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Mini Review

Traversal of human and animal trypanosomes across the blood-brain barrier

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The neurological complications of human African trypanosomiasis (HAT) in man caused by the unicellular protozoan parasites *Trypanosoma brucei gambiense* and *T. b. rhodesiense* are a consequence of the penetration of the blood-brain barrier (BBB) by trypanosomes that enter the central nervous system (CNS). Yet the mechanisms by which African trypanosomes cross the true BBB comprised of brain microvascular endothelial cells (BMECs) remain unclear. Human BBB models used to determine how African trypanosomes initially interact *in vitro* with the human BBB proper suggest that parasites cross the human BBB in part by generating Ca^{2+} activation signals in human BMECs through the activity of parasite cysteine proteases. *In vivo* murine models of HAT have suggested additional mechanisms of BBB traversal by trypanosomes, with recent compelling evidence for the important role of interferon-γ in facilitating this process. A clear understanding of how trypanosomes enter the CNS is critical for both understanding the neuropathogenesis of HAT and in developing more effective drug therapies for late-stage disease. *Journal of NeuroVirology* (2008) **14,** 344–351.

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Human African trypanosomiasis

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a major cause of mortality and morbidity in 36 countries sub-Saharan Africa, where 60 million people are at risk from the disease (Kennedy, 2004). The disease is caused by single-celled extracellular protozoan parasites of the genus *Trypanosoma,* and is always fatal if untreated (Atouguia and Kennedy, 2000). The disease is transmitted by the bite of the tsetse fly of the *Glossina* species. There are two forms of the disease, East African HAT, caused by *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and the West African variant, caused by *T. b. gambiense*. The tempo of the diseases caused by these two variants is different in that the progression of *rhodesiense* infection is rapid, with death within a few weeks, whereas the disease course of *gambiense* infection is much slower, with progression to eventual death taking many months to years (Atouguia and Kennedy, 2000).

In the early, or hemolymphatic, stage of the disease, which occurs 5 to 15 days after the bite of the tsetse fly, the parasites proliferate in the blood-stream, lymph nodes, liver, and spleen. Within a few weeks in the case of *rhodesiense* disease, or months in the case of *gambiense* disease, the parasites cross the blood-brain barrier (BBB) to invade the central nervous system (CNS) to cause the late, or encephalitic, stage. A variety of neurologic features characterize late-stage disease, including the characteristic sleep disorder with disruption of normal sleep-wake cycles, motor and sensory symptoms and signs, and then progressive coma, cerebral edema, and death

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(Atouguia and Kennedy, 2000). The most commonly used drug for late-stage HAT is the arsenical drug melarsoprol, which, unlike most drugs used for HAT, crosses the BBB. Although usually effective, melarsoprol is highly toxic, causing a severe post-treatment reactive encephalopathy (PTRE) in 10% of patients receiving it, half of whom will die, i.e., it has a 5% overall mortality rate (Pepin and Milord, 1994). The alternative drug for *gambiense* CNS disease is eflornithine (DFMO), which, although less dangerous than melarsoprol, still has a number of toxic side effects and is ineffective for *rhodesiense* disease (Kennedy, 2004). This very limited and highly toxic repertoire of drugs for late-stage HAT, none of which can be given orally, is very unsatisfactory. In addition, the biological and diagnostic criteria for defining CNS invasion in HAT is very problematic. Although the World Health Organization (WHO) criteria for late-stage HAT, namely the presence of trypanosomes in the cerebrospinal fluid (CSF) and/or >5 white blood cells (WBC)/μl, are accepted by many clinicians, there is not a consensus on this critical issue with some using a higher defining figure of 20 WBC/ μl (Kennedy, 2006; Lejon *et al*, 2003). Also, there is not always a clear match between the biological definition of what constitutes CNS disease and the ground for making therapeutic choices (Kennedy, 2004). The fact that CNS drug treatment is so toxic makes it essential to have available reliable and validated diagnostic criteria for late-stage HAT

In view of these two major issues with both defining when the late stage of HAT has been reached, and the current reliance of a few highly toxic drugs, it is imperative that we achieve a much greater understanding of how and when trypanosomes cross the BBB to reach the CNS. Here we first explain the nature of the BBB, especially in relation to trypanosomes, and then consider the understanding of this process that has been gained from both *in vitro* and *in vivo* approaches.

