

Vaccination against trypanosomiasis

Can it be done or is the trypanosome truly the ultimate immune destroyer and escape artist?

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Abbreviations: HAT, human African trypanosomiasis; TLF, trypanolytic factor; QTL, quantitative trait loci; TNF α , tumor necrosis factor α ; Tir, trypanosome infection response; VSG, variant surface glycoprotein; FP, flagellar pocket; ISG, invariant surface glycoprotein; GPI, glycosylphosphatidylinositol; DMG, dimyristoylglycerol; GIP, glycosyl-inositol-phosphate; CP, cysteine protease

To date, human African trypanosomiasis (HAT) still threatens millions of people throughout sub-Saharan Africa, and new approaches to disease prevention and treatment remain a priority. It is commonly accepted that HAT is fatal unless treatment is provided. However, despite the well-described general symptoms of disease progression during distinct stages of the infection, leading to encephalitic complications, coma and death, a substantial body of evidence has been reported suggesting that natural acquired immunity could occur. Hence, if under favorable conditions natural infections can lead to correct immune activation and immune protection against HAT, the development of an effective anti-HAT vaccine should remain a central goal in the fight against this disease.

In this review, we will (1) discuss the vaccine candidates that have been proposed over the past years, (2) highlight the main obstacles that an efficient anti-trypanosomiasis vaccine needs to overcome and (3) critically reflect on the validity of the widely used murine model for HAT.

Introduction

African trypanosomiasis, a parasitic infection caused by flagellated extracellular parasites that survive in the tissue fluids and the bloodstream, encompasses a number of diseases affecting both humans and animals.

Most trypanosome species are unable to infect man and hence are in the first place a burden for the economic development of endemic areas. This feature of the disease is due to the presence of two trypanolytic factors in human serum (TLF1 and TLF2) that provide a level of innate resistance, preventing human infections.^{1,2} *Trypanosoma congolense* and *Trypanosoma vivax* and to a lesser extent *Trypanosoma brucei brucei* and *Trypanosoma evansi* are responsible for the animal disease Nagana, (or depressed spirits in the Zulu language) which causes estimated losses of over US

\$1,300 million per year in resource-poor settings.³ All these parasites have a wide host range, which includes most economically important livestock species such as cattle and goat. *Trypanosoma equiperdum* is a disease that primarily affects horses, causing Dourine. Transmission of animal trypanosomiasis occurs mainly through its natural insect vector, the tsetse fly, but can also be accomplished through mechanical transmission by biting insects. The latter has allowed certain animal trypanosomes to propagate outside the African continent, as is the case for *T. evansi* and *T. vivax* that have become endemic to Asia and South America.⁴⁻⁶ Sexual transmission of trypanosomiasis so far has only been confirmed in the case of *T. equiperdum*.⁷

HAT (or sleeping sickness) is caused by either *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense*, combined accounting for over 10,000 reported human infection cases a year.⁸ Both these parasites have developed resistance mechanisms that neutralize the function of the human trypanolytic serum factors TLF1 and TLF2.⁹⁻¹¹ *T. b. gambiense*, commonly found in West and Central Africa, causes over 90% of the reported HAT cases. It evolves as a chronic disease characterized by a slow progression to the late/encephalitic stage and eventual death. In contrast, *T. b. rhodesiense*, present in East and Southern Africa, is responsible for acute infections with a rapid onset and a faster progression of disease. All human infective trypanosomes are transmitted by the tsetse fly (*Glossina sp*) in a clearly defined region of Africa, the so-called tsetse belt.¹² Important is that *T. b. rhodesiense* is primarily a zoonotic disease, where disease outbreaks can be attributed to the continuous presence of a livestock reservoir of the human infective parasite.

Despite the significant amount of yearly HAT victims, disease prevalence is at historic low levels when compared with the infection incidence recorded over the past 100 years¹³ due to efficient control campaigns that have been initiated and maintained over the last decade. Despite these efforts, the reality is that HAT is still a neglected disease that requires further research on prevention, diagnosis and treatment strategies. In particular now that the incidence has become low, detection of the remaining cases is crucial to prevent re-emergence of the disease. In addition, tackling

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the zoonotic nature of East-African HAT, and hence dealing with the animal reservoir of in particular *T. b. rhodesiense*, should be a priority. Taken that full eradication of the parasite reservoir from the African livestock and game population is unfeasible, vaccination against trypanosomiasis should be an integral part of the fight against HAT in infection endemic areas, as it is the only strategy that will provide protection against re-infection of the human population. In summary, the target locations would be in the first place areas where the animal reservoir represents a constant danger for human populations, and in particular, areas such as northern Uganda, which is already endemic for *T. b. gambiense* infection.¹⁴ In the circumstance of merging, treatment provision would be dramatically delayed due to the uncertainty regarding the type of disease (acute or chronic infection). Last but not least, any endemic area in the tsetse belt with a relatively high transmission rate (for example the Democratic Republic of Congo) should be considered as candidate for vaccination programs.

African trypanosomiasis: A Natural Killer Disease by Default or an ill-Adapted Parasitic Infection for Certain Hosts?

Trypanotolerance is a trait that confers the capacity to survive and remain productive upon trypanosome infection.¹⁵ As demonstrated by Naessens et al. trypanotolerance in cattle is a result of two independent mechanisms: (1) the capacity to control parasitemia, which is independent of the hematopoietic system and (2) the capacity to limit anemia, which is mediated by hemopoietic cells. The latter appears to be more important for survival and productivity. With regards to trypanotolerance in human trypanosomiasis, several reports suggest indeed the existence of such phenomenon. For example, a study on trypanosome infected patients in Côte d'Ivoire reported that after refusing treatment, patients that had been diagnosed with HAT both by microscopy and serology were found to be parasitologically negative when re-examined 7 years later. While some individuals even became 'sero-negative' showing no anti-trypanosome antibody titers in their serum over time, others remained 'seropositive', even though parasites could not be detected in blood, thus considered asymptomatic carriers.¹⁷ Despite the general description of HAT as a 'deadly disease', asymptomatic carriers have been reported in the literature for both *T. b. gambiense*^{18,19} and *T. b. rhodesiense*^{20,21} infections. Even though those differences in the outcome of the disease can be attributed to genetic variations in parasite virulence,²² it has been demonstrated in mouse models that the host genotype can play a crucially important role in severity of disease as well.²³ Furthermore, it has been observed that co-infections of parasite strains can play a major role in attenuated disease progression,²⁴ which could partially contribute to regional differences observed in HAT outcome. Indeed, as reported by Balmer et al.²⁴ co-infections in murine models with both high and lower virulent trypanosome stocks lead to a strong mutual competitive suppression early in infection. The level of suppression is related to the density of the co-infecting strains, and it is believed to be the result of an active inhibition rather than a numerical response due to limited

space.²⁴ Thus, competition for resources within the host, allelopathic interference by excreted factors and immune-mediated competition might be at play. Important to note is that during human HAT surveillance, co-infections of *T. brucei gambiense* and the normally non-human infective *T. congolense* have been reported.²⁵ Although this situation appears to occur only at very rare occasions, it is an interesting thought that regular exposure to *T. congolense* could provide human subjects with a certain level of protection against human pathogenic trypanosomes.

