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The Pathogenesis of African Trypanosomiasis

Etienne Pays,[1] Magdalena Radwanska,[2,3] and Stefan Magez[2,4,5]

[1]Laboratory of Molecular Parasitology, Université Libre de Bruxelles, Gosselies, Belgium; email: etienne.pays@ulb.be

[2]Laboratory for Biomedical Research, Ghent University Global Campus, Incheon, South Korea

[3]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium; email: Magdalena.Radwanska@ghent.ac.kr

[4]Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium; email: Stefan.Magez@vub.be

[5]Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium

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African trypanosomes, sleeping sickness, antigenic variation, variant surface glycoprotein, VSG, apolipoprotein L1, APOL1, kidney disease

Abstract

African trypanosomes are bloodstream protozoan parasites that infect mammals including humans, where they cause sleeping sickness. Long-lasting infection is required to favor parasite transmission between hosts. Therefore, trypanosomes have developed strategies to continuously escape innate and adaptive responses of the immune system, while also preventing premature death of the host. The pathology linked to infection mainly results from inflammation and includes anemia and brain dysfunction in addition to loss of specificity and memory of the antibody response. The serum of humans contains an efficient trypanolytic factor, the membrane pore-forming protein apolipoprotein L1 (APOL1). In the two human-infective trypanosomes, specific parasite resistance factors inhibit APOL1 activity. In turn, many African individuals express APOL1 variants that counteract these resistance factors, enabling them to avoid sleeping sickness. However, these variants are associated with chronic kidney disease, particularly in the context of virus-induced inflammation such as coronavirus disease 2019. Vaccination perspectives are discussed.

1. INTRODUCTION

Salivarian trypanosomiasis includes several diseases caused by protozoan trypanosome parasites that are mostly transmitted by the bite of hematophagous insects (**Table 1**). Human African trypanosomiasis (HAT) is largely confined to sub-Sahara. This disease is also called sleeping sickness, due to the clinical outcome of its meningoencephalitis stage. Animal trypanosomiasis (AT) represents diseases only in animals that are found in South America, Africa, Asia, and Oceania, with occasional infections also being reported in Europe. HAT is caused by the two *Trypanosoma brucei* subspecies *T. b. gambiense* and *T. b. rhodesiense*, both transmitted by tsetse flies, hematophagic insects

Table 1 Overview of the main pathogenic salivarian trypanosomes

Species	Subgenus	Main vector(s)	Disease	Main geographic location(s)	Main characteristics
Trypanosoma brucei gambiense	*Trypanozoon*	Tsetse fly (*Glossina*)	HAT	West and central sub-Saharan Africa	Resistance to APOL1 trypanolytic activity, causing 98% of HAT Typically slow progression of disease Asymptomatic infection caused by APOL1 variant G1
Trypanosoma brucei rhodesiense	*Trypanozoon*	Tsetse fly (*Glossina*)	HAT	East and southeast sub-Saharan Africa	Resistance to APOL1 trypanolytic activity, causing less than 2% of HAT Typically acute disease onset, with early neuropathology SRA-mediated parasite resistance overcome by APOL1 variants G1/G2
Trypanosoma brucei brucei	*Trypanozoon*	Tsetse fly (*Glossina*)	Nagana: AAT	Sub-Saharan Africa	Noninfective to humans due to lack of resistance to APOL1
Trypanosoma evansi	*Trypanozoon*	Biting flies: *Glossina*, *Tabanus*, *Stomoxys*, *Haematopota*, *Chrysops*, *Lyperosia* Vampire bat: *Desmodus rotundus*	Surra or mal de caderas (in South America): AT	South America, Africa, Asia, Oceania (occasional import into Europe)	Life cycle completion in tsetse flies prevented by partial loss of mitochondrial DNA Potential mechanical transmission by *Tabanus*, *Stomoxys*, and *Haematobia* spp. Rare cases of atypical human infection in Asia, due to APOL1 loss by mutations (or, alternatively, IgM depletion?)
Trypanosoma equiperdum	*Trypanozoon*	NA: sexual transmission	Dourine: AT of horse	Asia (occasional import into Europe)	Sexually transmitted variant of *T. evansi*
Trypanosoma congolense	*Nannomonas*	Tsetse fly (*Glossina*)	Nagana: AAT	Sub-Saharan Africa	Bloodstream parasite that does not infiltrate tissue, hence causing anemia
Trypanosoma vivax	*Dutonella*	Biting flies: *Glossina*, *Tabanus*, *Stomoxys*, *Haematobia*	Nagana: AT	Sub-Saharan Africa, South and Central America	Potential mechanical transmission by *Tabanus*, *Stomoxys*, and *Haematobia* spp., allowing the spread of infection

Abbreviations: AAT, animal African trypanosomiasis; APOL1, apolipoprotein L1; AT, animal trypanosomiasis; HAT, human African trypanosomiasis; IgM, immunoglobulin M; NA, not applicable; SRA, serum resistance-associated.

in which part of the life cycle takes place. With occurrence of *T. b. gambiense* in multiple countries of West and Central Africa, this parasite is responsible for 98% of all HAT infections. Since *T. b. gambiense* HAT is an anthroponotic disease mostly involving human-to-human transmission, it has been the main target of anti-HAT campaigns focusing on diagnosis and treatment, combined occasionally with insect trapping. Consequently, its incidence has dropped to fewer than 1,000 cases per year in 2019 and 2022, compared with 35,000 cases per year two decades earlier (1). In contrast, control of *T. b. rhodesiense* HAT is much harder to achieve as this is a zoonotic infection in which animal-to-human transmission can result in unpredictable disease outbreaks, with the main reservoir being present in livestock and game animals. As *T. b. rhodesiense* HAT has always been relatively rare, with an average of 80 cases/year reported for the entire sub-Saharan region for the last 10 years, the disease is mainly controlled by trying to limit the contact between livestock and parasite-harboring wildlife.

In addition to being a reservoir for *T. b. rhodesiense* HAT, animals are susceptible to infection with other trypanosomes. First, *T. evansi* is the most widely distributed animal trypanosome (2). As this parasite has adapted to transmission by multiple blood-consuming vectors that include hematophagic flies as well as vampire bats, it is no longer restrained by the need for tsetse fly presence and has been able to migrate out of Africa. Today, *T. evansi* infections are found in South America, Africa, the Arabian Peninsula, the Middle East, Asia, and some islands of Oceania. The second most widely distributed animal trypanosome is *T. vivax*, a livestock parasite found in South America as well as in sub-Saharan Africa. Also here, continued spread of the parasite is ensured by transport of infected animals combined with mechanical transmission, that is, parasite passage simply through the contact of blood-contaminated mouth parts of insects when feeding on a new host, rather than an obligatory developmental passage through the tsetse fly. A third pathogenic trypanosome that affects animals in Africa is *T. congolense*. This parasite is mainly transmitted by hematophagous flies of the families Glossinidae, Tabanidae, Chrysops, Atylotus, and Muscidae, all common in places where commercial livestock activities are practiced. Because *T. congolense* has a large wildlife host reservoir that seems tolerant to infection, parasite eradication appears to be impossible. Certain indigenous taurine breeds such as N'Dama cattle appear to resist the disease symptoms of trypanosomiasis much better than others and hence are considered trypanotolerant, but most livestock farmers prefer to invest in other breeds such as zebu or even import breeds such as Holstein Friesian cattle, which both have higher milk and meat productivity but are highly susceptible to trypanosomiasis. Interestingly, the true genetic reasons for occurrence of trypanotolerance have still not been unraveled. Finally, the animal-infective *T. brucei* subspecies *T. b. brucei* exhibits a relatively minor importance for the livestock economy. Besides these flyborne trypanosomes, only *T. equiperdum* causes a significant threat to the economy in different parts of the world. This sexually transmitted parasite is closely related to *T. evansi* but segregates from the latter by the difference in transmission mode.