The blood-brain barrier and trypanosomes

How trypanosomes enter the brain is a crucial question because the brain has a unique barrier that restricts the passage of molecules and pathogens into it. This barrier is called the blood-brain barrier (BBB), which is composed of tightly apposed, capillary endothelial cells held together by ''tight junctions'' that hold the BBB cells together in intimate contact. The spaces that occur between endothelial cells elsewhere (except the testes) in the body are not found at the BBB. This barrier also lacks the connective tissue matrix that is found in other organs of the body to which *T. brucei* seems to be attracted. Despite this, African trypanosomes gain access to the human brain.

There are some other points that highlight the importance of the BBB that are seldom considered when one thinks of how trypanosomes cause neuropathology. In the brain, there are three barriers that limit and regulate the exchanges that occur between the blood, neural tissue, or its fluid spaces (Abbott *et al*, 2006). There is the choroid plexus epithelium between blood and ventricular CSF, and the arachnoid barrier cells between blood and subarachnoid CSF (Abbott *et al*, 2006; Haines, 2006). But, it is the true BBB formed by brain microvascular endothelium and separating brain interstitial fluid from the blood—whose surface area of approximately 20 m^2 is 1000-fold greater than that of the arachnoid membrane or the blood-CSF barrier (Pardridge, 2002) and 5000-fold greater than those of the choroid plexus and circumventricular areas (Davis)—that exerts the greatest control over the brain's microenvironment (Abbott *et al*, 2006). Here an African trypanosome some 20 μm in length could have the most dramatic impact, because individual neurons and closely associated neuronal processes and the perivascular endfeet of astrocytic glia are rarely more than 8 to 20 μm from a brain capillary, but may be millimeters or centimeters away from a CSF compartment (Abbott *et al*, 2006). Furthermore, the microvasculature is so dense (with a mean intercapillary distance of 40 μm) (LeBowitz, 2005) that trypanosomes could easily access the entire brain by transmigrating a short distance across the BBB.

In vitro approaches

To date, the key molecular players involved in parasite-induced signaling and crossing of BBB are not understood. *In vitro* models of the BBB have served as very useful tools for identifying the cellular and molecular elements that are possible targets for intervention in the transmigration of pathogens into the CNS (Kim, 2006; Tuomanen, 1996; Zhang and Tuomanen, 1999). Extensive studies using human brain microvascular endothelial cells (HBMECs) to decipher how bacteria (*Escherichia coli*, group B *Streptococcus*, and *Streptococcus pneumoniae*, *Citrobacter* sp.), viruses (human immunodeficiency virus [HIV]), fungi (*Candida albicans*), rickettsiae (*Ehrlichia chaffeensis*–infected monocytes) (Park *et al*, 2003), and spirochetes (*Borrelia burgdorferi*) (Grab *et al*, 2005) cross the BBB clearly show that this *in vitro* model mimics many of the essential features of the human BBB *in vivo*.

The diversity of routes of entry used by microbial pathogens to subvert the BBB include (i) transcytosis (transcellular); (ii) opening of tight junctions (paracellular); (iii) the use of microbial products, possibly proteases and toxins, that directly induce

BBB breakdown; and/or (iv) in the case of intracellular pathogens invade the CNS using infected leukocytes as 'Trojan horses' by directly infecting a pool of leukocytes that recirculate through the brain; or by altering leukocyte gene expression such that the infected cells become capable of crossing the endothelium that lines the brain capillaries (Enanga *et al*, 2002; Kim, 2006; Nassif *et al*, 2002; Tuomanen, 1996; Zhang and Tuomanen, 1999).