The genes that confer tolerance in cattle or human are not yet identified, though considerable effort is currently being made to clarify the location of the implicated genes. Studies performed on *T. congolense* infected mice have led to the identification of three quantitative trait loci (QTL), which are regions in the genome that have an influence on a quantitative trait or phenotype (i.e., body weight, anemia and parasitemia control). Three such regions named trypanosome infection response (Tir1, Tir2, Tir3) have been defined by Kemp et al.²⁶ among which Tir1 showed the greatest effect on survival. Coincidentally, the tumor necrosis factor α (TNF α) gene is within a group of genes that co-localize to that particular locus, thus becoming an interesting candidate for resistance. As a matter of fact, TNF α has been reported to be a key mediator of host survival in experimental models,²⁷ as well as intervening in parasite control.^{28,29} Two other crucial immunoregulatory genes that have an impact on infection outcome have been found to map close to Tir3b. These are the interleukin 10 (IL-10) gene and a regulatory gene implicated in IL-10 synthesis, *Cypr2*.³⁰ Interestingly, IL-10 is implicated in the prevention of immunopathological lesions due to an exaggerated immune response.³¹ Upon *T. congolense* infection in particular, IL-10 activates a battery of genes involved in the control of inflammatory processes, thus preventing pathogenicity and promoting host resistance.³²

With reference to human cases, clinical profiles of *T. b. rhodesiense* infected patients from several HAT foci have been subject of study in recent years.³³⁻³⁵ However, while no genetic loci have yet been identified for trypanotolerance, it has been hypothesized that the ethnical origin of the subject might influence the outcome of the disease. People from Bantu origin, who have been exposed to trypanosomes for thousands of years, may thus be more resistant to infection. On the other hand, people of Nilotic ancestry, who are relatively new to the contact with trypanosomes, are more susceptible. However, with only a few contradictory reports on this subject available to date,^{20,36} no sound conclusion can be drawn as to one ethnicity being more resistant than the other.

Vaccine Candidates for HAT: Past and Present

As most mammals on the African continent are capable of dealing with trypanosomes as well as with other parasites, and as even certain human infections apparently manifest themselves as long-lasting well-controlled infections, it should be taken into account that under optimal conditions even the human immune system can be instructed to mount a protective anti-parasite response in case of exposure to trypanosomes. This,

together with the fact that eradication of the entire trypanosome reservoir is unthinkable, suggests that anti-trypanosome vaccination has to be the ultimate target in the fight against HAT. However, thus far, the goal of obtaining an effective vaccine candidate with potential use in a realistic field setting has not been achieved.

Initially, vaccine trails against trypanosomiasis started targeting the surface coat of the parasite. This coat is composed by 10 million copies of a single molecule: the variant surface glycoprotein (VSG). In principle, this surface protein would be an ideal vaccine candidate, if it were not for the antigenic variation strategy that the parasites have cunningly evolved. Before the basis of the evasion mechanism was elucidated, the first vaccination strategies focused on the abundant VSG molecules as targets.³⁷ Soon however, it became obvious that such an approach would never succeed due to (1) the innumerable possible molecules that the parasite can generate through gene rearrangements and (2) the fact that the main immunoglobulin response that they elicit is that of the IgM isotype, which is short lived.

Yet, while undergoing antigenic variation, trypanosomes are forced to maintain expression of some non-(or less) variable surface molecules in order to assimilate all the host factors they require for survival. A common architectural feature of trypanosomes is the flagellar pocket (FP); an invagination of the membrane at the base of the flagellum. This structure is readily involved in exocytic and endocytic processes, cell division and polarity, protein trafficking and more importantly, virulence and immune evasion.³⁸ Therefore, antigens present in the FP represent attractive candidates for vaccine development. A study from 1995 showed that immunization of cattle with an apparently invariant antigen localized at the FP provided them with a partial protection against infections, as reflected by the significant differences in disease incidence.³⁹ A similar experiment was performed in a murine model, where Balb/c susceptible mice were immunized with a FP preparation. A partial protection was also achieved in this case, since 60% of the mice survived the parasite challenge. The 40% that succumbed to infection exhibited a doubled survival time and a delayed parasitemia onset. In spite of these 'positive' findings, subsequent challenges with higher parasite load (inoculum of 10³ parasites or more) demonstrated that the induced protection was temporary and only gave borderline immunity to low dose infection. Hence the authors proposed that FP vaccination was not the correct way forward.⁴⁰

In a similar approach, targeting invariant trypanosome proteins, a number of molecular targets have been proposed, including the transferrin receptors ESAG6/7 and several invariant surface glycoproteins (ISGs).⁴¹⁻⁴⁴ According to recent data, vaccination with a DNA plasmid encoding a bloodstream-stage specific ISG enhances a partial protection of mice (40%),⁴⁵ with surviving animals showing increased levels of IgG2a antibodies. However, in view of the earlier published anti-FP vaccinations it should be indicated that also here partial protection was only reported for a low-dose parasite challenge.

Other structural molecules that have been proposed as vaccination candidates are the subcellular proteins of the cytoskeleton i.e., actin and tubulin. The former has been shown to play

a crucial role in bloodstream forms of *T. brucei*, not only due to its involvement in cell division, movement and morphology, but especially because it is indispensable for the formation of coated vesicles from the FP membrane.⁴⁶ Li et al. published an immunization study using recombinant *T. evansi* actin, which shows high homology with *T. equiperdum*, *T.b. brucei* and *T. cruzi*. Actin-immunized mice were protected to different extents according to the trypanosome species of challenge, and did not undergo autoimmune reactions.⁴⁷ These results are supported by another study of Li et al. in which mice immunized with recombinant β -tubulin of *T. evansi* were protected from lethal challenge with *T. evansi*, *T. equiperdum* and *T.b. brucei*.⁴⁸ It was suggested that this protection was antibody-mediated, and that these would reach their target by internalization through a yet to be discovered mechanism. However, particular care should be taken with the interpretation of these results as the parasite challenge was only done 6 days after the third vaccine boost, using a relatively low parasite dose of 1,000 parasites. Hence the functional implementation of immunological memory was not addressed by this study, and moreover the authors did not provide a sound explanation as to how antibodies could access their intracellular cytoskeleton protein target.

Similar to the vaccination approach by Li et al. anti-tubulin vaccination has been reported to result in partial protection against trypanosomiasis by Lubega et al.⁴⁹ Here, tubulin was targeted as a major component within the cytoskeleton with involvement in various intracellular functions, including the maintenance of cellular architecture, cell motility and transport.⁵⁰ Tubulin immunization of mice conferred sterile protection to 60–80% of the animals, including in heterologous challenges of *T. brucei*, *T. congolense* and *T.b. rhodesiense*.⁴⁹ However, as in the case of the studies of Li et al. the short period between vaccination boosts and parasite challenge does not allow to draw any conclusion about the actual functional involvement of immunological memory in the observed results, and neither was a sound hypothesis provided as to how the presumed protective antibodies actually conferred their protective intracellular mode of action. Finally, neither the actin nor tubulin vaccine studies resulted in a follow-up study in a more realistic field setting.