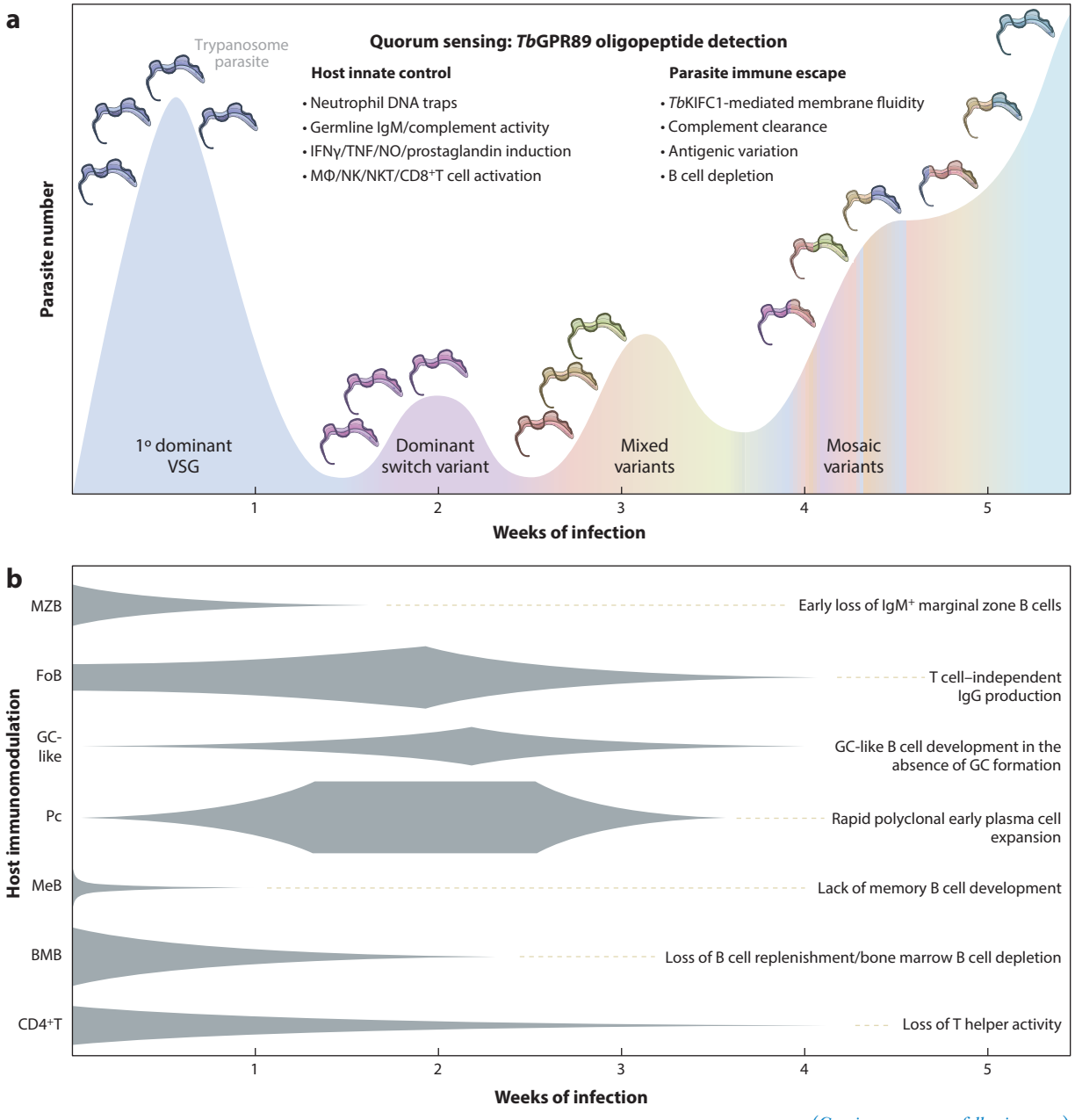
The mammalian immune system is unable to provide efficient protection against infection. While innate immunity can reduce early parasitemia, the adaptive response together with the complement pathway allow regular parasite clearance, but neither of these can provide sterile immunity. However, in the case of HAT there is a unique mechanism of innate immunity, provided by the human serum protein apolipoprotein L1 (APOL1). This protein kills trypanosomes except *T. b. gambiense* and *T. b. rhodesiense*, which acquired different APOL1 neutralization strategies. In turn, APOL1 mutations termed G1 and G2 allowed many African individuals to resist *T. b. rhodesiense* infection, but this came at the expense of higher probability to develop chronic kidney disease.

This review summarizes the different processes involved in the shaping of trypanosome infection (**Figure 1**), followed by a description of the linked host pathologies. Perspectives resulting from these analyses are presented at the end of the review.

2. PROCESSES INVOLVED IN THE CONTROL OF PARASITEMIA

2.1. General Innate Immunity

Innate immunity cannot fully protect against AT. In experimental settings, so-called resistant mice, such as the C57BL/6 strain, control parasitemia better than the BALB/c or C3H/He strains but are nevertheless fully susceptible to infection. This is due to various parasite defense activities that efficiently affect the innate immunity responses.



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Mechanisms shaping trypanosome infection, as determined in the mouse model. (*a*) Parasitemia control. After initial parasite escape from neutrophil DNA traps and germline immunoglobulins M (IgMs), which involves trypanosome motility and high surface fluidity triggering fast antibody clearance, early infection is controlled by a combination of parasite quorum sensing and modulation of the host innate immune responses by the inflammatory components interferon gamma (IFNγ), tumor necrosis factor (TNF), nitric oxide (NO), and prostaglandins, the expression of which results from activation of macrophages (MΦ), natural killer cells (NK) and natural killer T cells (NKT). After exposure of early antigenic variants expressing dominant variant surface glycoproteins (VSGs), continuous antigenic variation allows trypanosomes to escape antibodies, but trypanosomes also induce B cell depletion that impairs antibody synthesis. This results in the occurrence of parasitemia peaks with multiple trypanosome variants present simultaneously. Moreover, late infection is characterized by the expression of mosaic VSGs that exhibit reassortments between previously used amino acid sequences. Colors represent different trypanosome variants. (*b*) Trypanosome effects on the B cell compartment. Trypanosomes affect different B cells with different kinetics. The first cells to be lost are splenic IgM^+ marginal zone B cells (MZB). In contrast, follicular B cells (FoB) tend to increase in numbers, but they gradually disappear as infection progresses. Germinal center (GC) B cells occur after control of the first peak of infection, albeit in the absence of actual GC formation. These GC-like B cells also rapidly decline as infection progresses. The bulk of B cells observed during infection are plasma B cells (Pc). Their occurrence is part of a polyclonal response that is inefficient to improve parasitemia control. Successive waves of infection do not induce memory B cells (MeB), and the replenishment of the peripheral B cell compartment is hampered by the loss of bone marrow B cells (BMB) early on during infection. Finally, T cell suppression prevents T cells from helping the B cell compartment.

Upon infection, trypanosome invasion triggers a rapid expansion of germline immunoglobulin M (IgM) antibodies particularly recognizing high-mannose residues of the antigen that covers the entire parasite surface, the variant surface glycoprotein (VSG) (3, 4). Since IgMs are more efficient than IgGs at activating the complement system, trypanosome resistance to IgMs is required to avoid rapid and efficient complement attack. Accordingly, experimental trypanosome infections in B $cell^{-/-}$ and $IgM^{-/-}$ mice have revealed that germline IgMs at the start of infection are crucial to control the development of parasitemia (5, 6). *T. brucei* resistance to germline IgMs is ensured by the activity of the *Tb*KIFC1 kinesin. Indeed, experimental depletion of this kinesin is sufficient to completely inhibit trypanosome infection because the IgM/VSG complexes persist for too long on the parasite surface (6). The crucial parasite defense provided by *Tb*KIFC1 is linked to its cholesterol trafficking activity. Surface cholesterol depletion by *Tb*KIFC1 generates high plasma membrane fluidity that allows the fast movement of the IgM/VSG complexes over the parasite surface, directing these complexes toward the flagellar pocket where endocytosis and antibody degradation take place before efficient parasite killing can occur (6, 7).

Another arm of the host innate defense against trypanosomes is the early induction of interferon gamma (IFNγ) by natural killer (NK) and NKT cells, subsequently followed by $CD8^+$ T cells and finally maintained by $CD4^+$ T helper type 1 cells (8). IFNγ is required for the proinflammatory response of macrophages. Studies on inflammation linked to early stage trypanosomiasis have shown that this response is dependent on myeloid differentiation primary response 88 (MyD88) and Toll-like receptor 9 (TLR9) (9), indicating that recognition of unmethylated trypanosome DNA by the host innate immune response is crucial in the onset of activation. Accordingly, trypanosome parasite control can be improved in susceptible BALB/c mice by injection of CpG oligonucleotides, which not only increases the initial inflammatory response but also improves antitrypanosome B and T cell responses (10).

VSG-dependent inflammatory responses are mainly triggered by IFNγ-activated macrophages, after recognition of the polymannose carbohydrate core of the VSG glycosylphosphatidylinositol anchor (11). This triggers the production of tumor necrosis factor (TNF) and the expression and shedding of the TNF receptor (12). For both *T. brucei* and *T. congolense*, TNF has been shown to be crucial for peak parasitemia control (13, 14), in conjunction with nitric oxide (NO) and antibodies (15, 16). Counteracting this inflammatory response in the initial stage of infection appears to be the role of the multiple receptor-like adenylate cyclases

of the parasite. Internalization of adenylate cyclase–containing trypanosome membranes into macrophages, through phagocytosis of entire parasites (17) or released extracellular vesicles (18), promotes the synthesis of cyclic adenosine monophosphate that reduces TNF synthesis through activation of protein kinase A within macrophages (17). Thus, either lysed trypanosomes or vesicles released from the surface of live trypanosomes can condition macrophages to limit TNF synthesis early in infection. Similarly, a released kinesin heavy chain (*Tb*KHC1) can promote myeloid cells to synthesize the anti-inflammatory interleukin 10 (IL-10), thereby stimulating the growth-promoting activity of arginase-1 and conversely inhibiting the antiparasitic activity of the NO synthase (19). Finally, the parasite release of tryptophan-derived indole-3-pyruvate inhibits the production of inflammatory prostaglandins in activated macrophages, which contributes to promote the survival of both host and parasite (20).

