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Review Article

Rationale to Develop mRNA-based Vaccines for *Trypanosoma brucei* (a review)

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ABSTRACT

Human African trypanosomiasis and nagana diseases are caused by *Trypanosoma* species transmitted by infected *Glossina* flies. The disease is prevalent in African countries, particularly in rural areas where the vector is freely present and people acquire the infection during farming, fishing, hunting, or washing clothes. The control of tsetse fly and eradication of the reservoir from endemic areas is difficult. The two stages of the disease are responsible for different clinical symptoms. The drugs used for the treatment of infection are old and cause many side effects. The variant surface glycoprotein coating of the parasite is highly variant genetically and the parasite modifies coating during infection that leads to suppression or exhaustion of the immune system of the host that ultimately fails memory. The vaccines produced using the antigens from variant surface glycoprotein or flagellar pockets give partial protection and after some time become non-effective. The mRNA-based vaccines result in the production of memory cells therefore, the attention of the researcher may shift towards finding suitable candidates for mRNA-based vaccines. These mRNAs encode specific antigens by using the machinery of the host cells and after translation, are degraded by the cellular nucleases. The fragments of lysed mRNA also participate in the induction of strong immune responses. The purpose of this review is to create a roadmap for the development of mRNA vaccines against *T. brucei*.

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Introduction

Human African trypanosomiasis (sleeping sickness) is an extracellular parasitic disease caused by *Trypanosoma brucei* (unicellular flagellated protozoan) which is a fatal disease if not treated. In the 1960s this disease was almost eradicated from the world, but a resurgence occurred in the late 1990s due to the collapse of surveillance and preventive measure in countries where it was endemic (Silva et al. 2009; Maxfield and Bermudez

2023). In the past, three major outbreaks of trypanosomiasis occur between 1896 and 1906 which largely affected equatorial Africa, 1920 and late 1940s, and in the late 1990s. The first outbreak killed approximately 800,000 people (Franco et al. 2014; Pays et al. 2022). The two forms of trypanosomiasis namely sleeping sickness and the animal form of disease together known as nagana. This is largely prevalent in the rural areas of sub-Saharan Africa but also occurs in urban and peri-urban areas and is transmitted to people during the activities of hunting, fishing, farming, or clothes washing (Brun et al. 2010).

The causative agent of this disease has further three subspecies such as *Trypanosoma brucei gambiense* (*T. b. gambiense*) responsible for the low progressing form of the disease, *Trypanosoma brucei rhodesience* (*T. b. rhodesience*) responsible for a faster-progressing form of the disease, and *Trypanosoma brucei brucei* (*T. b. brucei*). The first one is endemic in western and central Africa, the second one is responsible for the acute form of the disease in eastern and southern Africa, and the last one causes disease in domestic and wild animals respectively (Büscher et al. 2017). The zoonotic form of trypanosomiasis is caused by *T. b. rhodesience* while the main reservoirs of *T. b. gambiense* are the humans which play a vital role in the cycle of transmission (Franco et al. 2014). The other species which infect animals such as *T. b. brucei* (serum-associated proteins are absent in it that protect it from the degradation by the human serum's apolipoprotein L1), *T. evansi* and *T. congolense* are also reported sporadically infecting humans (Manivel et al. 2019). Both male and female flies can transmit disease due to blood-feeding habits; hematophagous (Brun et al. 2010). Both vertical and horizontal transmission can occur. Transmission through the sexual route, mechanical route, blood transfusion, and congenital are also reported (Franco et al. 2014).

Tsetse flies are viviparous i.e., female flies lay eggs that develop inside the uterus into larvae which are deposited into the outer environment and burrow into the soil. Larvae molt into pupae and a month later emerge as adult flies. The newly hatched *Glossina* flies are non-infectious until they feed on the infected mammalian animals from where they get Trypanosoma; trypomastigote form (Franco et al. 2014). The ingested parasites undergo differentiation in the digestive tract of the fly and migrate to salivary glands where they become infective; in metacyclic form (refer to Fig. 1). Only 0.1% of flies become infected with the mature protozoan parasites and transmit it to healthy individuals during blood-sucking. The life span of the tsetse fly is 2-3 months and can infect many people and animals during feeding after every 2-4 days (up to 10 days when the host is unavailable or climatic conditions are adverse). Once infected *Glossina* remains infective throughout its lifespan. The transmission of Trypanosomes can be decreased by controlling the spread and contact of its vector among animals and humans (Büscher et al. 2017). The infective stage of trypanosome is its metacyclic form that can cause disease in mammalian vertebrates and is characterized by the presence of a variant surface glycoprotein coat that has a protective role to prevent them from the immune system of the host (Franco et al. 2014).