Other membrane-associated candidates for vaccine development that have been described are trypanosome trans-sialidases and cation pumps. Sialidases are membrane-associated enzymes that transfer sialic acid from sialylated glycoconjugates from the host's cell surface to acceptor molecules on the parasite's surface.⁵¹ Though the enzyme has been thoroughly studied in *T. cruzi*, it has not yet been investigated in *T. brucei*. Immunization with a plasmid encoding the catalytic and N-terminal domain of the enzyme conferred 60% of protection in mice when challenged with a low dose of 500 *T.b. brucei* parasites,⁵² hence obtaining similar results as the FP-vaccination results outlined above. With respect to the immune targeting of parasite cation pumps, essential for survival and cation homeostasis of the parasite, vaccination was shown to provoke a biased stimulation of pro-inflammatory cytokines, though it failed to induce long-term protection.⁵³

Taken the limited success of anti-trypanosome vaccinations described above, a number of research groups have taken an alternative approach in the past, and have addressed the possibility of

developing an anti-disease vaccine, targeting infection-associated pathology rather than the parasite itself. One of the main targets in this approach is the glycosylphosphatidylinositol (GPI) anchor that attaches the VSG molecules to the parasite's membrane. This anchor has been associated with the induction of TNF α activity. The relation between this cytokine and initiation of disease-associated immunopathology has long been recognized.⁵⁴⁻⁵⁶ Upon environmental stress, trypanosomes can cleave their VSG anchor by means of a phospholipase hydrolytic enzyme,⁵⁷ leaving the dimyristoylglycerol (DMG) moiety attached to the membrane and releasing the glycosyl-inositol-phosphate (GIP) fragment.⁵⁸ These two components of the GPI are able to elicit qualitatively different macrophage activations; the GIP (particularly, the galactose chain within it) is responsible of the induction of TNF α production, whereas the DMG part primes the macrophages, thus becoming sensitized to other inflammatory agents such as LPS, and driving the induction of IL-1 α secretion.⁵⁹ In turn, IL-1 α is implicated in TNF α production.⁶⁰ Interestingly, serum levels of LPS have been reported to be increased during trypanosome infection.⁶¹ Although the exact origin of the increased LPS levels remains to be elucidated, this makes the entire GPI-macrophage priming physiologically relevant during disease development. Liposome-based GPI treatment of mice previous to parasitic challenge provoked a shift in the cytokine pattern observable during infection. In fact, it results in an increased expression of anti-inflammatory cytokine IL-10, whereas secretion of pro-inflammatory mediators (TNF, IL-6 and IL-12) is impaired.⁶² This attenuation in inflammation enhanced a prolonged survival and alleviated the clinical symptoms of the infection (anemia, weight loss, liver damage, locomotor impairment), albeit it does not affect parasitemia development. In spite of these encouraging results and the presence of low but yet detectable antibodies against GPI, it was later demonstrated that the elicited protection did not relate to the B cell compartment, since the positive outcome was exhibited in B-cell deficient animals as well. Hence, conventional B-cell memory involvement was thus precluded (unpublished data Magez S et al.). Interestingly a similar study was performed in the context of malaria infections by Schofield et al.⁶³ This time, a synthetic molecule of GPI was utilized for immunization of mice. Resulting from its high immunogenicity, IgG antibodies could be detected in the sera. However, these antibodies were shown to be not only short-lived, but also dependent on the presence of parasite antigen.⁶⁴ In this case, while the GPI vaccination did alleviate some of the inflammatory clinical symptoms, it did not result in prolongation of survival for the infected host.

A second approach in anti-disease vaccination for trypanosomiasis involves congopain, a cysteine protease (CP) that appears to elicit a high IgG response in trypanotolerant, though not in susceptible cattle. A role in pathogenicity was suggested for this molecule back in 1993.⁶⁵ A first vaccination trial in cattle was reported in 2001.⁶⁶ Here, trypanosusceptible animals were immunized with two predominant families of CPs (CP1 and CP2) that differ in their functional characteristics.⁶⁷ Results from these experiments excluded any implication of CPs in early stages of infection, since no differences were observed between

immunized and non-immunized controls. Nevertheless, immunized cattle showed weight gain in posterior stages as well as less severe anemia. A role in immunosuppression was suggested for CP2, based on the more prominent IgG responses developed by cattle immunized against it, which mimics in a certain way the response of a trypanotolerant animal. Despite these promising results, there have been no further publications on the subject. A summary of the vaccine candidates presented above can be found in Table 1

The Main Pitfalls of Anti-trypanosome Vaccination: Antigenic Variation and Abrogation of B-cell Homeostasis and Memory

Despite all the anti-trypanosome trails reported and reviewed above, not a single 'promising' experimental result obtained in these studies has sparked off a positive field trial. Indeed, in reality it appears that trypanosomes have evolved two defense mechanisms that protect them from antibody-mediated elimination by the immune system. The first mechanism involves the capacity to modulate its own antigen 'appearance', while the second mechanism relies on undermining the hosts capacity to mount an efficient immune response and to maintain its immunological memory.

One of the most remarkable features of trypanosomes, as already mentioned above, is their ability to regularly switch their surface coat and hence evade immune destruction. This mechanism is known as antigenic variation.^{68,69}

Subsequent to the sequencing of the trypanosome genome, an enormous repertoire of VSG was identified, made up out of full-length VSG genes as well as a huge array of pseudogenes.⁷⁰ The latter coheres with the fact that many VSGs are expressed as mosaic products. In other words, the parasite greatly increases the variations of the expressed protein by creating a puzzle made of fragments of several genes. Trypanosomes exclusively express only one VSG out of that vast repertoire at the time. This, together with the fact that all *T. brucei* parasites survive extracellularly, would theoretically turn the parasite into an 'easy target' for antibody-mediated killing by the host immune system. Even though the latter occurs upon infection, by the time the host mounts an efficient response against the most frequently encountered VSG, VSG switching has already taken place and a different VSG-expressing population has arisen. Hence, the mechanism of antigenic variation allows the parasites to continually escape the immune system of the host and in conclusion, it appears that even if many VSGs share combined epitopes, this molecule is unable to be targeted by an efficient destructive antibody response.