TNF, an inflammatory cytokine first linked to *T. brucei*–induced cachexia in rabbits (21), is one of the major drivers of infection-associated pathology. During infection, the inflammatory activity of TNF is counteracted at a minimum of two levels. First, by shedding soluble TNF-P75 receptors from macrophages, peak cytokine concentrations can be complexed in the circulation, reducing the levels of cytokine available for triggering detrimental host responses (22). Second, induction of IL-10 production can dampen excessive immune stimulation by IFNγ, including TNF responses (13). While IL-10 is produced by many cells during infection, only hepatocyte-derived IL-10 appears to exert a crucial impact on the outcome of trypanosome infection (23).

Only a limited number of studies have addressed the innate immune responses at the level of the skin, where natural infection and transmission are initiated. While parasite-activated neutrophils induce the shedding of DNA nets (24, 25), trypanosomes can avoid this response through active motility driven by flagellum beating, together with TatD DNase–mediated disruption of extracellular DNA traps (26). Surprisingly, during natural transmission, skin neutrophil activation benefits trypanosomes (27). While we lack sufficient data to fully explain these observations, it is interesting to note that in cattle, parasite growth control relies on a hematopoietic-independent innate immune effector mechanism (28). The skin is a highly complex tissue that not only serves as a physical immune barrier against most pathogens but also provides various levels of defense in cases of barrier breakage, for example, by the bite of the tsetse fly. Dermal macrophages and dendritic cells, as well as NK and NKT cells, mast cells, αβ, and γδ T cells all deliver a specific contribution to immunity and wound healing. Moreover, natural infections are initiated by metacyclic salivary trypomastigotes from infected flies, which exhibit a much-reduced infectivity compared with bloodstream form trypanosomes that are usually injected intraperitoneally for mice infection in laboratory experiments (29). On the basis of available data, it appears that in mice, trypanosomes try to modulate the skin immune system into an IL-4/IL-10–based anti-inflammatory direction (30). In addition, saliva from infected tsetse flies reduces the anticoagulant function of naive saliva, affecting feeding time and hence increasing the likelihood for parasite transmission. In this respect, thrombin serum activity and platelet activation must also directly contribute to the innate immunity response (31, 32).

2.2. Human-Specific Innate Immunity (APOL1)

Unlike other mammalian hosts, humans and some primates such as gorillas can resist infection by the prototypal African trypanosome *T. b. brucei*. The two subspecies *T. b. rhodesiense* and *T. b. gambiense* can avoid this human resistance, causing sleeping sickness in East and West Africa, respectively. The identification of the human serum component conferring resistance to *T. b. brucei* resulted from the prior characterization of the *T. b. rhodesiense* factor abolishing this resistance. Expression of this parasite resistance factor was found to require the selective transcriptional activation of a specific polygenic site among several sites for expression of the VSG (33). In this

specific genomic site, a gene encoding a truncated version of the VSG was found to be necessary and sufficient to confer parasite resistance to human serum, and the protein encoded by this gene was termed serum resistance-associated (SRA) protein (33). The subsequent search for SRA-like proteins in *T. b. gambiense* led to the identification of another VSG-like factor of resistance to human serum, termed *T. b. gambiense*-specific glycoprotein (TgsGP) (34). Thus, the VSG was used as a building module for the generation of parasite adaptive proteins allowing expansion of the host range to humans, through their ability to neutralize the toxic activity of human serum (35). SRA-mediated resistance to this serum was found to depend on an N-terminal helix with the potential for interaction with other proteins. Therefore, SRA was used as a bait to capture the trypanolytic factor from human serum, which was identified as APOL1, an apolipoprotein mainly associated with high-density lipoproteins (36). SRA neutralizes APOL1 trypanolytic activity by direct interaction with its C-terminal leucine zipper (LZ) helix (36). In contrast, TgsGP does not interact with APOL1 but rather protects the parasite against APOL1 activity (34).

APOL1 is the most recent member of a rapidly evolving family of lipoproteins related to adaptation to pathogens (37). APOL1 is the only member to be secreted, and, therefore, it is the sole member to exhibit extracellular activity (38). Together with APOL3, APOL1 is highly expressed under inflammatory conditions, typically during type I interferon (IFN-I)-mediated response to infection, such as occurs with several viruses (39–41). In this response, APOL1 and APOL3 both participate in apoptosis (40, 41). In addition, APOL3 can kill intracellular bacteria (42). Both APOL1 and APOL3 are characterized by a strong binding to anionic lipids, particularly cardiolipin (41). When expressed in *Escherichia coli*, APOL1 exerts cytotoxicity linked to membrane depolarization due to ion pore-forming activity by a double-stranded hairpin helix resembling those of bacterial colicins and protein members of the apoptotic B cell lymphoma 2 family (43). In this experimental expression system, a hairpin-contiguous region termed membrane-addressing domain (MAD) is necessary for APOL1 toxicity (43). Membrane association of the hairpin-MAD tandem was also observed upon in vitro incubation of APOL1 with mitochondrial membranes (44). As bacterial and mitochondrial membranes share a high level of cardiolipin, association of the APOL1 hairpin with membranes could involve cardiolipin binding. Such activity is also expected to occur with APOL3, providing an explanation for the ability of APOL3 to induce membrane destabilization of intracellular bacteria (42). In trypanosomes or in vitro with synthetic planar lipid membranes, APOL1 needs acidic conditions to insert into membranes, due to the necessary protonation of negatively charged amino acids in the hairpin helix (45, 46). As such pH dependence is not shared by APOL3 that is exclusively intracellular (45), it could result from the APOL1 requirement of avoiding insertion in vesicular membranes during the intracellular traffic leading to APOL1 exocytosis and secretion.

Because APOL1 is taken up by the trypanosome into its digestive system (5), the low pH of endosomes allows APOL1 insertion in the parasite endosomal membranes, leading to transmembrane anionic flux linked to osmotic swelling of the lysosome (43, 44). However, APOL1 trypanolytic activity is not due to lysosome swelling but rather results from the transfer of APOL1-containing endosomal membranes to the mitochondrion (**Figure 2*a***). APOL1 triggers mitochondrial membrane permeabilization, leading to the traffic of the *Tb*EndoG endonuclease from the mitochondrion to the nucleus, *Tb*EndoG-mediated DNA degradation into nucleosomes, and resulting apoptotic-like death of the parasite (44). Two trypanosome proteins participate in this process: the *Tb*KIFC1 kinesin ensuring the transport of endosomes to the mitochondrion (44) and a trypanosome homolog of the fusogenic vesicle-associated membrane protein (VAMP8) known as *Tb*VAMP7B (47) presumably promoting membrane fusion between endosomes and the mitochondrion. APOL1 also induces increased fusion between mitochondrial membranes, but this phenomenon in itself is not responsible for trypanosome lysis (44).

