Review on Immunity to African Trypanosomes

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ABSTRACT

Trypanosomiasis is a disease of varying severity, it is caused by a unicellular flagellated protozoan parasite of the family Trypanosomatidae, and genus Trypanosoma. This study review the mouse models to examined and analyze infections with African trypanosomes which provides an insight on how the possible mechanisms by which African trypanosomes can be destroyed. With the introduction of gene targeted mouse, immune response in humans that are potentially suggestive of protective immunity, can be tested in mouse models to understand and verify the importance of particular immunological pathway. When comparing the susceptibility and resistance of mouse strains infected with African trypanosomes, the balance between Th1 cytokines such as TNF, IFNγ, and the induction of nitric oxide release in addition to IL 10 appear to be very important.

Key Words:

Trypanosomiasis; Immunity; Africa ;trypanosome lytic factors

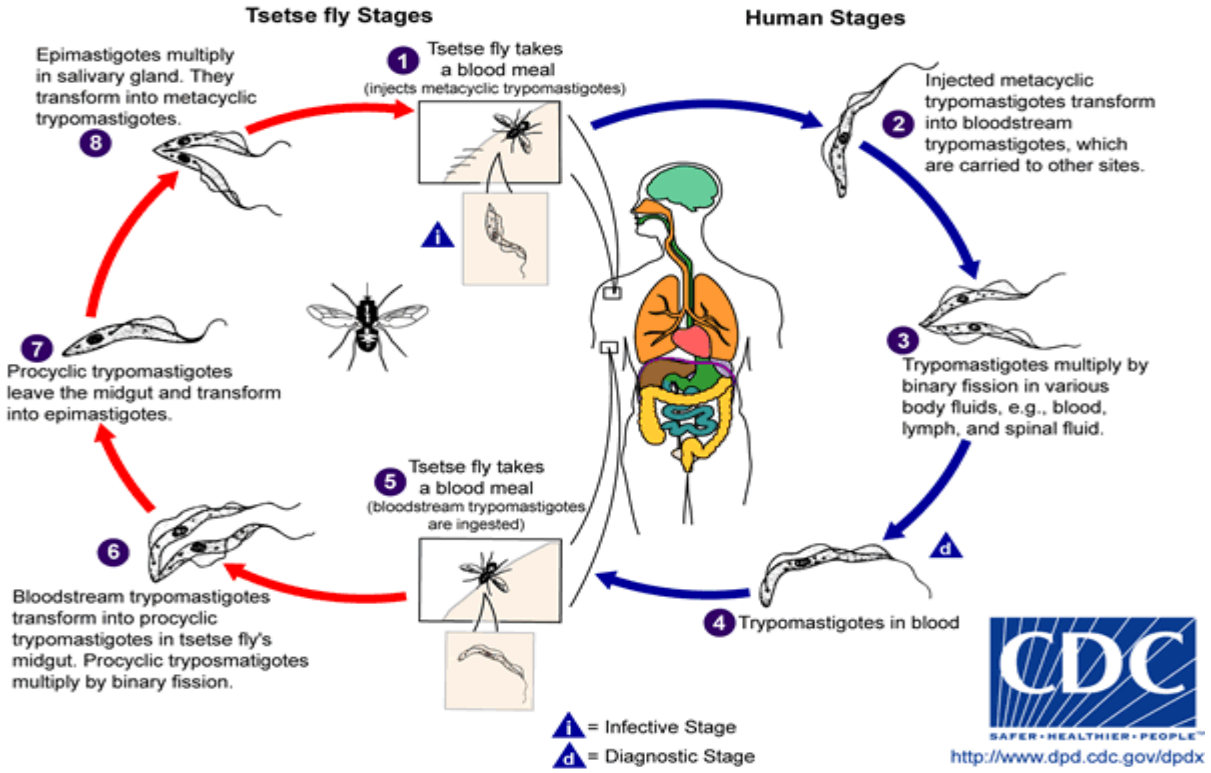
INTRODUCTION

Trypanosomiasis is a disease of varying severity, it is caused by a unicellular flagellated protozoan parasite of the family *Trypanosomatidae*, and genus *Trypanosoma* (Baral, 2009). Two subspecies of *Trypanosoma brucei*; the *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are the etiological agents of human African trypanosomiasis (Sternberg, 2004). *Trypanosoma brucei gambiense* are spread widely in about 24 countries in central and west Africa (World Health Organisation [WHO], 2013), while *Trypanosoma brucei rhodesiense* is found in 13 countries in south and east Africa (W.H.O, 2013). These pathogenic microorganisms are the most important extracellular protozoan parasites (Wakelin, 1996) due to their ability of colonising an unfavourable environment to most parasitic organisms (Wakelin, 1996). The disease is spread to humans by the bite of an infected tsetse fly of the genus *Glossina* (Moore, 2013), which cause a recurring transmission pattern to both human and various vertebrate hosts. However, studies reveal that transmission by congenital and blood borne forms are rare (Moore, 2013).

