Escape mechanisms of African trypanosomes: why trypanosomosis is keeping us awake

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SUMMARY

African trypanosomes have been around for more than 100 million years, and have adapted to survival in a very wide host range. While various indigenous African mammalian host species display a tolerant phenotype towards this parasitic infection, and hence serve as perpetual reservoirs, many commercially important livestock species are highly disease susceptible. When considering humans, they too display a highly sensitive disease progression phenotype for infections with *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense*, while being intrinsically resistant to infections with other trypanosome species. As extracellular trypanosomes proliferate and live freely in the bloodstream and lymphatics, they are constantly exposed to the immune system. Due to co-evolution, this environment however no longer poses a hostile threat, but has become the niche environment where trypanosomes thrive and obligatory await transmission through the bites of tsetse flies or other haematophagic vectors, ideally without causing severe side infection-associated pathology to their host. Hence, African trypanosomes have acquired various mechanisms to manipulate and control the host immune response, evading effective elimination. Despite the extensive research into trypanosomosis over the past 40 years, many aspects of the anti-parasite immune response remain to be solved and no vaccine is currently available. Here we review the recent work on the different escape mechanisms employed by African Trypanosomes to ensure infection chronicity and transmission potential.

Key words: African trypanosomes, escape mechanisms, immune modulation, inflammation, antigenic variation.

INTRODUCTION

Trypanosomosis is a parasitic disease caused by African trypanosomes. These unicellular protozoan parasites are mainly transmitted through the bite of a tsetse fly (*Glossina species*) and form a threat to human and animal health on the African continent.

Trypanosoma brucei (*T. b.*) is the only trypanosome species able to infect humans, and can be further subdivided into three subspecies according to host infectivity, pathogenicity and geographical occurrence. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are the causative agents of Human African Trypanosomosis (HAT) or *Sleeping Sickness*, while *T. b. brucei* is only infective for livestock and game, (Jackson *et al*. 2010). Other animal infective species are *Trypanosoma congolense* and *Trypanosoma vivax*, causing a wasting disease called Nagana, and *Trypanosoma evansi, Trypanosoma equiperdum* and *Trypanosoma suis* affecting various species of economically important livestock. Together, these infections cause massive economic damage to the sub-Saharan African continent, impacting on milk and meat production as well as agriculture labour potential under the form of tracking and transport power. Important to mention is that trypanosomes such as *T. vivax, T. evansi* and *T. equiperdum* are also classified as non-tsetse transmitted trypanosomosis, and now occur beyond the borders of the African continent, i.e. in South America, Asia and even occasionally in Europe (Silva *et al*. 1995; Reid and Copeman, 2000; Oliveira *et al*. 2009; Da Silva et al. *2011*; Desquesnes *et al*. 2013).

Tsetse fly transmitted infection begins with the injection of non-dividing metacyclic trypomastigotes into the host bloodstream during the blood meal of a tsetse fly (Fig. 1). In the mammalian blood, the metacyclic trypomastigotes resume cell division and differentiate into long slender bloodstream trypomastigotes, which multiply by longitudinal binary fission. Eventually, the long slender forms differentiate into short-living short stumpy forms by a mechanism involving cell density and the release of the stumpy induction factor (SIF). Differentiation to this short stumpy form limits parasite growth in the mammalian host and causes the parasitaemia levels to plateau (Vassella *et al*. 1997; Reuner *et al*. 1997; Tyler *et al*. 2001; Rico *et al*. 2013; Szöőr *et al*. 2013; Mony *et al*. 2014). The short stumpy trypomastigotes are then ingested

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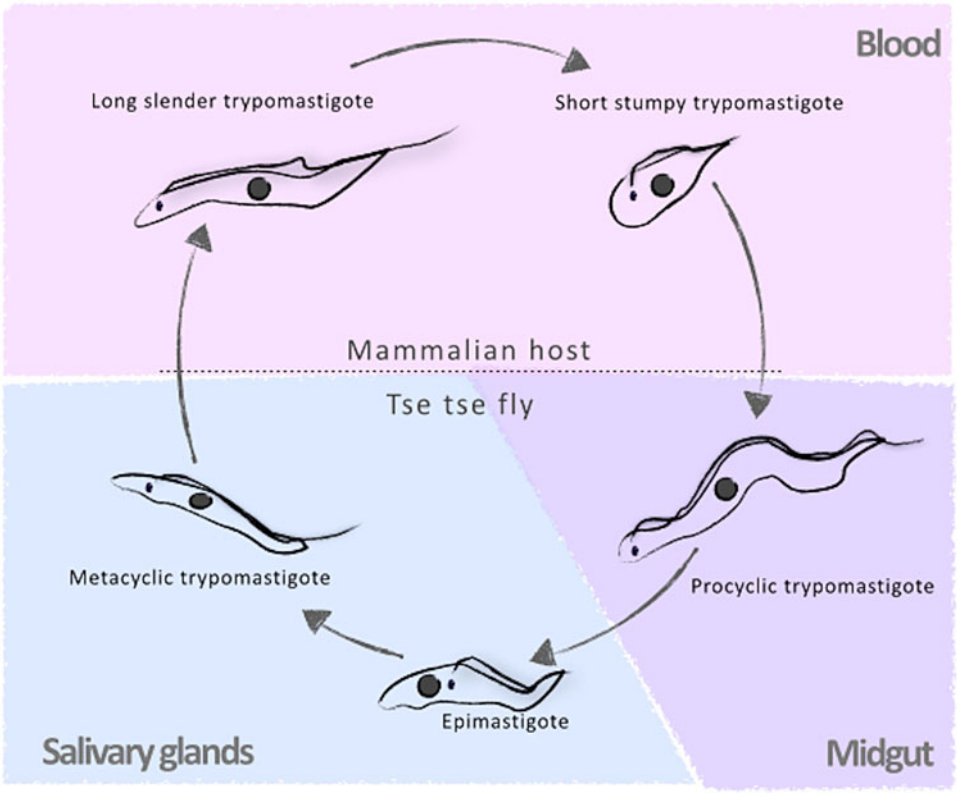