In order to study the mechanisms underlying human BBB traversal by bloodstream forms (BSFs) of African trypanosomes, a well-described *in vitro* model composed of the major functional unit of BBB has been used, i.e., brain microvascular endothelial cells (BMECs) (Stins *et al*, 1994, 1997b; Grab *et al*, 2004). In these studies, human BMECs (HBMECs) are typically $>99\%$ pure after testing nonendothelial specific markers, take up acetylated low-density lipoprotein and express factor VIII–Rag, carbonic anhydrase IV, *Ulex europaeus* agglutinin I, P-glycoprotein, γ-glutamyltranspeptidase, protease-activated receptors (PAR1 to PAR4), and tight junctional proteins (Grab *et al*, 2004, 2005; Kim *et al*, 2004; Persidsky *et al*, 1997; Stins *et al*, 1997a,b). In addition, HBMEC expression of several adherens junction (AJ) and tight junction (TJ) proteins contributes to the low level of permeability characteristic for these cells (Grab *et al*, 2005; Paemeleire *et al*, 1999).

To create *in vitro* models of the human BBB, HBMECs are grown collagen-coated Costar Transwell inserts (Grab *et al*, 2004; Matter and Balda, 2003) (Figure 1). The Transwell-based *in vitro* BBB model allows (i) separate access to the upper (blood side) and lower compartments (brain side); (ii) the assessment of HBMEC integrity before and after an experiment through transendothelial electrical resistance (TEER) measurements in Endohm Chambers reaching TEER values similar to epithelial cells (Grab *et al*, 2004; Matter and Balda, 2003); and (iii) enumeration of the number of parasites that traverse the HBMECs into the bottom compartment (Grab *et al*, 2004). Although Transwell inserts are typically incubated inside a microwell plate, as a rough approximation to real-time TEER measurements by electric cell-substrate impedance sensing (ECIS), the inserts can be continuously incubated in EndOhm chambers and TEER values taken directly at select time without disturbing the system.

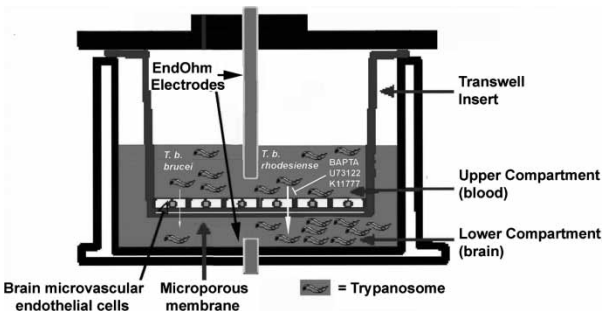

Figure 1 The *in vitro* human BBB model. When incubated with HBMECs grown on Transwell inserts, BSF *T. b. rhodesiense* cross HBMECs eightfold or more better than BSF *T. b. brucei*. In addition, BSF *T. b. rhodesiense* transmigration across HBMECs is inhibited when (i) the parasites are pretreated with K11777, a specific inhibitor of cathepsin L (i.e., brucipain), or (ii) when the HBMECs have been pretreated with BAPTA-AM (a chelator of intracellular calcium) or U73122 (a PLC-inhibitor).

Using this model, it was found that bloodstream form (BSF) trypanosomes attach firmly to HBMECs, whereas procyclic forms (present in the tsetse fly vector) bind poorly to the monolayer (Grab *et al*, 2004). Furthermore, procyclic forms do not cross HBMECs, whereas a human-infective bloodstream form parasite, formerly thought to be *T. b. gambiense* IL1852 but now reclassified as *T. b. rhodesiense* IL1852 based on the presence of the SRA gene (D. J. Grab, unpublished), cross HBMECs eightfold or more efficiently than animal-infective *T. b. brucei*. A transient decay and recovery in TEER (by ECIS) was observed during BSF parasite passage (Grab *et al*, 2004). It was proposed that parasite binding at or near intercellular junctions of the BBB precedes crossing via a paracellular route. In addition, we reported that the parasites induce oscillatory changes in the intracellular calcium ($[Ca^{2+}]_i$) of BMECs and proposed that signaling events triggered by BSF parasites might render the HBMEC permissive to *T. brucei* traversal (Grab *et al*, 2004).