These parasites are the etiological agents of sleeping sickness in humans (Magez & Caljon, 2011) observed during the late stage of the disease, but animals such as Cattle may also serve as reservoir hosts (W.H.O, 2013) where alot of economic loss are evident in endemic areas (W.H.O, 2013). A third subspecie, the *T.b brucei* and numerous other species including; *T. congolense, T. evansi, T.equipadum* and *T. vivax* are responsible for animal forms of the disease otherwise called Nagana and

Surra (Baral, 2009; Singh et al., 2013). Both the human and animal forms of the disease affects the Central Nervous System during the late stage of infection (Baral, 2009; W.H.O, 2013). Infections by *T.b rhodesiense* in the south and central Africa is characterised by high fever, skin rash, headache, thrombocytopenia, and more rarely renal failure and cardiac dysfunction which are both manifested between one to three weeks of infection (Moore, 2013). On the other hand, however, infections by *T.b gambiense* are characterised by fever, malaise, headache, facial oedema, inflammation of the lymph nodes (lymphadinopathy), pruritis and infection of parts of the brain, the Central Nervous System (CNS), resulting in endocrine disorders and change in behaviour which are less severe when compared to those seen in infections by *T.b rhodesiense* (Moore, 2013; Baral, 2009 & W.H.O, 2013). The symptoms presented by *T.b rhodesiense* are non specific, while those presented by *T.b gambiense* can only be observed months after transmission.

During blood meal, the "*metacyclic trypomastigote*" form of the parasite are inoculated together with the salivary secretions of the vector into the blood stream of the vertebrate host where they undergo asexual reproduction to proliferate and increase in number (this is the diagnostic stage of the parasite). These trypomastigotes are ingested by another vector when it comes for a blood meal on an infected individual, which are then passed on to the insect's midgut where an asexual reproduction occur to produce numerous "*procyclic trypomastigostes*" that leave the midgut and transform to "*epimastigotes*" which migrate to the insect's salivary gland to multiply and tranform into the "*infective metacyclic trypomastigotes*" (Center for Disease Control [CDC], 2012) .



Extracted from CDC website.

Because the characterisation of new drugs and understanding the immunological aspects of parasitic infections using human is unethical, and animal models remain to be the best choice in understanding

immunity due to their availability and simplicity in handling, and matching physiology to humans, this paper will analyse how different species of African trypanosomes are controlled *In-vivo* using laboratory mouse as experimental models.

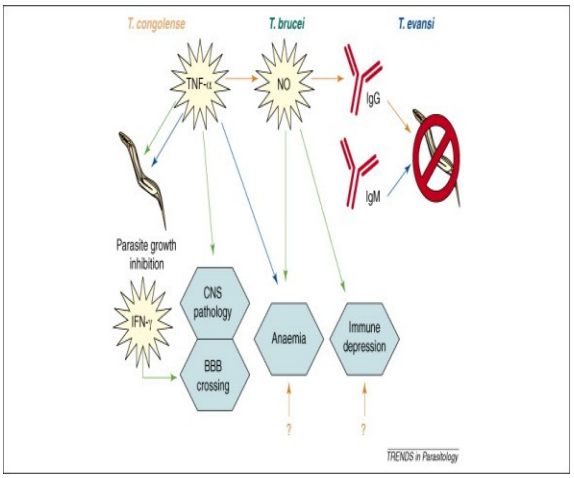
2.0 OVERVIEW OF THE IMMUNE RESPONSE TO AFRICAN TRYPANOSOMES

The host immune system that is triggered due to infections by *T.brucei* subspecies and other Trypanosome species are the B and T lymphocytes. These immune cells responds to the parasite's Variant Surface Glycoprotein (VSG) (Sternberg, 2004). Upon injection into the circulating blood, the trypanosome's VSG peptide-MHC class II activates the lymphocytes' antigen presenting cells (APCs) which include the dendritic cells and macrophages to respond by producing a Th1 cytokine response (Sternberg, 2004). These reaction include the discharge of gamma interferons (IFN-γ), whose action are to activate tissue macrophages to produce trypanocidal effects which include reactive nitrogen intermediates (RNI), reactive oxygen intermediates (ROI), tumor necrosis factor alpha (TNF-α) together with numerous other molecules capable of destroying the parasite in extracellular tissues (Baral et al., 2006).

