Genetic and immunological basis of human African trypanosomiasis
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Human African trypanosomiasis, or sleeping sickness, results from infection by two subspecies of the protozoan flagellate parasite Trypanosoma brucei, termed Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense, prevalent in western and eastern Africa respectively. These subspecies escape the trypanolytic potential of human serum, which efficiently acts against the prototype species Trypanosoma brucei brucei, responsible for the Nagana disease in cattle. We review the various strategies and components used by trypanosomes to counteract the immune defences of their host, highlighting the adaptive genomic evolution that occurred in both parasite and host to take the lead in this battle. The main parasite surface antigen, named Variant Surface Glycoprotein or VSG, appears to play a key role in different processes involved in the dialogue with the host.

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Introduction
Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense are respectively responsible for a chronic and an acute form of sleeping sickness, the first being widespread in western and central Africa, and the second in eastern and southern Africa. While humans are regarded as the main reservoir for T.b. gambiense transmission, human infection by T.b. rhodesiense only occurs occasionally. As both trypanosome species are transmitted by Glossina (tsetse) flies, the risk of sleeping sickness is determined by the occurrence of contacts between humans and infected tsetse flies. In 1998, almost 40 000 cases were reported, but it is estimated that 300 000 cases were undiagnosed and therefore untreated (https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)). Trypanosomiasis in domestic animals (Nagana) is also a very serious animal health issue that impacts on animal mortality, growth and productivity with consequent negative impacts on socio-economic development throughout sub-Saharan Africa.

Growing successively in Glossina flies and mammalian hosts, African trypanosomes have developed complex mechanisms for molecular adaptations to quite different environments [1]. Among the various factors involved in these adaptations, the organization of the surface membrane is particularly important to counteract both innate and adaptive responses of the host. Contrary to strategies used by other pathogens to evade host surveillance, such as antigen mosaicism characteristic of the American trypanosome Trypanosoma cruzi, antigenic variation in African trypanosomes involves periodic changes of a uniform, densely packed coat containing 10^7 copies of a single antigen called VSG, which covers the entire parasite surface including the flagellum.

The sole site for membrane traffic to/from the surface is the flagellar pocket, a specialized invagination of the surface membrane at the base of the flagellum. The pocket membrane is coated with VSG, but also contains various surface receptors whose elongated structure allows insertion within this coat [2,3].

T. brucei infection begins in the skin, where the parasites interact with adipocytes and neutrophils [4], and then follows in the bloodstream, where the dialogue with the immune system can persist in long-lasting chronic infection. Whereas T. brucei-triggered innate immunity involves strong inflammatory and anti-inflammatory responses notably due to carbohydrate components of the VSG such as high-mannose [5,6], adaptive immunity mainly involves antibodies directed against VSG epitopes [7]. In addition, efficient human innate immunity is conferred by the primate-specific trypanolytic factor Apolipoprotein L1 (APOL1) [8].

APOL1 trypanolytic activity
Unlike the other five members of the APOL family, which are intracellular proteins, APOL1 is a serum protein bound to high-density lipoprotein particles [9]. In these particles APOL1 is associated with a protein related to haptoglobin (haptoglobin-related protein, or Hpr), which like Hp can bind hemoglobin (Hb). This complex, termed Trypanosome Lytic Factor 1 or TLF1, may be
associated with germline IgMs, which recognize pathogen signatures such as high-mannose carbohydrates, and accumulate upon infection \[^{10**,11}\]. This IgM-associated TLF1 is called TLF2 \[^{10**}\]. TLF1 uptake occurs through Hpr/Hb binding to the Hp/Hb receptor \(Tb\text{HbHbR}\), normally responsible for providing growth-promoting heme to the parasite \[^{12}\], whereas uptake of TLF2 involves non-specific IgM binding to VSG \[^{10**,11}\]. Because of competition between Hp/Hb and Hp/Hb, TLF1 uptake is only efficient in the absence of HpHb, such as occurs in extravascular environments like the skin at the beginning of infection, or in hypohaptoglobinemic serum \[^{13}\]. In contrast, TLF2 uptake is unaffected by the presence of HpHb, and probably represents the major physiological mode of APOL1 uptake by bloodstream parasites \[^{10**,11-13}\]. In both cases APOL1 moves through the endocytic system, and generates endolysosomal ilnic pores due to acidic pH-dependent membrane insertion \[^{14-17}\]. Subsequent transfer of APOL1-containing membranes to the mitochondrion by the C-terminal kinesin \(Tb\text{KIFC1}\) triggers trypanolysis \[^{14}\]. This occurs through mitochondrial membrane permeabilization and release of the mitochondrial \(Tb\text{EndoG}\) endonuclease to the nucleus, which cleaves DNA into nucleosomal units as occurs during apoptosis in higher eukaryotes \[^{14}\]. It was hypothesized that APOL1-carrying endosomes also recycle to the plasma membrane, inducing cation conductance that disrupts ionic homeostasis \[^{15,16}\]. However, the presence of APOL1 in the plasma membrane, as well as the mechanism for this transport, has not been demonstrated so far.

