HHS Public Access

Author manuscript

*Trends Parasitol*. Author manuscript; available in PMC 2015 December 22.

Published in final edited form as:

*Trends Parasitol*. 2012 December ; 28(12): 539–545. doi:10.1016/j.pt.2012.09.002.

# Trypanosome resistance to human innate immunity: targeting Achilles' heel

**Natalie A. Stephens**[a], **Rudo Kieft**[a], **Annette MacLeod**[b], and **Stephen L. Hajduk**[a,*]

[a]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602 USA

[b]Wellcome Trust Centre for Molecular Parasitology, Institute of Biodiversity Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G61 1QH, UK

## Abstract

Trypanosome lytic factors (TLFs) are powerful, naturally-occurring toxins in humans that provide sterile protection against infection by several African trypanosomes. These trypanocidal complexes predominantly enter the parasite by binding to the trypanosome haptoglobin/hemoglobin receptor (HpHbR), trafficking to the lysosome, causing membrane damage and ultimately, cell lysis. Despite TLF-mediated immunity, the parasites that cause human African Trypanosomiasis (HAT), *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, have developed independent mechanisms of resistance to TLF killing. Here we describe the parasite defenses that allow trypanosome infections of humans and discuss how targeting these apparent strengths of the parasite may reveal their Achilles' heel, leading to new approaches in the treatment of HAT.

### Keywords

African trypanosomes; innate immunity; high density lipoproteins; haptoglobin; apolipoprotein L-1; haptoglobin related protein; serum resistance associated protein; trypanosome lytic factor

## Evolution of parasite resistance

The transmission of African trypanosomes by tsetse flies (*Glossina* spp.) provides these hemoflagellates with a broad sampling of mammalian environments. Within non-primates, these extracellular parasites nimbly escape the humoral and cellular immune responses by periodic changes to the composition of a major surface antigen, the variant surface glycoprotein (VSG) (1). The process of antigenic variation is both necessary for parasite survival and is considered sufficient to sustain long-term infection. In antelope and other

*corresponding author: Stephen L. Hajduk, Mailing address: Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602 USA, shajduk@bmb.uga.edu, Phone: 706-542-1676.

wild ungulates infection is transferred from host to tsetse fly without noticeable sign of disease in either. In many domesticated animals, most notably cattle, infections lead to pathology and the chronic wasting disease Nagana. When these African trypanosomes are introduced into most primates, including humans, antigenic variation alone is insufficient to ensure infection. In the human blood, lymph and tissue fluids, trypanosomes must also survive the onslaught of novel and extremely potent innate immune molecules called Trypanosome Lytic Factors (TLF). This defense mechanism in humans potentially evolved to provide protection against infection by the highly prevalent African trypanosomes, *Trypanosoma brucei brucei*, *Trypanosoma congolense and Trypanosoma vivax*, which infected the wild ungulate herds of the African savannah.

The human infectious African trypanosomes, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are classified as sub-species of *T. b. brucei*. However, while *T. b. brucei* and *T. b. rhodesiense* are highly similar it is clear that *T. b. gambiense* is more distantly related and has been further sub-divided into group 1 and group 2 based on their pathophysiological characteristics and genetic differences (2). Group 1 *T. b. gambiense* parasites are more genetically homogeneous and invariably resistant to human serum, whereas group 2 parasites are genetically heterogeneous and have variable expression of the human serum resistance phenotype, often losing resistance in the absence of selection pressure (2). As might be anticipated, these parasites have evolved diverse mechanisms to circumvent the activity of human TLF. Just as humans have undergone alterations in response to parasite infection, trypanosomes have adjusted to the changing host environment, thus establishing a dynamic equilibrium driven by the cause and effect of infection. In response to the slow processes of host evolution, these parasites have developed defense mechanisms that include either neutralization or avoidance of TLF. Indeed, the prolonged interactions between the human host and trypanosomes appear to have resulted in combinations of inhibition and avoidance mechanisms. In this review, we highlight the most recent developments in the elucidation of parasite resistance mechanisms against human TLF. Further, we explore attempts to shift the equilibrium patterns of resistance back in favor of the host by targeting trypanosome defense mechanisms that may render the parasite susceptible to TLF.

## Mechanisms of trypanosome killing by TLF

An understanding of how African trypanosomes infect humans first requires a consideration of the cellular and molecular mechanisms leading to TLF killing. Transmission of African trypanosomes, by the bite of infected tsetse flies, results in a rapid and abrupt encounter of parasite with the host defenses. Two molecules with trypanocidal activity have been identified in human blood (3–5). TLF-1 and TLF-2 are compositionally related, both containing apoA-I, apoL-I and Hpr (4,6–8). However, TLF-2 also contains significant amounts of IgM (4,6). Moreover, these toxins are physically distinct, differing both in size and lipid content. TLF-1 is a minor subclass of human HDL (~40% lipid) of approximately 500 kDa, whereas TLF-2 is a lipid poor (< 2%) protein complex of approximately 2 mDa (4).

One of the earliest observations of *T. b. brucei* killing by TLF-1 and normal human serum (NHS) was made by Rifkin (9). Initially, trypanosome morphology and motility were unaffected by addition of TLF-1, however, after ~30 minutes, at 37°C, many of the trypanosomes, while still highly motile, begin to swell and eventually become non-motile and ghost-like in appearance. The morphological progress of NHS and TLF-1 killing is identical and has been reported by several groups (9–13). A distinctly different sequence of morphological changes associated with NHS killing has also been described (14–16). These investigators reported that human serum treatment resulted in the development of a large cytosolic vacuole that expanded over time eventually leading to breakage of the trypanosome plasma membrane and death of the parasite (14–16).

The initial interaction of *T. b. brucei* with TLF-1 is facilitated by high affinity binding to a haptoglobin hemoglobin receptor (*Tb*HpHbR) located on the trypanosome surface within the flagellar pocket (17–19). This receptor likely evolved to satisfy the trypanosome's need to scavenge heme by binding Hp/Hb complexes in the blood of infected mammals (18). Haptoglobin is an acute phase protein produced by the liver of all mammals in response to injury and inflammation (20). Haptoglobin binds Hb, released into the circulation by hemolysis, with high affinity leading to detoxification when the Hp/Hb complexes are bound by the CD163 macrophage scavenger receptor (21,22). In humans, the evolution of a second Hb binding protein, Hpr, explains the high affinity of TLF-1 for *T. b. brucei* (18,22,23). With greater than 90% amino acid sequence identity to Hp (24), Hpr/Hb facilitates TLF-1 binding to the *Tb*HpHbR. Unlike the Hp/Hb in blood, TLF-1-associated Hpr/Hb is not cleared by the mammalian scavenger receptor and has been proposed to accumulate in the circulation of humans (21,23).