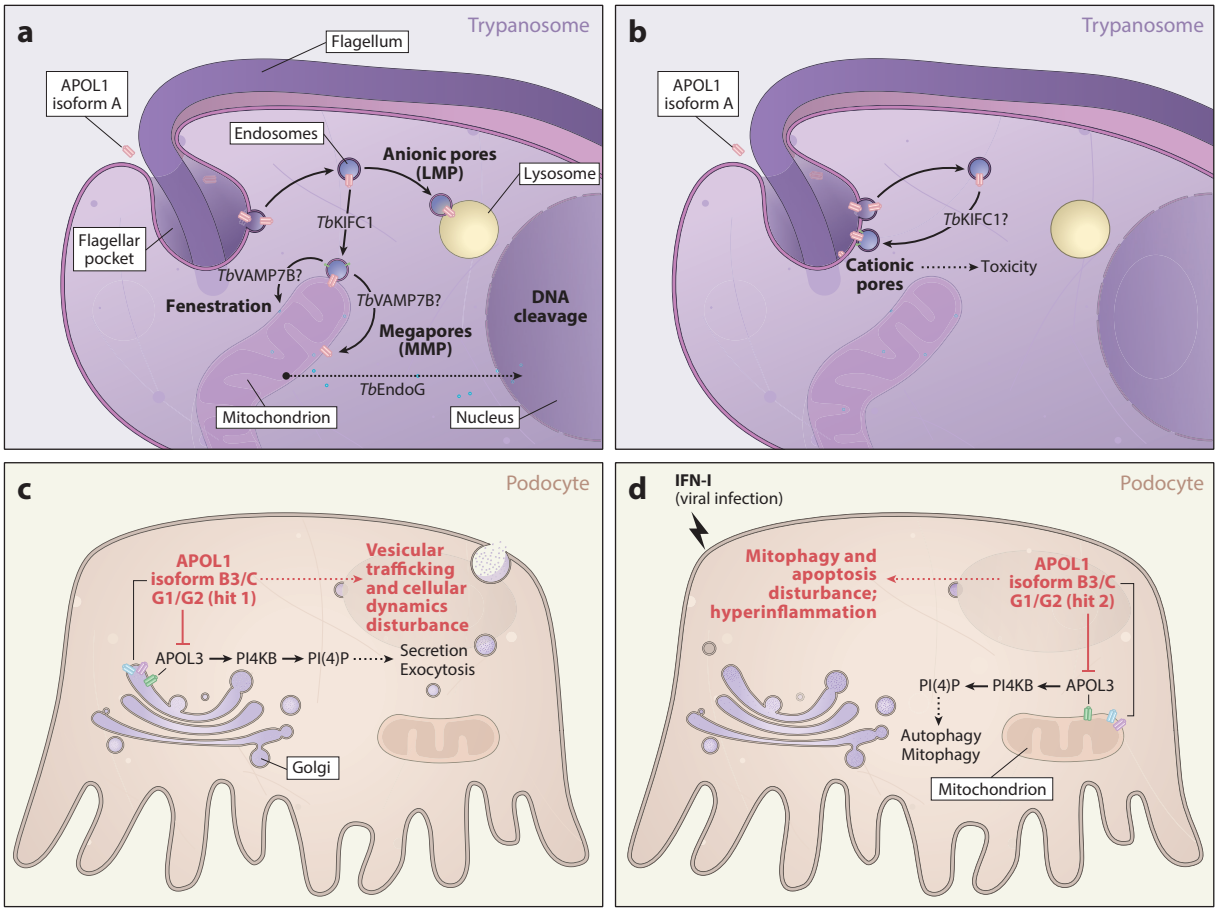


Figure 2

Mechanisms of toxicity induced by the trypanolytic factor apolipoprotein L1 (APOL1). (*a*) Trypanolysis model 1 (44). APOL1 insertion into endolysosomal membranes induces lysosomal membrane permeabilization (LMP) accompanied by endolysosomal osmotic swelling due to transmembrane anionic flux. In addition, *Tb*KIFC1 kinesin–mediated recycling of APOL1-containing endosomes to the mitochondrion triggers the formation of megapores (mitochondrial membrane permeabilization, or MMP) that allow the *Tb*EndoG endonuclease to reach the nucleus and degrade the DNA. APOL1 also triggers mitochondrial membrane fusion (fenestration). (*b*) Trypanolysis model 2 (48). Recycling of APOL1-containing endosomes to the plasma membrane triggers the formation of cationic channels that disrupt ionic homeostasis and cause general toxicity. (*c*) Podocyte dysfunction (hit 1). C-terminal mutations increase the interaction of intracellular APOL1 isoform B3 or C with APOL3, thereby interfering with the APOL3 control of phosphatidylinositol-4-phosphate [PI(4)P] synthesis by the phosphatidylinositol-4-kinase IIIB (PI4KB) at the Golgi (41). PI(4)P is involved in vesicular trafficking (membrane elongation, fission, and fusion). (*d*) Podocyte dysfunction (hit 2). Under the type I interferon (IFN-I) response to viral infection, PI4KB is recruited to mitochondrion contact sites with the endoplasmic reticulum (MERCs) to initiate membrane elongation, fission, and fusion linked to autophagy. G1/G2-mediated APOL3 inactivation at MERCs affects mitochondrial membrane dynamics, triggering a reduction of the autophagic flux that increases the damage due to infection. Alteration of mitochondrion function could induce mitochondrial DNA release, triggering hyperinflammation and sepsis (143), as occurs with coronavirus disease 2019 (126).

In vitro in synthetic membranes, APOL1 insertion at low pH generates a weak transmembrane flux of anions, but consecutive switching to neutral conditions triggers a high flux of cations (48). Thus, the APOL1 pore is pH gated. Based on this observation, it was proposed that in vivo, APOL1-containing endosomes recycle to the plasma membrane, activating a flux of cations leading to disruption of ion homeostasis (48) (**Figure 2*b***). However, so far the demonstration

of transmembrane APOL1 recycling to the plasma membrane is still lacking. Using propidium iodide as a pore marker, no plasma membrane pore was identified during trypanolysis by APOL1 (44). Instead, the cation flux by APOL1 could occur at the parasite mitochondrion, given the evidence that APOL1 disrupts the mitochondrial membrane potential (44).

In summary, trypanolysis by APOL1 results from the acidic pH-dependent insertion of this protein in endosomal membranes, followed by intracellular traffic of these membranes to neutral pH compartments such as the mitochondrion, which triggers apoptotic-like death of the parasite.

2.3. Adaptive Immunity

As salivarian trypanosomes are extracellular pathogens, one would expect the host adaptive immunity to play a key role in controlling infection through the activation of both B and T cells, the induction of antibodies, and the buildup of immunological memory. However, trypanosomes successfully counteract the host weaponry, being able to (*a*) evade antibody-dependent and complement-mediated destruction, (*b*) hamper activation of the T cells exerting helper functions, (*c*) trigger B cell dysfunction, and (*d*) cause failure of B cell memory in recognizing reappearing parasite surface epitopes over time.

Trypanosomes are covered with a quasi-uniform coat of VSG that is fully exposed to immune detection (49). After early recognition of VSG high-mannose residues by germline IgMs (4), IgGs are directed against surface-exposed epitopes of the N-terminal VSG domain (50). Fast and efficient clearance of these VSG/antibody complexes results from the particularly high fluidity of the trypanosome plasma membrane, owing to active cholesterol depletion by the *Tb*KIFC1 kinesin (7). During development of parasitemia, the VSG coat undergoes continuous antigenic variation (50). As the parasite can use a collection of more than a thousand VSG genes and pseudogenes to express one VSG coat at a time, the host antibody response is always one step behind the parasite in mounting an epitope-matched response against newly arising VSGs (51). Thus, trypanosomes continuously elicit and escape the antibody responses.

The host ability to successfully deploy complement-mediated cytotoxicity against the parasite would be the next strategy to conquer proliferating trypanosomes. However, complement-deficient animals such as AKR mice that lack the C5 complement factor do not suffer from any exuberant parasitemia and control successive waves of infections (52). Thus, peak parasitemia clearance does not require the active participation of the full complement pathway. Nevertheless, complement-mediated lysis assays have been used in in vitro experiments as a diagnostic tool to show the presence of antibodies in the serum of HAT patients. Thus, while complement can aid trypanosome lysis under in vitro laboratory conditions, during animal infection trypanosomes are well capable of protecting themselves from this host defense system. They do so by clearing activated complement factors from the serum and inhibiting the complement cascade itself (52, 53). In addition, the sheer thickness of the VSG coat makes it spatially difficult for deposited complement components to reach the trypanosome plasma membrane, making it hard for a C9 membrane attack complex to be formed and exert pore-forming activity. Trypanosome clearance rather relies on antibody-dependent cellular phagocytosis, with the complement receptor of the immunoglobulin superfamily playing a major role through C3b and iC3b opsonins binding in the presence of antibodies (54).

With IgMs playing a pivotal role in early-stage trypanosome elimination, one would expect that subsequent generation of affinity-matured IgGs should be able to further aid parasitemia control. However, although trypanosomiasis is marked by the rapid occurrence of IgGs, there is no clear indication that these antibodies provide an efficient antitrypanosome defense. On the contrary, recent results obtained with activation-induced cytidine deaminase–deficient ($AID^{-/-}$) mice, which

are unable to undergo B cell somatic hypermutation and class switch recombination, have shown that inhibiting the production of affinity-matured IgGs has a beneficial effect in the control of *T. evansi* infection (55). In the absence of IgG, AID$^{-/-}$ mice exhibited a superior defense level against *T. evansi* as compared with fully immune-competent wild-type mice. The AID$^{-/-}$ mice have higher titers of germline IgMs that can exert in vivo and in vitro trypanocidal activity (55). Hence, a rapid class switch from IgMs to IgGs during an early infection may facilitate trypanosome escape from detrimental IgM-mediated elimination, resulting in host susceptibility during the chronic phase. In this context, it would be interesting to investigate the role of IgMs in rare non-classic *T. evansi* infections of APOL1-competent humans. Those patients could be suffering from depressed levels of IgMs.

The in-depth mechanism of trypanosomiasis-induced differentiation of mature B cells was assessed by single-cell RNA sequencing (scRNAseq) (55). Results showed that upon infection of C57BL/6 mice, mature splenic B cells rapidly differentiate into AID^{+} germinal center-like B cells that produce T cell–independent IgG2c antibodies. This occurs simultaneously with the depletion of immature bone marrow B cells, preventing mature B cell replenishment in the periphery and impairing host ability to generate antibodies against new parasite variants (55–57). Both *T. brucei* and *T. evansi* infections cause severe destruction of splenic B cell follicles and the marginal zone (MZ), giving rise to progressing immunopathology (55, 57). The destruction of B cell follicles prevents the interactions between the follicular B, T, and dendritic cells, which are needed for the generation of high-affinity matured and class-switched antibodies as well as conventional memory B cells (MBCs). In contrast, *T. evansi* infection induces the generation of short-lived atypical memory B cells (atMBCs) (55). Previously, atMBCs have been associated with autoimmune pathology as well as the production of protective IgG2a/c antiviral responses. Thus, during *T. evansi* infection, atMBCs could contribute to the rapid IgG2c antitrypanosome response and give rise to the autoimmune complications characteristic of trypanosomiasis pathology (58). Finally, trypanosomiasis triggers a profound state of T cell suppression in the host, by inhibiting both IL-2 production and IL-2R expression. This contributes to suppression of immunological T cell help, further reducing the B cell maturation process (59).