Because phospholipase C-β (PLC-β) increases HBMECs permeability through the activation of the actin cytoskeleton (stress fibers), inhibition of this enzyme should lead to a tightening of cellular junctions, which in turn would be expected to hinder paracellular crossing of the parasites. As shown in Figure 2A, HBMECs on Transwell inserts pretreated for 30 min with the PLC-β inhibitor U73122, to block the $[Ca^{2+}]_i$ signaling response to trypanosomes, maintained control EndOhm TEER values during a 3-h transmigration experiment. Real-time TEER changes by ECIS also confirmed that pretreatment of HBMECs with U73122 tightened the barrier to the trypanosomes (Figure 2B). Unlike U73122, the inactive isomer U73343 (Stemkowski *et al*, 2002), failed to attenuate the HBMEC TEER response to the parasites.

It was later found that the ability to cross HBMECs required trypanosome cysteine proteases and that the ability to cross this barrier was abrogated by K11777, an irreversible inhibitor of cathepsin L–like cysteine proteases (brucipain), but not by the cathepsin B inhibitor CA074 (Nikolskaia *et al,* 2006) (Figure 1). Furthermore, K11777-treated trypanosomes failed to elicit calcium fluxes in BMECs, suggesting that generation of activation signals for the BBB was critically depended on brucipain activity. Strikingly, crossing of *T. b. brucei* across the BBB was enhanced upon incubation with brucipain-rich supernatants derived from *T. b. rhodesiense*. (Nikolskaia *et al*, 2006). The effects of the

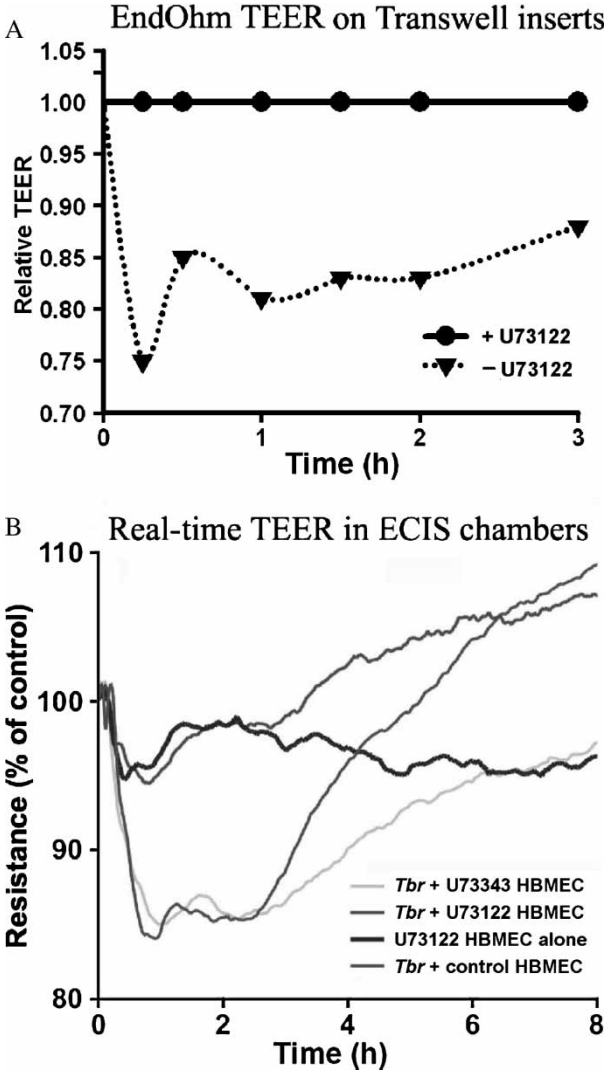

Figure 2 Changes in HBMEC TEER in response to trypanosomes is mediated by PLC. (**A**) Confluent HBMECs grown on Transwell inserts were pretreated 30 min with 5 μM U73122 (+U73122) or 0.5% DMSO (-U73122). The figure shows the relative change in HBMEC TEER of a single Transwell inserts in response to *T. b. rhodesiense* IL1852 monitored without interruption in individual EndOhm chambers. (**B**) Real-time TEER changes by ECIS also confirmed that pretreatment of HBMEC with U73122 as described in Panel A tightened the barrier to the trypanosomes. Note the initial 20% drop in TEER followed by a recovery period when HBMEC are incubated with trypanosomes (bold light gray line). Compare this to the steady increase of TEER (monolayer tightening) over the controls when the parasites were incubated with HBMEC pretreated with U73122 (bold black line). Unlike U73122, the inactive isomer U73343, failed to attenuate the HBMEC TEER response to the parasites (bold medium gray line). U73122 alone (thin black line) was not toxic having little affect on HBMEC TEER relative to controls. Each line represents the average of duplicate determinations.