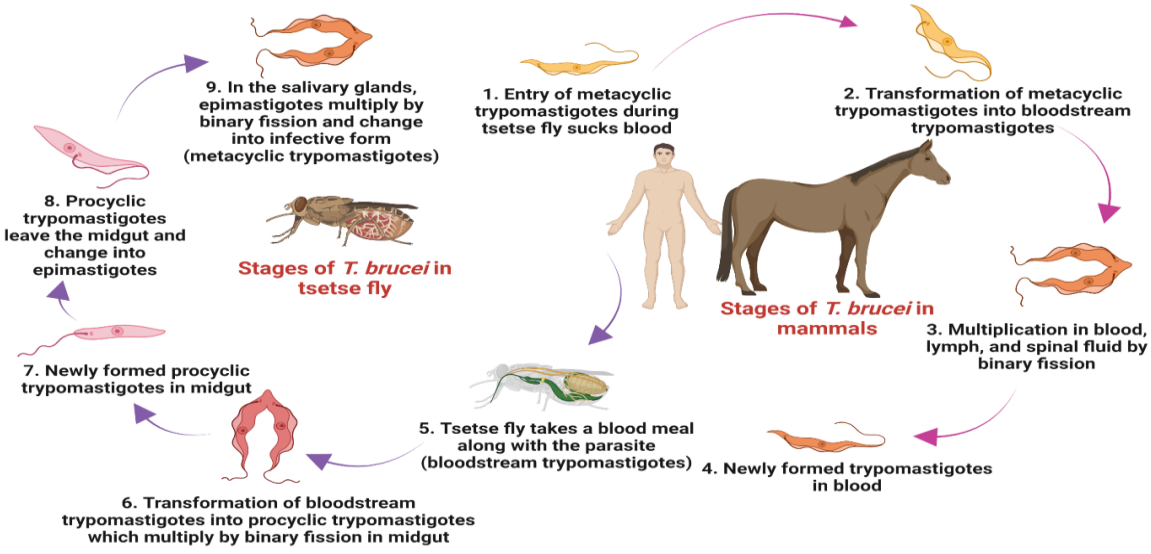

Fig.1: Life cycle of *Trypanosoma brucei*, created with BioRender

A local reaction of the bite of tsetse fly occurs which are carrying mature *T. b. gambiense* known as Trypanosomal chancre. Clinically there are two forms of this disease namely the haemolymphatic stage and the meningoencephalatic stage. The protozoa can actively cross the blood-brain barrier and lead to coma and death if therapy is not started (Simarro et al. 2008). The symptoms of the first stage of trypanosomiasis include fever of chronic origin and intermittent, pruritis, headache, splenomegaly, hepatomegaly, and lymphadenopathy. While the second stage is characterized by the presence of neuropsychiatric disorders (tremors, paralysis, improper motor coordination, emotional lability, stereotypic and aggressive behavior, confusion, deficit attention, and dementia) and disturbances of sleep (Fairlamb 2003). Nagana disease also known as African animal trypanosomiasis is caused by *T. b. brucei, T. congolense, T. evansi*, and *T. vivax* (Greca and Magez 2011). Domestic animals are severely affected by these trypanosomes which often become fatal as compared to wild animals and are clinically characterized by the presence of fever, alopecia, emaciation, lacrimation, edema, listlessness, anemia, weight loss, infertility, abortion, and paralysis (Melfi et al. 2023). With the advancement of the disease, the emaciated animals become unfit for work and hence named "N'gana"; Zulu language word that means useless or powerless (Steverding 2008). Death of some animals occurs in the acute form of the disease and the animals who are survived remain infected for months to years and act as a reservoir for the transmission of the disease (Melfi et al. 2023).

The detection of the parasite in the blood smear of infected animals is the standard method for the diagnosis of disease. For this purpose, many techniques can be employed to detect the parasite such as direct microscopy of the blood smear, concentration techniques, and inoculation of other healthy animals to check for the appearance of the symptoms (Büscher et al. 2017). Alternative to these methods is mostly based on the detection of antigen-antibody response such as enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescent antibody test, the card agglutination test for trypanosomiasis specifically for *T. evansi* (Melfi et al. 2023).

Table 1: The drugs used for the treatment of trypanosomiasis in humans and animals

Drug	Species	Stage	Route	Dose rate (mg/kg/day)	Year of first use	References
Suramin	*T. b. gambiense* and *T. b. rhodesiense*	1st	Intravenous	4-5 (day 1), 20 once a week x 5 weeks	1920s	(Melfi et al. 2023; Legros et al. 2002; Fairlamb 2003 Brun et al. 2010; Büscher et al. 2017)
Pentamidine	*T. b. gambiense*	1st	Intramuscular	4	1940	
Melarsoprol	*T. b. gambiense* and *T. b. rhodesiense*	2nd	Intravenous	2.2	1949	
Eflornithine	*T. b. gambiense*	2nd	Intravenous	400 mg/kg/day	1981	
Nifurtimox	*T. b. gambiense* and *T. b. rhodesiense*	2nd	Orally	15 mg/kg/day	1977	

The drugs used for the treatment of trypanosomiasis are old, have a toxic effect, availability is limited, and to some extent are resistant. The treatment protocols include stage-specific use of drugs (Melfi et al. 2023). For the therapy of the first stage of trypanosomiasis (Table 1), suramin and pentamidine are used with a dose rate of 5mg/kg on the first day, 10mg/kg on the third day, 20mg/kg on 5, 11, 23, and 30 days by slow intravenous injection and 4mg/kg/day daily or after one day by intramuscular injection in humans respectively (Fairlamb 2003). For the treatment of second-stage infection, three drugs are used viz melarsoprol (2.2mg/kg/day), nifurtimox (15mg/kg/day for 4 days in 3 doses), eflornithine (400mg/kg/day for 7-14 days in 4 daily infusions) (Legros et al. 2002). Eflornithine is a commercially available drug for veterinary use (Agbo et al. 2003). This review discusses the important aspects of mRNA vaccine development, the mechanism of induction of the immune response of the host against the vaccines, *T. brucei's* capability of antigenic variations, and perspective.