The impact of antigenic variation on the capacity of the host to generate specific MBCs requires further attention, as successful generation of MBCs would ensure protection against reinfection. As VSG molecules carry an array of B cell epitopes that could give rise to the generation of MBCs, a simplistic view would be that every time a new B cell epitope is generated, the parasite is able to escape the "old" B cell repertoire. However, the efficacy of the mammalian immune system is driven by the opposite principle: As long as there are common epitopes present, immunological memory can be recalled at the level of both T cells and B cells, kick-starting a fully mature antibody production. Accordingly, successful antigenic variation would require every individual VSG to be radically different from all previously expressed VSGs. However, as trypanosome infection progresses into the chronic phase, parasites use mosaic VSGs consisting of combined epitopes of previously used VSGs (60). Hence, even in the absence of any reoccurring B cell epitope, the appearance of conserved T cell epitopes on different VSGs would ensure that the immune system can rely on immunological memory helper functions to quickly generate an efficient B cell response. Such T cell epitopes were shown to be present in the structurally conserved parts of VSG molecules (61). To overcome this problem, trypanosomes acquired yet another immune evasion strategy, which is a profound destruction of immunological memory. While suspected for a long time, recent scRNAseq data have confirmed that the short-term appearance of a given VSG during a single parasitemia peak is not enough to generate long-lived conventional MBCs, at least not in experimental mouse models (55). In addition, trypanosome infection has a detrimental impact on existing immunological memory (62–64). This has mainly been shown in models where the

effect of trypanosomiasis was studied on nonrelated vaccine responses against bacterial and viral pathogens, showing that the recall capacity of the immune system is ablated after trypanosome infection (57).

In conclusion, trypanosomes not only continuously escape the antibody responses but also actively affect the B cell compartment, depriving the host of mature B cells able to respond to new VSGs and preventing the generation of memory B cells.

2.4. Parasite Quorum Sensing

Trypanosomes have adopted a system of quorum sensing to continuously limit parasitemia levels and prevent premature killing of the host. The mechanism ensuring self-control of *T. brucei* parasites involves the differentiation from the proliferative long slender form to a nondividing stumpy form prepared for survival inside the tsetse midgut. Sensing of di-and tripeptide levels in the environment by the oligopeptide transporter *Tb*GPR89 signals to the parasite that the level of tissue damage is reaching critical levels and subsequently drives the population growth arrest (65). This parasitemia control is independent of VSG switching, explaining how *T. evansi* has lost the slender–stumpy transformation cycle without losing its VSG switching competence (66). This control is also independent of the antibody response since it is unaffected by genetic abrogation of B cell development (6, 13) and is not affected by T cells either (67). Finally, the quorum sensing could explain why mouse strains with a high inflammatory background such as C57BL/6 mice have significantly lower parasitemia levels than those of low-inflammatory strains, because in these strains severe tissue pathology produces higher levels of oligopeptides. Thus, increased tissue damage calls for an earlier stop of parasite proliferation, independently of innate and adaptive immune responses.

3. HOST PATHOLOGIES LINKED TO INFECTION

3.1. Anemia and Iron Metabolism

In both animals and humans, anemia is one of the pathological outcomes of chronic trypanosomiasis. In livestock animals, the induction of anemia has been best described for cattle (68). African cattle breeds can be classified as trypanotolerant or susceptible, a characteristic that is dependent not on parasitemia control but rather on the way these animals deal with anemia. Under experimental infection by *T. congolense*, West African taurine breeds such as the N'Dama cattle (*Bos taurus*) do not show signs of severe anemia or hemophagocytosis. In contrast, Zebu cattle (*Bos indicus*), introduced into sub-Saharan Africa only 1,000 years ago, suffer from trypanosomiasis-induced anemia independently of the parasitemia load (69). Comparative studies between these two breeds have shown that anemia results from disproportionate innate immune response to infection. This conclusion was confirmed using N'Dama chimeras carrying susceptible hematopoietic cells of Boran origin. During *T. congolense* infection, these animals lose their capacity to maintain normal red blood cell (RBC) levels but control parasitemia in the same manner as tolerant N'Dama cattle (29).

In the case of humans, trypanosomiasis-linked anemia is poorly documented. However, field studies (70) and reports on non-African tourist infections (71) indicated that *T. b. rhodesiense* induces hemolytic anemia. With *T. b. gambiense*, chronic disease symptoms are diverse, and there is no direct link between parasitemia and patient hematocrit levels. Studies comparing the pathogenicity and virulence of *T. b. gambiense* isolates in BALB/c mice have confirmed the pathology heterogeneity observed in the field, without identification of the mechanism responsible for anemia (72).

While the chimera N'Dama/Boran cattle experiments have shown that infection-induced anemia largely occurs through T cell–independent processes, in mice the pathology was strongly correlated to the presence of an activated T cell compartment and the production of IFNγ by

CD8 T cells (9). While IFNγ is known to inhibit erythroblast differentiation and erythropoiesis through activities of TNF family proteins (73), the role of TNF itself in the induction of trypanosomiasis-associated anemia is ambiguous, as TNF-deficient mice suffer anemia following infection by *T. congolense* but not by *T. b. brucei* and *T. evansi* (13, 74). In mice, abrogating excessive infection-associated inflammation by shedding TNF-P75 receptors improves anemia control (24). Similarly, the induction of anti-inflammatory IL-10 production, mainly by hepatocytes, also alleviates anemia (5).

The main immunological reasons for the induction of anemia include (*a*) accelerated erythrophagocytosis involving adhesins such as galectin-3, (*b*) a block of extramedullary erythropoiesis involving cytokines such as macrophage migration inhibitory factor, and (*c*) inhibition of RBC maturation, all resulting in the activation of macrophages (75). This process leads to disruption of the host iron metabolism and reduced iron availability in the circulation (76). Trypanosome infection leads to rapid alterations in the levels of heme oxygenase-1 involved in extracting Fe^{2+} from phagocytosed RBCs, the membrane proteins Nramp-1 and Nramp-2 involved in Fe^{2+} transport to the cytosol, the ferritin heavy chain involved in Fe^{2+} storage, ferroportin-1 responsible for Fe^{2+} release after erythrophagocytosis, ceruloplasmin responsible for Fe^{2+} to Fe^{3+} conversion, and finally transferrin (Tf), the main transporter of Fe^{3+} needed for continued erythropoiesis (75). In addition, expression of the Tf receptors Tf-R1 and Tf-R2 as well as the heme/haptoglobin scavenger receptor CD163 are affected by *T. brucei*, albeit the latter was not observed with *T. congolense* (77). Most recently, the peptide hormone hepcidin, which regulates the surface expression of the Fe^{2+} transporter ferroportin (78), was identified as a key player in the induction of *T. brucei*–associated anemia (79). Upregulation of hepcidin results in abrogation of the Fe^{2+}/Fe^{3+} recycling and hampers the host to produce iron-loaded Tf, subsequently blocking erythropoiesis and hence leading to anemia (80). In contrast, the hepcidin repressor erythroferrone does not appear to contribute to anemia (79).

Studies on trypanosomiasis-induced anemia have also focused on accelerated RBC phagocytosis. During *T. congolense* infection, erythrophagocytosis appears to be a main contributor to anemia (81). Recognition of RBCs by the host phagocytic system is accelerated by the action of trypanosome sialidases, responsible for the modification of the target cell surface. Induction of antibodies neutralizing this activity reduced the severity of infection-associated anemia (82). Enhanced erythrophagocytosis of desialylated RBCs is also considered the cause of *T. vivax*–induced anemia (83). In contrast, no such activity was reported during *T. brucei* infection, and here susceptibility of the RBC membrane to peroxidation and rapid alteration of cell membrane stability appear to prime cells for accelerated phagocytosis (84). In addition, it has been suggested that during *T. brucei* infection the transfer of VSGs to the membranes of RBCs could make the latter a target for anti-VSG antibodies, subsequently resulting in lysis or phagocytosis (85). This transfer of VSGs to RBCs was confirmed when studying the in vitro biological effect of trypanosome extracellular vesicles (19). Moreover, autoimmune antibodies directed against RBC surface-exposed phosphatidylserine coincided with problems in anemia recovery (58). However, results obtained with antibody-deficient μMT animals indicated that, at least in mice, the presence or absence of antibodies during infection has no impact on infection-associated anemia (6).

In many infection models, strict regulation of the iron metabolism is crucial. Fe^{2+} iron is toxic because of its reactivity with oxygen that generates hydroxyl radical and reactive oxygen species. This activity is blocked through the storage of Fe^{2+} by ferritin, in combination with the activity of ferroxidase, which converts reactive iron into nucleated iron (Fe^{3+}) that no longer catalyzes the production of free radicals. However, these toxic compounds are beneficial in the immunological fight against microorganisms, and iron is also a crucial component in molecules such as hemoglobin, myoglobin, and particularly the enzymes of the oxidative phosphorylation pathway.