conditioned medium, which correlated with ability to evoke ($[Ca^{2+}]_i$) fluxes, were cancelled by K11777, but not by the cathepsin B inhibitor CA074. Collectively, these *in vitro* studies implicated brucipain as a critical driver of African trypanosomes transendothelial migration of the human BBB.

These data indicate that cysteine proteases (particularly brucipain) secreted by African trypanosomes induce Ca^{2+} signaling in the endothelial cells, and this event is required for subsequent parasite traversal of the endothelial cell monolayer (Nikolskaia *et al*, 2006). Considering the critical role of Ca^{2+} in nuclear factor (NF)-κB) activation (Steinhoff *et al*, 2005), it is possible that the parasite cysteine proteases also induce NF-κB proinflammatory signaling in HBMECs. Using human marrow endothelial cells, which exhibit cell properties similar to brain endothelial cells, Girard showed that *T. b. gambiense* could activate endothelial cells to release interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α, (Girard *et al*, 2005). Screening for expression of human cytokines and chemokines by Luminex multiplex fluorescent bead technology, we find similar changes in IL-6, IL-8, CCL2, and TNF-α protein expression by HBMECs when incubated with *T. b. rhodesiense* (D. J. Grab, unpublished) This is of interest given (i) putative roles of IL-6 or TNF-α in neuroprotection or pathology (Girard *et al*, 2005); (ii) *T. b. rhodesiense's* ability to induce the synthesis of IL-6 and IL-8 in human bone marrow endothelial cells (Courtioux *et al*, 2006; Lejon *et al*, 2002); and (iii) *T. b. rhodesiense's* ability to increase human CSF levels of IL-8 and CCL2 (Chambers, 2003).

So what is the role of brucipain in CNS invasion? It is possible that brucipain triggers protease-acitvated receptors (PARs), which are G-protein-coupled receptors (GPCRs) that signal in response to multiple extracellular proteases (Traynelis and Trejo, 2007). There are four known PARs and all are activated by serine proteases such as thrombin (PAR1, PAR3, and PAR4) and trypsin (PAR2). Our HBMECs express all four PARs (Figures 1 and 2) and PAR1 and PAR2 can trigger calcium-mediated HBMECs transmembrane signaling (Kim *et al*, 2004). Interestingly, gingipains, cysteine proteases of *Porphyromonas gingivalis*, can activate PARs on neutrophils and oral epithelial cells and lead to calcium signaling and IL-6 production (Lourbakos *et al*, 2001). Because activating signals (increases in $[Ca^{2+}]_i$ and PLC activation) were observed during the interaction of African trypanosomes with HBMECs, we hypothesize that the activation of PAR1 and/or PAR2 by the parasite may also play a role in increasing HBMEC permeability, enabling subsequent crossing.

In vivo approaches

Studies of how trypanosomes get into the human brain have been hampered by the fact that the presence of parasites in the brain at autopsy has

been reported only infrequently (Mott, 1907). This may be a consequence of most patients having received some form of trypanocidal therapy prior to death, and that the histological demonstration of parasites is only possible if the brain is fixed within 4 to 5 h after death (Calwell, 1937). Because most autopsy specimens are obtained after 5 h after death, the chances of finding trypanosomes are significantly diminished. The question of where trypanosomes enter the brain has therefore relied on direct animal studies and/or by inference from pathologic findings in human and animal tissues. It is clearly not possible to carry out mechanistic BBB studies in human patients.