TLF-1 is rapidly taken up by trypanosomes following binding to the *Tb*HpHbR, traffics via endosomes to the trypanosome lysosome, and is activated at acidic pH leading to lysosomal membrane destabilization and ultimately lysis of *T. b. brucei* (3,18,25,26). The interaction of TLF-1 with the lysosomal membrane is likely facilitated by apoL-I, which is an ion channel forming protein that integrates into anionic membranes at low pH (15,27). *In vitro* studies, with negatively charged liposomes, further support the role of apoL-1 in TLF-1 binding to lysosomal membranes (27). Direct evidence for the role of apoL-1 in *T. b. brucei* killing has come from deletion and mutational analysis of recombinant apoL-1(15,28). The direct role of Hpr in trypanosome killing is less clear. One study has shown that the unprocessed N-terminal signal peptide of Hpr, which is necessary for Hpr association with HDL, is selectively toxic to bloodstream form African trypanosomes (29). In addition, a role for Hpr/Hb mediated lipid peroxidation has been proposed based on accumulation of peroxidated lipids in TLF-1 treated *T. b. brucei* (8). While the direct involvement of Hpr-Hb as a toxin remains unresolved, its role as the ligand for receptor-mediated uptake of the TLF-1 particle is well established. Thus, TLF-1 killing requires the targeting of the molecule to the lysosome, and the interaction of the TLF-1 proteins with the anionic lysosomal membrane (15,25,27,30).

Much less is known about how TLF-2 kills *T. b. brucei*. Since both Hpr and apoL-I are present in this particle, it is possible that TLF-2 may function in a manner similar to TLF-1. However, previous studies suggest this may not be the case. It has been reported that TLF-2

killing was not inhibited by the addition of Hp, a potent competitive inhibitor of TLF-1 uptake, leading to the suggestion that TLF-2 has a different mode of internalization from TLF-1 (4,8). It has also been proposed that TLF-2 uptake may be linked to apoL-I interaction with the VSG coat of *T. b. brucei* or that TLF-2-associated IgM may bind *T. b. brucei*, as it is the only protein component that distinguishes both classes of TLF (31). No results supporting either mechanism of TLF-2 binding to *T. b. brucei* have been reported. In addition, recent studies have revealed that TLF-2 killing of *T. b. brucei* is partly dependent on the *Tb*HpHbR receptor for uptake, further suggesting that there are commonalities between the uptake mechanisms of TLF-1 and TLF-2 (6).

## Mechanisms of trypanosome resistance to human serum

In order to survive in the human bloodstream African trypanosomes have evolved two primary mechanisms of resistance against the trypanolytic serum complexes: (i) neutralization, and (ii) avoidance. Although *T. b. rhodesiense* and group 2 *T. b. gambiense* appear to use a singular mechanism of resistance to TLF-1, group 1 *T. b. gambiense* seem to have more than one mode of resistance against TLF-1 killing. As mentioned above, little is known about how TLF-2 kills trypanosomes, so we will focus on the mechanisms of trypanosome resistance to TLF-1 since we can only speculate about mechanisms of resistance to TLF-2.

## Mechanisms of resistance: neutralization

*T. b. rhodesiense* is highly human infectious and causes rapidly developing pathology and death. Genomic analyses have shown that *T. b. brucei* and *T. b. rhodesiense* are virtually indistinguishable suggesting that *T. b. rhodesiense* only recently diverged from a ‘brucei-ancestor’ (32). Resistance to human innate immunity in *T. b. rhodesiense* is relatively unstable, being lost when cells are maintained in the absence of NHS or TLF-1 selection, suggesting that the resistance phenotype has come at a cost to the parasite (13,33,34).

### What is the molecular basis for human infectivity by T. b. rhodesiense?

Nearly 25 years ago a comparative analysis of mRNA transcripts in human infectious and non-infectious lines of *T. b. rhodesiense* revealed that human infectious cells expressed a serum resistance associated (SRA) gene, while serum-sensitive forms did not (33–35). Human serum-resistant *T. b. rhodesiense* lost *SRA* expression when grown in the absence of NHS and became sensitive to lysis. When NHS sensitive cells were re-exposed to NHS, a revertant population, which had regained *SRA* expression and NHS resistance, had emerged (34). Identical results were obtained when purified TLF-1 was used in place of NHS (33). Transfection of TLF-1 susceptible *T. b. brucei* with *SRA* showed that *SRA* expression was sufficient to confer resistance to TLF-1 and NHS killing (30,34,36). To date, there have been no other proteins identified as mediators of TLF-1 resistance in *T. b. rhodesiense*. Thus, it appears that expression of a single protein, SRA, is sufficient to confer resistance to TLF-1.

## What is SRA?

SRA belongs to the VSG family of trypanosome proteins (37). Variant surface glycoproteins in African trypanosomes are encoded by hundreds of chromosome internal, silent genes and a limited number of genes at both transcriptionally active and silent telomeric 'expression sites' (ES). Monoallelic expression of VSGs is accomplished by RNA polymerase I transcription of a single ES. *SRA* is an expression site associated gene (ESAG), which is co-transcribed, with the expressed VSG, from the active ES. Switching to a previously silent ES, lacking *SRA*, results in loss of *SRA* expression and increased susceptibility to NHS or TLF-1 (34). Based on sequence analysis, SRA appears to be a truncated VSG containing a large internal deletion (38). Post-translational modifications, including N-linked glycosylation and glycophosphatidylinositol (GPI) anchoring, similar to those of the VSGs, as well as the transferrin receptor, another VSG family protein, have been predicted (38). N-linked glycosylation has been verified (30), and while highly likely, GPI anchoring of SRA remains unconfirmed. Protein modeling also suggested that the tertiary structure of SRA would be similar to VSGs and that it would also exist as a dimer (38). Despite these predictions, the homo-dimerization of SRA has not been reported, nor has a binding partner been identified. The cellular localization of SRA was also shown to be very different from that of VSG. While VSGs and transferrin receptor localize to the cell surface and flagellar pocket respectively (39–43), localization studies of SRA revealed a predominately intracellularly localized protein (26,30,36). Initially, SRA was identified as a resident lysosomal protein (30). However, recent studies showed that SRA was largely non-lysosomal, confined to vesicular components of the endosomal network localized between the nucleus and the flagellar pocket of *SRA* transfectant cell lines (26,36).