According to Vanhamme (2004) and Baral (2009) the human serum and those of other primates have the potentials of killing trypanosomes *In-vitro*, and these effects are termed trypanosome lytic factors (TLF), which are said to be powerful, naturally occuring toxins in the human serum capable of providing sterile protection against infection by several African trypanosome species (Natalie et al., 2012). The TLF provide this protection by entering the trypanosme through binding to the parasite's haptoglobin haemoglobin receptor (HpHbR), thereby trafficking the lysosome, thus resulting in lysosomal membrane damage, which leads to the eventual release of toxins from the lysosome that are harmful to the parasite resulting into cell lysis (Pays & Vanhollebeke, 2009). Additional description of TLF in the human serum reveal that the factor is associated with high density lipoproteins (HDL), and endocytosis of the particles of HDL by the trypanosome is required for lysis (Baral, 2009). Two TLF complexes (TLF-1 and TLF-2) have been shown to be present in the human serum, each of which has a different composition. On one hand, TLF-1, is a 500kDa high density lipoprotein complex made up of apolipoprotein A1 (apoA1), apolipoprotein AII (apoAII), apolipoprotein L1(apoL1), haptoglobin related protein (Hpr), human cathelicidin antimicrobial peptide 18 (hCAP 18), GPI-specific phospholipase D (GPI-PLD) and paraoxanase (Hadjuk et al., 1989 and Smith et al., 1995 cited in Baral, 2009). On the other hand, TLF-2 is a 1000kDa poor lipid immunocomplex, composed of all the constituents of TLF-1 except (apo AII), and paraoxanase, but contain, in addition, an immunoglobulin M (IgM) (Molina-portela et al., 2005; Pays & Vanhollebeke, 2009).

Of all the components of TLF-1 and TLF-2, Hpr was thought for long as the active trypanolytic component of the TLF (Lugli et al., 2004), because it was considered to be recognised by the variant surface glycoprotein of the trypanosome, due to the fact that contending aggregate of the related protein haemoglobin can hinder trypanolysis *In- vitro* (Pays & Vanhollebeke, 2009), and that Hpr is not found in the serum of chimpanzees which lack trypanolytic ability (Lugli et al., 2004). Numerous studies are against these idea, noting that apoL-1 is the main trypanolytic factor of the normal human serum (NHS) (Poelvoorde et al., 2004; Vanhamme & Pays, 2004; Vanhamme et al., 2003; Baral et al., 2006; Perez-Morga et al., 2005). However, other scientific scholars are of the opinion that apoL-1

must be combined with Hpr for which HDL provide the platform for maximum trypanolytic activity to be achieved (Shiflett et al., 2005). Moreover, Molina-Portela et al. (2008) indicates that Hpr, apoA-1 and apo L-1 acting together have the maximum killing potential of the parasite. These idea was supported by a recent study on some close relatives to human (the baboons) showing that the homologous gene sequences of Hpr and apoL-1 are present in the sera of baboons, and when expressed together with apoA-1, maximum protection is provided against infection by *T. b. rhodesiense* (Thomson et al., 2010).



"Diagram of major differences among known pathogenesis and defence mechanisms for different 'African trypanosomiasis' agents. The most remarkable differences in trypanosome pathogenesis and host defence mechanisms are schematically represented for T. congolense (orange arrows), T. brucei (green arrows) and T. evansi (blue arrows). These include: (i) the absence of involvement of TNF or NO in anaemia and immune-depression development, and the absence of Blood Brain Barriers crossing and CNS pathology for the strictly intravascular T. congolense compared with the major pathological role of TNF, NO and interferon-γ (IFN-γ) in T. brucei infection; (ii) the direct effect of TNF on T. brucei growth but lack of major involvement of TNF in global defence against T. brucei and T. evansi in contrast to T. congolense; (iii) and the preponderance of IgM in T. evansi clearance compared to the pivotal role of IgG in T. congolense infection".

Extracted from (Antoine-Moussiaux et al., 2008).

