimental trypanosomiasis. Scand. J. Immunol. **41:** 49–52

47 Eneroth A., Bakhiet M., Olsson T. and Kristensson K. (1992) Bi-directional signals between *Trypanosoma brucei brucei* and dorsal root ganglion neurons. J. Neurocytol. **21:** 846–852

48 Hill K. L., Hutchings N. R., Grandgenett P. M. and Donelson J. E. (2000) T lymphocyte-triggering factor of African trypanosomes is associated with the flagellar fraction of the cytoskeleton and represents a new family of proteins that are present in several divergent eukaryotes. J. Biol. Chem. **275:** 39369–39378

49 Daulouede S., Bouteille B., Moynet D., De Baetselier P., Courtois P., Lemesre J. L. et al. (2001) Human macrophage tumor necrosis factor (TNF-*α*) production induced by *Trypanosoma brucei gambiense* and the role of TNF-*α* in parasite control. J. Infect. Dis. **183:** 988–991

50 Okomo-Assoumou M. C., Daulouede S., Lemesre J. L., N'Zila-Mouanda A. and Vincendeau P. (1995) Correlation of high serum levels of tumor necrosis factor alpha with disease severity in human African trypanosomiasis. Am. J. Trop. Med. Hyg. **53:** 539–543

51 Hertz C. J., Filutowicz H. and Mansfield J. M. (1998) Resistance to African trypanosomes is interferon-γ dependent. J. Immunol. **161:** 6775–6783

52 Bakhiet M., Jansson L., Buscher P., Holmdahl R., Kristensson K. and Olsson T. (1996) Control of parasitemia and survival during *Trypanosoma brucei brucei* infection is related to strain dependent ability to produce interleukin-4. J. Immunol. **157:** 3518–3526

53 Baetselier P., Namangala B., Noel W., Brys L., Pays E. and Beschim A. (2001) Alternative versus classical macrophage activation during experimental African trypanosomiasis. Int. J. Parasitol. **31:** 575–587

54 Namangala B., Noel W., Baetselier P., Brys L. and Beschim A. (2001) Relative contribution of interferon-γ and interleukin-10 to resistance to murine African trypanosomiasis. J. Infect. Dis. **183:** 1794–1800

55 De Viers H. E., Kuiper J., De Boer A. G., Van Berkel T. J. C. and Breimer D. D. (1997) The blood-brain barrier in neuro-inflammatory diseases. Am. Soc. Pharmacol. Exp. Ther. **49:** 143–155

56 Vincendeau P., Daulouede S., Veyret B., Darde M. L., Bouteille B. and Lemesre J. L. (1992) Nitric oxide mediated cytostatic activity on *Trypanosoma brucei gambiense* and *Trypanosoma brucei brucei*. Exp. Parasitol. **75:** 353–360

57 Sternberg J. M. and McGuigan F. (1992) Nitric oxide mediates suppression of T-cell response in murine *Trypanosoma brucei* infection. Eur. J. Immunol. **22:** 2741–2744

58 Hurst R. D., Azam S., Hurst A. and Clark J. B. (2001) Nitric-oxide-induced inhibition of glyceraldehyde-3-phosphate dehydrogenase may mediate reduced endothelial cell monolayer integrity in an in vitro model blood-brain barrier. Brain Res. **894:** 181–188

59 Radi R., Beckman J. S., Bush K. M. and Freeman B. A. (1991) Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys. **288:** 481–487

60 Radi R., Beckman J. S., Bush K. M. and Freeman B. A. (1991) Peroxynitrite oxidation of sulfhydryls: the cytotoxic potential of superoxide and nitric oxide. J. Biol. Chem. **266:** 4244–4250

61 Boje K. M. K. and Lakhaman S. S. (2000) Nitric oxide redox species exert differential permeability effects on the blood-brain barrier. J. Pharmacol. Exp. Ther. **293:** 545–550

62 Reist M., Marshall K.A., Jenner P. and Halliwell B. (1998) Toxic effect of sulphite in combination with peroxynitrite on neuronal cells. J. Neurochem. **71:** 2431–2438

63 Keita M., Vincendeau P., Buguet A., Cespuglio R., Vallat J. M., Dumas M. et al. (2000) Inducible nitric oxide synthase and nitrotyrosine in the central nervous system of mice chronically infected with *Trypanosoma brucei brucei*. Exp. Parasitol. **95:** 19–27