Taken the fact that many VSGs are expressed as mosaic proteins of previously 'used' VSGs, it remains remarkable that this system of antigenic variation seems to be so effective in escaping immune recognition. The reason why this is the case, is most likely linked to the second defense system that trypanosomes have developed, i.e., the abrogation of B-cell homeostasis and the destruction of the host's immunological memory. Together, these immune dysfunctions result in the lack of buildup of anti-VSG memory, and hence allow the parasite to use over time

Table 1. Summary of vaccine candidates reported in literature

Type of vaccine	Antigen	Antigen preparation	Boosts	Time lapse last boost-challenge	Parasite load	Immunological outcome	Reference
Intramuscular	FP	Parasite isolated	3	14 d or more	Natural exposure in a field	Partial protection	Mkunza et al. Vaccine 1995 ³⁹
I.p.	FP	Parasite isolated	3	NI	500 x 10 ³	Partial/no protection	Radwanska et al. Parasite Immunol 2000 ⁴⁰
I.p.	ISG65, ISG75	Recombinant protein	3	11 d	10 ⁴	No protection	Ziegelbauer et al. J Biol Chem 1993 ⁴⁴
I.p.	ISG	Plasmid DNA	1	175 d	500	Partial protection	Lança et al. Exp Parasitol 2011 ⁴⁵
Subcutaneous	Actin	Recombinant protein	3	6 d	10 ³	Partial protection	Li et al. Parasitol Res 2009 ⁴⁷
Subcutaneous	Tubulin	Recombinant protein	3	6 d	10 ³	Partial protection	Li et al. Parasite Immunol 2007 ⁴⁸
Subcutaneous	Tubulin	Parasite isolated	3	NI	10 ³	Partial protection	Lubega et al. Exp Parasitol 2002 ⁴⁹
Intramuscular	Sialidase	Plasmid DNA	1	175 d	500	Partial protection	Silva et al. Parasitol Res 2009 ⁵²
I.p.	Cation ATPases	Recombinant protein	3	6 weeks	10 ⁶	No protection	Ramey et al. Am J Trop Med Hyg 2009 ⁵³
I.p.	GPI	Liposomes	2	3 weeks	5 x 10 ³	Partial protection	Stijlemans et al. J Immunol 2007 ⁶²
Subcutaneous	CP	Recombinant protein	4	1 mo	Experimental tsetse fly challenge	Partial protection	Authie et al. Int J Parasitol 2001 ⁶⁶

I.p.: intraperitoneal; FP: flagellar pocket; ISG: invariant surface glycoprotein; GPI: glycosylphosphatidylinositol; CP: cysteine protease; NI: not indicated.

very similar VSG molecules, or even re-use a surface coat protein that already has been encountered by the host.

A number of studies reported four decades ago, already described the following key facts of *T. brucei* infection: (1) The immune system is unable to react against unrelated new antigens upon infection;⁷¹ (2) there is a non-specific activation of immunoglobulin production;⁷² (3) the cellular components of the humoral response are no longer coordinated upon infection;⁷³ (4) despite a sustained plasma cell hyperplasia, failure of antibody production (both IgM and IgG) at the cellular level occurs at 2–4 weeks post infection.⁷⁴ Those findings were directly associated to the presence of living trypanosomes, since the immune competence was restored after treatment,⁷⁵ and define the basis of immunosuppression, a hallmark of trypanosome infections.

Further insight into B cell dysfunction was gained through the study of morphological and qualitative changes in secondary lymphoid organs.⁷⁶ The first peak of parasitemia and a marked splenomegaly were observed simultaneously, the latter due to plasma cell proliferation in the white pulp and erythrocyte expansion in the red pulp of the spleen. These events were followed by a gradual disorganization of the white pulp caused by the increasing number of red blood cells, whereas the amount

of plasma cells grew smaller with time. Under no circumstances were germinal centers observed. As confirmed by Radwanska et al.⁷⁷ not only is the architecture of the spleen severely affected by *T. brucei* infection, but the study of the cellular composition revealed a drastic disappearance of marginal zone B cells as well. This cell subset is the main mediator of T-cell independent immune responses. Finally, the same authors showed that B-cell memory was gravely impaired, due to either depletion or hindered reactivation. Important to stress is that the destruction of the B-cell memory compartment appears to affect not only anti-trypanosome responses, but immunological memory in general. Indeed, using a combined model of DTPa vaccine (against diphtheria, tetanus and pertussis) and trypanosome challenges, Radwanska et al.⁷⁷ showed that the latter destroys even unrelated vaccine induced memory responses such as the DTPa induced protection against a subsequent *B. pertussis* challenge. Using this knowledge, it was later shown that during experimental trypanosome infections in mice, exposure to a particular VSG does not provide the host with the capacity to mount a protective memory response against this given VSG. Indeed, re-challenge with a previously encountered trypanosome stock is possible within weeks after encountering the same VSG antigenic variant.⁷⁸

Murine Model for African Trypanosomiasis: Introducing Artifacts or Providing Good Laboratory Working Models?

Despite the fact that the mouse model has been widely used in the study of African trypanosomiasis, the question remains whether it is indeed a valid model to study host-parasite interactions and functional infection-associated immune alterations. However, the crucial contributions of the murine model to the understanding of trypanosomiasis can certainly not be denied. First, the use of inbred mouse strains has enabled the study of genetic determinants of susceptibility.⁷⁹ Survival monitoring and the ability to control parasite load in homogeneous backgrounds has enhanced the ranking of a wide range of strains from less to more susceptible to infection. Second, gene knockout technology has permitted the identification of key immune mediators during the infections, such as TNF α ,^{27,80} IFN γ ,^{81,82} IL-10⁸³ or nitric oxide, and their relative importance according to the species of the infecting trypanosome. In addition, mouse studies have provided valuable insight into a number of crucial aspects related to infection: the mechanism of antigenic variation,^{9,84} the B-cell compartment dysfunction⁷⁷ as well as the impact of innate immunity on infection-associated complications.^{30,85}

However, inbred mouse strains do not mimic a reality where the population at risk is undoubtedly heterogeneous. Furthermore, mice lack various host-specific interaction molecules such as for example the trypanolytic factors present in normal human serum. As a matter of fact, TLF activity is restricted to primates with the ApoL1.⁸⁶ Finally, mice exhibit excessive parasitemia levels as compared with natural hosts, drastically altering the ratio between the number of circulating parasites and immune cells. This ratio might greatly impact on the way the parasite deregulates the immune system.

Models that circumvent the above-mentioned mouse artifacts do exist, but in turn bring their own issues and limitations. A number of studies performed on vervet monkeys have been described, offering a model of infection that is much closer to human infections.⁸⁷⁻⁸⁹ Working with non-human primates for infectious disease studies requires a unique infrastructure, and in terms of fundamental genetic approaches has huge limitations as compared with the mouse model. However, recently published data emphasize the advantage of the use of monkeys, whose infection accurately mimics that of humans.⁹⁰ For example, infections started by a single tsetse fly bite showed different pre-patent periods in monkeys, consistent with the heterogeneity reported for HAT. Host factors are thus clearly involved in the outcome of the disease, beyond the intrinsic properties of the parasite that determine the virulence. Surprisingly, individuals with longer survival times showed more fluctuations in parasitemia levels. The similarity of this animal model and the human disease has also permitted some insight into the hematological consequences of the infection. For example, the anemia observed in vervet monkeys was of the microcytic hypochromic type, unlike the normocytic one usually reported in mice. Unfortunately, it is currently difficult to establish which of the two is most representative of the

human disease, due to the lack of means to analyze blood samples in the field and the presence of other infections or nutritional conditions.⁹¹ Consistent with previous data, a marked leucopenia could be observed in the animals around the same period when parasites could first be detected in peripheral blood. Strikingly, multiple peaks of white blood cell counts could be observed in subsequent days in all infected monkeys, suggesting that despite the immunosuppression described elsewhere for trypanosomiasis,^{72,73,75} some myeloid precursor cells are still able to proliferate in response to the multiple waves of parasitemia. Combined, these observations suggest that non-murine models might be a better way forward in future vaccine research, as they would circumvent intrinsic artifacts that are related to experimental trypanosome infections in the mouse model.