## How does SRA inhibit TLF-1 killing?

Both resistant and susceptible *T. b. rhodesiense* cell lines endocytose TLF-1 (44). However, in resistant *T. b. rhodesiense* the level of TLF-1 accumulation was reduced and the intracellular localization of TLF-1 appeared distinct for that seen in TLF-1 sensitive cell lines (36,44). Consistent with these results, recent studies confirmed that *T. b. rhodesiense* expresses a functional *Tbr*HpHbR that allows for TLF-1 binding and endocytosis (18). Therefore, *T. b. rhodesiense* resistance to TLF-1 is not a consequence of 'avoidance' of binding and uptake but rather involves the 'neutralization' of TLF-1 following internalization by these parasites.

It has long been assumed that SRA binding to TLF-1 was the underlying mechanism of inhibition. *In vitro* binding studies with recombinant SRA revealed a specific and high affinity binding to apoL-1 (30). The interaction of SRA and apoL-1 is facilitated by a coiled-coil, anti-parallel interaction of α-helices at the C-terminal and N-terminal regions of apoL-I and SRA, respectively (30). Deletion and mutational analyses of the C-terminus of apoL-I have also revealed that the loss of this region, or disruption of the α-helix ablates binding to SRA (15,28). While mutation of the ApoL-I C terminus does not inhibit trypanosome lysis, deletion of this region results in a loss of ApoL-I lytic activity (28,45).

The cellular site of SRA binding to TLF-1 was determined by immunofluorescence microscopy analysis of SRA-expressing cells following incubation with TLF-1. In these

studies, both SRA and TLF-1 were shown to exhibit a steady-state colocalization in early endosomes immediately following endocytosis of TLF-1 (26). However, when TLF-1 trafficking was examined in the presence of lysosomal protease inhibitors both TLF-1 and SRA accumulated in the lysosome (26). Based on these results it was suggested that SRA/TLF-1 complexes form in early endosomes and traffic to the lysosome where the SRA/TLF-1 complex is rapidly degraded (26).

### What is the mechanism of TLF-1 resistance in *T. b. rhodesiense*?

One possible mechanism is that SRA/apoL-1 binding simply inhibits apoL-1 mediated insertion into the lysosomal membrane (30). Blocking apoL-1 membrane insertion may prevent ion channel formation, lysosomal swelling and cell lysis. An alternative model is based on the finding that, at acidic pH, all TLF-1 proteins bind to membranes (27). If the interaction of TLF-1 with membranes were mediated by apol-1 binding, then SRA binding to apoL-1 may block TLF-1 interactions with the lysosomal membrane thus leaving the particle vulnerable to accelerated degradation by lysosomal proteases. This model would explain how interactions of SRA with apoL-1 neutralizes both apoL-1 and Hpr toxicity. While some of the specific details of this model remain untested, it is proposed that SRA mediates human infectivity through the binding and neutralization of TLF-1 immediately following uptake in an early endosomal compartment. Once the binary SRA/TLF-1 complex forms, it traffics to the lysosome but is unable to bind lysosomal membranes thus leaving TLF-1 associated apolipoproteins susceptible to lysosomal degradation (Figure 1) (26).

Collectively, the studies and proposed hypotheses of resistance from several laboratories point to an SRA-mediated mechanism of inhibition of TLF-1 that involves three key elements: (i) binding of TLF-1 via apoL-I/SRA interaction, (ii) neutralization of TLF-1 by inhibition of membrane association and (iii) clearance of the toxin by rapid lysosomal degradation.

### Is SRA responsible for human infectivity by *T. b. gambiense*?

The simple answer is no. Neither group 1 nor group 2 *T. b. gambiense* have the *SRA* gene and transfection of a *T. b. gambiense* SRA-like gene into *T. b. brucei* does not protect against NHS killing (46). Moreover, in group 1 *T. b. gambiense* it is likely that decreased uptake of TLF-1 is a major factor in the resistance phenotype (47).

Recent studies showed that TLF-1 uptake and delivery to the lysosome are similar in susceptible and resistant lines of group 2 *T. b. gambiense* (48). In the latter, cells are also highly resistant to both NHS and recombinant apoL-I, which is internalized and lysosomally localized (48). Therefore, group 2 *T. b. gambiense* resistance is not due to reduced HpHbR expression, and is more likely to be due to a mechanism analogous to SRA in *T. b. rhodesiense*. Further studies are needed identify this inhibitory molecule.

## Mechanisms of resistance: avoidance

Since TLF-1 internalization is required for killing of *T. b. brucei*, lack of binding and uptake provides perhaps the simplest and most effective means by which the parasite is able to infect humans. The first evidence for a TLF-1 avoidance mechanism in trypanosomes came

from *in vitro* growth selection studies with a TLF-1 sensitive line of *T. b. brucei* (49). In these studies, *T. b. brucei* was grown in the presence of sub-lethal concentrations of TLF-1 that selected for TLF-1 resistant cells. Surviving cells were expanded and successively passaged in media containing increasing concentrations of TLF-1 until a highly resistant stable cell line was obtained (49). Analysis of this cell line revealed a dramatic reduction in TLF-1 uptake suggesting that endocytosis of TLF-1 might be affected. The resistance phenotype was unstable since growth in the absence of selective pressure by TLF-1 results in emergence of cells that had reverted back to being susceptible to TLF-1 [21]. Resistance to TLF-1 was evaluated in a number of cell lines derived from the initial TLF-1 resistant clonal line and no correlation was seen in the VSG expressed or the active expression site (47). However, all of the TLF-1 resistant *T. b. brucei* cell lines showed a reduction in expression of the *Tb*HpHbR gene. *Tb*HpHbR mRNA levels were reduced at least 20-fold in the TLF-1 resistant cells suggesting that downregulation of the *Tb*HpHbR gene was selected for in these *in vitro* studies (47). TLF-1 resistant cells were also analyzed for survival in NHS and TLF-2 and were found to be at least 100-fold more resistant than *Tb*HpHbR-expressing isogenic cell lines, indicating that resistance to both TLF complexes is at least partly dependent on *Tb*HpHbR expression (6). While increased resistance to NHS and TLF-2 resulting from *Tb*HpHbR downregulation was noted, this resistance was considerably reduced in comparison to that observed with TLF-1 treatment (6). Furthermore, at concentrations of TLF-1 comparable to that found in human serum (>10μg/ml), loss of receptor expression was not sufficient to provide complete protection against cell lysis (6). These studies, using TLF-1 resistant *T. b. brucei*, demonstrated for the first time an SRA independent mechanism for resistance and while resistance was incomplete these studies suggested that the *HpHbR* expression levels might play a role TLF-1 resistance in group 1 *T. b. gambiense*.