3.0 MOUSE MODEL AS A TOOL FOR UNDERSTANDING INNATE IMMUNITY AGAINST AFRICAN TRYPANOSOMIASIS.

In order to evaluate and compare the susceptibility and resistance of infections associated with pathogenesis of trypanosomiasis and test new drug therapies, small animal models were used. Studies reveal that the laboratory mice C57Bl/6 were used because they mimic the disease in cattles, and results indicate that they are relatively resistant to numerous waves of parasitaemia because they clear the infection and survive 30-120 days post-infection (Singh et al., 2013). On the other hand, BALB/C mice were found to be highly susceptible and gave up between 3-10 days post-infection without controlling the first wave of parasitaemia (Oguuremi & Tabel, 1995 cited in Singh et al., 2013).

Macrophages being an antigen presenting cells play a key role in initiating early defence against several pathogenic microorganisms through phagocytosis and secretion of proinflammatory cytokines, and the effect of macrophages on parasitic microorganisms were shown to be associated with the changes in their inducible nitric oxide synthase (iNOS) gene expression and the production of nitric oxide (NO) (Singh et al., 2013). The role of T-cells and the cytokines they produce are therefore important in evaluating these idea. T-cell suppression appear to be one of the initial assurances of trypanosomiasis (Tabel et al., 2008). The difference in the pathogenecity of genetically different strains of *T. brucei*

have also been attributed to T-cell signalling (Morrison et al., 2010). Both CD8+ and CD4+ T-cells were implicated in initiating defence against infection by African trypanosomes. An interesting hypothesis suggest that IFNγ production may possibly be initiated by CD8+ T-cells during early infection with trypanosomes (Magez & Caljon, 2011), while in the chronic state of infection, IFNγ would be secreted by a delayed CD4+ T-cell populations that has arisen from continous antigenic stimulation (Magez & Caljon, 2011).

3.1 THE ROLE OF INTERFERON gamma (IFNγ) ON IMMUNITY AGAINST AFRICAN TRYPANOSOMES.

IFNγ and TNF were shown to be the most important cytokines in clearing infections caused by African trypanosomes (Beutler et al., 1985; Beutler *et al.,* 1986 cited in (Magez & Caljon, 2011). IFNγ "*knock out*" mice was revealed to be susceptible to infection with *T. brucei rhodesiense*, resulting in high parasitaemia and eventual death (Hertz et al., 1998). B10.Br and C57BL/6 mice were shown to be resistant and epressed Th1 cytokine in response to VSG stimulation, while C3H mice was revealed to be susceptible because they expressed poor or no Th1 response. Hertz et al., (1998) again show that neither C3H the susceptible nor B10.Br and C57BL/6 being the resistant forms express Th2 response to the parasites antigen. In the same vain however, C57BL/6-IFNγ knock out mice were revealed to be as susceptible as C57BL/6 scid mice, whereas C57BL/6-IL-4 knock out mice remained to be resistant equally as the wild strain because they express IL-2 and IFNγ. This was examined to determine the potential role of IFNγ and IL-4 in hosts resistance (Hertz et al., 1998). However, reistance and susceptibility are linked to the genetic make up of the mouse model (Rani et al., 2013). In a culture analysis of the spleen, IFNγ when produced in excess appear to hinder the parasite's antigen-driven and mitogen driven T-cell production (Darji et al., 1996). When C57BL/6-IFNγ knock out mice were injected with spleen from wild type strain, results revealed that both the wild type and C57BL/6-IFNγ knock out controlled the infection with VSG specific antibody responses, although the C57BL/6-IFNγ knock out show high level of parasitaemia (Hertz et al., 1998).

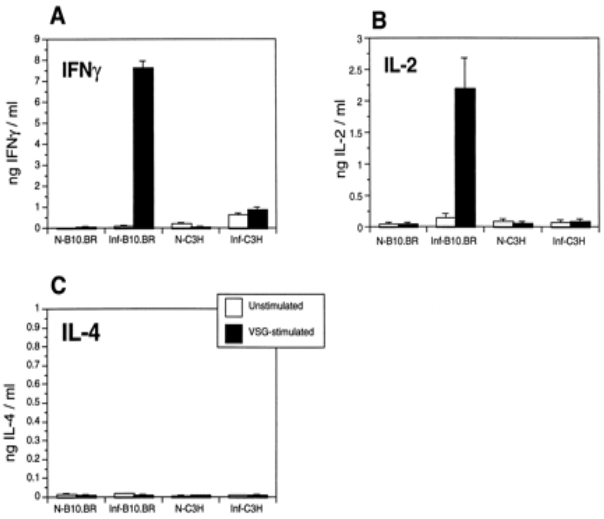


FIGURE 1 *"T cell cytokine responses in B10.BR/SgSnJ and C3HeB/FeJ mice infected with T. brucei rhodesiense. 1. ELISA assays for IFN-γ (A), IL-2 (B), and IL-4 (C) PC from normal and 2-wk-infected mice were cultured in tissue culture medium alone or medium with VSG (50 μg/ml). Culture supernatant fluids were harvested after 24 h of incubation and assayed for cytokines; values ± SEM are shown. CTLL-2 bioassays for IL-2 and IL-4 were consistent with the ELISA results"*