Fig. 1. *Trypanosoma brucei* lifecycle: The life cycle of the extracellular protozoan parasite *T. brucei* begins when trypanosomes are injected into the blood of a mammal by a tsetse fly. When injected into the mammalian host, metacyclic parasite's first transform in a long slender trypomastigote form that multiplies by binary fission. Next, it differentiates into a non-proliferating short stumpy trypomastigote form. When ingested by the insect vector, short-stumpies differentiate into the procyclic form, and colonize the midgut of the tsetse fly. After migration to the salivary glands, they assume epimastigote forms. Finally they differentiate into the metacyclic trypomastigote form, which is able to infect mammals.

when the tsetse fly takes a blood meal. In the fly, the parasites differentiate into procyclic forms that colonize the midgut, where they further multiply and differentiate into mesocyclic trypomastigotes that migrate to the salivary glands of the tsetse fly. In the salivary glands the parasite transforms into a proliferating epimastigote form. Finally these multiply and differentiate into non-proliferating metacyclic trypomastigotes that acquire a variant surface glycoprotein (VSG) coat and detach from the epithelium to be injected into the mammalian host (Matthews *et al*. 2004; Roditi and Lehane, 2008; Field and Carrington, 2009; Lacomble *et al*. 2010; Langousis and Hill, 2014).

HAT is a disease that occurs in distinct geographic locations in Africa. Occurring mostly in West- and Central Africa, *T. b. gambiense* is responsible for 98% of all human infections (Fig. 2). It causes a chronic disease, which progresses gradually and only has fatal outcome after several years of infection. In contrast *T. b. rhodesiense* infection occurs primarily in East Africa and causes an extremely virulent disease, resulting in death within weeks or months after infection. In addition to the human reservoir, both *T. b. gambiense* and *T. b. rhodesiense* have an animal reservoir, which hampers control and eradication of the disease (Njiokou *et al*. 2010; Simarro *et al*. 2011; WHO, 2012). Over the last two decades, a huge effort has been made to bring down the number of actual *Sleeping Sickness* cases, without however bringing new therapies to the field. Today, renewed and sustained control programmes have resulted in a dramatic drop in actual case reports, reducing the number of infections to less than 10 000 reported patients in 2010. Nevertheless the WHO estimates that 57 million people are still at risk of contracting *T. b. gambiense* HAT (WHO, 2012; Simarro *et al*. 2012). Most important for future control strategies is the fact that no vaccination strategies against trypanosomosis exist, due to specific and non-specific parasitic defence mechanisms summarized below. Hence to date, and in the foreseeable future, HAT control must mainly rely on the intense combination vector control, diagnosis and treatment.

Sleeping Sickness encompasses two disease stages: during the early haemolymphatic stage the parasites proliferate in blood and lymphatic system, while in the late meningoencephalitic stage, the parasites penetrate the blood-brain barrier and invade the central nervous system (Sternberg, 2004; Blum *et al*. 2006; MacLean *et al*. 2010). While accurate

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Fig. 2. Distribution of HAT on the African continent: *T. b. gambiense*, depicted in blue, occurs primarily in West- and Central Africa and is responsible for 98% of current HAT infections. *Trypanosoma b. rhodesiense*, depicted in pink, occurs primarily in East Africa. Based on data from 'Report of a WHO meeting on elimination of African trypanosomiasis' 2012.

trypanosomosis diagnosis is hard by itself, due to generally low parasite numbers, the correct stage determination of the disease poses further difficulties, and is based mainly on CSF determination of lymphocyte counts (Ngoyi *et al*. 2013). Hence, there is an urgent need for new disease staging methods that are more specific, easy-to-use and reliable under field conditions, as administration of late stage drugs to early stage patients can lead to drug toxicity complications. For example, the current drug used for late stage treatment during *T. b. rhodesiense* HAT Melarsoprol, has a high treatment failure and high patient lethality and should never be administered to first-stage HAT patents (Blum, 2001).