Thus, limiting iron availability to microorganisms is expected to hamper cellular growth, and induction of anemia has been proposed as a form of nutritional immunity, with hepcidin being a key regulator in the host defense against infection (86). However, bloodstream form trypanosomes do not use oxidative phosphorylation to generate energy and do not depend on the bulk of iron-containing electron transport molecules generating ATP in other aerobic organisms (66). Therefore, it is unlikely that strong iron scavenging occurs during trypanosomiasis as a host strategy to starve the parasite, possibly explaining why, in this case, parasitemia levels and anemia levels are not correlated.

We conclude that in trypanosomiasis, anemia is merely the result of an ill-regulated immune response in trypanosusceptible mammals, including humans in the case of acute *T. b. rhodesiense* infection.

3.2. Neuropathology

HAT is often referred to as sleeping sickness. This name is derived from the late stage of infection, where patients suffer from daytime somnolence and impaired consciousness before death. However, other symptoms are linked to progression of the disease. Trypanosomes first invade the blood and lymphoid tissue, hence the reference to the hemolymphatic stage of infection (stage 1). Here, the nonspecific symptoms resemble those of malaria disease, including intermittent fever, headaches, fatigue, and joint stiffness or pain. Gradually, these symptoms will worsen as the victim starts to suffer a general state of inflammation, which will affect the brain where astrocyte activation and inflammatory cell infiltration will lead to the onset of the second stage of the disease, called the encephalitic stage, in which parasites migrate into the central nervous system (CNS) (stage 2). Neurological symptoms include irritability, behavioral and sensory disturbances, and motoric disorders such as the occurrence of tremors, uncontrolled muscle movements and slurred speech, myelopathy and myositis, and, finally, coma preceding death. Progression of stage 1 into stage 2 depends on both parasite and host genetics (87). First, disease progression in *T. b. gambiense* infection can take years. Due to the chronic nature, it is hard to retrieve reliable data on how long the preclinical period of the disease lasts, and information about this disease aspect is most often obtained through self-reporting and recollection of clinical symptoms. Interestingly, combined symptoms such as walking disabilities, speech disorder, and abnormal movements, all corresponding with the stage 2 characteristic white blood cell (WBC) infiltration in the cerebrospinal fluid (CSF), only surpass the 20% patient mark after a period of 24 months. This is much slower than *T. b. rhodesiense* HAT progression, which leads to a lethal outcome in up to 80% of victims within 6 months. However, this number is strongly dependent on location, highlighted by a study showing an 87% disease progression to the lethal stage in patients identified in eastern Uganda, strongly contrasting with findings from central Malawi where a mere 7% of HAT patients suffered from CNS invasion by the parasite over a period of several months (88). Second, stage progression is different in tourists as compared with local people infected with the same parasites. Finally, even in the case of early-stage *T. b. rhodesiense* infections, victims can exhibit late-stage symptoms such as sleeping disorders and mental confusion (88). On the other side of the spectrum, long-term *T. b. gambiense* patients appear trypanotolerant, having seropositive diagnosis scores but being at the same time aparasitemic and asymptomatic (89). As discussed in Section 3.3 below, this infection pattern can result from expression of a variant version of APOL1 termed G1.

Even though the link between sleeping sickness symptoms and CNS trypanosome invasion was made over a century ago, the exact route through which this happens remains a matter of debate (90). Most research focused on how human trypanosomes pass the blood–brain barrier (BBB), as parasite brain infiltration and activated lymphocyte infiltration occur simultaneously.

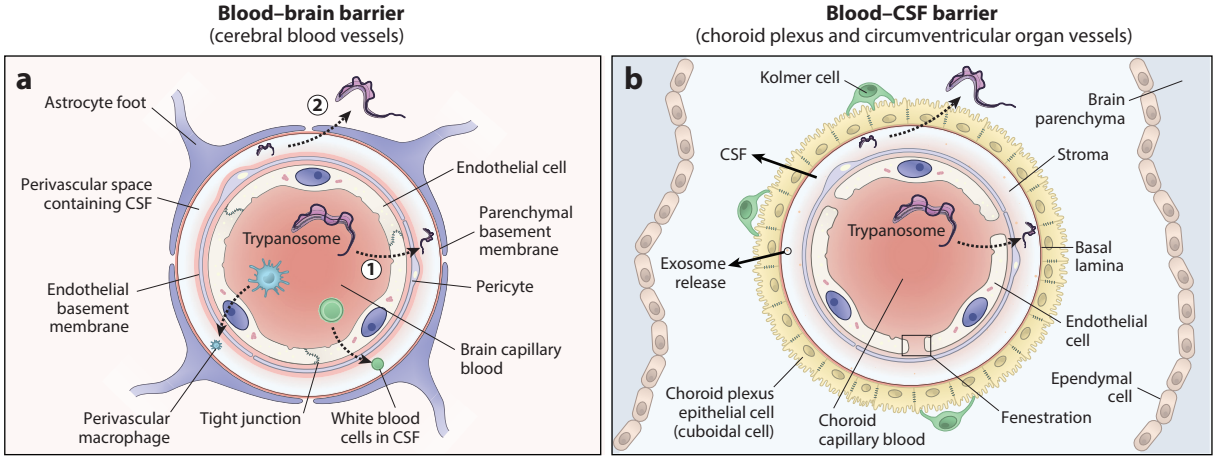


Figure 3

Trypanosome journey into the brain. (*a*) Crossing the blood–brain barrier (BBB). Trypanosomes pass the BBB of the cerebral blood vessels in a two-stage process. First (①), parasites pass from the blood into the cerebrospinal fluid (CSF) in a process that coincides with the passage of inflammatory T cells. This process requires the temporary disruption of the tight junctions that anneal the endothelial vessel cells. (②) As an interferon gamma–driven inflammation progresses, parasites are subsequently able to pass the parenchymal basement membrane and enter the cerebral tissue. (*b*) Crossing the blood–CSF barrier. This barrier is not protected by a tight junction cell wall but exhibits a fenestrated endothelial cell lining through which parasites can pass into the CSF. For CSF to be released from the choroid plexus and circumventricular organ vessels, it must pass a basal lamina and tight junction layer of epithelial cells through vesicular transport. Trypanosomes use the same vesicular exosome release pathway to enter the CSF circulation.

Interestingly, trypanosomes can pass the human brain microvascular endothelial cell layer by creating only a temporary disruption of barrier integrity (91), which has significant consequences for treatment of stage 2 infection as this requires specific drugs that are able to transverse an intact BBB. However, during progression of the disease, deterioration of the BBB occurs in nearly half of the patients that have entered the encephalitic stage (92). One of the issues when studying parasite infiltration in the brain is the fact that parasites are virtually never observed in postmortem biopsies. Hence, knowledge to date is derived from various animal models using both primates and rodents, using either non-human-infective *T. b. brucei* parasites or experimental models with *T. b. gambiense* and *T. b. rhodesiense*. According to these studies, the passage of trypanosomes through the BBB is a two-step process (**Figure 3**). First, trypanosomes pass the brain microvascular endothelial cells that are firmly connected through tight junctions, making up for the endothelial basement membrane. When successful, this allows parasites to enter the perivascular space filled with CSF. However, to penetrate the actual brain parenchyma, trypanosomes need to traverse the parenchymal basement membrane that is made up of astrocyte end feet (93). The trypanosome-secreted cysteine protease cathepsin L (brucipain) plays a major role here (94). This second step takes place only after inflammatory activation of perivascular macrophages and microglia, and it requires the presence of activated T cells. Indeed, results obtained in gene-deficient mice including $TLR^{-/-}$, $MyD88^{-/-}$, $RAG^{-/-}$, and $IFN\gamma^{-/-}$ or $IFN\gamma R^{-/-}$ mice have shown that in the absence of activated T cells, CNS infiltration of parasites is hindered (95), even though these knockout (KO) mice all have much higher blood parasitemia levels. Furthermore, the process requires activation of the matrix metalloprotease MMP9, involves both upregulation of TNF and IFNα/β expression, and is CXCL10 and ICAM-1 regulated (96).

In murine models, more severe trypanosomiasis-induced neuroinflammation correlated with increased TNF and IFNγ plasma levels, while milder CNS inflammation coincided with increased

IL-10 and IL-6 levels (97). In addition, comparative studies using both C57BL/6 and 129 Sv/Ev mice revealed no correlation between blood parasitemia levels and brain parenchyma invasion, linking the latter only to the inflammatory state of the host (98). Analyzing *T. b. brucei* infection in rats revealed a unique dual role for inducible NO synthase, which is highly expressed in neuroglial cells and neurons in different brain regions (99). While NO is an immunological defense molecule actively involved in trypanosome destruction, it is also important to maintain the integrity of the BBB, reducing parasite infiltration into the CNS (100). The overall neuroinflammatory response is linked not only to T cell inflammation but also to brain parenchyma infiltration of macrophages, B cells, plasma cells, and atypical Ig-containing plasma cells, called Mott cells (87).