Current vaccine scenario

Vaccines are used to control and prevent diseases. The development of an anti-trypanosome vaccine is the ultimate target for the control of disease but for the formation of a vaccine no effective vaccine candidate has been identified (Greca and Magez 2011; Abbasi et al. 2023). The trypanosomes contain a coating of variant surface glycoprotein (VSG). Initially, this protein was considered ideal for the development of vaccines provided the antigenic variation does not occur. But later, it was realized that it could not be used as a vaccine candidate because through the rearrangement of genes parasites can produce many molecules and the immune response elicited by VSG has a short duration due to IgM production (Radwanska et al. 2008). Trypanosomes contain a flagellum that is involved in cell division, endocytosis, exocytosis, virulence, and evasion of host immune responses (Field and Carrington 2009; de Liz et al. 2023). In 1995, a study was conducted in which it was proved that vaccination of cattle with VSG from the flagellar pocket (FP) of the parasite elicits partial immunity against the infection (de Liz et al. 2023). The protection induced by FP was short-term and could not protect against the heavy burden of parasites therefore it could not serve as an effective candidate for the development of a vaccine (Radwanska et al. 2000). The cytoskeletal subcellular proteins (tubulin and actin) were also proposed to be used as a candidate for the development of the vaccine. These subcellular proteins play a key role in cell division, morphology, and movement and therefore have an important role in the bloodstream forms of the extracellular parasite (García-Salcedo et al. 2004). The immunity induced by this type of antigen does not provide memory therefore it is also regarded as a neglected vaccinal antigen.

In another study by Lubega et al. (2002), an anti-tubulin vaccination approach was proposed. This vaccine mainly targets the subcellular protein of the cytoskeleton of the trypanosomes i.e., tubulin. It induced partial protection against the infection of about 60-80% along with cross-protection of other subspecies of the genus *Trypanosoma*. The duration between the booster dose and subsequent challenge of the parasitic dose did not allow us to conclude whether it could provide long-term immunity with the production of memory cells or not (Lubega et al. 2002). Cation pumps and trans-sialidases of trypanosomes were also studied as a candidate for vaccine development. Sialidases are the enzymes (membrane-associated) that transfer sialic acids from the cell surface sialylated glycoconjugate of the host to the parasite acceptor surface molecules (Triller et al. 2023). Protection (60%) was induced with the vaccine containing plasmids encoding the N-terminal and catalytic domain of the sialidases and the immunization that targets the cation pumps of the parasites also provoked the immune response, but it did not provide long-lasting immunity (Triller et al. 2023).

Anti-disease vaccine development

The success of the development of vaccines against trypanosomiasis was limited therefore many researchers started to find alternate methods for the control of the disease. In this approach, the main purpose is to target the pathology associated with the infection. Glycosylated phosphatidyl inositol (GPI) lipid acts as an anchor that places the variant surface glycoproteins on the membrane of the trypanosomes. GPI anchor also activates the tumor necrosis factor-alpha (TNFα) which is associated with the immunopathology of the disease (Chandra et al. 2023). Environmental stress leads to the cleavage of VSG from the trypanosomes with the help of a hydrolytic enzyme; phospholipase (Radwanska et al. 2008). This hydrolytic enzyme splits GPI into glycosylated inositol phosphate (GIP) and dimyristoyl glycerol (DMG) with the first one being released while the second one remained attached to the surface of the membrane of parasites (Danazumi et al. 2022). These two components participate in the activation of immune cells. DMG primes the macrophages while GIP induces the production of TNFα making parasites sensitive to lipopolysaccharide (LPS) along with the secretory induction of interleukin-1α (Moon et al. 2022). During the infection with trypanosomes, all these things result in an increased IL-10 expression (anti-inflammatory cytokine). These also result in the disruption of pro-inflammatory mediators (IL-6, IL-12, and TNF) secretions. The production of IL-10 alleviates the disease symptoms (Stijlemans et al. 2007). The vaccination with GPI decreased the symptoms of inflammation associated with trypanosomiasis but it did not increase the survival ability of the host infected with trypanosomes.

The second anti-disease vaccine approach involved the use of the enzyme congopain (cysteine protease) from *Trypanosoma congolense*. It could elicit the production of IgG antibodies in trypanotolerant animals. The immunized cattle challenged with trypanosomes showed an increase in weight gain and less anemia as compared to non-immunized infected cattle (Pereira et al. 2022). Despite all these promising results elicited using anti-disease vaccination no further study was conducted to elaborate more on the beneficial effects of these enzymes used as a vaccine.