IMMUNE EVASION MECHANISMS DEVELOPED BY BOTH THE MAMMALIAN HOST AND THE TRYPANOSOMES

As indicated above, trypanosomes are obligatory extracellular parasites that dwell in the blood and lymphatics of their mammalian host. While in general the host will be unable to eliminate the infections, various host–parasite interactions do occur that have evolved in such a way that parasite survival is ensured, and that prolonged host survival allows successful population transmission. Hence, both the mammalian host, as well as the trypanosomes have selectively acquired a number of defence strategies that allow optimizing the race for survival and transmission. These defence mechanisms include toxic serum factors, antibodies and cytokines from the host's side, and antigenic variation, immune modulation and immune destruction form the parasite's side.

Innate human trypanolytic factors and parasite defence mechanisms

While HAT is to be considered a very serious human infection, it has to be stressed that humans are safe from infection by most trypanosomes, due to the presence of trypanolytic serum factors that are capable of killing most trypanosomes with exception of HAT-causing species *T. b. gambiense* and *T. b. rhodesiense*. These factors can be considered as part of the innate immunity, as they are not specifically induced during infection but are an intrinsic component of normal human serum (NHS), although their actual mode of action is to quickly lyse trypanosome upon entry in a non-immune way (Alsford *et al*. 2014). The factors responsible for this innate resistance are called 'trypanolytic factors' TLF1 and TLF2 (Rifkin, 1978). The activity of both factors is considered to be mediated by the presence of apolipoprotein apoL1. This molecule is part of a larger apolipoprotein family, and also provides trypanosome resistance to gorillas and baboons. Albeit chimpanzees seem to have lost the apoL1 encoding gene, rendering them highly susceptible to trypanosomosis in general (The Chimpanzee Sequencing and Analysis Consortium, 2005; Thomson *et al*. 2014). Interestingly, compared to human apoL1, the old world monkey homologue was shown to be more potent, rendering baboons even resistant to the human pathogenic *T. b. rhodesiense* parasite (Lugli *et al*. 2004).

TLF-1 forms complexes with high-density lipoprotein (HDL), apolipoprotein L1 (apoL1), Haptoglobin-related protein (Hpr), apolipoprotein A1 (apoA1) and haptoglobin (Hp) while TLF-2 forms lipid-poor complexes with polyclonal IgM antibodies, apoL1, apoAi1 and Hpr (Rifkin, 1978; Tomlinson and Raper, 1996; Raper *et al*. 1999, 2001; Vanhamme *et al*. 2003; Vanhollebeke and Pays, 2010*a*; Pays *et al*. 2014). It is generally accepted that TLF-2 is the main lytic factor in NHS (Raper *et al*. 1996), although it is still not clear how TLF2 is recognized/bound by the parasite. Even a recent screen using an RNAi approach did not yield an answer as to how trypanosomes take-up TLF2 (Lecordier *et al*. 2014). The presence of a low affinity receptor (Drain *et al*. 2001) as well as a potential scavenger receptor (Green *et al*. 2003) have been reported, however without providing adequate information that would allow the exact understanding of TLF2 functioning. In addition, preliminary data from our own group have indicated that a lectin-like interaction involving the complex

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TLF2/IgM carbohydrate side-chains might be involved (Magez *et al*. unpublished data). However, taken the complexity and instability of TLF2, the exact mode of TLF2 uptake remains to be elucidated, posing a challenge in the full understanding of the NHS anti-trypanosome activity. In contrast to the TLF2 situation, the uptake of TLF1 is much better understood. Here, the haptoglobin-related protein Hpr facilitates uptake of TLF1 *via* the trypanosome haptoglobin–haemoglobin receptor (HpHbR) responsible for parasite haeme supply (Vanhollebeke *et al*. 2008). This receptor is however unable to discriminate between haptoglobin and haemoglobin (Hp–Hb) complexes and TLF1–Hpr–Hb (HDL) complexes. This results in the fact that in serum of 'healthy' individuals, TLF1 uptake might be virtually absent, as Hb concentrations usually exceed those of Hpr by a factor 100 (Vanhollebeke and Pays, 2010*b*). When endocytosed (trough TLF2 and/or TLF1), apoL1 exhibits pore-forming activities in the lysosome, leading to osmotic imbalance and a disruption of the lysosomal membrane. This in turn causes uncontrolled swelling and parasite death (Hager *et al*. 1994; Pays *et al*. 2006). Despite the vast body of literature available to date on the biological activity of TLF1, a critical note needs reminding: detailed analysis of genetically modified parasites that lack the receptor HpHbR shows that they are still fully susceptible to TLF2 lysis, and alteration of their TLF1 mediated lysis pattern is only observed in *in vitro* culture. In addition, these mutants are fully sensitive to NHS lysis in physiological concentrations and an altered phenotype was observed only in conditions in which extreme low serum conditions are used, i.e. less than 1% NHS (Vanhollebeke *et al*. 2008). These findings confirm that under normal physiological conditions, the role of TLF1 and the HpHb receptor might be minimal.