**Trypanosome resistance to APOL1**

The two possible modes of resistance to APOL1, either APOL1 inactivation or resistance to APOL1 activity, have been respectively developed by the two distinct \(T.b\) \(brucei\) clones that can infect humans \[^{11}\]. In the case of \(T.b\) \(rhodesiense\), the specific Serum-Resistance Associated protein (SRA) binds to the C-terminal helix of APOL1, thereby inhibiting the APOL1 pore-forming activity \[^{8,18}\]. Interestingly, SRA is derived from a VSG that acquired the ability to interact with APOL1 through truncation of its surface-exposed domain and resulting localization to endocytic compartments instead of the cell surface \[^{11,19}\]. High SRA expression is required for \(T.b\) \(rhodesiense\) resistance to APOL1, probably explaining why the SRA gene is transcribed by the highly processive RNA polymerase I, rather than the usual RNA polymerase II \[^{20}\].

Resistance of \(T.b\) \(gambiense\) to APOL1 involves another VSG-derived protein termed \(T.gambiense\)-specific glyco-protein, or TgsGP \[^{11,21}\]. This protein stiffens endosomal membranes where APOL1 should normally insert during its journey within the trypanosome, therefore hindering APOL1 pore-forming activity \[^{21}\]. However, TgsGP-mediated protection cannot resist high APOL1 uptake occurring in the absence of HpHb, such as in hypohaptoglobinemic serum \[^{21}\]. Given that human adaptation to malaria is linked to frequent hypohaptoglobinemia in Western Africa, \(T.b\) \(gambiense\) probably adapted to this condition by limiting APOL1 uptake through L210S mutation in \(Tb\text{HbHbR}\), which reduces the affinity for the ligand \[^{21}\]. Thus, \(T.b\) \(gambiense\) likely responded to a human adaptation to another parasite, \(Plasmodium\), illustrating an unexpected dialogue between parasites through the host.

**The APOL1 arms race**

Given the direct interaction of SRA with the C-terminal helix of APOL1, experimental disruption of this helix by mutagenesis can allow APOL1 to escape neutralization by SRA, enabling these APOL1 C-terminal variants to kill \(T.b\) \(rhodesiense\) \[^{18}\]. Interestingly, such C-terminal APOL1 variants, able to kill \(T.b\) \(rhodesiense\), were found to be naturally widespread in Western Africa \[^{22}\]. These variants, termed G1 and G2, are respectively characterized by two separate mutations and a double amino acid deletion in the APOL1 C-terminal helix. Their abundance in Western Africa could possibly account for the disappearance of \(T\) \(rhodesiense\) from this part of the continent \[^{11}\]. Thus, G1 and G2 allowed humans to restore their lead in the resistance to African trypanosomes, except for \(T.b\) \(gambiense\).

However, like resistance to malaria is linked to sickle cell anemia, the price to pay for resistance to sleeping sickness is a propensity to develop chronic kidney disease \[^{22}\].