Reasons for the failure of the anti-trypanosome vaccine

Nature has awarded a special defensive mechanism to trypanosomes that protects them from antibody-mediated killing by host immunity (Tabel et al. 2013). The first mechanism is their ability to make changes in their antigens and the second mechanism is their ability to hind from the immune system of the host and fail the host immune system

to develop immunological memory. The antigenic variation of the trypanosomes involves the switching of their surface coat VSG to evade the host immune response (Vanhamme et al. 2001). Trypanosomes express only one VSG on their membranes making them an easy target for the antibodies but during the infection, they switch their VSG type into another type like a puzzle so that the immune system remains ineffective for their elimination. VSG appears as a mosaic that seems to be useful in dodging immune recognition. The parasite also destroys immunological memory (dysfunction of B cells) and makes them use similar molecules over time (Urquhart et al. 1973). Trypanosomes produce their antigenic variable VSG at a rate greater than the host immune destruction thus resulting in parasitemia recurrence, evasion of the host tissues, and enhancing the immunopathological changes in susceptible mammalian animals (Black and Mansfield 2016).

Challenges for identifying the appropriate candidate for vaccine development

The regimen for controlling the infection solely relies on chemotherapy and vector control measures. No vaccine is currently available for the prevention of disease. Moreover, the trypanosomes showed an increased development of resistance to the drugs used against them. Therefore, it is essential to identify the targets for the development of new drugs and vaccines. Vaccines can provide the most effective tools for preventing trypanosomiasis. The advancement in molecular biology has enabled researchers to identify the targets and detailed characteristics of *T. brucei* by using genetic markers (Agbo et al. 2001). Certain genetic methods are used to identify the survival ability and virulence of an organism such as crossing and knockout of the genes, or induced mutation. The molecule of trypanosomes proposed as a target antigen for the development of a vaccine must be genetically analyzed to assess the variation in the genes encoding that antigen (Agbo et al. 2003). The genes recognized from *T. brucei* did not match with the genes of any other organism identified so far. Therefore, the function of proteins encoded by these genes is still unknown and requires their characterization biochemical analysis or genetic analysis (Monica et al. 2022).

Expressed sequence tag (EST) technique can be used to rapidly identify the genes of *T. brucei*. The data collected from all the life cycle stages of a parasite can be useful to silence a particular gene *in vivo* so that a new candidate for the development of a vaccine or drug can be found. Moreover, these genes have no counterparts in humans therefore the vaccine or drug developed will be highly specific against the parasite (MacLeod et al. 2000). RNA interference or small interfering RNA can be used for knocking down genes rather than knockout. This technique is used to recognize the expression of trypanosome genes and their function which offer the easiest way to identify the target for the development of drugs and vaccine (Greef et al. 1989). The *T. brucei* has a complex of genes that encode the outer coating of the parasite known as variant surface glycoprotein. These genes are responsible for the evasion of the host response and obstacles to the ideal vaccinal candidate. The immune response of the host is not effective against VSG because of rapid immunological distinct variants shifting (Rudenko et al. 1998). The antigenic activity of excretory-secretory (ES) protein plays a key role in the induction of immune response in the host organisms. *T. b. brucei* contains 109 specific ES proteins which can serve as a potential target for the development of drugs or vaccines. The candidates extracted from the ES proteins of the parasite are used to identify the epitopes of T and B cells (Manivel et al. 2019). The flagellar pocket of the pathogen contains five membrane proteins involved in the exchange of cations (Na^+ and Ca^{2+}). These membranous proteins are not studied extensively for the identification of novel vaccinal or drug targets (Ramey et al. 2009).

How does the mRNA vaccine work?

A vaccine is a foreign body that provokes the immune response of the host and results in the production of antibodies. When an individual is exposed to an active infection, they fight against them and provide protective immunity (Wang et al. 2021). mRNA vaccine is a new and novel concept in molecular biology and immunology. The mRNA of the pathogen is administered to the host where it is taken up by the somatic cells and expresses a specific antigen by using the translatory machinery of the host cell (Pollard et al. 2013). The mRNA-encoding antigen induces the immune response and later gets destroyed by the host cell. The advantages of the mRNA vaccine include the safe production of antibodies in phase I human clinical trials. It is rapidly degraded by the RNases of the host cell and their fragments result in excessive activation of the host immune system (Wang et al. 2021). Moreover, it is a non-replicating vector having the characteristics of no antibiotic resistance, high immunogenic response, and integration in the genome. The handlining of the mRNA vaccine is easy and its manufacturing is rapid (Pollard et al. 2013). Different delivery platforms are available that protect mRNA from the cytoplasmic nuclease of the host cells and promote its translation by the host ribosomes. It is an intermediate hereditary substance in the central dogma carrying the genetic information necessary for the formation of proteins (Crommelin et al. 2021).