As mentioned, *T. b. rhodesiense* and *T. b. gambiense* are able to resist lysis by NHS. Hence in contrast to other trypanosomes, these parasites must have acquired resistance mechanisms that provide a defence against both TLF1 and TLF2, or against the common active compound, i.e. apoL1. Resistance of *T. b. rhodesiense* is not constitutive, but is induced upon the activation of transcription of a gene encoding resistance, termed serum resistance associated or *sra* (De Greef *et al*. 1989; Van Xong *et al*. 1998). SRA resembles a truncated version of VSG, which is located in the endocytic pathway (Shiflett *et al*. 2007). Resistance to TLF-1 is conferred by SRA interaction with apoL1 in the lysosome (Vanhamme *et al*. 2003). As *T. b. rhodesiense* is resistant to NHS lysis containing both TLF1 and TLF2, it is presumed that SRA has a similar manner of inhibiting apoL1 entering the parasite through TLF-2 uptake. One critical note that needs to be made here is the fact that recently more evidence is emerging that also human infective *T. b. rhodesiense* parasites exist that do not have the SRA (Enyaru *et al*. 2006). Hence, as usual in the biology of host–parasite interaction, the full picture of NHS resistance might be more complicated than initially proposed.

As compared to *T. b. rhodesiense*, the situation in *T. b. gambiense* is even more complex. Important to mention here is that two different types of *T. b. gambiense* parasites have been characterized. Tbg Type 1 is the most common of the two and is characterize by a constitutive resistance to NHS. Tbg Type 2 on the other hand is characterized by an inducible level of NHS resistance, much like what is observed in *T. b. rhodesiense*. As both *T. b. gambiense* types lack SRA, and so far no common mechanisms for the resistance phenotype has been described, it appears that throughout evolution trypanosomes have acquired multiple times independent mechanisms to resist the aopL1 activity of NHS (Capewell *et al*. 2011). With respect to Type 1 Tbg it was recently shown that a truncated VSG-like *T. b. gambiense*-specific glycoprotein (TgsGP), located in the endocytic compartment is crucial for apoL1 resistance, as depletion of the TgsGP gene rendered *T. b. gambiense* susceptible to TLF1, apoL1 and NHS lysis (Capewell *et al*. 2013; Uzureau *et al*. 2013). In contrast to SRA, this mechanism does not involve direct apoL1 neutralization despite the fact that TgsGP and apoL1 co-localized, but rather has a function is altering membrane fluidity in the endocytic compartments (Uzureau *et al*. 2013). Interestingly, TgsGP is absent from Tbg Type 2, indicating that *T. b. gambiense* needs to rely on multiple independent mechanisms to ensure NHS resistance (Radwanska *et al*. 2002; Gibson *et al*. 2010). A second resistance feature is a reduction in apoL1 sensitivity through cysteine protease activity and lower early endosomal pH (Uzureau *et al*. 2013). Finally, a third resistance mechanism proposed to be involved here is the reduced uptake of TLF1, due to a single amino acid substitution in the HpHb receptor, which ablates binding and subsequent endocytosis (DeJesus *et al*. 2013; Higgins *et al*. 2013; Uzureau *et al*. 2013). Indeed, Tbg Type 1 intrinsic NHS resistance coincides with the reduced capacity of TLF1 uptake and while this might not be relevant in 'normal' NHS conditions (see above), it has been speculated that in co-infection condition were malaria-induced hypohaptoglobinaemia occurs, reduced TLF1 uptake could help trypanosomes to overcome NHS lysis due to reduced intracellular TLF1 accumulation (Pays *et al*. 2014). Recent evidence comparing NHS resistant and NHS sensitive *T. b. gambiense* Type 2 strains has however show that here the phenotype is independent of TLF1–HpHb binding and uptake capacity. In addition, it was

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shown that Tbg Type 2 NHS resistance is independent of the expression site used, differentiating this activity mechanistically from the BES-associated SRA activity observed in *T. b. rhodesiense* NHS resistance (Capewell *et al*. 2011). Taken these most recent data, one could pose the critical question is to whether the reduced uptake of TLF1 by Tbg Type 1 measured *in vivo* really correlates with NHS resistance, or merely coincides, while having little or no biological relevant *per se* in an *in vivo* setting.