The mechanism allowing G1 and G2 to cause kidney disease has been debated for years \[^{23}\]. While many studies pointed to non-specific cytotoxicity of G1 and G2, a recent report concluded that cytotoxicity is due to APOL1 overexpression, and that the disease results from unfolding of an intracellular isoform of the G1/G2 variants, triggering their interaction with APOL3 \[^{24**}\]. Such interaction inactivates APOL3-mediated stimulation of phosphatidylinositol-4-phosphate [PI(4)P] synthesis by the Golgi kinase PI4KB \[^{24**}\] (Figure 1). The reduction of PI(4)P levels in G1/G2-expressing podocytes would account for the increased mobility and reduced autophagy that characterize podocytes of kidney disease patients \[^{24**,25}\]. Interestingly, a non-sense variant of APOL3 was found to exhibit positive selection in African populations, while also causing nephropathy like the APOL1 G1/G2 variants \[^{26}\]. Because of its inability to be secreted, APOL3 cannot kill bloodstream trypanosomes despite its intrinsic lytic potential \[^{17}\]. Since the interaction of APOL1 variants with APOL3 leads to inactivation of both partners \[^{24**}\], the selective advantage of APOL3 KO could result from the loss of APOL3-mediated neutralization of the trypanolytic APOL1 variants.
Early parasite defences: the key roles of motility and \textit{TbKIFC1}

In the skin, neutrophils are rapidly recruited to the site of infection, which paradoxically facilitates the development of trypanosome colonization [4,27]. Indeed, trypanosomes efficiently resist the neutrophil-mediated engulfment and elimination [27]. This faculty is linked to parasite motility following TatD DNase-mediated disruption of neutrophil extracellular DNA traps [28], and trypanosomes completely lacking propulsive motility cannot infect mice despite efficient clearance of antibody-VSG complexes [29]. Conversely, reduction of the rate of antibody-VSG clearance in motile trypanosomes fully prevents infection [30**]. The fast clearance results from lateral mobility of the glycosyl-phosphatidylinositol-anchored VSG, linked to high plasma membrane fluidity ensured by the cholesterol-trafficking activity of the \textit{TbKIFC1} kinesin [30**]. Thus, fast antibody clearance is absolutely required for trypanosome infectivity, and \textit{TbKIFC1} is an essential virulence factor needed for this purpose (Figure 2).

Paradoxically, the requirement of \textit{TbKIFC1} for clearance of IgM-VSG complexes is also likely to be the driving force for TLF2 internalization, and thus trypanosome killing, because TLF2 IgMs bind to the VSG [10**,11]. Therefore, as also occurs for TLF1-associated APOL1 uptake, which is linked to the capture of the growth factor...
heme, TLF2-associated APOL1 uptake may depend on a mechanism normally intended to help trypanosome survival, and thus could be another version of the Trojan horse story.

The parasite armoury against innate immunity: parasite-released factors

Trypanosome infection triggers the rapid synthesis of inflammatory components such as TNF-α and reactive nitrogen/oxygen species like nitric oxide (NO) [4]. The precise role of TNF-α in infection has been controversial. This cytokine is important for parasite control at the beginning of infection, and yet trypanosome growth is unaffected in established infections despite considerable induction of TNF-α synthesis. This paradox probably results from the fact that only membrane-bound TNF-α affects the parasite [31]. Counteracting this inflammatory response in early stages of infection appears to be the role of the multiple receptor-like adenylate cyclases (ACs) of the parasite [32]. Internalization of AC-containing trypanosome membranes into macrophages, through phagocytosis of either entire parasites [32] or released extracellular vesicles [33], promotes the synthesis of cyclic AMP that reduces TNF-α synthesis through activation of protein kinase A within macrophages [32]. Thus, lyzed trypanosomes or vesicles released from the surface of live trypanosomes can condition macrophages to stop synthesizing TNF-α early in infection. Similarly, a released kinesin heavy chain (7bKHC1) can condition myeloid cells to synthesize the anti-inflammatory cytokine IL-10, thereby stimulating the growth-promoting activity of arginase-1 and conversely inhibiting the anti-parasite activity of NO synthase [34].

Trypanosomes also secrete metabolites that modulate host responses, which may be especially important within the central nervous system. Trypanosomes constitutively secrete aromatic ketoacids such as indole pyruvate, which inhibit production of the pro-inflammatory cytokine IL-1β by macrophages [35]. Moreover, these metabolites additionally suppress IL-6 and TNF-α production by glial cells, and are potent inducers of heme oxygenase 1, an activity associated with anti-inflammatory and anti-oxidant pathways [36]. These effects may orchestrate pro-inflammatory and anti-inflammatory responses to produce a 'chemical lullaby' within the host, to lessen systemic pathologies associated with persistent infection. Such long-lasting infection is required for the transfer of trypanosomes between hosts, given their inefficient mode of transmission through the tsetse fly.