Most of our knowledge in this area has come from rat and mouse models of chronic trypanosome infection, with particular contributions from the laboratory of K. Kristensson. In the infected mouse, *T. b. rhodesiense* has been observed in highly vascularized areas of the brain (e.g., cerebral cortex, the hippocampus, and the nuclei of the brain stem), suggesting that meningoencephalitis may start from the capillaries (Fink and Schmidt, 1979). Trypanosomes have also been found in areas of the brain where the endothelial BBB is incomplete (Lonsdale-Eccles and Grab, 2002; Schmidt and Bafort, 1987; Schultzberg *et al*, 1988). In experimental animals infected with the rodent pathogenic strain *T. b. brucei,* parasites appear early during infection in the circumventricular areas and the choroid plexus (Lonsdale-Eccles and Grab, 2002; Schultzberg *et al*, 1988) that lack a BBB. This localization correlates with some of the neurologic features of the disease. However, even this barrier-deficient area is not fully permeable, and the trypanosomes must bypass epithelial cells in order to penetrate the CNS through the choroid plexus. At later stages, the parasites penetrate the true BBB and enter the brain parenchyma, as revealed by double immunohistochemical labeling of parasites and brain endothelial cells in a rat model of the chronic disease (Mulenga *et al*, 2001). In experimental mouse models of HAT, once the parasites have crossed the BBB into the brain, there is a marked neuroinflammatory response that mirrors the neuropathology seen in the late stage of human disease. Thus, there is a meningoencephalitis with diffuse infiltration of white matter with perivascular lymphocytes, plasma cells, and macrophages, and activated astrocytes and macrophages are also evident (Kennedy, 2004).

An important question is the extent of BBB damage during trypanosome infection because it seems logical to assume that the parasites would enter the CNS more easily and in greater numbers if the BBB is damaged or disrupted by the infection with a high parasite load. In a study of BBB integrity in a rat model of late-stage HAT using a fluorescent marker technique, it was found that in this chronic trypanosome infection, there was extensive damage to the BBB and vasogenic edema (Philip *et al*, 1994). Parasites had accumulated around a damaged choroid plexus and also in the brain and spinal cord. However, a more recent study of experimental *T. b. brucei* infection in rats demonstrated that the parasites were able to cross the BBB but this was not associated with a generalized loss of tight junction-specific proteins such as occluding and zonula occludens 1, indicating a lack of significant BBB damage (Mulenga *et al*, 2001). There was, however, up-regulation of the endothelial cell adhesion molecules ICAM-1 (intercellular cell adhesion molecule 1) and VCAM-1 (vascular endothelial adhesion molecule 1). These latter observations are consistent with recent *in vitro* data showing that *T. b. gambiense* can activate endothelial cells through the release of soluble activating factors, one consequence of which was an increases endothelial cell expression of ICAM-1, E-selectin, and VCAM-1 (Girard *et al*, 2005).

In murine models of HAT there is abundant evidence for the central role of different cytokines in generating the neuroinflammatory reponse (Kennedy, 2004, 2006), and it is likely that during this process there is a balance between pro-inflammatory cytokines such as interferon-γ (IFN-γ), interleukin-1(IL-1), and tumor necrosis factor-α (TNF-α), and counterinflammatory cytokines such as IL-10 (Sternberg *et al*, 2005). A question is whether any of these cytokines has any direct effect in determining entry of trypanosomes into the CNS. Insight into this issue was recently provided by a seminal study in knockout mice in which the gene for IFN-γ had been disrupted (Masocha *et al*, 2004). This group showed that following systemic infection, trypanosomes accumulated in the perivascular regions, and were located, or 'trapped' between the endothelial and the parenchymal basement membranes. It was also found that the parasites accumulated around endothelial basement membranes that contained the laminin α4 chain but not around those expressing the α5 chain. These findings showed that lymphocyte-derived IFN-γ is required for trypanosome traversal across cerebral blood vessels, and that the specific laminin composition of the vessels' basement membranes determines the site of BBB penetration (Masocha *et al*, 2004). Precisely how IFN-γ facilitates BBB traversal by the parasites has yet to be determined, but possibilities include that it might induce chemokines, leukocyte chemotaxis, or matrix metalloproteases (Masocha *et al*, 2007). Recently a very credible human correlate of this finding was discovered in HAT patients in the Ugandan field (Maclean *et al*, 2007). It was found that patients who showed an increased frequency of progression to and the severity of the meningoencephalitic stage had higher plasma IFN-γ concentrations compared with those who did not exhibit such neurological features. Thus the magnitude of the systemic IFN-γ response may determine the time course and possibly severity of the CNS disease. This would be entirely consistent

with the enhancement of trypanosome traversal of the BBB mediated by IFN-γ.