Additional innate host–parasite interactions Upon successful infection of a mammalian host by trypanosomes, a range of innate immune responses will be initiated that serve to hamper parasite growth. These mechanisms are in large connected to macrophage activation, inflammatory cytokine secretion and iNOS activation. These mechanisms have been reviewed recently in detail by Beschin *et al*. 2014. With respect to the specific innate immune aspect of trypanosome–host interactions, recent discoveries of the biological significance of the *ESAG4* gene family need to highlighted here. *ESAG4*, belongs to a large gene subfamily encoding approximately 80 members of *T. brucei* adenylate cyclases, of which most are expressed constitutively (Alexandre *et al*. 1990, 1996). These transmembrane receptor-like enzymes are activated under stress and among other, are implicated in cytokinesis (Salmon *et al*. 2012*a*). Recently it was shown that adenylate cyclases inhibit the early host innate immune response by inhibiting TNF production of liver-associated myeloid cells. By generating a double negative mutant for *ESAG4*, adenylate cyclase activity was reduced by 50%, which resulted in reduced parasite growth and significantly longer host survival time (Salmon *et al*. 2012*b*). To underline the crucial importance of TNF in this event, experiments were repeated in TNF deficient mice, showing an abrogation of the phenotype. The diversification and abundance of adenylate cyclases in trypanosomes could be indicative for the fact that this trait to aid in growth and manipulate the immune system is essential. As adenylate cyclase synthesis is directly implicated in the inhibition of the early innate immune response, one might consider their expression as an escape mechanism. Indeed, due to the inhibition of TNF, a higher peak parasitaemia can favour transmission to the insect vector at this stage. However, transmission to the insect vector is also ensured by prolonged survival. In this aspect adenylate cyclase production must be tightly regulated in a natural host–parasite interaction setting, as accelerated parasite growth could in turn result in early host death, limiting subsequent parasite transmission potential.

Antigenic variation: a trypanosomes defence against the host adaptive immunity

The surface of the long slender bloodstream form is densely packed with 10^7 copies of a single VSG attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor. These VSGs are highly immunogenic and enable the host to mount an effective humoral anti-VSG response, putting the parasite under continuous immune pressure. In order to deal with this pressure, trypanosomes have evolved a system called antigenic variation. This system comprises a frequent switching of the entire VSG coat, allowing the continuous evasion of new antibody attacks. Simplified, during the first ascending phase of a parasitaemia wave the majority of the parasites express the same VSG and are consequently of the major variable antigenic type (VAT) (Fig. 3). Approximately 0×1–1% of trypanosome divisions then produces a new VAT, thus expressing a different VSG (Robinson *et al*. 1999; Hall *et al*. 2013). These new 'antigenically distinct' trypanosomes multiply and overgrow the first VAT, giving rise to a subsequent parasitaemia wave. This process is repeated multiple times and results in the development of a chronic infection (Pays *et al*. 2001; Baral, 2010; Schwede and Carrington, 2010; Hall *et al*. 2013). Recently, studies by Hall *et al*. involving *T. brucei* VSG cDNA sequencing have shown that an African trypanosome infection comprises much more diverse parasite populations than originally described. Indeed, obtained results showed that an individual growth peak in mice can contain at least 15 distinct variants (Hall *et al*. 2013). It is estimated that this number is even greater in natural hosts.

Transcription of VSG occurs from specialized subtelomeric transcription units known as the bloodstream expression sites (BES). BESs are large poly-cistronic transcription units, which additionally harbour genes termed expression site-associated genes (ESAGs) (Kooter *et al*. 1987; Pays *et al*. 2001; Vanhamme *et al*. 2001*a*; Borst, 2002; Berriman *et al*. 2002), including the transmembrane proteins adenylyl cyclase (*ESAG4*) mentioned above (Pays *et al*. 2001) and the heterodimeric surface receptor for host transferrin (*ESAG7/6*). The trypanosome has an estimated 10–40 BESs harbouring VSGs. In addition the parasite can access ~1000 silent (pseudo) VSG genes scattered in the genome (Vanhamme *et al*. 2001*b*). These silent genes and pseudogenes can appear into the BES by gene conversion mechanisms. VSG expression is monoallelic, hence the appearance of a uniform VSG coat on the parasite surface. Recombination events responsible for a switch in VSG include telomere exchange, duplicative gene conversion and segmental or partial gene conversion (Vanhamme *et al*. 2001*b*; Morrison *et al*. 2009; Horn and McCulloch,

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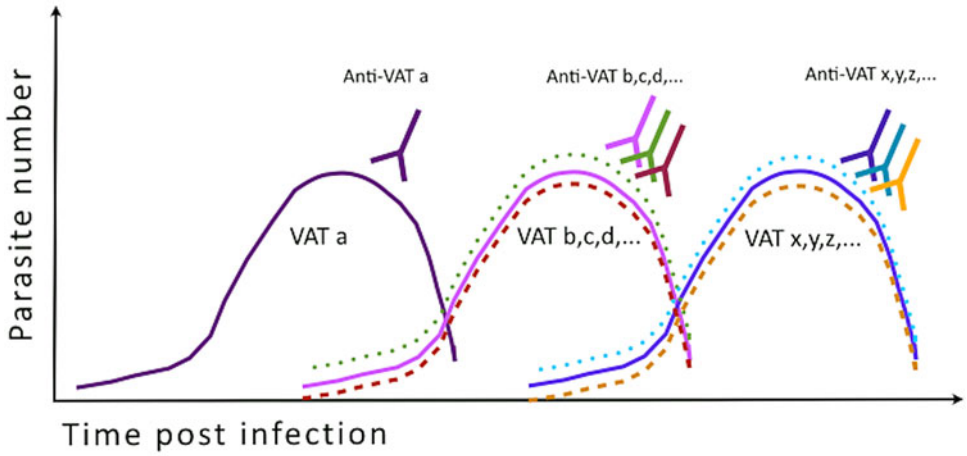