When an mRNA encoding a specific antigen for the disease is inoculated into the host elicits both humoral immunity and cell-mediated immunity (Xu

et al. 2020). The innate immune system plays a pivotal role in the protection of the host from pathogenic diseases. This system detects pathogen-associated molecular patterns (PAMPs) from the exogenous substances through pattern recognition receptors (PRRs). Antigen-presenting cells contain a high number of PRRs but are also present on the surface of other cells (Crommelin et al. 2021). After this proinflammatory chemokines and cytokines are expressed by the intracellular signaling pathway cascade. The cells differently sense the self RNA and non-self RNA. To induce adaptive immune response, there are two PRR types present that detect PAMPs either in the blood or extracellular fluid. Toll-like receptors (TLRs) recognize the RNA inside the endosomes (Kato et al. 2008). The self-single-stranded RNA is recognized by TLR-7 and TLR-8 while the self-double-stranded RNA is recognized by TLR-3 and induces the production of myeloid differentiation marker 88 and interferon-β and proinflammatory cytokines respectively (Crommelin et al. 2021). The second type of PRR namely retinoic acid-inducible gene-I-like receptors, nucleotide oligomerization domain-like receptors, RNA-dependent protein kinase, and oligoadenylate synthetase receptors are involved in the recognition of exogenous or non-self RNA. These are involved in the production of interferons, and proinflammatory cytokines (Kato et al. 2008).

Host immune responses during trypanosomiasis

The immune responses include innate immune response and adaptive immune response which provide non-specific and specific immunity respectively. The body's immune response against extracellular *T. brucei* is innate and adaptive immunity which is discussed below (Rottenberg et al. 1993).

Innate immunity

When the tsetse fly draws blood from the host, it injects the parasite into the bloodstream of the host through its salivary secretions. Initially, trypanosomatids face the innate immune response of the host as a first barrier (Tabel et al. 2013). Human and other primates' serum contains two types of trypanolytic factor (TLF) namely TLF-1 and TLF-2 that aid in the primary immune response. Both these factors comprise apolipoprotein L-I and haptoglobin-related protein (Uzureau et al. 2013). The inflammatory response is initiated by the different factors of the parasite. The classical activation of the macrophages occurs through the DNA of a deadly parasite that results in the production of IL-12, nitric oxide (NO), and TNF (Baral 2010). The involvement of TLR showed that the DNA of trypanosomes plays a role in the advancement of the disease. The GPI anchor also encounters macrophages leading to proinflammatory cytokines production (Tachado and Schofield 1994). Hence the first line of defense against trypanosomiasis includes the classical activation of macrophages which secrete immunomodulatory molecules such as NO, TNF, IL-1, and IL-6 (Pan et al. 2006).

Adaptive immune response

During the early stage of the disease, the inflammatory response of the host is beneficial but at the late or chronic stage, it becomes pathologic. So, it becomes essential for the body to limit inflammation by down-regulating the production of macrophages. IL-4, IL-10, and IL-13 are the second type of cytokines which modulate the macrophages result in anti-inflammatory types and increase the survival of the individuals (Namangala et al. 2009). This shifting of response from type 1 to type 2 is necessary for the host and its capacity depends on the pathology of the organism (Baral 2010). IL-4 is associated with the control of *T. b. gambiense* and IL-10 and IL-13 are associated with the control of the pathology of brains in the case of human African trypanosomiasis (Sternberg et al. 2005). During the infection, polyclonal B cell production starts which protects the animals from the successive infection in a trypanotolerant host. The victim infected with the homologous trypanosomes remains protected. The antigenic variant property of the VSG of trypanosomes results in the exhaustion of antibody production which ultimately leads to failure of the clearance of the parasitemia (Hudson and Terry 1979).

Future directions

At present, for the control of infection three strategies are adopted (i) vector control, (ii) vaccination, and (iii) treatment of the infected host (Maxfield and Bermudez, 2023). Vaccines for the prevention of trypanosomiasis have a goal to make animals and humans immunized from the pathogen (Favaro et al. 2023). At present millions of people and animals are at risk of getting human African trypanosomiasis and nagana disease and the drugs used for the therapy cause a post-therapeutic change; encephalopathy (Pays et al. 2023). The entire eradication of the reservoir parasites from endemic areas seems to be impossible. The vaccine is the sole method that is also economically feasible to provide long-lasting immunity in individuals. The immunological control of the disease is also possible because some people who live in endemic areas become trypanotolerant which gives the idea that the immunological intervention could limit the morbidity and mortality of the disease (Favaro et al. 2023). Previously the attempts made for the development of an effective vaccine failed due to the genetic variant property of trypanosomes. They contain a protective coating made up of variant surface glycoproteins. The parasite makes changes in this coating upon exposure to the immune system of the host (Krishna et al. 2023).

mRNA-based vaccines are promising because they are cost-efficient, have a low risk of mutation, rapid production, and easy storage. In past years, many mRNA-based vaccines had entered clinical trials and proved protective (Le et al. 2022). Due to the failure of all conventional methods of vaccination that provide partial or even no immunity, now it's time to move for the development of a mRNA-based vaccine. The advancement in molecular biology and immunology can be beneficial for the identification of new target antigens. In the future, the mRNA vaccine can give promising protection from infection and will help in preventing the disease.

Conclusion

It is evident that many studies have been conducted to find the appropriate target antigen for the development of the vaccine, but none has achieved 100% success. This disease poses a serious health risk to both animals and humans living in the endemic areas. Tsetse flies act as a vector for the transmission of parasites. Trypanosomes have antigenic variation characteristics that prevent the memory of the host. Therefore, the identification of new candidates for vaccines is necessary. Due to the many benefits of the mRNA vaccine, it could draw the attention of researchers for further investigations. The mRNA-based vaccinal approach can prevent the disease but till now no study was conducted regarding the concept.