## How does group 1 *T. b. gambiense* escape TLF-1 killing?

Since group 1 *T. b. gambiense* lack *SRA* an alternative mechanism of resistance is to avoid the toxin by decreased uptake. This is the mechanism selected for *in vitro* in the TLF-1 resistant *T. b. brucei* lines described above. Consistent with this possibility, group 1 *T. b. gambiense*, Eliane strain, does not take up TLF-1 (47). The lack of TLF-1 uptake can be explained, at least to some extent, by the reduced levels of *T. b. gambiense* HpHb receptor (*TbgHpHbR*) expression. *TbgHpHbR* mRNA was reduced 5- to 50-fold relative to *HpHbR* mRNA levels in TLF-1 sensitive *T. b. brucei* (18,47). Furthermore, it appears that reduced expression of the *TbgHpHbR* is a general trait of *T. b. gambiense* since mRNA levels were reduced 10- to 20-fold in six geographically distinct *T. b. gambiense* isolates (47). It is likely that reduced expression of *TbgHpHbR* was selected for during *T. b. gambiense* adaptation to primates. However, since human infectivity requires complete protection against TLF-1, other mechanisms in addition to reduced expression of *TbgHpHbR*, may be necessary. Analysis of the sequence of the *TbgHpHbR* from several isolates of group 1 *T. b. gambiense* revealed the accumulation of mutations. Five conserved, nonsynonymous, point mutations are present in the *TbgHpHbR* when compared to the *T. b. brucei* and *T. b. rhodesiense HpHbR* sequences (Figure 2) (47). The overall conservation of these changes to the *TbgHpHbR*, in particular a single serine to leucine change at amino acid 210, suggests that mutations may have contributed to the development of *T. b. gambiense* resistance to TLF-1

(50). Indeed, transfection of the *TbgHpHbR* gene variant into a *HpHbR* null background *T. brucei* strain demonstrated that the *TbgHpHbR* variant of the gene is not functional [44]. Therefore, it seems likely that both downregulation of expression and mutation led to a loss of a functional TLF-1 receptor in group 1 *T. b. gambiense* (Figure 3).

### Is the loss of HpHpR sufficient to confer human infectivity to group 1 *T. b. gambiense*?

Receptor mediated endocytosis plays an important role in the delivery of TLF-1 to the trypanosome lysosome ultimately leading to trypanosome killing. The high-affinity of the trypanosome *HpHbR* for TLF-1 (~3–5 nM $K_d$) suggests that this receptor is critical to TLF-1 killing (17). However, trypanosomes that infect humans must escape a continuous onslaught of TLF-1 and any mechanism of resistance needs to provide either complete avoidance or inhibition of TLF-1 activity. It appears that SRA is able to do this providing complete protection against TLF-1 for *T. b. rhodesiense.* However, it is unlikely that loss of the *HpHbR* alone in *T. b. gambiense* is sufficient to provide protection against TLF-1 and human infectivity. Two alternative uptake mechanisms for TLF-1 have been described, either of which could deliver TLF-1 to the lysosome in group 1 *T. b. gambiense*. Though relatively inefficient, requiring high concentrations (~1mM $K_d$), African trypanosomes have an SRB-1 like HDL scavenger receptor that can facilitate TLF-1 uptake (51). In addition, fluid phase endocytosis may also provide a mechanism for TLF-1 uptake in the absence of functional HpHbR. TLF-1 resistant *T. b. brucei*, expressing low levels of HpHbR, remained susceptible to TLF-1 but only at concentrations 1000-fold higher than *HpHbR* expressing cells (6). Thus, it is likely that group 1 *T. b. gambiense* avoids TLF-1 by loss of HpHbR function but also have evolved a non-SRA inhibitor that has yet to be discovered.

## Concluding remarks

African trypanosomes are undoubtedly a model of adaptability and survival. These parasites have evolved to survive not only adaptive immunity and non-specific innate immunity of complement, but also the highly specialized trypanolytic factors in the human bloodstream. Faced with a multi-component toxin that seems to have been designed to ensure efficient uptake and targeting to specialized membranes, and armed with two potent trypanolytic proteins, the parasite has responded with the development of highly effective resistance mechanisms that enable avoidance and neutralization of the toxins lethal to trypanosome. While there has been significant progress in elucidating these resistance mechanisms, several major questions remain unanswered (Box 1). Ongoing efforts to resolve these unknowns have led to several strategies for combating HAT and Nagana that are tailored to specific subspecies (Box 2). For *T. b. rhodesiense,* SRA/apoL-I binding is critical to trypanosome resistance to TLF-1, therefore the disruption of this interaction will be a key target for potential therapeutics. The two main approaches to disruption of the SRA/apoL-I interface address either the mutation of apoL-I, or the use of small molecule/peptide inhibitors to binding (Box 2). Group 1 *T. b. gambiense* resistance through the loss of receptor-mediated uptake of trypanolytic factors, provides another potential target for combating HAT. Through conjugation to targeting molecules or alternate ligands, TLFs may be delivered to the lysosome, and thus bring about trypanosome lysis independent of the HpHbR (Box 2). Finally, as wild game and domesticated cattle provide a substantial

reservoir for African trypanosomes, the introduction of a population of resistant transgenic animals would lead to a decrease in the pool of infectious parasites and may lead to a decline in transmission and subsequently, the spread of infection (Box 2). With continued breakthroughs in the field, a holistic understanding of the multiple mechanisms of TLF resistance should ultimately facilitate the development of a general therapeutic agent against both the human and veterinary diseases.