64 Hu J., Ferreira A. and Van Eldik L. J. (1997) S100 beta induces neuronal cell death through nitric oxide release from astrocytes. J. Neurochem. **69:** 2294–2301

65 Landino L. M., Crews B. C., Timmons M. D., Morrow J. D. and Marnett L. J. (1996) Peroxynitrite, the coupling product of nitric oxide and superoxide, activates prostaglandin biosynthesis. Proc. Natl. Acad. Sci. USA **93:** 15069–15074

66 Pentreath V. W., Rees K., Owolabi O. A., Philip K.A. and Doua F. (1990) The somnogenic T-lymphocyte suppressor prostaglandin D2 is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. Trans. R. Soc. Trop. Med. Hyg. **84:** 795–799

67 Hayaishi O. (1989) Prostaglandin D2 and sleep. Ann. N.Y. Acad. Sci. **559:** 374–381

68 Hayaishi O. (1991) Molecular mechanisms of sleep-wake regulation: roles of prostaglandins D2 and E2. FASEB J. **5:** 2575–2581

69 Buguet A. and Cespuglio R. (1999) Sleeping sickness: a disease of clock with nitric oxide involvement. In: Progress in Human African Trypanosomiasis, Sleeping Sickness, pp. 191–201, Dumas M., Bouteille B. and Buguet A. (eds), Springer, Paris

70 Buguet A., Tapie P. and Bert J. (1999) Reversal of the sleep/wake cycle disorder of sleeping sickness after trypanocide treatment. J. Sleep Res. **8:** 225–235

71 Philip K. A., Dascombe M. J., Fraser P. A. and Pentreath V. W. (1994) Blood-brain barrier damage in experimental African trypanosomiasis. Ann. Trop. Med. Parasitol. **88:** 607–616

72 Roldan E., Rodriguez C., Navas G., Parra C. and Brieva J. A. (1992) Cytokine network regulating terminal maturation of human bone marrow B cells capable of spontaneous and high rate Ig secretion in vitro. J. Immunol. **149:** 2367–2371

73 Mayhan W. G. (1998) Effect of lipopolysaccharide on the permeability and reactivity of the cerebral microcirculation: role of inducible nitric oxide synthase. Brain Res. **792:** 353–357

74 Adams J. H., Haller L., Boa F. Y., Doua F., Dago A. and Konian K. (1986) Human African trypanosomiasis (*Trypanosoma brucei gambiense*): a study of 16 fatal cases of sleeping sickness with some observations on acute reactive arsenical encephalopathy. Neuropathol. Appl. Neurobiol. **12:** 81–94

75 Cattand P., Miezan B. T. and De Raadt. P. (1988) Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. Bull. WHO **66:** 83–86

76 Magnus E., Vervoort T. and Van Meirvenne N. (1978) A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. Ann. Soc. Belg. Med. Trop. **58:** 169–176

77 Truc P., Jamonneau V., Cuny G. and Frezil J. L. (1999) Use of polymerase chain reaction in human African trypanosomiasis stage determination and follow-up. Bull. WHO **77:** 745–748

78 Kyambadde J. W., Enyaru J. C. K., Matovu E., Odiit M. and Carasco J. F. (2000) Detection of trypanosomes in suspected sleeping sickness patients in Uganda using the polymerase chain reaction. Bull. WHO **78:** 119–124

79 Miezan T. W., Meda H. A., Doua F., Dje N .N., Lejon V. and Buscher P. (2000) Single centrifugation of cerebrospinal fluid in a sealed pasteur pipette for simple, rapid and sensitive detection of trypanosomes. Trans. R. Soc. Trop. Med. Hyg. **94:** 293

80 Burri C., Nkunku S., Merolle A., Smith T., Blum J. and Brun R. (2000) Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomised trial. Lancet **355:** 1419–1425

81 Kuzoe F. A. S. (1993) Current situation of African trypanosomiasis. Acta Trop. **54:** 153–162

82 Hunter C. A., Jennings F. W., Adams J. J. H., Murray M. and Kennedy P. G. E. (1992) Subcurative chemotherapy and fatal post-treatment reactive encephalopathies in African trypanosomiasis. Lancet **339:** 956–958

83 Haller L., Adams H., Merouze F. and Dago A. (1986) Clinical and pathological aspects of human African trypanosomiasis (*Trypanosoma brucei gambiense*) with particular reference to reactive arsenical encephalopathy. Am. J. Trop. Med. Hyg. **35:** 94–99