Extracted from (Hertz et al., 1998).

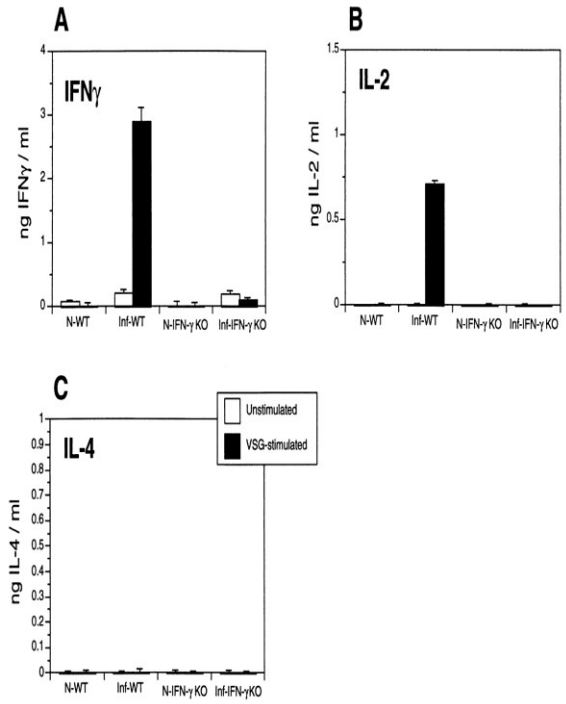


FIGURE 2 *"T cell cytokine responses of C57BL/6 wild-type (WT) and C57BL/6 IFN-γ knockout mice infected with T. brucei rhodesiense. ELISAs for IFN-γ (A), IL-2 (B), and IL-4 (C). PC from normal (N) and 2-wk-infected (Inf) mice were cultured in medium alone or medium with VSG (50 μg/ml). Culture supernatant fluids were harvested after 24-h incubation and assayed for cytokine levels (±SEM). The CTLL-2 bioassays for IL-2 and IL-4 were consistent with ELISA results, except that low levels of IL-2 (but not IL-4) were detectable in the culture fluids of VSG-stimulated PC from IFN-γ KO mice".*

Extracted from (Hertz et al., 1998).

IFNγ was shown to induce macrophages to produce inducible nitric oxides which inturn causes the release of reactive nitrogen intermediates such as nitric oxides which has full trypanotoxic effect resulting in lysis (Rani et al., 2013). Although, Rani et al., (2013) reveal that the molecular mechanism leading to *Trypanosoma congolense* induced nitric oxide release from macrophages were not known.

3.2 THE ROLE OF TUMOR NECROSIS FACTOR (TNF) ON IMMUNITY AGAINST AFRICAN TRYPANOSOMES.

TNF was shown to play a detrimental role on host immunity against African trypanosomes, in that during the first TNF "*knock out*" mouse experiment, results revealed that neutralysing anti TNF antibodies might block the repressive action employed by infection-derived macrophages, suggesting the crucial role of this cytokine in parasite control (Magez et al., 1999). In different mouse models whose TNF were knocked out using anti TNF antibodies and later on injected with *T.b rhodesiense* and *T. congolense* respectively, the outcome revealed that both mice were susceptible to infection, but if both TNF and nitric oxide are left intact, and *T. b rhodesiense* on one extreme and *T.congolense* on the other are injected *In-vivo*, the parasite are cleared clarifying that TNF in conjunction with nitric oxide exert full typanotoxic potential (Magez et al., 2006; Magez et al., 2007). TNF was also revealed to play a part in inducing the suppression of T-cell (Magez and Caljon, 2011). However, Magez et al. (1999) used C57BL/6-TNF-α knock out mice and C57BL/6 wild type mice to demonstrate the role of TNF-α and monitor its effects in clearing levels of parasitaemia with *T. brucei* parasites. Their results revealed that after both C57BL/6-TNF-α knock out and C57BL/6 wild type mice were infected with the parasite intraperitonially, C57BL/6-TNF-α knock out exhibited high levels of parasitaemia when compared to the wild type.