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References

Maxfield L and Bermudez R, 2023. Trypanosomiasis. In: StatPearls Treasure Island (FL): StatPearls Publishing; 2023 Jan.

de Liz LV, Stoco PH and Sunter JD, 2023. Cell-to-flagellum attachment and surface architecture in kinetoplastids. Trends in Parasitology 39(5):332-344. doi: 10.1016/j.pt.2023.02.009.

Chandra M, Đaković S, Foti K, Zeelen JP, van Straaten M, Aresta-Branco F, Tihon E, Lübbehusen N, Ruppert T, Glover L, Papavasiliou FN and Stebbins CE, 2023. Structural similarities between the metacyclic and bloodstream form variant surface glycoproteins of the African trypanosome. PLoS Neglected Tropical Diseases17(2):e0011093. doi: 10.1371/journal.pntd.0011093.

Pays E, Radwanska M and Magez S, 2023. The Pathogenesis of African Trypanosomiasis. Annual Review of Pathology 18:19-45. doi: 10.1146/annurev-pathmechdis-031621-025153.

Krishna CK, Franke L, Erdmann R and Kalel VC, 2023. Isolation of Glycosomes from Trypanosoma brucei. Methods in Molecular Biology 2643:33-45. doi: 10.1007/978-1-0716-3048-8_3.

Abbasi Shiran J, Ghanbari M, Mohammadnejadi E and Razzaghi-Asl N, 2023. Structural Insight into Privileged Heterocycles as Anti-*Trypanosoma cruzi* and *brucei* Agents. Current Topics in Medicinal Chemistry 23(9):736-752. doi: 10.2174/1568026623666230201103843.

Favaro A, Bolcato G, Comini MA, Moro S, Bellanda M and Sturlese M, 2023. Drugging the Undruggable *Trypanosoma brucei* Monothiol Glutaredoxin 1. Molecules 28(3):1276. doi: 10.3390/molecules28031276.

Agbo EC, Majiwa PA, Büscher P, Claassen E and te Pas MF, 2003. *Trypanosoma brucei* genomics and the challenge of identifying drug and vaccine targets. Trends in Microbiology 11(7):322-9. doi: 10.1016/s0966-842x(03)00151-3.

Agbo EC, Majiwa PA, Claassen EJ and Roos MH, 2001. Measure of molecular diversity within the *Trypanosoma brucei* subspecies Trypanosoma brucei brucei and Trypanosoma brucei gambiense as revealed by genotypic characterization. Experimental Parasitology 99(3):123-31. doi: 10.1006/expr.2001.4666.

Silva Pereira S, De Niz M, Serre K, Ouarné M, Coelho JE, Franco CA and Figueiredo LM, 2022. Immunopathology and *Trypanosoma congolense* parasite sequestration cause acute cerebral trypanosomiasis. Elife 11:e77440. doi: 10.7554/eLife.77440.

Baral TN, 2010. Immunobiology of African trypanosomes: need of alternative interventions. Journal Biomedicine Biotechnology 2010:389153. doi: 10.1155/2010/389153.

Black SJ and Mansfield JM, 2016. Prospects for vaccination against pathogenic African trypanosomes. Parasite Immunology 38(12):735-743. doi: 10.1111/pim.12387.

Brun R, Blum J, Chappuis F and Burri C, 2010. Human African trypanosomiasis. Lancet 375(9709):148-59. doi: 10.1016/S0140-6736(09)60829-1.

Büscher P, Cecchi G, Jamonneau V and Priotto G. Human African trypanosomiasis. Lancet 390(10110):2397-2409. doi: 10.1016/S0140-6736(17)31510-6.

Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W and Mastrobattista E, 2021. Addressing the Cold Reality of mRNA Vaccine Stability. Journal of Pharmaceutical Science 110(3):997-1001. doi: 10.1016/j.xphs.2020.12.006.

De Greef C, Imberechts H, Matthyssens G, Van Meirvenne N and Hamers R, 1989. A gene expressed only in serum-resistant variants of *Trypanosoma brucei rhodesiense*. Molecular and Biochemical Parasitology 36(2):169-76. doi: 10.1016/0166-6851(89)90189-8

Sá M, Costa DM and Tavares J, 2022. Imaging Infection by Vector-Borne Protozoan Parasites Using Whole-Mouse Bioluminescence. Methods in Molecular Biology 2524:353-367. doi: 10.1007/978-1-0716-2453-1_29.

Fairlamb AH, 2003. Chemotherapy of human African trypanosomiasis: Current and future prospects. Trends in Parasitology 19(11): 488–494. https://doi.org/10.1016/j.pt.2003.09.002

Field MC and Carrington M, 2009. The trypanosome flagellar pocket. Nature Reviews Microbiology 7(11):775-86. doi: 10.1038/nrmicro2221.