84 Pepin J. and Milord F. (1994) The treatment of human African trypanosomiasis. Adv. Parasitol. **33:** 1–47

85 Burri C., Baltz, T., Giroud C., Doua F., Welker H. A. and Brun R. (1993) Pharmacokinetic properties of the trypanocidal drug melarsoprol. Chemotherapy **39:** 225–234

86 Burri C., Onyango J. D., Auma J. E., Burudi E. M. and Brun R. (1994) Pharmacokinetics of melarsoprol in uninfected vervet monkeys. Acta Trop. **58:** 35–49

87 Bronner U., Brun R., Doua F., Ericsson O., Burri C., Keiser J. et al. (1998) Discrepancy in plasma melarsoprol concentrations between HPLC and bioassay methods in patients with *T. gambiense* sleeping sickness indicates that melarsoprol is metabolized. Trop. Med. Int. Health **3:** 913–917

88 Keiser J., Ericsson O. and Burri C. (2000) Investigations of the metabolites of the trypanocidal drug melarsoprol. Clin. Pharmacol. Ther. **67:** 478–488

89 Friedheim E. A. H. (1949) MelB in the treatment of human trypanosomiasis. Am. J. Trop. Med. Hyg. **29:** 173–180

90 Burri C. and Keiser J. (2001) Pharmacokinetic investigations in patients from northern Angola refractory to melarsoprol treatment. Trop. Med. Int. Health. **6:** 412–420

91 Brun R., Schumacher R., Schmid C., Kunz C. and Burri C. (2001) The phenomenon of treatment failures in human African trypanosomiasis. Trop. Med. Int. Health. **6:** 906–914

92 Legros D., Evans S., Maiso F., Enyaru J. C. K. and Mbulamberi D. (1999) Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda. Trans. R. Soc. Trop. Med. Hyg. **93:** 439–442

93 Matovu E., Enyaru J. C. K., Schmid C., Seebeck T. and Kaminsky R. (2001) Melarsoprol refractory *T. b. gambiense* from Omugo, north-western Uganda. Trop. Med. Int. Health **6:** 407–411

94 Bacchi C. J., Nathan H. C., Hunter S. H., McCann P. P. and Sjoerma A. (1980) Polyamine metabolism: a potential therapeutic target in trypanosomes. Science **210:** 332–224

95 Iten M., Mett H., Evans A., Enyaru J. C., Brun R. and Kaminsky R. (1997) Alterations in ornithine decarboxylase characteristics account for tolerance of *Trypanosoma brucei rhodesiense* to D,L-alpha-difluoromethylornithine. Antimicrob. Agents Chemother. **41:** 1922–1925

96 Milord F., Loko L., Ethier L., Mpia B. and Pepin J. (1993) Eflornithine concentrations in serum and cerebrospinal fluid of 63 patients treated for *Trypanosoma brucei gambiense* sleeping sickness. Trans. R. Soc. Trop. Med. Hyg. **87:** 473–477

97 Pepin J., Khonde Z., Maiso F., Doua F., Jaffar S., Ngampo S. et al. (2000) Short-course eflornithine in Gambian trypanosomiasis: a multicentre randomized controlled trial. Bull. WHO **78:** 1284–1295

98 Hickman J.G., Huber F. and Palmisano, M. (2001) Human dermal safety studies with eflornithine HCl 13.9% cream (Vaniqa), a novel treatment for excessive facial hair. Curr. Med. Res. Opin. **16:** 235–244

99 Sands M., Kron M. A. and Brown R. B. (1985) Pentamidine, a review. Rev. Infect. Dis. **7:** 625–634

100 Bronner U., Doua F., Ericsson O., Gustafsson L. L., Miezan T. W., Rais M. et al. (1991) Pentamidine concentrations in plasma, whole blood and cerebrospinal fluid during treatment of *Trypanosoma gambiense* infection in Cote d'Ivoire. Trans. R. Soc. Trop. Med. Hyg. **85:** 608–611

101 Doua F., Miezan T. W., Sanon Singaro J. R., Boa Y. F. and Baltz T. (1996) The efficacy of pentamidine in the treatment of early – late stage *Trypanosoma brucei gambiense* trypanosomiasis. Am. J. Trop. Med. Hyg. **55:** 586–588