Fig. 3. Simulating the revised concept of antigenic variation during mammalian *T. brucei* infection. In contrast to the previously published models of parasitaemia waves consisting of a single VAT it is now believed that parasitaemia waves comprise much more diverse parasite populations, as each individual growth peak in mice can contain at least 15 distinct variants.

2010; Rudenko, 2011). These three mechanisms involve the exchange of genetic material between an active and a silent BES, while remaining under the original active BES promoter and remaining in the transcription body, the unique transcription apparatus where properly processed mRNA for VSG production is generated (Navarro and Gull, 2001). This mechanism of VSG switching is believed to be a crucial adaptation to long-term survival in a given host, as all the ESAG gene products remain the same, while only the VSG within the given BES is altered. Using this gene rearrangement, also antigenically distinct mosaic VSGs can be fabricated from silent VSG genes and pseudogenes by means of partial gene conversion, a process termed mosaicism. VSG expression follows a loose hierarchy and mosaicism occurs increasingly as the infection proceeds, contributing immensely to antigenic variation and infection chronicity (Hall *et al.* 2013). Hence, the potential for trypanosome antigenic variation is enormous, and constitutes the major immune escape mechanism.

Besides VSG switching to gene rearrangement, a switch in active VSG BES transcription can also result in a new VSG variant expression. However, in this case all the ESAGs located on the BES are altered as well, meaning that the expression of for example the transferrin receptor will be altered to a new homologue as well. The latter BES switch mechanism has most likely evolved as an adaptation that allows the infection of a wide host range of mammals (Pays *et al.* 2001; Morrison *et al.* 2009). It involves multiple mechanisms including transcription silencing chromatin remodelling and regulation of pre-mRNA elongation, although the exact mode of action still remains to be elucidated (Hughes *et al.* 2007; Figueiredo *et al.* 2008; Li *et al.* 2009; Landeira *et al.* 2009).

Additional VSG-mediated defence mechanisms

Besides antigenic variation, it seems that trypanosomes might have evolved a number of addition mechanisms that provide a certain level of protection against antibody-mediated attack. First, while complement-mediated lysis is a well-established *in vitro* method to determine the VSG-specificity of a given antibody response, it is not clear whether this system effectively operates *in vivo*. Indeed, mice that lack the C5 component of the complement cascade exhibit parasitaemia control patters that are similar to fully immune competent mice. Taken the thickness of the VSG coat (approx. 200 Å) and the vast N-linked and GPI-associated carbohydrate barrier, one could argue that the final complement complex would be unable to efficiently target the lipid membrane of the parasite. Secondly, trypanosomes have developed an endocytosis mechanism that allows antibody clearance from the VSGs. This means that VSG-antibody complexes are endocytosed in the flagellar pocket and antibodies are degraded in the lysosome after which the VSG is recycled back to the surface coat (Barry, 1979; McLintock *et al.* 1993; Engstler *et al.* 2007). This mechanism would provide a way to escape antibody-mediated immune attack at low to moderate antibody concentrations and could therefore pose as an escape mechanism promoting the survival of individual cells, possibly supporting their transmission to the insect vector (Engstler *et al.* 2007).

Immune modulation: undermining the long-term immunity of the host

As if all the above described escape mechanisms were not sufficient, trypanosomes have invested in yet another way to ensure infection chronicity

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and hence successful parasite transmission. Trypanosomes modulate the host immune system in various ways so that the capacity of the host to mount an efficient immune response is undermined.

Infection-induced immune suppression is long considered as a hallmark of Trypanosomosis. Early studies on African trypanosomes show that the parasite overwhelms the host immune system with a massive antigenic load. This was shown to be associated with immune depression and polyclonal lymphocyte activation, and occurs during rodent, livestock and human infections (Ormerod, 1970; Goodwin *et al*. 1972; Mansfield and Wallace, 1974; Diffley, 1983; Oka *et al*. 1988). The polyclonal lymphocyte activation depletes antigen-reactive lymphocyte populations and can exhaust and supress B and T cells in the induction of antigen specific immunity against subsequent trypanosome variants or even unrelated antigens.