Conclusion

Vaccination against trypanosomiasis should remain the ultimate goal in the fight against this disease, both in case of animal trypanosomiasis and HAT. This assumption is based on two main observations: first, taken that it is an impossible task to eradicate the entire parasite reservoir of endemic areas, only vaccination will provide a long lasting economically viable option to prevent human casualties and vast economic losses due to livestock infections. Second, taken that trypanotolerance occurs in many mammals endemic to regions where trypanosomiasis occurs, and that even in human infections immunological control of the infection has been reported, it appears that immune intervention to prevent the deadly outcome of trypanosomiasis should be somehow a feasible target.

To date, not a single experimental anti-trypanosome vaccination protocol has made it to a stage where preliminary promising results have been reported in a field setting. One of the problems of experimental trypanosomiasis research might be the general use of murine models for basic research. While these models do serve their purpose in a certain context, it has become clear that with respect to B-cell function and memory maintenance during infection, mice suffer from trypanosomiasis-associated defects that might be specific for the model, and do not necessarily reflect the immunological situation of the natural host. Indeed, the fact that mice can be re-infected with previously encountered trypanosome variants suggest that antigenic variation only has a limited importance in the defense of the parasite against the destruction by the host (mouse) immune system. In contrast, the fact that trypanosomes very efficiently destroy vaccine induced immunological memory in a mouse setting, might be an exaggeration of what is observed in natural hosts, associated to the distorted parasite/immune cell ratio in mice, that exhibit abnormally high circulating parasite numbers throughout infection. This last issue raises a very important question with respect to vaccine development and experimental vaccine trials: could it be that the murine model for trypanosomiasis is prone to failure when it comes to testing the effect of B-cell immunity and B-cell memory? If so, it could be that many of the 'failed' murine vaccine studies using various conserved trypanosome surface molecules are actually

negatively biased by the intrinsic immune deficiency occurring during trypanosome infections in mice. Possibly, using other, more relevant models for infection, could lead to other outcomes in vaccine trials. While there is no 'easily accessible' alternative for initial murine experiments, maybe such alternatives have to be considered in order to increase the success of