102 Bronner U., Gustafsson L. L., Doua F., Ericsson O., Miezan T. W., Rais M. et al. (1995) Pharmacokinetics and adverse reactions after a single dose of pentamidine in patients with *Trypanosoma gambiense* sleeping sickness. Br. J. Clin. Pharmacol. **39:** 289–295

103 Seed J. R. (2001) African trypanosomiasis research: 100 years of progress, but questions and problems still remain. Int. J. Parasitol. **31:** 434–442

104 Nichols A. C., Yielding K. L. and Agbe S. A. O. (2000) A chlorodiazirine analog of pentamidine with anti-trypanosomal activity. J. Parasitol. **86:** 177–180

105 Pepin J., Milord F., Meurice F., Ethier L., Loko L. and Mpia B. (1992) High-dose nifurtimox for arseno-resistant *Trypanosoma brucei gambiense* sleeping sickness: an open trial in central Zaire. Trans. R. Soc. Trop. Med. Hyg. **86:** 254–256

106 Bisser S., Van Nieuwhonove S., Lejon V., N'Siessi X., Preux P. M. and Buscher P. (2000). New therapeutic regimen for sleeping sickness: description, results and lessons from the clinical trial established in Bwamanda (Equateur, RDC). Proceedings of the Trypanosomiasis and Leishmaniasis Seminars and 3rd COST-B9 Meeting in Anti-Protozoal Chemotherapy, Bruges

107 Bouteille B., Marie-Daragon A., Chauvière G., De Albuquerque C., Enanga B, Darde M. L. et al. (1995) Effect of megazol on *Trypanosoma brucei brucei* acute and subacute infections in Swiss mice. Acta Trop. **60:** 73–80

108 Bouteille B., Millet P., Enanga B., Mezui Me J., Keita M., Jauberteau MO. et al. (1999) Experimental models for new chemotherapeutic approches to human African trypanosomiasis. In: Progress in Human African Trypanosomiasis, Sleeping Sickness, pp. 253–280, Dumas M., Bouteille B. and Buguet A. (eds), Springer, Paris

109 Barrett M. P., Fairlamb A. H., Rousseau B., Chauvière G. and Perié J. (2000) Uptake of the nitroimidazole drug megazol by African trypanosomes. Biochem. Pharmacol. **59:** 615–620

110 Mäser P., Sutterlin C., Kralli A. and Kaminsky R. (1999) A nucleoside transporter from *Trypanosoma brucei* involved in drug resistance. Science. **285:** 242–244

111 Enanga B., Keita M., Chauvière G., Dumas M. and Bouteille B. (1998) Megazol combined with suramin: a chemotherapy regimen which reversed the CNS pathology in a model of human African trypanosomiasis in mice. Trop. Med. Int. Health **3:** 736–741

112 Enanga B., Boudra H., Chauvière G., Labat C., Bouteille B., Dumas M. et al. (1999) Pharmacokinetics, metabolism and excretion of megazol, a new potent trypanocidal drug in animals. Drug Res. **49:** 441–447

113 Enanga B., Mezui Me Ndong J., Boudra H., Debrauwer L., Bouteille B., Chauvière G. et al. (2000) Pharmacokinetics, metabolism and excretion of megazol in a *Trypanosoma brucei gambiense* primate model of human African trypanosomiasis: preliminary study. Drug Res. **50:** 158–162

114 Enanga B., Labat C., Boudra H., Chauviere G., Keita M., Bouteille B. et al. (1997) Simple high-performance liquid chromatographic method to analyse megazol in human and rat plasma. J. Chromatogr. B. **696:** 261–266

115 Buxbaum E. (1999) Co-operative binding sites for transported substrates in the multiple drug resistance transporter Mdr1. Eur. J. Biochem. **265:** 64–70

116 Ferrera R. C. C. and Ferrera L. C. S. (1986) CL 64,855, a potent anti-*Trypanosoma cruzi* drug, is also mutagenic in *Salmonella* microsome assay. Mem. Inst. Oswaldo Cruz **81:** 49–52

117 Watanabe M., Nishino T., Takio K., Sofuni T. and Nohmi T. (1998) Purification and characterisation of wild-type and mutant 'classical' nitroreductases of *Salmonella typhimurium*: L33R mutation greatly diminishes binding of FMN to the nitroreductase of *S. typhimurium*. J. Biol. Chem. **273:** 23922–23928