In both mice and cattle, B cells seem to play an important role in host protection, despite their limitation in VSG specificity (Corsini *et al*. 1977; Campbell *et al*. 1977; de Gee *et al*. 1983; Guirnalda *et al*. 2007; Magez *et al*. 2008). Although additional host factors contribute to parasite control (see further), B cells seem to be essential for post-peak parasite removal and prolonged survival (Magez *et al*. 2008). Mouse models of human and animal trypanosomosis show that multiple Trypanosome species cause a sustained loss in splenic and bone marrow B cell populations (Baltz *et al*. 1981; Radwanska *et al*. 2008; Bockstal *et al*. 2011*a*; Obishakin *et al*. 2014; La Greca *et al*. 2014). In the spleen, microarchitecture is disrupted and different B cell subsets are undergoing apoptosis (Radwanska *et al*. 2008; Bockstal *et al*. 2011*b*). In addition, B cell lymphopoiesis in the bone marrow is affected, preventing replenishment of the splenic mature B cell pool (Bockstal *et al*. 2011*b*). The trypanosome hereby prevents the induction of a protective memory response, an additional insurance for infection chronicity. Vaccination experiments against unrelated pathogens have also shown that trypanosomes destroy previously induced vaccine-induced memory. Indeed, vaccine efficacy was abolished after the host was infected with *T. brucei* (Onah and Wakelin, 2000; Radwanska *et al*. 2008). If these findings regarding B cell destruction would also hold true for field Trypanosomosis, this would complicate not only anti-Trypanosomosis vaccination, but generally any vaccination programme in Sub-Saharan Africa, implying the need of re-vaccinating HAT patients after treatment.

In light of these results, Lejon *et al*. (2014) conducted a field trial in Democratic Republic of Congo on *T. b. gambiense* infected individuals. In this study, HAT patients had higher percentages of peripheral memory T and B cells than healthy controls. In addition they investigated the immunological memory by measuring anti-measles antibodies of vaccinated subjects before and after anti-trypanosomosis treatment. Anti-measles antibodies were significantly lower in HAT patients compared to controls, and although they remained lower after treatment, the levels were above the cut off value assumed by the manufacturer to provide protection. As the authors state themselves, antibody quantification is a sub-optimal tool for the investigation of immunological memory, as they do not reflect the presence of antibody-secreting memory B cells and could be elevated in spite of immunological suppression (Onah and Wakelin, 2000). In addition, polyclonal B cell activation can replace the measles-specific antibodies by low-affinity cross-reactive antibodies and hence a functional characterization is necessary to determine if the antibodies maintain their protective capacity. Despite the previously mentioned shortcomings of this study, these results could indicate that destruction of the B cell memory compartment might not be as big an issue in humans as it is in mice, at least in the case of *T. b. gambiense* infection. This would be encouraging news for vaccination campaigns throughout HAT-endemic regions in Africa. Further investigation into a functional antibody assay should confirm these results. In addition, this phenomenon needs to be investigated in the more virulent *T. b. rhodesiense* infections, as previous results indicated a correlation between immune depression and parasite load (Obishakin *et al*. 2014).

Murine models have also shown that aside from B cells, VSG-specific Th1 cells and IFNγ regulate another major component of host resistance to African trypanosomes (Hertz *et al*. 1998; Paulnock *et al*. 2010). The parasites first activate macrophages and dendritic cells through the production of pathogen-associated molecular patterns (PAMPS) which comprise the GPI anchors of shed VSG and CpG DNA (Mansfield and Paulnock, 2005). Particularly splenic dendritic cells seem to be an important cell subset for the induction of VSG-specific T cell responses (Dagenais *et al*. 2009). Th1 cells are an important source of IFNγ, which is necessary to activate macrophages for the production of trypanolytic factors (Magez *et al*. 1999; Drennan *et al*. 2005). In addition the trypanosomes induce macrophages to produce suppressor effector molecules like prostaglandins, which inhibit the VSG-specific T cells to proliferate (Schleifer and Mansfield, 1993) and constitute another way for the parasite to ensure its survival.

CONCLUSION AND DISCUSSION

African trypanosomes have evolved multiple mechanisms to ensure their survival in the host and consequently establish a chronic infection (Fig. 4). These immune evasion mechanisms have prevented the