## References


1. Mansfield JM, Paulnock DM. Regulation of innate and acquired immunity in African trypanosomiasis. Parasite Immunol. 2005; 27:361–371. [PubMed: 16179030]
2. Gibson WC. Will the real Trypanosoma b. gambiense please stand up. Parasitol Today. 1986; 2:255–257. [PubMed: 15462856]
3. Hajduk SL, Moore DR, Vasudevacharya J, Siqueira H, Torri AF, Tytler EM, Esko JD. Lysis of Trypanosoma brucei by a toxic subspecies of human high density lipoprotein. J Biol Chem. 1989; 264:5210–5217. [PubMed: 2494183]
4. Raper J, et al. Characterization of a novel trypanosome lytic factor from human serum. Infect Immun. 1999; 67:1910–1916. [PubMed: 10085035]
5. Rifkin MR. Identification of the trypanocidal factor in normal human serum: high density lipoprotein. Proc Natl Acad Sci U S A. 1978; 75:3450–3454. [PubMed: 210461]
6. Bullard W, et al. Haptoglobin-hemoglobin receptor independent killing of African trypanosomes by human serum and trypanosome lytic factors. Virulence. 2012; 3:72–76. [PubMed: 22286709]
7. Shiflett AM, et al. Human high density lipoproteins are platforms for the assembly of multi-component innate immune complexes. J Biol Chem. 2005; 280:32578–32585. [PubMed: 16046400]
8. Smith AB, et al. Killing of trypanosomes by the human haptoglobin-related protein. Science (New York, N.Y. 1995; 268:284–286.
9. Rifkin MR. Trypanosoma brucei: biochemical and morphological studies of cytotoxicity caused by normal human serum. Exp Parasitol. 1984; 58:81–93. [PubMed: 6745390]
10. Hager KM, et al. Endocytosis of a cytotoxic human high density lipoprotein results in disruption of acidic intracellular vesicles and subsequent killing of African trypanosomes. J Cell Biol. 1994; 126:155–167. [PubMed: 8027174]
11. Shiflett AM, et al. African trypanosomes: intracellular trafficking of host defense molecules. J Eukaryot Microbiol. 2007; 54:18–21. [PubMed: 17300512]
12. Yorke W, et al. Studies in Chemotherapy II: The action in vitro of normal human serum on the pathogenic trypanosomes, and its significance. Ann Trop Med Parasitol. 1930; 24:115–163.
13. Hawking F. The resistance to human plasma of Trypanosoma brucei, T. rhodesiense and T. gambiense. II. Survey of strains from East Africa and Nigeria. Trans R Soc Trop Med Hyg. 1976; 70:513–520. [PubMed: 841655]
14. Pays E, et al. The trypanolytic factor of human serum. Nature reviews. 2006; 4:477–486.
15. Perez-Morga D, et al. Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. Science. 2005; 309:469–472. [PubMed: 16020735]
16. Vanhollebeke B, et al. Human serum lyses Trypanosoma brucei by triggering uncontrolled swelling of the parasite lysosome. J Eukaryot Microbiol. 2007; 54:448–451. [PubMed: 17910690]
17. Drain J, et al. Haptoglobin-related protein mediates trypanosome lytic factor binding to trypanosomes. J Biol Chem. 2001; 276:30254–30260. [PubMed: 11352898]
18. Vanhollebeke B, et al. A haptoglobin-hemoglobin receptor conveys innate immunity to Trypanosoma brucei in humans. Science. 2008; 320:677–681. [PubMed: 18451305]
19. Widener J, et al. Hemoglobin is a co-factor of human trypanosome lytic factor. PLoS Pathog. 2007; 3:1250–1261. [PubMed: 17845074]
20. Stadnyk A, Gauldie J. The acute phase protein response during parasitic infection. Immunol Today. 1991; 12:A7–A12. [PubMed: 1712594]

21. Nielsen MJ, et al. A unique loop extension in the serine protease domain of haptoglobin is essential for CD163 recognition of the haptoglobin-hemoglobin complex. J Biol Chem. 2007; 282:1072–1079. [PubMed: 17102136]
22. Kristiansen M, et al. Identification of the haemoglobin scavenger receptor. Nature. 2001; 409:198–201. [PubMed: 11196644]
23. Nielsen MJ, et al. Haptoglobin-related protein is a high-affinity hemoglobin-binding plasma protein. Blood. 2006; 108:2846–2849. [PubMed: 16778136]
24. Maeda N. Nucleotide sequence of the haptoglobin and haptoglobin-related gene pair. The haptoglobin-related gene contains a retrovirus-like element. J Biol Chem. 1985; 260:6698–6709. [PubMed: 2987228]
25. Bishop JR, et al. Insight into the mechanism of trypanosome lytic factor-1 killing of Trypanosoma brucei brucei. Mol Biochem Parasitol. 2001; 118:33–40. [PubMed: 11704271]
26. Stephens NA, Hajduk SL. Endosomal localization of the serum resistance-associated protein in african trypanosomes confers human infectivity. Eukaryotic cell. 2011; 10:1023–1033. [PubMed: 21705681]
27. Harrington JM, et al. Membrane permeabilization by trypanosome lytic factor, a cytolytic human high density lipoprotein. J Biol Chem. 2009; 284:13505–13512. [PubMed: 19324878]
28. Lecordier L, et al. C-terminal mutants of apolipoprotein L-I efficiently kill both Trypanosoma brucei brucei and Trypanosoma brucei rhodesiense. PLoS Pathog. 2009; 5:e1000685. [PubMed: 19997494]
29. Harrington JM, et al. The plasma membrane of bloodstream-form African trypanosomes confers susceptibility and specificity to killing by hydrophobic peptides. J Biol Chem. 2010; 285:28659–28666. [PubMed: 20615879]
30. Vanhamme L, et al. Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature. 2003; 422:83–87. [PubMed: 12621437]
31. Vanhollebeke B, Pays E. The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. Mol Microbiol. 2010; 76:806–814. [PubMed: 20398209]
32. Gibson W. Will the real Trypanosoma brucei rhodesiense please step forward? Trends Parasitol. 2002; 18:486–490. [PubMed: 12473364]
33. Milner JD, Hajduk SL. Expression and localization of serum resistance associated protein in Trypanosoma brucei rhodesiense. Mol Biochem Parasitol. 1999; 104:271–283. [PubMed: 10593181]
34. Xong HV, et al. A VSG expression site-associated gene confers resistance to human serum in Trypanosoma rhodesiense. Cell. 1998; 95:839–846. [PubMed: 9865701]
35. De Greef C, et al. A Gene Expressed only in serum-resistant variants of Trypanosoma brucei rhodesiense. Molecular and Biochemical Parasitology. 1989; 36:169–176. [PubMed: 2528066]
36. Oli MW, et al. Serum resistance-associated protein blocks lysosomal targeting of trypanosome lytic factor in Trypanosoma brucei. Eukaryotic cell. 2006; 5:132–139. [PubMed: 16400175]
37. De Greef C, Hamers R. The serum resistance-associated (SRA) gene of Trypanosoma brucei rhodesiense encodes a variant surface glycoprotein-like protein. Mol Biochem Parasitol. 1994; 68:277–284. [PubMed: 7739673]
38. Campillo N, Carrington M. The origin of the serum resistance associated (SRA) gene and a model of the structure of the SRA polypeptide from Trypanosoma brucei rhodesiense. Mol Biochem Parasitol. 2003; 127:79–84. [PubMed: 12615339]
39. Engstler M, et al. Kinetics of endocytosis and recycling of the GPI-anchored variant surface glycoprotein in Trypanosoma brucei. J Cell Sci. 2004; 117:1105–1115. [PubMed: 14996937]
40. Grunfelder CG, et al. Endocytosis of a glycosylphosphatidylinositol-anchored protein via clathrin-coated vesicles, sorting by default in endosomes, and exocytosis via RAB11-positive carriers. Mol Biol Cell. 2003; 14:2029–2040. [PubMed: 12802073]
41. Kabiri M, Steverding D. Studies on the recycling of the transferrin receptor in Trypanosoma brucei using an inducible gene expression system. Eur J Biochem. 2000; 267:3309–3314. [PubMed: 10824117]