Danazumi AU, Iliyasu Gital S, Idris S, Bs Dibba L, Balogun EO and Górna MW, 2022. Immunoinformatic design of a putative multi-epitope vaccine candidate against *Trypanosoma brucei gambiense*. Computational and Structural Biotechnology Journal20:5574-5585. doi: 10.1016/j.csbj.2022.10.002.

Franco JR, Simarro PP, Diarra A and Jannin JG, 2014. Epidemiology of human African trypanosomiasis. Clinical Epidemiology 6:257-75. doi: 10.2147/CLEP.S39728.

García-Salcedo JA, Pérez-Morga D, Gijón P, Dilbeck V, Pays E and Nolan DP, 2004. A differential role for actin during the life cycle of *Trypanosoma brucei*. EMBO Journal 23(4):780-9. doi: 10.1038/sj.emboj.7600094.

La Greca F and Magez S, 2011. Vaccination against trypanosomiasis: can it be done or is the trypanosome truly the ultimate immune destroyer and escape artist? Human Vaccines 7(11):1225-33. doi: 10.4161/hv.7.11.18203.

Hudson KM and Terry RJ, 1979. Immunodepression and the course of infection of a chronic *Trypanosoma brucei* infection in mice. Parasite Immunology 1(4):317-26. doi: 10.1111/j.1365-3024.1979.tb00717.x.

Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T and Akira S, 2008. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. Journal of Experimental Medicine 205(7):1601-10. doi: 10.1084/jem.20080091.

Le T, Sun C, Chang J, Zhang G and Yin X, 2022. mRNA Vaccine Development for Emerging Animal and Zoonotic Diseases. Viruses 14(2):401. doi: 10.3390/v14020401.

Legros D, Ollivier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J and Büscher P, 2002. Treatment of human African trypanosomiasis--present situation and needs for research and development. Lancet Infectious Diseases 2(7):437-40. doi: 10.1016/s1473-3099(02)00321-3.

Lubega GW, Byarugaba DK and Prichard RK, 2002. Immunization with a tubulin-rich preparation from Trypanosoma brucei confers broad protection against African trypanosomosis. Experimental Parasitology 102(1):9-22. doi: 10.1016/s0014-4894(02)00140-6.

MacLeod A, Tweedie A, Welburn SC, Maudlin I, Turner CM and Tait A. Minisatellite marker analysis of *Trypanosoma brucei*: reconciliation of clonal, panmictic, and epidemic population genetic structures. Proceedings of the National Academy of Science USA 97(24):13442-7. doi: 10.1073/pnas.230434097.

Moon S, Janssens I, Kim KH, Stijlemans B, Magez S and Radwanska M, 2022. Detrimental Effect of Trypanosoma brucei brucei Infection on Memory B Cells and Host Ability to Recall Protective B-cell Responses. Journal of Infectious Diseases 226(3):528-540. doi: 10.1093/infdis/jiac112. PMID: 35363871.

Manivel G, Meyyazhagan A, Durairaj D R and Piramanayagam S, 2019. Genome-wide analysis of Excretory/Secretory proteins in *Trypanosoma brucei brucei*: Insights into functional characteristics and identification of potential targets by immunoinformatics approach. Genomics 111(5):1124-1133. doi: 10.1016/j.ygeno.2018.07.007.

Namangala B, De Baetselier P and Beschin A, 2009. Both type-I and type-II responses contribute to murine trypanotolerance. Journal of Veterinary Medical Science 71(3):313-8. doi: 10.1292/jvms.71.313.

Melfi F, Carradori S, Campestre C, Haloci E, Ammazzalorso A, Grande R and D'Agostino I, 2023. Emerging compounds and therapeutic strategies to treat infections from *Trypanosoma brucei*: an overhaul of the last 5-years patents. Expert Opinion in Therapeutic Patents 33(3):247-263. doi: 10.1080/13543776.2023.2193328.

Okomo-Assoumou MC, Daulouede S, Lemesre JL, N'Zila-Mouanda A and Vincendeau P, 1995. Correlation of high serum levels of tumor necrosis factor-alpha with disease severity in human African trypanosomiasis. The American Journal of Tropical Medicine and Hygiene 53(5):539-43. doi: 10.4269/ajtmh.1995.53.539.

Pan W, Ogunremi O, Wei G, Shi M and Tabel H, 2006. CR3 (CD11b/CD18) is the major macrophage receptor for IgM antibody-mediated phagocytosis of African

trypanosomes: diverse effect on subsequent synthesis of tumor necrosis factor alpha and nitric oxide. Microbes and Infections 8(5):1209-18. doi: 10.1016/j.micinf.2005.11.009.

Pollard C, De Koker S, Saelens X, Vanham G and Grooten J, 2013. Challenges and advances towards the rational design of mRNA vaccines. Trends in Molecular Medicine 19(12):705-13. doi: 10.1016/j.molmed.2013.09.002.

Radwanska M, Guirnalda P, De Trez C, Ryffel B, Black S and Magez S, 2008. Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. PLoS Pathogy 4(5):e1000078. doi: 10.1371/journal.ppat.1000078.

Radwanska M, Magez S, Dumont N, Pays A, Nolan D and Pays E, 2000. Antibodies raised against the flagellar pocket fraction of *Trypanosoma brucei* preferentially recognize HSP60 in cDNA expression library. Parasite Immunology 22(12):639-50. doi: 10.1046/j.1365-3024.2000.00348.x.