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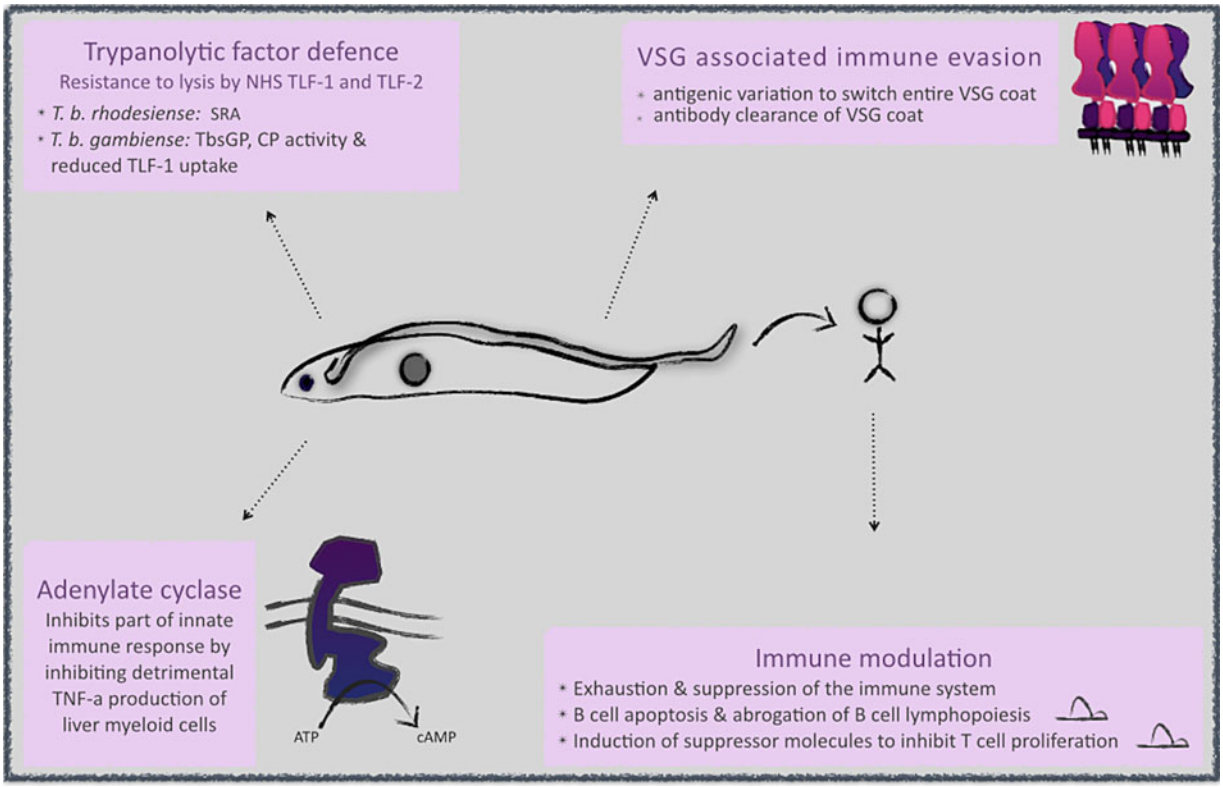

Fig. 4. Schematic overview of the escape mechanisms used by African trypanosomes. (I) VSG associated immune evasion strategy (antigenic variation) encompasses the most important escape mechanism used by trypanosomes. In addition, antibody clearance by the VSG coat can aid escape on the single cell level. (II) In contrast to other trypanosome species, human-infective trypanosomes can resist lysis by human serum factors TLF-1 and TLF-2 via different strategies. (III) Adenylate cyclase, produced by the trypanosome can inhibit a part of the innate immune response. (IV) Trypanosomes modulate their hosts immune response by exhausting and supressing the host immune system.

design of a prophylactic vaccine so far. As the main reservoir for *T. b. rhodesiense* are African domestic animals like cattle and African wildlife, the full eradication of the parasite from this reservoir is impossible. Therefore the only way to protect the human population against re-infection is through prophylactic vaccination. Over the last decades different vaccination strategies have been designed, but not a single one obtained 100% sterile immunity or made its way to a field trial (La Greca and Magez, 2011). Due to the parasite's antigenic variation system, vaccination against VSG is impossible. Vaccination protocols involving invariant antigens such as invariant surface glycoproteins like the transferrin receptor ESAG6/7 (Lança *et al*. 2011), the flagellar pocket proteins (Mkunza *et al*. 1995; Radwanska *et al*. 2000) only rendered animals partially protected against low parasite dose challenge. In addition to the antigenic variation system, immunosuppression, and in particular depletion of immune memory, could be another way that parasites ensure infection chronicity. Trypanosomes could have invested in these additional evasion strategies to block any previous antibody reaction against their newly synthesized (mosaic) VSGs, are antigenically distinct (Hall *et al*. 2013).

In animal Trypanosomosis it would be useful to protect the host from disease-associated complications, as many animals can harbour infection without developing severe symptoms, suggesting the deadly outcome in human infections to be a consequence of the host reaction. An alternative to sterile immunity is therefore anti-disease vaccination, to target the infection-associated pathology. This strategy has given some positive results in an experimental setting against trypanosome cysteine proteases (Authie *et al*. 2001), but has so far not resulted in a field application.

Given that to date, experimental vaccine attempt have not resulted in any promising results, it must be mentioned that the use of murine model for Trypanosomosis in initial experimental settings might not represent the ideal host–parasite context for research regarding vaccination against trypanosomes. However, this model has given us valuable insights into host parasite interactions and biology of antigenic variation. Hence, future efforts are needed to validate the use of these mouse models in trypanosome vaccine research, and, alternative models that better reflect the parasite–host interaction will need to be evaluated as well.

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