finding a future anti-trypanosome vaccination approach, taken the very specific features of host-parasite interactions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Raper J, Fung R, Ghiso J, Nussenzweig V, Tomlinson S. Characterization of a novel trypanosome lytic factor from human serum. *Infect Immun* 1999; 67:1910-6; PMID:10085035.
- Barth P. A new method for the isolation of the trypanocidal factor from normal human serum. *Acta Trop* 1989; 46:71-3; PMID:2566265; DOI:10.1016/0001-706X(89)90019-3.
- Shaw A. The economics of African trypanosomiasis. In the trypanosomiasis edited by Maudlin I, Holmes P, Miles M, Wallingford: CABI Publishing 2004.
- Desquesnes M, Dia ML. Mechanical transmission of *Trypanosoma congolense* in cattle by the African tabanid *Atylotus agrestis*. *Exp Parasitol* 2003; 105:226-31; PMID:14990316; DOI:10.1016/j.exppara.2003.12.014.
- Desquesnes M, Dia ML. Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanid *Atylotus fuscipes*. *Vet Parasitol* 2004; 119:9-19; PMID:15036572; DOI:10.1016/j.vetpar.2003.10.015.
- Otte MJ, Abuabara JY. Transmission of South American *Trypanosoma vivax* by the neotropical horsefly *Tabanus nebulosus*. *Acta Trop* 1991; 49:73-6; PMID:1678579; DOI:10.1016/0001-706X(91)90033-G.
- Brun R, Hecker H, Lun ZR. *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Vet Parasitol* 1998; 79:95-107; PMID:9806490; DOI:10.1016/S0304-4017(98)00146-0.
- WHO. Human African trypanosomiasis: number of new cases drops to historically low level in 50 years. Available at: http://whoint/neglected_diseases/integrated_media/integrated_media_hat_june_2010/en/indexhtml 2010.
- Xong HV, Vanhamme L, Chamekh M, Chimfwembe CE, Van Den Abbeele J, Pays A, et al. A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* 1998; 95:839-46; PMID:9865701; DOI:10.1016/S0092-8674(00)81706-7.
- De Greef C, Hamers R. The serum resistance-associated (SRA) gene of *Trypanosoma brucei* rhodesiense encodes a variant surface glycoprotein-like protein. *Mol Biochem Parasitol* 1994; 68:277-84; PMID:7739673; DOI:10.1016/0166-6851(94)90172-4.
- Kieft R, Capewell P, Turner CM, Veitch NJ, MacLeod A, Hajduk S. Mechanism of *Trypanosoma brucei* gambiense (group 1) resistance to human trypanosome lytic factor. *Proc Natl Acad Sci USA* 2010; 107:16137-41; PMID:20805508; DOI:10.1073/pnas.100704107.
- Simarro PP, Cecchi G, Paone M, Franco JR, Diarra A, Ruiz JA, et al. The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *Int J Health Geogr* 2010; 9:57; PMID:21040555; DOI:10.1186/1476-072X-9-57.
- Courtin F, Jamonneau V, Duvallet G, Garcia A, Coulibaly B, Doumenge JP, et al. Sleeping sickness in West Africa (1906-2006): changes in spatial repartition and lessons from the past. *Trop Med Int Health* 2008; 13:334-44; PMID:18397396; DOI:10.1111/j.1365-3156.2008.02007.x.
- Waiswa C, Kabasa JD. Experiences with an in-training community service model in the control of zoonotic sleeping sickness in Uganda. *J Vet Med Educ* 2010; 37:276-81; PMID:20847337; DOI:10.3138/jvme.37.3.276.
- Murray M, Morrison WI, Whitelaw DD. Host susceptibility to African trypanosomiasis: trypanotolerance. *Adv Parasitol* 1982; 21:1-68; PMID:6762064; DOI:10.1016/S0065-308X(08)60274-2.
- Naessens J, Leak SG, Kennedy DJ, Kemp SJ, Teale AJ. Responses of bovine chimaeras combining trypanosomosis resistant and susceptible genotypes to experimental infection with *Trypanosoma congolense*. *Vet Parasitol* 2003; 111:125-42; PMID:12531289; DOI:10.1016/S0304-4017(02)00360-6.
- Jamonneau V, Ravel S, Garcia A, Koffi M, Truc P, Laveissiere C, et al. Characterization of *Trypanosoma brucei* s.l. infecting asymptomatic sleeping-sickness patients in Cote d'Ivoire: a new genetic group? *Ann Trop Med Parasitol* 2004; 98:329-37; PMID:15228714; DOI:10.1179/000349804225003406.
- Jamonneau V, Ravel S, Garcia A, Koffi M, Truc P, Laveissiere C, et al. Characterization of *Trypanosoma brucei* s.l. infecting asymptomatic sleeping-sickness patients in Cote d'Ivoire: a new genetic group? *Ann Trop Med Parasitol* 2004; 98:329-37; PMID:15228714; DOI:10.1179/000349804225003406.
- Wery M, Burke J. Human "healthy carriers" of *Trypanosoma (brucei type)* discovered by immunofluorescence test in the Republique Democratique du Congo. *Trans R Soc Trop Med Hyg* 1972; 66:332-3; PMID:4558826; DOI:10.1016/0035-9203(72)90209-X.
- Buyst H. The epidemiology of sleeping sickness in the historical Luangwa valley. *Ann Soc Belg Med Trop* 1977; 57:349-59; PMID:345980.
- MacLean L, Chisi JE, Odiit M, Gibson WC, Ferris V, Picozzi K, et al. Severity of human african trypanosomiasis in East Africa is associated with geographic location, parasite genotype and host inflammatory cytokine response profile. *Infect Immun* 2004; 72:7040-4; PMID:15557627; DOI:10.1128/IAI.72.12.7040-4.2004.
- Morrison LJ, Tait A, McLellan S, Sweeney L, Turner CM, MacLeod A. A major genetic locus in *Trypanosoma brucei* is a determinant of host pathology. *PLoS Negl Trop Dis* 2009; 3:557; PMID:19956590; DOI:10.1371/journal.pntd.0000557.
- Clayton CE. *Trypanosoma brucei*: influence of host strain and parasite antigenic type on infections in mice. *Exp Parasitol* 1978; 44:202-8; PMID:658218; DOI:10.1016/0014-4894(78)90099-1.
- Balmer O, Stearns SC, Schotzau A, Brun R. Intraspecific competition between co-infecting parasite strains enhances host survival in African trypanosomes. *Ecology* 2009; 90:3367-78; PMID:20120806; DOI:10.1890/08-2291.1.
- Truc P, Jamonneau V, N'Guessan P, N'Dri L, Diallo PB, Cuny G. *Trypanosoma brucei* ssp and *T. congolense*: mixed human infection in Cote d'Ivoire. *Trans R Soc Trop Med Hyg* 1998; 92:537-8; PMID:9861372; DOI:10.1016/S0035-9203(98)90904-X.
- Kemp SJ, Iraqi F, Darvasi A, Soller M, Teale AJ. Localization of genes controlling resistance to trypanosomiasis in mice. *Nat Genet* 1997; 16:194-6; PMID:9171834; DOI:10.1038/ng0697-194.
- Agez S, Radwanska M, Drennan M, Fick L, Baral TN, Allie N, et al. Tumor necrosis factor (TNF) receptor-1 (TNFR1) signal transduction and macrophage-derived soluble TNF are crucial for nitric oxide-mediated *Trypanosoma congolense* parasite killing. *J Infect Dis* 2007; 196:954-62; PMID:17703428; DOI:10.1086/520815.
- Agez S, Lucas R, Darji A, Songa EB, Hamers R, De Baetselier P. Murine tumour necrosis factor plays a protective role during the initial phase of the experimental infection with *Trypanosoma brucei* brucei. *Parasite Immunol* 1993; 15:635-41; PMID:7877840; DOI:10.1111/j.1365-3024.1993.tb00577.x.
- Agez S, Geuskens M, Beschin A, del Favero H, Verschuere H, Lucas R, et al. Specific uptake of tumor necrosis factor-alpha is involved in growth control of *Trypanosoma brucei*. *J Cell Biol* 1997; 137:715-27; PMID:9151676; DOI:10.1083/jcb.137.3.715.
- Tabel H, Kaushik RS, Uzonna JE. Susceptibility and resistance to *Trypanosoma congolense* infections. *Microbes Infect* 2000; 2:1619-29; PMID:11113381; DOI:10.1016/S1286-4579(00)01318-6.
- Mege JL, Meghari S, Honstetter A, Capo C, Raoult D. The two faces of interleukin 10 in human infectious diseases. *Lancet Infect Dis* 2006; 6:557-69; PMID:16931407; DOI:10.1016/S1473-3099(06)70577-1.
- Boschaerts T, Guillemins M, Noel W, Herin M, Burk RF, Hill KE, et al. Alternatively activated myeloid cells limit pathogenicity associated with African trypanosomiasis through the IL-10 inducible gene selenoprotein P. *J Immunol* 2008; 180:6168-75; PMID:18424738.
- MacLean L, Odiit M, MacLeod A, Morrison L, Sweeney L, Cooper A, et al. Spatially and genetically distinct African Trypanosome virulence variants defined by host interferon-gamma response. *J Infect Dis* 2007; 196:1620-8; PMID:18008245; DOI:10.1086/522011.
- Sternberg JM, MacLean L. A spectrum of disease in human African trypanosomiasis: the host and parasite genetics of virulence. *Parasitology* 2010; 137:2007-15; PMID:20663245; DOI:10.1017/S0031182010000946.
- MacLean LM, Odiit M, Chisi JE, Kennedy PG, Sternberg JM. Focus-specific clinical profiles in human African Trypanosomiasis caused by *Trypanosoma brucei* rhodesiense. *PLoS Negl Trop Dis* 2010; 4:906; PMID:21151878; DOI:10.1371/journal.pntd.0000906.
- Kuepfer I, Hrary EP, Allan M, Edielu A, Burri C, Blum JA. Clinical Presentation of *T. b. rhodesiense* Sleeping Sickness in Second Stage Patients from Tanzania and Uganda. *PLoS Negl Trop Dis* 2011; 5:968; PMID:21407802; DOI:10.1371/journal.pntd.0000968.
- Cornelissen AW, Bakkeren GA, Barry JD, Michels PA, Borst P. Characteristics of trypanosome variant antigen genes active in the tsetse fly. *Nucleic Acids Res* 1985; 13:4661-76; PMID:4022771; DOI:10.1093/nar/13.13.4661.
- Field MC, Carrington M. The trypanosome flagellar pocket. *Nat Rev Microbiol* 2009; 7:775-86; PMID:19806154; DOI:10.1038/nrmicro2221.
- Mkunza F, Olaho WM, Powell CN. Partial protection against natural trypanosomiasis after vaccination with a flagellar pocket antigen from *Trypanosoma brucei* rhodesiense. *Vaccine* 1995; 13:151-4; PMID:7625108; DOI:10.1016/0264-410X(95)93128-V.
- Radwanska M, Agez S, Dumont N, Pays A, Nolan D, Pays E. Antibodies raised against the flagellar pocket fraction of *Trypanosoma brucei* preferentially recognize HSP60 in cDNA expression library. *Parasite Immunol* 2000; 22:639-50; PMID:11123756; DOI:10.1046/j.1365-3024.2000.00348.x.