42. Schell D, et al. A transferrin-binding protein of Trypanosoma brucei is encoded by one of the genes in the variant surface glycoprotein gene expression site. Embo J. 1991; 10:1061–1066. [PubMed: 1845367]
43. Strickler JE, Patton CL. Trypanosoma brucei brucei: inhibition of glycosylation of the major variable surface coat glycoprotein by tunicamycin. Proc Natl Acad Sci U S A. 1980; 77:1529–1533. [PubMed: 6929505]
44. Hager KM, Hajduk SL. Mechanism of resistance of African trypanosomes to cytotoxic human HDL. Nature. 1997; 385:823–826. [PubMed: 9039915]
45. Molina-Portela MP, et al. Distinct roles of apolipoprotein components within the trypanosome lytic factor complex revealed in a novel transgenic mouse model. J Exp Med. 2008; 205:1721–1728. [PubMed: 18606856]
46. Berberof M, et al. A receptor-like flagellar pocket glycoprotein specific to Trypanosoma brucei gambiense. Mol Biochem Parasitol. 2001; 113:127–138. [PubMed: 11254961]
47. Kieft R, et al. Mechanism of Trypanosoma brucei gambiense (group 1) resistance to human trypanosome lytic factor. Proc Natl Acad Sci U S A. 2010; 107:16137–16141. [PubMed: 20805508]
48. Capewell P, et al. Differences between Trypanosoma brucei gambiense groups 1 and 2 in their resistance to killing by trypanolytic factor 1. PLoS Negl Trop Dis. 2011; 5:e1287. [PubMed: 21909441]
49. Faulkner SD, et al. In vitro generation of human high-density-lipoprotein-resistant Trypanosoma brucei brucei. Eukaryot Cell. 2006; 5:1276–1286. [PubMed: 16896212]
50. Symula RE, et al. Trypanosoma brucei gambiense Group 1 Is Distinguished by a Unique Amino Acid Substitution in the HpHb Receptor Implicated in Human Serum Resistance. PLoS Negl Trop Dis. 2012; 6:e1728. [PubMed: 22802982]
51. Green HP, et al. Evidence for a Trypanosoma brucei lipoprotein scavenger receptor. J Biol Chem. 2003; 278:422–427. [PubMed: 12401813]
52. Thomson R, et al. Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes. Proc Natl Acad Sci U S A. 2009; 106:19509–19514. [PubMed: 19858474]
53. Baral TN, et al. Experimental therapy of African trypanosomiasis with a nanobody-conjugated human trypanolytic factor. Nat Med. 2006; 12:580–584. [PubMed: 16604085]
54. Lukes J, Raper J. Prophylactic antiparasitic transgenesis for human parasitic disease? Mol Ther. 2010; 18:1745–1747. [PubMed: 20885434]
55. Stijlemans B, et al. High affinity nanobodies against the Trypanosome brucei VSG are potent trypanolytic agents that block endocytosis. PLoS Pathog. 2011; 7:e1002072. [PubMed: 21698216]
56. Goringer HU. Parasite-specific aptamers as biosynthetic reagents and potential pharmaceuticals. Trends Parasitol. 2012; 28:106–113. [PubMed: 22300805]

## Box 1

### Unanswered questions

**Has group 1 T. b. gambiense evolved a mechanism for TLF-1 neutralization?**

This is important to resolve since the down regulation and mutations of *Tbg*HpHbR may be insufficient to prevent killing by TLF-1.

**What is the mechanism of resistance of group 1 T. b. gambiense to TLF-2?**

While sharing some properties, TLF-1 and TLF-2 are physically distinct and may interact and kill trypanosomes differently (4,6,18,48). It is possible that these organisms evolved a distinct resistance mechanism for TLF-2.

**What is the mechanism of SRA inhibition of TLF-1?**

Despite identification of SRA as a critical resistance protein in *T. b. rhodesiense* over two decades ago the molecular mechanism of SRA-mediated resistance remains undetermined. The instability of TLF-1 in SRA expressing cells suggests that SRA binding to apoL-I may initiate the rapid degradation of the toxin.

**What is the mechanism of group 2 T. b. gambiense resistance to TLF-1?**

This group of trypanosomes most closely resembles *T. b. rhodesiense* pathology, host range and response to TLF-1, but lack SRA. The TLF-1 inhibitor in group 2 *T. b. gambiense* may reveal yet another distinct way that these parasites have evolved to live in the human host.