The parasite strategy against adaptive immunity: VSG variation

Antigenic variation and subsequent anti-VSG responses continuously limit infection, contributing to shape chronic infection. Not only VSG sequence variation resulting from total or partial gene replacement, but also variable post-translational VSG modifications such as O-glycosylation, contribute to antibody escape [37]. Antigenic variation requires monoallelic expression of the VSG: from a repertoire of several hundred VSG genes, only one is expressed at any one time. Failure to follow this rule prevents successful infection due to inefficient antibody escape [38]. This requirement is ensured by the existence of only one VSG expression site body (ES body) in the nucleus. Experimental generation of two simultaneously active VSG ES results in their dynamic colocalization within a single ES body [39]. The molecular machinery underlying the maintenance of a single ES body has been the subject of intense research, with the recent observations that factors such as histone variants or the telomere-binding protein RAP1 and its binding component PI(3,4,5)P3 delineate transcriptionally active versus inactive VSG ES in subtelomeric chromatin domains [40–42]. However, presumably the most significant breakthrough in this field was the discovery of a new protein complex responsible for allelic exclusion, containing the chromatin VSG-exclusion-1 (VEX1) protein and VEX2, an ortholog of the nonsense-mediated-decay heli-case UPF1 [43,44**]. VEX1 and VEX2 assemble in an
RNA polymerase-I transcription-dependent manner, and following DNA replication VEX1 compartmentalisation in the ES body is specifically maintained by the DNA replication-associated chromatin assembly factor CAF-1 [44**]. More specifically, productive monogenic VSG mRNA synthesis was found to result from spatial coupling between VEX1 in the mRNA splicing locus and VEX2 in the VSG-coding locus [45**], explaining how functional transcription in the active VSG ES depends on the selective recruitment of an RNA elongation/processing machinery [46,47].

Moreover, VSG ES inactivation was found to involve components of a transcription termination complex recruited to base J, a specific thymine modification linked to transcription silencing [48]. In addition to these factors, SNF2PH, a SUMOylated plant homeodomain (PH)-transcription factor, promotes bloodstream-specific transcription [49].

Changing the selected VSG ES to another in the ES body is a mechanism triggering antigenic variation, and this can result from DNA damage frequently observed in the VSG ES due to its open chromatin configuration and high transcription rate. Indeed, in the ES body the repeats flanking the VSG gene are transcribed into long-noncoding RNAs that form RNA:DNA hybrids (R-loops), and if not removed by RNase H these R-loops cause double-strand breaks that increase VSG ES switching [50–54]. Importantly, the mechanism linking DNA damage to VSG ES switching was found to involve ATR, a DNA damage-signalling protein kinase [55**]. ATR loss was found to alter the localization of both RNA polymerase I and VEX1 [55**]. Furthermore, a putative translocation DNA polymerase able to bypass telomeric DNA damage, termed 7bPoII, was also found to be involved in the control of antigenic variation [56].

In addition to VSG ES switching, DNA recombination such as gene conversion occurring between the active VSG gene and any other silent VSG gene can trigger antigenic variation [51,57]. Given the involvement of homologous recombination in this process, the order of VSG switching is influenced by the relative level of sequence homology between the donor and target gene [57]. However, two recent observations complicate this view. At least during early infection, the VSG hierarchy appears to be also dependent on the VSG size [58]. Presumably due to the increased metabolic cost of producing longer VSGs, the growth rate of trypanosomes expressing shorter VSGs favours their accumulation during switching to new VSGs. Subsequent elimination of fast-growing trypanosomes then allows slower-growing parasites with longer VSGs to accumulate [58]. Another relevant observation pertains to the mechanism of antigenic variation in the T. brucei-related trypanosome Trypanosoma vivax, a livestock pathogen. In this parasite, recombination plays little role in diversifying VSG sequences [59]. How this parasite manages nevertheless to develop antigenic variation is unclear.