41. Steverding D, Stierhof YD, Chaudhri M, Ligtenberg M, Schell D, Beck-Sickingler AG, et al. ESAG 6 and 7 products of *Trypanosoma brucei* form a transferrin binding protein complex. *Eur J Cell Biol* 1994; 64:78-87; PMID:7957316.
42. Jackson DG, Windle HJ, Voorheis HP. The identification, purification and characterization of two invariant surface glycoproteins located beneath the surface coat barrier of bloodstream forms of *Trypanosoma brucei*. *J Biol Chem* 1993; 268:8085-95; PMID:8463323.
43. Ziegelbauer K, Overath P. Identification of invariant surface glycoproteins in the bloodstream stage of *Trypanosoma brucei*. *J Biol Chem* 1992; 267:10791-6; PMID:1587855.
44. Ziegelbauer K, Overath P. Organization of two invariant surface glycoproteins in the surface coat of *Trypanosoma brucei*. *Infect Immun* 1993; 61:4540-5; PMID:8406850.
45. Lança AS, de Sousa KP, Atouguia J, Prazeres DM, Monteiro GA, Silva MS. *Trypanosoma brucei*: immunisation with plasmid DNA encoding invariant surface glycoprotein gene is able to induce partial protection in experimental African trypanosomiasis. *Exp Parasitol* 2011; 127:18-24; PMID:20599996; DOI:10.1016/j.exppara.2010.06.017.
46. García-Salcedo JA, Perez-Morga D, Gijón P, Dilbeck V, Pays E, Nolan DP. A differential role for actin during the life cycle of *Trypanosoma brucei*. *EMBO J* 2004; 23:780-9; PMID:14963487; DOI:10.1038/sj.emboj.7600094.
47. Li SQ, Yang WB, Ma LJ, Xi SM, Chen QL, Song XW, et al. Immunization with recombinant actin from *Trypanosoma evansi* induces protective immunity against *T. evansi*, *T. equiperdum* and *T. b. brucei* infection. *Parasitol Res* 2009; 104:429-35; PMID:18923843; DOI:10.1007/s00436-008-1216-9.
48. Li SQ, Fung MC, Reid SA, Inoue N, Lun ZR. Immunization with recombinant beta-tubulin from *Trypanosoma evansi* induced protection against *T. evansi*, *T. equiperdum* and *T. b. brucei* infection in mice. *Parasite Immunol* 2007; 29:191-9; PMID:17371456; DOI:10.1111/j.1365-3024.2006.00933.x.
49. Lubega GW, Byarugaba DK, Prichard RK. Immunization with a tubulin-rich preparation from *Trypanosoma brucei* confers broad protection against African trypanosomiasis. *Exp Parasitol* 2002; 102:9-22; PMID:12615162; DOI:10.1016/S0014-4894(02)00140-6.
50. Schneider A, Sherwin T, Sasse R, Russell DG, Gull K, Seebeck T. Subpellicular and flagellar microtubules of *Trypanosoma brucei* contain the same alpha-tubulin isoforms. *J Cell Biol* 1987; 104:431-8; PMID:3818788; DOI:10.1083/jcb.104.3.431.
51. Schenkman S, Jiang MS, Hart GW, Nussenzweig V. A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells. *Cell* 1991; 65:1117-25; PMID:1712251; DOI:10.1016/0092-8674(91)90008-M.
52. Silva MS, Prazeres DM, Lança A, Atouguia J, Monteiro GA. Trans-sialidase from *Trypanosoma brucei* as a potential target for DNA vaccine development against African trypanosomiasis. *Parasitol Res* 2009; 105:1223-9; PMID:19582478; DOI:10.1007/s00436-009-1542-6.
53. Ramey K, Eko FO, Thompson WE, Armah H, Igetsembe JU, Stiles JK. Immunolocalization and challenge studies using a recombinant *Vibrio cholerae* ghost expressing *Trypanosoma brucei* Ca(2+) ATPase (TBCA2) antigen. *Am J Trop Med Hyg* 2009; 81:407-15; PMID:19706905.
54. Sileghem M, Flynn JN, Logan-Henfrey L, Ellis J. Tumour necrosis factor production by monocytes from cattle infected with *Trypanosoma* (*Duttonella*) *vivax* and *Trypanosoma* (*Nannomonas*) *congolense*: possible association with severity of anaemia associated with the disease. *Parasite Immunol* 1994; 16:51-4; PMID:7908735; DOI:10.1111/j.1365-3024.1994.tb00304.x.
55. Okomo-Assoumou MC, Daulouede S, Lemesre JL, N'Zila-Mouanda A, Vincendeau P. Correlation of high serum levels of tumor necrosis factor-alpha with disease severity in human African trypanosomiasis. *Am J Trop Med Hyg* 1995; 53:539-43; PMID:7485714.
56. Magez S, Radwanska M, Beschin A, Sekikawa K, De Baetselier P. Tumor necrosis factor alpha is a key mediator in the regulation of experimental *Trypanosoma brucei* infections. *Infect Immun* 1999; 67:3128-32; PMID:10338530.
57. Rolin S, Hanocq-Quertier J, Paturiaux-Hanocq F, Nolan D, Salmon D, Webb H, et al. Simultaneous but independent activation of adenylate cyclase and glycosylphosphatidylinositol-phospholipase C under stress conditions in *Trypanosoma brucei*. *J Biol Chem* 1996; 271:10844-52; PMID:8631899; DOI:10.1074/jbc.271.18.10844.
58. Fox JA, Duzsenko M, Ferguson MA, Low MG, Cross GA. Purification and characterization of a novel glycan-phosphatidylinositol-specific phospholipase C from *Trypanosoma brucei*. *J Biol Chem* 1986; 261:15767-71; PMID:3782089.
59. Magez S, Stijlemans B, Radwanska M, Pays E, Ferguson MA, De Baetselier P. The glycosyl-inositol-phosphate and dimyristoylglycerol moieties of the glycosylphosphatidylinositol anchor of the trypanosome variant-specific surface glycoprotein are distinct macrophage-activating factors. *J Immunol* 1998; 160:1949-56; PMID:9469458.
60. Philip R, Epstein LB. Tumour necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, gamma-interferon and interleukin-1. *Nature* 1986; 323:86-9; PMID:3092113; DOI:10.1038/323086a0.
61. Pentreath VW. Endotoxins and their significance for murine trypanosomiasis. *Parasitol Today* 1994; 10:226-9; PMID:15275456; DOI:10.1016/0169-4758(94)90122-8.
62. Stijlemans B, Baral TN, Guillems M, Brys L, Korf J, Drennan M, et al. A glycosylphosphatidylinositol-based treatment alleviates trypanosomiasis-associated immunopathology. *J Immunol* 2007; 179:4003-14; PMID:17785839.
63. Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. *Nature* 2002; 418:785-9; PMID:12181569; DOI:10.1038/nature00937.
64. Boutlis CS, Gowda DC, Naik RS, Maguire GR, Mgone CS, Bockarie MJ, et al. Antibodies to *Plasmodium falciparum* glycosylphosphatidylinositols: inverse association with tolerance of parasitemia in Papua New Guinean children and adults. *Infect Immun* 2002; 70:5052-7; PMID:12183552; DOI:10.1128/IAI.70.9.5052-7.2002.
65. Authié E, Duvallet G, Robertson C, Williams DJ. Antibody responses to a 33 kDa cysteine protease of *Trypanosoma congolense*: relationship to 'trypanotolerance' in cattle. *Parasite Immunol* 1993; 15:465-74; PMID:8233561; DOI:10.1111/j.1365-3024.1993.tb00632.x.
66. Authié E, Boulange A, Muteti D, Lalmanach G, Gauthier F, Musoke AJ. Immunisation of cattle with cysteine proteinases of *Trypanosoma congolense*: targeting the disease rather than the parasite. *Int J Parasitol* 2001; 31:1429-33; PMID:11595229; DOI:10.1016/S0020-7519(01)00266-1.
67. Boulanger A, Serveau C, Brillard M, Minet C, Gauthier F, Diallo A, et al. Functional expression of the catalytic domains of two cysteine proteinases from *Trypanosoma congolense*. *Int J Parasitol* 2001; 31:1435-40; PMID:11595230; DOI:10.1016/S0020-7519(01)00267-3.
68. Pays E, Vanhamme L, Perez-Morga D. Antigenic variation in *Trypanosoma brucei*: facts, challenges and mysteries. *Curr Opin Microbiol* 2004; 7:369-74; PMID:15288623; DOI:10.1016/j.mib.2004.05.001.
69. Vanhamme L, Pays E, McCulloch R, Barry JD. An update on antigenic variation in African trypanosomes. *Trends Parasitol* 2001; 17:338-43; PMID:11423377; DOI:10.1016/S1471-4922(01)01922-5.
70. Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, et al. The genome of the African trypanosome *Trypanosoma brucei*. *Science* 2005; 309:416-22; PMID:16020726; DOI:10.1126/science.1112642.
71. Goodwin LG. The pathology of African trypanosomiasis. *Trans R Soc Trop Med Hyg* 1970; 64:797-817; PMID:5495630; DOI:10.1016/0035-9203(70)90096-9.
72. Urquhart GM, Murray M, Murray PK, Jennings FW, Bate E. Immunosuppression in *Trypanosoma brucei* infections in rats and mice. *Trans R Soc Trop Med Hyg* 1973; 67:528-35; PMID:4785461; DOI:10.1016/0035-9203(73)90083-7.
73. Terry RJ, Freeman J, Hudson KM, Longstaffe JA. Immunoglobulin M production and immunosuppression in trypanosomiasis: a linking hypothesis. *Trans R Soc Trop Med Hyg* 1973; 67:263; PMID:4544655; DOI:10.1016/0035-9203(73)90167-3.
74. Jennings FW, Murray PK, Murray M, Urquhart GM. Protein catabolism in trypanosomiasis. *Trans R Soc Trop Med Hyg* 1973; 67:277; PMID:4784083; DOI:10.1016/0035-9203(73)90191-0.
75. Murray PK, Jennings FW, Murray M, Urquhart GM. The nature of immunosuppression in *Trypanosoma brucei* infections in mice. II. The role of the T and B lymphocytes. *Immunology* 1974; 27:825-40; PMID:4548043.
76. Morrison WI, Murray M, Bovell DL. Response of the murine lymphoid system to a chronic infection with *Trypanosoma congolense*. I. The spleen. *Lab Invest* 1981; 45:547-57; PMID:7033658.
77. Radwanska M, Guirnalda P, De Trez C, Ryffel B, Black S, Magez S. Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. *PLoS Pathog* 2008; 4:1000078; PMID:18516300; DOI:10.1371/journal.ppat.1000078.
78. Magez S, Schwegmann A, Atkinson R, Claes F, Drennan M, De Baetselier P, et al. The role of B-cells and IgM antibodies in parasitemia, anemia and VSG switching in *Trypanosoma brucei*-infected mice. *PLoS Pathog* 2008; 4:1000122; PMID:18688274; DOI:10.1371/journal.ppat.1000122.
79. Antoine-Moussiaux N, Magez S, Desmecht D. Contributions of experimental mouse models to the understanding of African trypanosomiasis. *Trends Parasitol* 2008; 24:411-8; PMID:18684669; DOI:10.1016/j.pt.2008.05.010.
80. Iraqi F, Sekikawa K, Rowlands J, Teale A. Susceptibility of tumour necrosis factor-alpha genetically deficient mice to *Trypanosoma congolense* infection. *Parasite Immunol* 2001; 23:445-51; PMID:11489168; DOI:10.1046/j.1365-3024.2001.00401.x.
81. Shi M, Wei G, Pan W, Tabel H. Experimental African trypanosomiasis: a subset of pathogenic, IFN-gamma-producing, MHC class II-restricted CD4+ T cells mediates early mortality in highly susceptible mice. *J Immunol* 2006; 176:1724-32; PMID:16424202.
82. Magez S, Radwanska M, Drennan M, Fick L, Baral TN, Brombacher F, et al. Interferon-gamma and nitric oxide in combination with antibodies are key protective host immune factors during *Trypanosoma congolense* Tc13 Infections. *J Infect Dis* 2006; 193:1575-83; PMID:16652287; DOI:10.1086/503808.
83. Namangala B, Noel W, De Baetselier P, Brys L, Beschin A. Relative contribution of interferon-gamma and interleukin-10 to resistance to murine African trypanosomiasis. *J Infect Dis* 2001; 183:1794-800; PMID:11372033; DOI:10.1086/320731.