Ramey K, Eko FO, Thompson WE, Armah H, Igietseme JU and Stiles JK, 2009. Immunolocalization and challenge studies using a recombinant Vibrio cholerae ghost expressing *Trypanosoma brucei* Ca(2+) ATPase (TBCA2) antigen. The American Journal of Tropical Medicine and Hygiene 81(3):407-15.

Rottenberg ME, Bakhiet M, Olsson T, Kristensson K, Mak T, Wigzell H and Orn A, 1993. Differential susceptibilities of mice genomically deleted of CD4 and CD8 to infections with *Trypanosoma cruzi* or *Trypanosoma brucei*. Infection and Immunity 61(12):5129-33. doi: 10.1128/iai.61.12.5129-5133.1993.

Rudenko G, Cross M and Borst P, 1998. Changing the end: antigenic variation orchestrated at the telomeres of African trypanosomes. Trends in Microbiology 6(3):113-6. doi: 10.1016/s0966-842x(97)01200-6.

Triller G, Vlachou EP, Hashemi H, van Straaten M, Zeelen JP, Kelemen Y, Baehr C, Marker CL, Ruf S, Svirina A, Chandra M, Urban K, Gkeka A, Kruse S, Baumann A, Miller AK, Bartel M, Pravetoni M, Stebbins CE, Papavasiliou FN and Verdi JP, 2023. A trypanosome-derived immunotherapeutics platform elicits potent high-affinity antibodies, negating the effects of the synthetic opioid fentanyl. Cell Rep. 2023 Jan 30;42(2):112049. doi: 10.1016/j.celrep.2023.112049.

Silva MS, Prazeres DM, Lança A, Atouguia J and Monteiro GA, 2009. Trans-sialidase from *Trypanosoma brucei* as a potential target for DNA vaccine development against African trypanosomiasis. Parasitological Research 105(5):1223-9. doi: 10.1007/s00436-009-1542-6.

Simarro PP, Jannin J and Cattand P, 2008. Eliminating human African trypanosomiasis: where do we stand and what comes next? PLoS Medicine 5(2):e55. doi: 10.1371/journal.pmed.0050055.

Sternberg JM, Rodgers J, Bradley B, Maclean L, Murray M and Kennedy PG, 2005. Meningoencephalitic African trypanosomiasis: Brain IL-10 and IL-6 are associated with protection from neuro-inflammatory pathology. Journal of Neuroimmunology 167(1-2):81-9. doi: 10.1016/j.jneuroim.2005.06.017.

Steverding D, 2008. The history of African trypanosomiasis. Parasite and Vectors 1(1):3. doi: 10.1186/1756-3305-1-3.

Stijlemans B, Baral TN, Guilliams M, Brys L, Korf J, Drennan M, Van Den Abbeele J, De Baetselier P and Magez S, 2007. A glycosylphosphatidylinositol-based treatment alleviates trypanosomiasis-associated immunopathology. Journal of Immunology 179(6):4003-14. doi: 10.4049/jimmunol.179.6.4003.

Tabel H, Wei G and Bull HJ, 2013. Immunosuppression: cause for failures of vaccines against African Trypanosomiases. PLoS Neglected Tropical Diseases 7(3):e2090. doi: 10.1371/journal.pntd.0002090.

Tachado SD and Schofield L, 1994. Glycosylphosphatidylinositol toxin of *Trypanosoma brucei* regulates IL-1 alpha and TNF-alpha expression in macrophages by protein tyrosine kinase mediated signal transduction. Biochemical and Biophysical Research Communications 205(2):984-91. doi: 10.1006/bbrc.1994.2763.

Urquhart GM, Murray M, Murray PK, Jennings FW and Bate E, 1973. Immunosuppression in *Trypanosoma brucei* infections in rats and mice. Transactions of the Royal Society of Tropical Medicine and Hygiene 67(4):528-35. doi: 10.1016/0035-9203(73)90083-7.

Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homblé F, Grélard A, Zhendre V, Nolan DP, Lins L, Crowet JM, Pays A, Felu C, Poelvoorde P, Vanhollebeke B, Moestrup SK, Lyngsø J, Pedersen JS, Mottram JC, Dufourc EJ, Pérez-Morga D and Pays E, 2013. Mechanism of *Trypanosoma brucei* gambiense resistance to human serum. Nature 501(7467):430-4. doi: 10.1038/nature12516.

Vanhamme L, Pays E, McCulloch R and Barry JD, 2001. An update on antigenic variation in African trypanosomes. Trends in Parasitology 17(7):338-43. doi: 10.1016/s1471-4922(01)01922-5.

Wang Y, Zhang Z, Luo J, Han X, Wei Y and Wei X, 2021. mRNA vaccine: a potential therapeutic strategy. Molecular Cancer 20(1):33. doi: 10.1186/s12943-021-01311-z.

Xu S, Yang K, Li R and Zhang L, 2020. mRNA Vaccine Era-Mechanisms, Drug Platform and Clinical Prospection. International Journal of Molecular Science 21(18):6582. doi: 10.3390/ijms21186582.