The Kristensson group has followed up these *in vivo* observations by studies with the antibiotic drug minocycline (Masocha *et al*, 2007). The rationale for this is that trypanosomes and leukocytes share features in terms of their penetration of the BBB (Masocha *et al*, 2007). It was found that administration of this drug to mice infected with *T. b. brucei* resulted in a reduction in the penetration of leukocytes and trypanosomes into the brain parenchyma as well as a clinical improvement compared with nontreated mice (Masocha *et al*, 2006). Thus trypanosome invasion of the CNS is indeed similar to that of leukocytes, which is consistent with the notion that trypanosomes might cross the BBB either with or actually inside leukocytes, although trypanosomes are classically extracellular.

In further, very recent studies, these workers showed that minocycline caused a delay in the relapses that occurred when infected mice were treated with suramin, a drug used for early-stage disease (Amin *et al*, 2008), suggesting that minocycline was reducing parasite traversal of the BBB. In this study, the BBB in infected mice was visualized directly by immunocytochemistry, and it was found that suramin could effectively treat the infection even when only a low level of parasite neuroinvasion had occurred.

Another mouse model of HAT has been used by workers in Glasgow for over 20 years (Kennedy, 1999, 2006). Mice infected with *T. b. brucei* develop a chronic infection in which parasites are detectable in the CNS by 21 days. Subcurative therapy with the drug berenil then leads to an exacerbation of CNS disease, with neuropathologic features that show strong similarities with the human disease, especially the PTRE. This model has allowed the identification of key factors that generate the neuroinflammatory response, has identified potential therapeutic targets, and has allowed testing of both existing and novel drugs. In the present context, the most relevant is the identification of the key role of the neuropeptide substance P (SP) in generating CNS inflammation, including astrocyte activation (Kennedy *et al*, 1997, 2003). Because SP has been shown to have a role in damaging and activating the BBB vascular component in the presence of proinflammatory cytokines (Annunziata *et al*, 2002), it is possible that SP may produce its deleterious effects at least in part through its effect on damaging the BBB and thereby allowing traversal of increased numbers of parasites into the CNS. However, our current view is that SP probably has a major role in modulating a variety of immune responses, including cytokine release (Kennedy, 2004) so this issue requires further clarification.

We have recently begun to visualize BBB breakdown in this mouse model directly by *in vivo* 7-T small-bore magnetic resonance imaging (MRI) (P. Kennedy and colleagues, in preparation), and this should allow the identification and quantitation of BBB breakdown following a variety of experimental paradigms, which we hope will have great relevance to the human disease and the search for better drug therapy for HAT

Conclusions

At the present time, safe drugs for the treatment of HAT are sadly lacking. Melarsoprol given to patients when the parasites are thought to have entered the CNS kills 5% of all patients receiving it, but if left untreated, 100% of patients with HAT will die. Furthermore, the incidence of melarsoprol-refractory infections is increasing and alternative treatment regimens are urgently required Analysis of BBB structure and function in experimental trpanosomiasis infection is of great current importance (a) to better understand how trypanosomes enter the CNS as the initial stage of the neuroinflammatory process and (b) to increase the pace of new drug development studies. One of the key, if not the key problem, in treating HAT is the failure or limited ability of most drugs, including the novel compounds, to cross the BBB. Without this property, no new drug will ever be able to treat late-stage disease. If the main factors determining BBB parasite traversal were understood, then in due course it should be possible to manipulate the properties of existing drugs, or design new drugs, that can cross the BBB efficiently. This area of trypanosomiasis research is now a major and urgent priority for scientists working in this area.

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