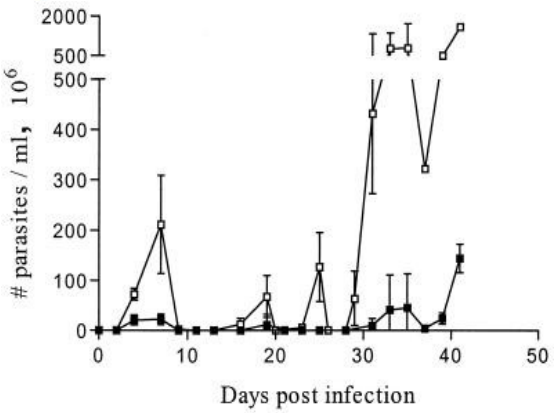


FIGURE 3 "*Parasitaemia development of pleomorphic T. brucei AnTat 1.1 parasites in C57BL/6 wild-type (■) and C57BL/6-TNF-α knock out (□) mice. Ten mice per group were infected at day 0 by intraperitoneal injection of 5,000 parasites*".

Extracted from Magez et al. (1999)

This result indicate the potential role played by TNF in reducing parasite load. TNF-α was also revealed to be highly trypanolytic to *T. brucei in vitro*, and reduce waves of parasitaemia *in vivo* (Magez et al., 1999).

3.3 THE ROLE OF NITRIC OXIDE ON IMMUNITY TO AFRICAN TRYPANOSOMIASIS.

Although, several literatures were shown to present conflicting results regarding the exact role of nitric oxide in African trypanosomiasis, even when considering the fact that different parasite strain may produce diiferent outcomes (de Sousa et al., 2011), Sternberg et al. (1994) cited in de Sousa et al. (2011) indicates that the biochemical reduction of the action of NO synthase yields an enhanced control of the first trend of parasitaemia in *T. brucei* infections. Although (de Sousa et al., 2011) indicates that the parasite is not susceptible to nitric oxide mediated killing *in vivo,* though *LouTat* 1 a clone of the parasite was shown to be sensitive to NO and die in its presence in a culture *in vitro*, but addition of N^G -Monomethyl L-arginine (L-NMMA) was shown to cause the inhibition of nitric oxide liberation and annul its cytotoxic impact on trypanosomes (Schleifer & Mansfield 1993 cited in de Sousa et al., 2011). However, the role of NO in T. brucei infections were shown using an iNOS knock out mice, which was revealed to behave as fully immuno-competent mice, but treatment with L-NMMA causes an induced immunosuppression and anaemia (de Sousa et al., 2011).

3.4 THE ROLE OF IL-10 ON IMMUNITY AGAINST AFRICAN TRYPANOSOMES.

Studies reveal that the early inflammatory response instigated to control parasite proliferation had to be neutralized by an increased production of IL-10 in order to achieve a long-lasting low pathogenic effect (Guilliams et al., 2007). An IL-10 "*knock out*" mice have been shown so far to be the *"most trypanosusceptible mice described ever"* (Namangala et al., 2001). This is an indication that appropriate initiation of IL-10 response is an outright requisite for the continued existence of the mouse model during infection with trypanosome species (Magez & Caljon, 2011).

In an attempt to prevent excessive IFNγ production, IL-10 was shown to be liable for keeping the stability amongst pathogenic and protective immune response during infection with *T. brucei,* suggesting the serious role played by IL-10 in the enhanced survival of experimental mouse models (de Sousa et al., 2011). Being a Th2 cytokine, IL-10 was implicated in the downregulation of Th1 cytokine responses and early inflammatory immune response.

IL-10 knock out mice were revaled to control first peak of parasitaemia in T.brucei infections (de Sousa 2008).

4.0 CONCLUSIONS

The use of mouse models to study and analyze infections with African trypanosomes provides an insight of how the possible mechanisms by which African trypanosomes can be destroyed. With the introduction of gene targeted mouse, immune response in humans that are potentially suggestive of protective immunity,it can be tested in mouse models to understand and verify the importance of particular immunological pathway.

When comparing the susceptibility and resistance of mouse strains infected with African trypanosomes, the balance between Th1 cytokines such as TNF, IFNγ, and the induction of nitric oxide release in addition to IL 10 appear to be very important.

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