## Box 2

### Targeting the trypanosome's Achilles' heel: therapeutics

African trypanosomes are highly successful parasites having evolved defense mechanisms for evasion of host adaptive and innate immunity. Trypanosome resistance to TLF-1 was a necessary adaptation to life in primates, further strengthening the parasites' protective armor and acting as a powerful virulence mechanism. Understanding the molecular basis for TLF-1 resistance, clearly a strength of these parasites, may uncover unexpected vulnerabilities as did the arrow that pierced the seemingly invincible Achilles' heel.

TLF-1 is an ideal drug. It is a powerful innate killing factor, naturally occurring in all humans and providing sterilizing protection from infection by most African trypanosomes. It is completely non-toxic to mammals yet is devastating to trypanosomes. Several approaches are worth considering.

**Targeting the apoL-I/SRA interface: engineering of apoL-1**

The importance of apoL-1/SRA binding was demonstrated in an elegant series of studies with recombinant C-terminal deletion mutants of apoL-I in which the binding to SRA was ablated (28,52). This mutated apoL-1 has been shown to be effective both *in vitro* and *in vivo* in lowering parasitemia of *T. b. rhodesiense* infection in mice either by transgenic gene expression or targeted nanobody-mediated delivery (28,52,53).

**Targeting the apoL-I/SRA interface: inhibitors of binding**

Small molecules or peptide mimics that bind the apoL-1 interaction domain of SRA may prevent the inhibition of TLF-1 and allow lysosomal membrane attack (14,53,54).

**Modification of receptor/ligand interactions for TLF-1**

The loss of HpHbR mediated binding of TLF-1 contributes to the infectivity of group 1 *T. b. gambiense*. Studies using nanobodies and RNA aptamers have shown that trypanosome targeting and internalization can be specified in these reagents (55,56). Coupling surrogate ligands to TLF-1 would allow delivery to the lysosome in a receptor independent fashion.

**Trypanosome resistant transgenic cattle**

The economic impact of the veterinary disease, Nagana, is driving recent efforts to evaluate the feasibility of developing transgenic cows (14,52,54). While these strategies cannot be put into practice directly in humans, the eradication of the parasite from a major animal reservoir would have a tremendous impact in reducing human disease.

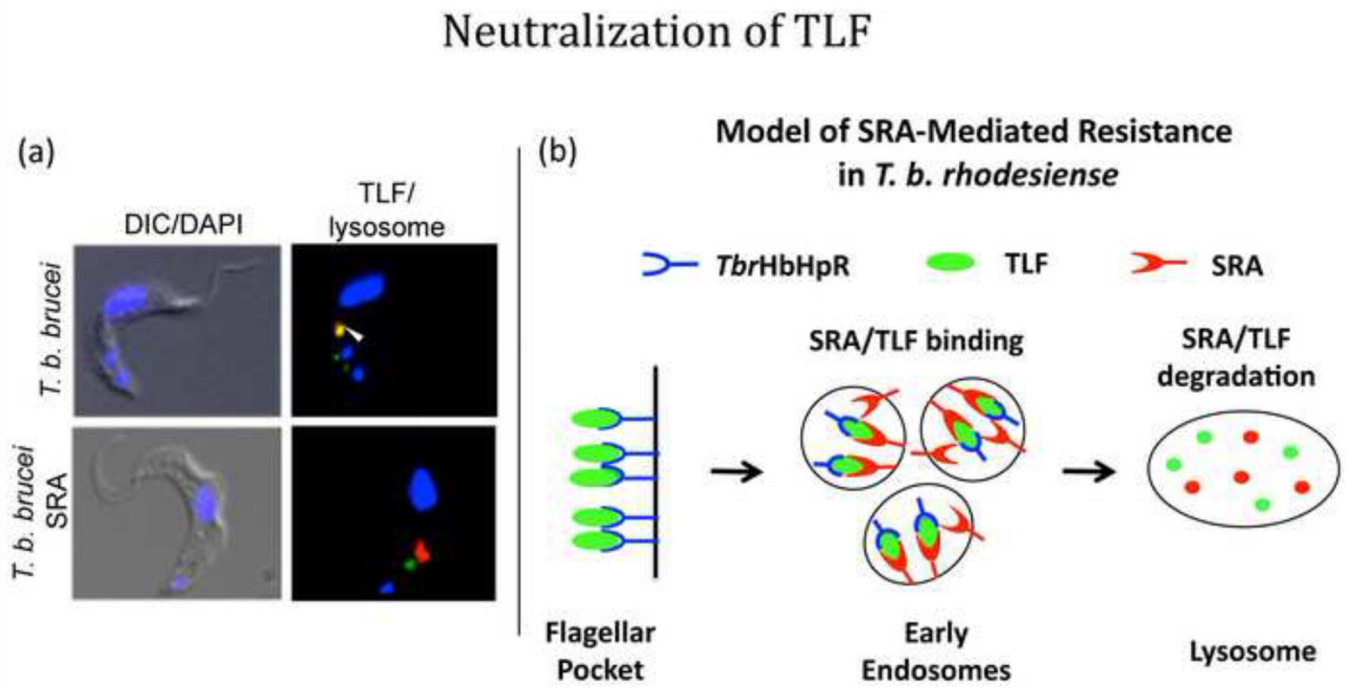
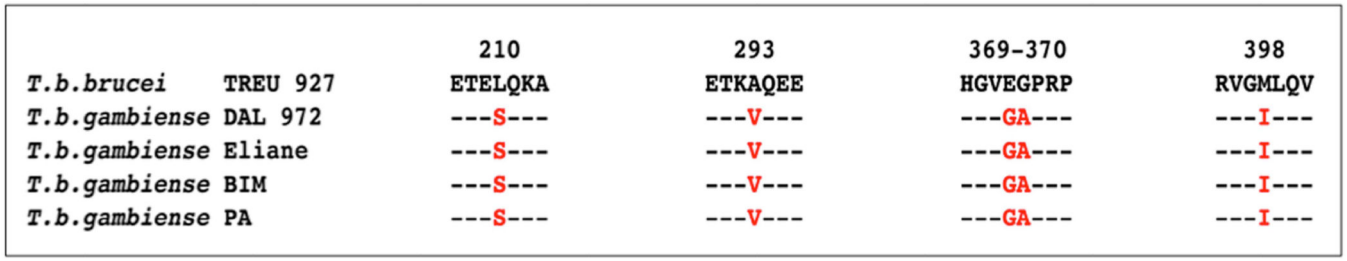