118 Redin G. S., Kuck N. A. and Forbes M. (1968) 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole, a new antimicrobial agent. VI. Antibacterial efficacy in mice and in vitro. Antimicrob. Agents Chemother. **8:** 534–537

119 Burden E. J. and Racette E. (1968) 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole, a new antimicrobial agent. IX. Action against hemoflagellate infections in laboratory animals. Antimicrob. Agents Chemother. **8:** 545–547

120 Pardridge W. M. (1999) Non-invasive drug delivery to the human brain using endogenous blood-brain barrier transport systems. Pharm. Sci. Technol. Today **2:** 49–59

121 Tuomanen E. (1993) Breaching the blood-brain barrier. Sci. Am. **268:** 80–84

122 Terasaki T. and Hosoya K. I. (1999) The blood-brain barrier efflux transporters as a detoxifying system for the brain. Adv. Drugs Deliv. Rev. **36:** 195–209

123 Cordon-Cardo C., O'Brien J. P. O., Casals D., Rittman-Grauer L., Biedler J. L., Melamed M. R. et al. (1989) Multidrug resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc. Natl. Acad. Sci. USA **86:** 695–698

124 Huisman M. T., Smit J. W., Wiltshire H. R., Hoetelmans R. M. W., Beijnen J. H. and Schinkel A. H. (2001) P-Glycoprotein

limits oral availability, brain and fetal penetration of Saquinar even with high doses of Ritonavir. Mol. Pharmacol. **59:** 806–813

125 Groothuis D. R. (2000) The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. Neurol. Oncol. **2:** 45–59

126 Edwards R. H. (2001) Drug delivery via the blood-brain barrier. Nat. Neurosci. **4:** 221–222

127 Dagenais C., Rousselle C., Pollack G. M. and Scherrmann J. M. (2000) Development of an in situ mouse brain perfusion model and its application to mdr1a P-glycoprotein-deficient mice. J. Cereb. Blood Flow Metab. **20:** 381–386

128 Letrent S. P., Pollack G. M., Brouwer K. R. and Brouwer K. L. (1999) Effects of a potent and specific P-glycoprotein inhibitor on the blood-brain barrier distribution and antinociceptive effect of morphine in the rat. Drug Metab. Dispos. **27:** 827–834

129 Piquette-Miller M., Pak A., Kim H., Anari R. and Shahzamani A. (1998) Decerased expression and activity of P-glycoprotein in rat liver during acute inflammation. Pharmacol. Res. **15:** 706–711

130 Jennings F. W. (1991) Chemotherapy of CNS-trypanosomiasis: combination chemotherapy with 5-nitroimidazole (MK 436), an arsenical (Cymelarsan) and suramin. Trop. Med. Parasitol. **42:** 157–168

131 Clarkson A. B. J., Bienen E. J., Bacchi C. J., McCann P. P., Nathan H. C., Hutner S. H. et al. (1984) New drug combination for experimental late-stage African trypanosomiasis: DL-alpha-difluoromethylornithine (DFMO) with suramin. Am. J. Trop. Med. Hyg. **33:** 1073–1077

132 Paemeleire K. and Leybaert L. (2000) ATP-dependent astrocyte-endothelial calcium signaling following mechanical damage to a single astrocyte in astrocyte-endothelia co-cultures. J. Neurotrauma **17:** 345–358

133 Emereich D. F., Dean R. L., Osborn N. and Bartus R. T. (2001) The development of the bradykinin agonist Labradimil as a means to increase the permeability of the blood-brain barrier. Clin. Pharmacokinet. **40:** 105–123

134 De Boer, A. G., and Sutanto, W. (eds) Drug Transport Across the Blood Brain Barrier: In Vitro and in Vivo Techniques, Harwood, Amsterdam

135 Gaillard P. J., Voorwinden L. H., Nielsen J. L., Ivanov A., Atsumi R., Engman H. et al. (2001) Establishment and functional characterization of an in vitro model of the blood-brain barrier, comprising a co-culture of brain capillary endothelial cells and astrocytes. Eur. J. Pharmacol. Sci. **12:** 215–222

136 Keseru G. M. and Molnar L. (2001) High-throughput prediction of blood-brain partitioning: a thermodynamic approach. J. Chem. Inf. Comput. Sci. **41:** 120–128

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