Despite the wealth of information on the link between inflammation and trypanosome CNS infiltration, some observations nevertheless call into question any direct relation. For example, trypanosomes can be detected in the brain as early as 4 days postinfection, long before neurological complications or increased CNS cytokine levels occur (101). Not all areas of the brain are protected by the BBB, and although the hematogenous route remains the most plausible entry point for trypanosomes to the brain, entry to other locations cannot be excluded. Indeed, at the level of the fenestrated vessels of the choroid plexus, the barrier function is provided by the blood–CSF barrier (BCSFB), and trypanosomes could cross into the CSF through this route (102) (**Figure 3**). This allows trypanosomes to disseminate into the subarachnoid space and reach the pia mater. Migration of parasites into the pia mater from the CSF has been proposed to be a survival strategy of the parasite, as CSF constitutes a rather hostile environment that does not sustain long-term parasite survival. However, at the level of the meninges, a third CSF barrier is found, that is, the arachnoid barrier, that shields the dura from the subarachnoid CSF (103). Finally, a third possible route for trypanosomes to accomplish neuroinvasion is through circumventricular organs (104). These organs are isolated from the brain by ependymal tanycytes, cells responsible for producing CSF. While these cells allow the transfer of chemicals from the CSF to the CNS, they should be able to restrict the passage of trypanosomes to the brain unless IFNγ-driven inflammation disrupts the BCSFB (105).

As outlined above, trypanosomiasis-associated neuropathology is divided into two clinical stages. The stage determination of sleeping sickness is based on CSF lymphocyte counts. Conventional stage assessment requires the invasive procedure of lumbar puncture. The WHO criteria for defining stage 2 disease are the presence of more than 5 WBCs/μL CSF and/or the presence of trypanosomes. However, some guidelines have shifted this cutoff to 20 WBCs/μL, defining the range in between 5 and 20 cells as an intermediate stage, where stage 1 drug treatment still can be efficient to cure the disease (106). To define more specific staging markers, other factors have been considered such as the CSF levels of IgMs, cytokines, or chemokines. However, individual patients can show high marker variations, rendering these markers difficult to apply as a final decisive criterion for treatment. In the past, this has been problematic, as the drug treatment of HAT has depended on the disease staging. For early-stage *T. b. gambiense* infection, the standard treatment has been intravenous injection of pentamidine, while treatment for stage 2 infection is NECT, a nifurtimox-eflornithine combination therapy consisting of intravenous injection of the first drug, combined with oral treatment of the second. However, this issue has now become more of an academic consideration, as the introduction of a new oral therapy for *T. b. gambiense* HAT with fexinidazole can be used for treatment of both infection stages. Today, the recommendation is that only when CSF WBCs are exceeding 100 cells/μL should the NECT regimen be maintained, but in practice lumbar puncture assessment can be omitted as long as there are no clear signs of severe stage 2 symptoms (107). As for *T. b. rhodesiense* HAT, NECT is not efficacious, and fexinidazole trials have begun only recently. Hence, this disease is still treated by intravenous injection of suramin, independent of stage determination. Unfortunately, suramin treatment causes

severe toxic side effects and has a high treatment-associated fatality rate due to the development of posttreatment reactive encephalitis (108).

Important efforts have been made over the years to understand the molecular background of both daytime somnolence and nocturnal insomnia, the sleeping sickness characteristics. Attention has mainly focused on the regulation of the circadian rhythm and the melatonin fluctuation rhythm. To start with the latter, melatonin is a regulatory hormone produced by the pineal gland, with a production peak in the middle of the night, following indirect signals from the suprachiasmatic nuclei (SCN) of the hypothalamus, considered to be the master clock. As melatonin production is inhibited by light-triggered signals that travel from the retina to the SCN and olivary pretectal nucleus along the retinohypothalamic tract, the hormone has been linked to regulation of the sleep-wake cycle. HAT does not alter melatonin levels per se, albeit during infection the peak production of melatonin shifts to a slightly earlier time point during the night (109). The same holds true for the rhythm of the body temperature. These observations have prompted several groups to investigate how trypanosomes could affect the circadian rhythm of their host (110). This rhythm is regulated by light intensity reaching the retina and the information transmitted to the SCN of the hypothalamus, coordinating a molecular gene induction rhythm in synchronization with the solar day. How gene regulation related to the host circadian rhythm is functionally affected during HAT remains to be elucidated in detail, but it is known that *T. b. gambiense* HAT causes significant damage to the SCN of the mouse *Mastomys natalensis* (111). In C57BL/6 mice, *T. b. brucei* causes an alteration in the nighttime running behavior that is directly controlled by the master clock in the SCN (112). This disruption occurs independently of blood parasitemia, correlating with *T. b. rhodesiense* HAT observations indicating that sleeping sickness symptoms can occur before stage 2, when blood parasitemia levels are low. In the same mouse model, expression of the period-controlling *Per2* gene was shortened, but only toward the late stage of infection. In addition, severe infection-induced sleep disturbance was related to changes in the homeostasis of adenosine signaling in the brain (113). However, in experimental rat infection models no alteration in the cyclic expression of *Per2* was observed, suggesting that SCN alterations might be mainly due to mechanisms that are independent of the molecular regulation of the clock machinery (114). In this respect, trypanosomes can reach the median eminence (ME) and arcuate nucleus (ARC) of the hypothalamus, both involved in circadian release of hormones (115). The ME/ARC region also shows a strong expression of the IFN receptor IFNγR, making it susceptible to trypanosomiasis-associated inflammation, subsequently affecting the sleep-wake cycle (116). HAT also has a significant impact on the circadian profiles of plasma cortisol and prolactin levels, both related to sleep regulation in healthy individuals (117). In addition, HAT affects renin activity, an enzyme with a nocturnal plasma oscillation rhythm that is closely related to the NREM-REM sleep cycle (118). Finally, the levels of the sleep-promoting prostaglandin PGD2 and of the CXCL10 chemokine are increased in the CSF of HAT patients, coincident with alterations of the sleep-wake cycle (119, 120). However, the presence of these molecules could result only from overall inflammation, without any functional involvement in the deregulation of sleep rhythms (121).

In conclusion, both brain invasion by African trypanosomes and neuropathology induced by trypanosomiasis are clearly linked to inflammation, but the mechanisms of these processes are still far from completely elucidated.

3.3. Human Pathology Linked to Resistance to Trypanosomes

Since the building of the APOL1-driven innate immunity against *T. b. brucei*, a rapidly evolving arms race occurred between trypanosomes and humans (35). Between 4,000 and 10,000 years ago, two APOL1 variants termed G1 and G2 spread in Africa, presumably linked to their ability to kill *T. b. rhodesiense* in both heterozygous and homozygous genotypes (122). Whereas G2 consists

of a double amino acid deletion in the C-terminal LZ helix of APOL1, G1 is characterized by the strict association of two distant missense residues in the C-terminal domain. Both G1 and G2 variants exhibit resistance to interaction with the APOL1-neutralizing SRA protein of *T. b. rhodesiense*, although G1 is less resistant to SRA interaction than G2 (122). When tested in vitro, both variants efficiently killed various *T. b. rhodesiense* strains but not *T. b. gambiense*, and in G1 the combination of both distant missense residues was required to achieve complete trypanolysis (122). Thus, the G1 and G2 variants can confer resistance to *T. b. rhodesiense* owing to their ability to avoid neutralization by SRA. However, field studies did not reveal a robust protection of G1 individuals against *T. b. rhodesiense* infection (123). Moreover, the G1 and G2 variants also exhibited strong opposing associations with the outcome of *T. b. gambiense* disease. G1 was associated with undetectable parasitemia, while G2 was associated with faster infection (123). So far, the reason for these contrasting effects is still obscure. Given that the resistance of *T. b. gambiense* to APOL1 involves TgsGP-mediated membrane stiffening of early endosomes to prevent insertion of the APOL1 hairpin helix (34), it is possible that as presented in human serum, G1 and G2 are respectively less and more sensitive to this restriction of endosomal membrane access, thereby increasing or reducing the *T. b. gambiense*-killing potential of APOL1.

While protecting against trypanosomiasis, at the homozygous genotype the G1 and G2 variants are linked to increased risk of developing nondiabetic chronic kidney disease, particularly following viral infections such as human immunodeficiency virus (HIV)-associated nephropathy (HIVAN) or coronavirus disease 2019 (COVID-19)-associated nephropathy (COVAN) (124–126). Nondiabetic chronic kidney disease includes focal segmental glomerulosclerosis, hypertension-attributed end-stage kidney disease, collapsing glomerulopathy, and sickle cell nephropathy. The G1/G2-linked pathology involves the detachment of podocytes from glomeruli following podocyte foot process effacement. Transgenic expression of either wild-type APOL1 or G1/G2 variants in mice reconstituted the G1/G2-specific kidney disease (127). In these mice, podocytes exhibited perturbed vesicular trafficking and reduction of autophagic flux (127).