84. Dubois ME, Demick KP, Mansfield JM. Trypanosomes expressing a mosaic variant surface glycoprotein coat escape early detection by the immune system. *Infect Immun* 2005; 73:2690-7; PMID:15845470; DOI:10.1128/IAI.73.5.2690-7.2005.
85. Magez S, Stijlemans B, Baral T, De Baetselier P. VSG-GPI anchors of African trypanosomes: their role in macrophage activation and induction of infection-associated immunopathology. *Microbes Infect* 2002; 4:999-1006; PMID:12106794; DOI:10.1016/S1286-4579(02)01617-9.
86. McEvoy SM, Maeda N. Complex events in the evolution of the haptoglobin gene cluster in primates. *J Biol Chem* 1988; 263:15740-7; PMID:3170608.
87. Gichuki C, Brun R. Animal models of CNS (second stage) sleeping sickness. In: *Handbook of Animal Models of Infection* (Zak O, SandeEds M) Academic Press, New York 1999; 795-800.
88. Ouwe-Missi-Oukem-Boyer O, Mezui-Me-Ndong J, Boda C, Lamine I, Labrousse F, Bisser S, et al. The vervet monkey (*Chlorocebus aethiops*) as an experimental model for *Trypanosoma brucei* gambiense human African trypanosomiasis: a clinical, biological and pathological study. *Trans R Soc Trop Med Hyg* 2006; 100:427-36; PMID:16325877; DOI:10.1016/j.trstmh.2005.07.023.
89. Schmidt H, Sayer P. *Trypanosoma brucei* rhodesiense infection in vervet monkeys. II. Provocation of the encephalitic late phase by treatment of infected monkeys. *Tropenmed Parasitol* 1982; 33:255-9; PMID:7164167.
90. Thuita JK, Kagira JM, Mwangangi D, Matovu E, Turner CM, Masiga D. *Trypanosoma brucei* rhodesiense transmitted by a single tsetse fly bite in vervet monkeys as a model of human African trypanosomiasis. *PLoS Negl Trop Dis* 2008; 2:238; PMID:18846231; DOI:10.1371/journal.pntd.0000238.
91. Jenkins G, Facer CA. Haematology of African trypanosomiasis. In: Tizard I, Ed. *Immunology and Pathogenesis* Boca Raton: CRC press 1985; 134.

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