| Table 1 | Mechanisms allowing African trypanosomes to evade host immunity |
|---------|---------------------------------------------------------------|
| **Host defence components** | **Parasite response** |
| **Specific innate immunity** | - Human serum toxin (APOL1) targeted to the parasite through different Trojan horse strategies (VSG and 7bHpHsR) [10**,11–13] |
| | - APOL1 C-terminal G1/G2 variants, which resist SRA of T. rhodesiense [22,24**] |
| | - APOL3 nonsense variant, relieving APOL3-mediated G1/G2 inactivation [26,24**] |
| | - APOL1 neutralization (T. rhodesiense SRA) [8]; |
| | - Replacement of T. rhodesiense by T. gambiense in western Africa [11] |
| **General innate immunity** | - Neutrophil DNA traps |
| | - Germline IgMs |
| | - Inflammatory response (TNF-α, NO, IL-1β, IL-6) |
| | - DNA cleavage (TatD DNases) [28] |
| | - Parasite motility (flagellum) [29] |
| | - High membrane fluidity ensuring fast clearance of IgM-VSG complexes (TbKIFC1) [30**] |
| | - Inhibition of early TNF-α synthesis (adenylate cyclases) [32] |
| | - NO synthase inhibition (TbKHC1) [34] |
| | - IL-1β/IL-6 inhibition (indole pyruvate) [35,36] |
| | - High membrane fluidity ensuring fast clearance of IgG-VSG complexes (TbKIFC1) [30**] |
| | - VSG antigenic variation (VEX1/VEX2: expression site selection; ATR: expression site switching) [44**,45**,55**] |
| | - Receptor embedding within the VSG coat [3,61] |
| | - Receptor saturation shielding the ligand site |
| | - N-glycan coating |
| | - Antigenic variation of surface-exposed regions [83] |

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The parasite strategy against adaptive immunity: variation and shielding of surface receptors

The best characterized receptors for growth factors in bloodstream forms of *T. brucei* are those for transferrin (*TfTIR*) and Hp-Hb (*TfHpHbR*), respectively responsible for iron and heme uptake [3,12,60,61]. Recently, a receptor for mammalian factor H (*TfFHR*), only expressed in tsetse-transmissible and fly gut forms, has been characterised [62]. All of these receptors possess a three-helical fold that allows for an elongate structure compatible with the densely packed VSG coat. In all cases, the binding site is present at the membrane distal end, ensuring that it is accessible to macromolecular ligands. Either N-glycosylation (*TfTIR*) or a structural kink (*TfHpHbR*) is thought to prevent molecular crowding of the receptor by VSGs, and facilitates ligand access to the binding site. Given the necessary exposure at the cell surface, these receptors must have evolved mechanisms to escape antibody detection. Given the reported receptor affinities and concentrations of circulating ligand, receptor saturation may render the exposed binding site inaccessible to antibody. This is certainly the case for *TfTIR* and *TfFHR* [60,61] and possibly for *TfHpHbR* [3,11,13]. Since the *TfTIR* is encoded by a collection of different VSG-like genes present in the various VSG ESs, antigenic variation of this receptor occurs upon VSG ES switching [47,60,63]. However, recent structural studies suggest that immune avoidance is the driving force for *TfTIR* diversification. In accordance with early studies demonstrating the influence of polymorphic surface-exposed sites on ligand affinity [63], it was concluded that variable regions are the most exposed to immune detection, and that N-linked glycans serve to shield a large part of the membrane-distal region not involved in ligand binding [61].

Conclusions

Not surprisingly, African trypanosomes have developed multiple mechanisms to counteract the host defences (Table 1). At the beginning of infection, a combination of swimming motility and membrane fluidity is necessary for the parasite to avoid immediate elimination due to the early recruitment of neutrophils and induction of germine IgMs. The response to innate immunity involves the release of various anti-inflammatory parasite components, possibly through the shedding of extracellular vesicles. Many pathogens, for example, *Anaplasma, Borrelia, Neisseria, Mycoplasma* and *Plasmodium*, employ antigenic variation as an evasion strategy, but in African trypanosomes this system has evolved to an extreme level [64], probably because only extensive antigenic variation allows long-lasting chronic infection, which is vital for successful transmission to the insect vector. Trypanosome antigenic variation necessitates the activity of many factors ensuring both monallelic VSG expression and continuous changes of selectively activated VSG gene. Despite the complexity of this system, major progresses have been performed during the period of this review, with the identification of several key actors and the precise identification of their mode of action. Structural studies have also revealed answers to the longstanding issue of how parasite receptors for macromolecules can interact with their ligand without being vulnerable to antibody attack. Finally, in the case of human infection, the particular story of the trypanolytic factor APOL1 has revealed how parasites and host can entertain a continuous arms race with price to pay on both sides. Clearly, studying the dialogue between trypanosomes and humans largely extends beyond the fields of parasitology and immunology.

Conflict of interest statement

Nothing declared.

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