Given the toxic activity of APOL1 following uptake in trypanosomes or ectopic expression in various cell types, many studies analyzed the relative toxicity of the G1 and G2 variants, concluding that these variants are more toxic than wild-type APOL1 (128, 129). However, APOL1 toxicity results from acidic pH-dependent insertion of the double-stranded hairpin helix into membranes, and in human cells intracellular APOL1 is not detected in acidic compartments. Moreover, following ectopic expression, cytotoxicity has been observed even with wild-type APOL1 (130, 131). Thus, either improper processing linked to ectopic expression might expose toxic APOL1 components that would trigger a stress response (132) or mislocalized APOL1 could be targeted to the acidic environment of endosomes, causing toxic transmembrane insertion (130). Significantly, natural APOL1 and APOL3 overexpression induced by the IFN-I inflammatory pathway does not confer cytotoxicity but rather triggers programmed apoptosis in a minority of cells (40, 41). Moreover, intracellular APOL1 (isoform B3 and/or C) is processed differently from the secreted version (isoform A) and is also differently oriented with respect to intracellular membranes, being located on the cytoplasmic side of Golgi and endoplasmic reticulum (ER) membranes (41, 131). Thus, the acidic pH-dependent toxic pore-forming activity exerted by APOL1 isoform A in trypanosomes or in various cell types following experimental ectopic expression could be distinct from the genuine intracellular activity exerted by APOL1 (131).

Independently of APOL1 G1 and G2, an APOL3 null variant was also linked to kidney disease (133). Accordingly, transgenic invalidation of APOL3 (APOL3 KO) resulted in a podocyte phenotype like that of G1/G2 podocytes, with alterations of the actomyosin cytoskeleton evoking the linkage between some actomyosin mutations and kidney disease (41, 134). Therefore, kidney disease does not necessarily only result from APOL1-driven toxicity but also occurs in

the absence of APOL3. The APOL3 KO phenotype is mimicked by C-terminal truncation of APOL1 (APOL1Δ) (41). Thus, either the loss or sequence alteration of the C-terminal LZ helix of APOL1 mimics the phenotype associated with deletion of APOL3. Both APOL1Δ and G1 or G2 variants exhibit increased *trans*-interaction with APOL3. As this increased interaction with APOL3 is linked to reduced internal *cis*-interaction between the C-terminal LZ helix and another similar LZ sequence in the N-terminal region, APOL1 *trans*-interaction with APOL3 appears to result from reduced *cis*-interaction between the two APOL1 LZs (41). Thus, kidney disease could result from inactivation of APOL3 due to increased interaction with APOL1 risk variants, which would induce alteration of actomyosin activities in podocytes.

In contrast to APOL1, APOL3 binds to neuronal calcium sensor 1, an activator of the Golgi phosphatidylinositol-4-kinase IIIB (PI4KB), as well as with the PI4KB inhibitor calneuron 1 and PI4KB itself (38, 41). In vitro, APOL3 can increase phosphatidylinositol-4-phosphate [PI(4)P] synthesis by PI4KB (41). Accordingly, APOL3 KO podocytes are characterized by a reduction of Golgi PI(4)P levels, indicating inactivation or absence of PI4KB at the Golgi (41). Such inactivation or absence is also observed in G1, G2, and APOL1Δ podocytes (41). Thus, APOL1 C-terminal variants inhibit the PI4KB-activating function of APOL3 at the Golgi, presumably following direct interaction. Given the role of PI4KB in membrane elongation, fission, and fusion (38, 135, 136), the resulting decrease of PI(4)P level in the Golgi could affect actomyosin control of vesicular trafficking, altering the adhesion and motility properties of podocytes (**Figure 2*c***).

The G1/G2-linked risk of kidney disease is considerably increased by the IFN-I response to viral infection, such as occurs in HIVAN and COVAN (124–126). This cannot simply result from toxicity due to APOL1 overexpression because mimicking viral infection (and ensuing APOL1 overexpression) by podocyte incubation with the IFN-I inducer polyinosinic:polycytidylic acid does not trigger general cytotoxicity (39–41). Following viral infection, the IFN-activated immunity-related GTPase family M protein promotes the traffic of Golgi-derived vesicles to mitochondrion contact sites with the ER (MERCs) (137). The Golgi-to-ER traffic of PI4KB occurs in vesicles carrying the early autophagy marker ATG9A, triggering the folding and expansion of membranes for autophagy, leading to the formation of autophagosomes (138). Significantly, many viruses including coronaviruses hijack PI4KB for the generation of autophagosome-derived ER membrane pockets that build virus replication platforms (38, 138). Since autophagy protects infected cells from the toxicity due to viral infection (138, 139), inhibition by G1/G2 of the autophagy-promoting activity of PI4KB at MERCs could aggravate the pathology linked to infection (**Figure 2*d***).

APOL1 and APOL3 influence not only vesicular trafficking and autophagy but also mitochondrion dynamics. Increased mitochondrial fission is observed in both APOL3 KO and APOL1Δ podocytes (41), and the G1/G2 variants trigger increased mitochondrial fission (140). These observations suggest that inhibition of APOL3 increases mitochondrial fission; thus, APOL3 promotes mitochondrial fusion. Accordingly, in trypanosomes APOL1 and APOL3 induce mitochondrial fusion (44, 45), possibly with the involvement of a homolog of the fusogenic v-SNARE protein VAMP8 (47), and in podocytes APOL1 interacts with VAMP8 (141). The fact that APOL1 risk variants increase mitochondrial fission provides an explanation for the induction of mitochondrial dysfunction by these variants (142) and may be related to the effects of APOLs on PI4KB, since PI4KB is required for mitochondrial fission (136). The involvement of these activities in autophagy, particularly mitophagy, but also apoptosis, is probable. In support of this conclusion, alteration of mitophagy was proposed to account for exacerbated sepsis linked to G1/G2 (143), although delayed neutrophil apoptosis could also participate in this effect (38).

In conclusion, the effects of APOL1 C-terminal variants on mitochondrial dynamics, particularly following increased expression induced by viral infection, could crucially contribute to the

G1/G2-linked pathology. Not only is mitophagy affected, but more importantly the damage to the mitochondrial membrane and consecutive cytoplasmic release of mitochondrial DNA could induce hyperinflammation and sepsis, as is typically observed for COVID-19.

4. VACCINE FAILURE AND FUTURE PROSPECTS

Today, not a single antitrypanosome vaccine has proved successful under field conditions, despite the use of highly immunogenic invariant antigens such as the invariant surface glycoprotein 75 or the recently discovered invariant flagellum antigen from *T. vivax* (IFX) (144–146). Hence, even if a successful vaccine candidate were to be discovered for HAT or AT, it should overcome the speed at which the trypanosome manages to ablate functional memory. To test this, experimental approaches should include strategies in which vaccine-induced antibodies are allowed to completely decline to background levels prior to challenge, to test true immunological recall capacity. Based on classic knowledge from other models, it would be expected that the time taken to trigger the subsequent recall response would be 4 to 7 days, during which the trypanosome would already be capable of triggering detrimental effects on the MZ and bone marrow, where long-lived memory cells are residing (57). Once that process has been initiated, it would be hard for the host immune system to bring together the components for memory reactivation. In addition, after successful drug treatment of trypanosomiasis-affected livestock or humans, revaccination with previous vaccines against unrelated diseases might be required to rebuild the forgotten memory and ensure the health of the recovered individual. Thus, approaches independent of immunological memory need to be considered for combating HAT and AT. Today, the use of antibody therapy as a passive vaccination backup approach is undergoing a revival in cases of low vaccine efficacy. This has been highlighted by the recent fight against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), where vaccine efficacy strongly depends on the immune status of individuals, disease predisposition, and senescence of the immune system. Here, antibody engineering offers an opportunity to tailor therapeutic antibodies into various formats, coupling them to drugs, activating complement factors, and generating antibody variants with an extended half-life. Their use also provides several other advantages such as high specificity, complement activation, and few off-target effects besides being safe and showing limited interference with other drugs (147). Recently implemented HAT surveillance strategies such as improved diagnostics, drug treatment, and vector control significantly reduced the number of cases in humans despite the absence of a vaccine (108). In this context, future antibody therapies targeting invariant antigens could offer a backup alternative to drugs when needed in acute HAT treatment. However, the best option for the long-term control of the trypanosome animal reservoir would remain a vaccination strategy for livestock. Hence, research into the immune-destructive pathology of trypanosomiasis remains crucial, as it is pivotal to tackle this aspect of infection before a memory-inducing immune intervention can be considered.

SUMMARY POINTS

1. African trypanosomes can develop long-lasting chronic infection in mammals, causing sleeping sickness in humans and nagana disease in cattle.
2. African trypanosomes escape innate immunity through a combination of high mobility and high membrane fluidity ensuring fast clearance of antibody complexes, together with control of the inflammatory response by released parasite factors.

3. African trypanosomes escape adaptive immunity through a combination of growth self-control by quorum sensing and continuous variation of the main surface antigen (variant surface glycoprotein), together with impairment of specificity and memory of the B cell response.
4. In humans, specific antitrypanosome immunity is ensured by the trypanolytic activity of apolipoprotein L1 (APOL1), but the *Trypanosoma brucei* subspecies *T. b. rhodesiense* and *T. b. gambiense* can resist APOL1, causing sleeping sickness in East and West Africa, respectively.
5. In West Africa, the recent spreading of the APOL1 variants G1 and G2 has allowed humans to resist infection by *T. b. rhodesiense*, but this is associated with high propensity to develop kidney disease, particularly in the context of viral infection.
6. Sleeping sickness results from brain injuries linked to chronic inflammation.
7. Vaccination prospects are discussed in this review, considering the disruption of adaptive immunity by the parasite.

DISCLOSURE STATEMENT

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Errata

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