**Figure 1.**

SRA-mediated inhibition of TLF. **(a)** Fluorescence microscopy showing uptake of Alexa 488-conjugated TLF (green). The lysosome is shown by anti-*Tb*CatL staining (red). The nucleus and kinetoplast are shown by DAPI staining (blue). Cells are shown by DIC imaging. Colocalization is shown by the white arrowhead (26). **(b)** Schematic diagram depicting neutralization resistance mechanism. SRA binds TLF via apoL-I within the early endosomes. The SRA/TLF complex traffics to the lysosome where apoL-I insertion into the lysosomal membrane is prevented by SRA binding. Prevention of apoL-I insertion in turn prevents membrane association of all TLF proteins. Without membrane association, the particle is more susceptible to lysosomal proteolysis and accelerated degradation results in clearance of the toxin (black arrow indicates direction of vesicle trafficking).



| | | 210 | 293 | 369-370 | 398 |
|---|---|---|---|---|---|
| *T.b.brucei* | TREU 927 | ETELQKA | ETKAQEE | HGVEGPRP | RVGMLQV |
| *T.b.gambiense* | DAL 972 | ---S--- | ---V--- | ---GA--- | ---I--- |
| *T.b.gambiense* | Eliane | ---S--- | ---V--- | ---GA--- | ---I--- |
| *T.b.gambiense* | BIM | ---S--- | ---V--- | ---GA--- | ---I--- |
| *T.b.gambiense* | PA | ---S--- | ---V--- | ---GA--- | ---I--- |

**Figure 2.**

Alignment of mutations in the trypanosome HpHbR receptor. Five mutations have been identified in several different *T. b. gambiense* strains (red).

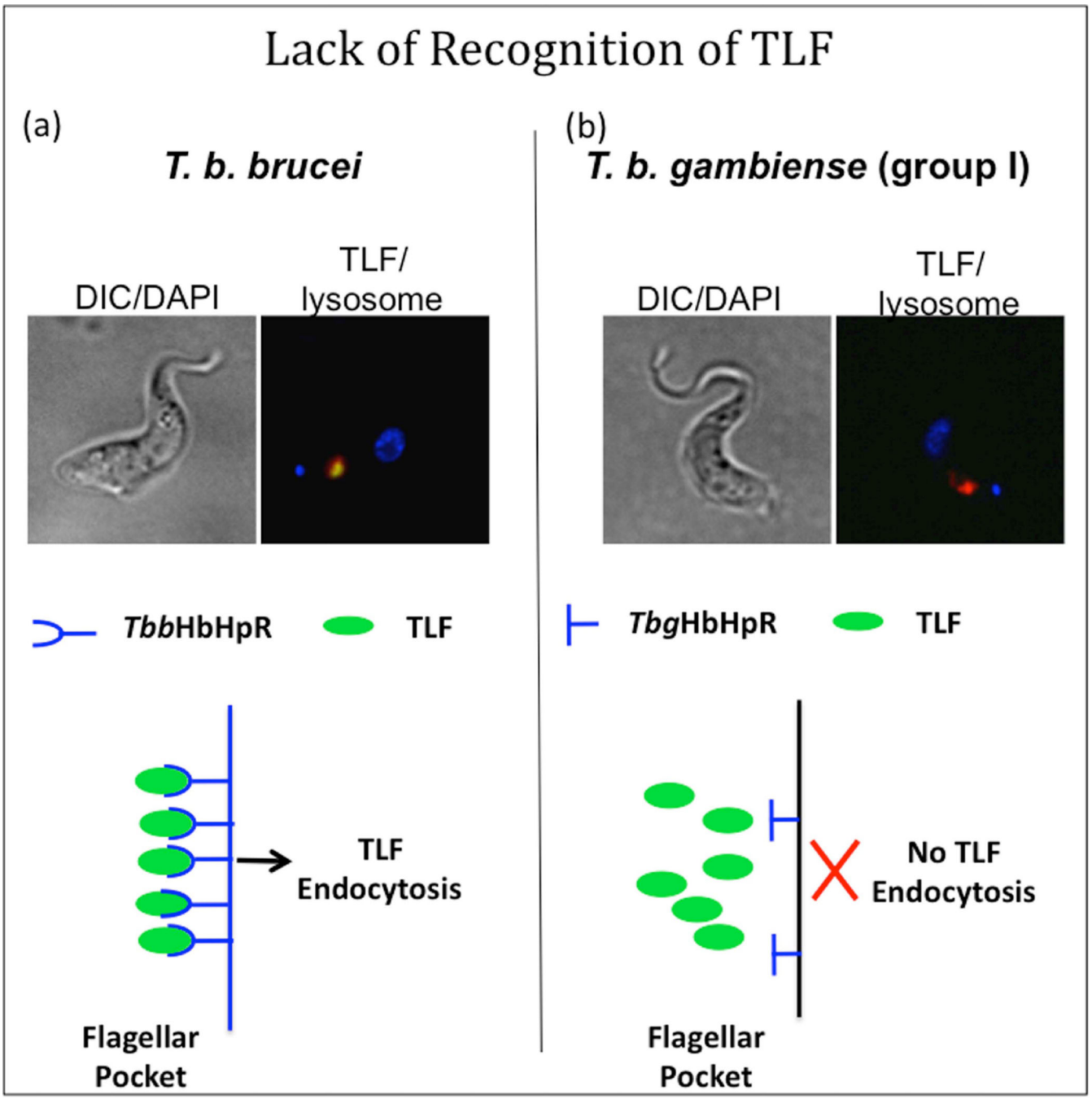



**Figure 3.**

*Tbg*HpHbR-Mediated Inhibition of TLF. **(a)** Fluorescence microscopy showing uptake of Alexa 488-conjugated TLF (green) (47). Schematic diagram depicting TLF uptake in sensitive cells via *Tbb*HpHbR. *Tbb*HpHbR is expressed in the flagellar pocket and binds Hb-bound TLF with high affinity, allowing TLF uptake. **(b)** Fluorescence microscopy showing lack of uptake of Alexa 488-conjugated TLF (green) (47). Schematic diagram depicting lack of recognition resistance mechanism. *Tbg*HbHpR is downregulated and has mutations which prevent TLF binding and endocytosis. In the fluorescent images, the lysosome is shown by

lysotracker staining (red). The nucleus and kinetoplast are shown by DAPI staining (blue). Cells are depicted by phase contrast (47).