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IMMUNE RESPONSE OF HOSTS AND PROSPECTS OF VACCINE DEVELOPMENT AGAINST AFRICAN TRYPANOSOMES: THE REVIEW

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ABSTRACT

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. Although hosts acquire infection principally via the bite of infected tsetse flies, other haematophagus insects like Tabanids and Stomoxysspecies also transmit trypanosomiasis mechanically. African trypanosomes are exposed to the host immune system from the time of infection. Antigenic variation is the immune evasion strategy that has evolved in African trypanosomes. Currently, there are no effective vaccines against African trypanosomiasis, neither for humans nor for livestock. Initially, vaccine trials against African trypanosomiasis, started targeting the surface coat of the parasite. In principle, this surface protein would be an idea vaccine candidate, if it were not for the antigenic variation strategy that the parasites have cunningly evolved. While a vaccine against African trypanosomiasis is not an immediate prospect, but there are several promising avenues for immunological exploration, namely, trypanosomes attenuated in in vitro and in vivo culture systems, genetic engineering, cross-reacting subcellular fractions, variant antigen types and metacyclic antigen. . It is likely, if any one of these areas is rewarding, that the resulting vaccine will be more successfully exploited, at least initially, in trypanotolerant animals. Since discovery of more efficacious drug is slow and the development of resistance of the existing drug, vaccination is viewed as the most promising sustainable method of controlling African trypanosomiasis.

KEYWORDS: *Immune Response, Vaccine, Trypanosomes.*

1. 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 (Swallow, 2000). This disease occurs in about 10 million km2 in 37 sub-Saharan countries corresponding almost to one- third of Africa's total land area. Trypanosomiasis in humans, also known as sleeping sickness, is caused by two sub-species of *trypanosomabrucei: T. b. gambiense*, which is agent of late-onset, chronic form that is endemic in Western and Central Africa, and *T. b. rhodesiense* which is responsible for an early-onset, acute disease found in Eastern and Southern Africa (Solano et al., 2003). African animal trypanosomiasis (nagana) is a group of disease of ruminants, camels, equines, swine and carnivores caused by different trypanosome species. The major pathogenic species in African cattle are *T. congolense*, *T. vivix*, and to lesser extent, *T. b. brucei* (Taylor and Authie, 2004). Although hosts acquire infection principally via the bite of infected tsetse flies, other haematophagous insects like *Tabanids* and *Stomoxys* species also transmit trypanosomiasis mechanically (Eisler*et al*., 2004).

Unfortunately, since this disease persists almost exclusively in the most marginalized communities of undeveloped countries, very little resource is spent to lighten their tremendous social and economic burden. Human African trypanosomiasis represents a major public health threat in Africa and together with nagana, the animal form of African trypanosomiasis, is considered a main obstacle for development of rural regions of the continent (Simarro*et al*,. 2008).

African trypanosomes are single cell, extracellular blood parasites. Shared characteristics of the different species of African trypanosomes include the ability to produce almost unlimited antigenic variation of their variant surface glycoprotein (VSG) and to induce a predominantly T-cell independent antibody response to the VSG, profound immunosuppression, polyclonal B – cell activation and persistent hypocomplementemia in infected mammalian hosts (Pan *et al*,. 2006). Infection of mammalian host leads to cycle of parasitemia associated with new VSGs.

Each new VSG initially elicits a strong immunoglobulin M (IgM) anti- VSG response which leads to phagocytosis of the trypanosomes, predominantly by macrophages of the liver (Naessens, 2006).

Currently there are no effective vaccines against African trypanosomiases, neither for humans nor for livestock. The present day methods for the control of African trypanosomiasis, namely, systemic case detection and treatment, tsetse control, do not more than limit the disease although both these approaches have been shown to be effective where they have been vigorously applied (Delespaux*et al*,. 2008). The disadvantage attending the use of trypanocidal drugs include lack of availability of effective drugs, drug resistance and in heavy tsetse fly challenge area, the frequency with which treatment has to be applied, often to economically unacceptable levels (Pays *et al*., 2008). Thus, there is little doubt that the introduction of an effective vaccine, if used strategically along with established control methods, would make an enormous contribution to the control of African trypanosomiasis, not only by increasing productivity in endemic trypanosome areas but also by opening up for exploitation of the vast areas of the African continent largely devoid of livestock because of trypanosomiasis (Venugopal, 2010).

Therefore; the objectives of this review are

- To highlight the immune response of hosts against African trypanosomes focusing on common antigens
- To indicate available vaccine development approaches and types against African trypanosomes
- To give overview of the opportunities and obstacles in the development of vaccine against African trypanosomes and the way forward.

2. AFRICAN TRYPANOSOMES

Trypanosome belongs to phylum *Sarcomastigophora*, order *Kinetoplastida,* family *Trypanosomatidae*, and genus *Trypanosoma*. They are unicellular haemoflagellated protozoan parasites characterized by one nucleus and one flagellum, either free or attached to the parasites body by mean of an undulating membrane (Bourn *et al.,* 2005). Trypanosomes also usually contain a small, compact kinetoplast, a disc shaped DNA -containing organelle, situated within a large mitochondria (Brun*et al*., 1998). A certain collection of salivarian trypanosome can be further classified in the trypanozoon groups. Within this subgenus, there are three major trypanosome species; *T. brucei, T. equiperdum T. evansi. T.brucei* can be additionally divided in to three subspecies, of which *T.bruceirhodesiense* and *T. bruceigambiense*is the causative agent of the debilitating sleeping sickness disease. *T. b. brucei* is not pathogenic to the humans and together with *T. congolense* (belongs to the

subgenus, Nannomonas) and *T. vivax*(belongs to subgenus Duttonella), is associated with trypanosomiasis of domestic animals and livestock (Hoare, 1972).

2.1. Morphological Characterization

The different trypanosomes species differ in in morphological characteristics as described by (Maudlin et al., 2004). All trypanosomes species have a size range of 15- 55µm and typical live in the blood, lymph, and tissues of their hosts. Bloodstream form of trypanosome are covered by a protective surface coat consisting of variant surface glycoprotein (VSG) linked in turn to the plasma membrane surface by means of glycosyl-phosphatidyyl- inositol (GPI) anchors (Vickerman, 1985). African trypanosomes are characterized on the basis of their size, shape, position of the nucleus, size and location of the kinetoplast, host range, and geographical distribution. Generally they are elongated, spindle shaped organisms with a single flagellum (Morrison et al., 2009). The flagellum originates from the basal body near the kinetoplast and runs the length of the trypanosomes. The pellicle, the layer bordering the cytoplasm, while maintaining a definite shape, is a flexible enough to permit a certain degree of body movement. The pellicle and the cytoplasm are pinched up in to a thin sheet of a tissue along the length of the body forming the undulating membrane (Soltys and Woop, 1997).

2.2. Life Cycle

Trypanosomes are single celled parasites with a two host life cycle: mammalian and arthropod (Brun*et al*., 2009). With the exception of *T. equiperdium*and *T. evansi* the majority of trypanosome species undergo a developmental phase in insect vectors, the tsetse fly (Vickerman, 1985). The cycle starts when blood from a trypanosome infected animal is ingested by tsetse fly. It is within the insect vectors that the trypanosomes undergo a chain of events involving differentiation, multiplications and biochemical alterations, such as swapping their energy metabolism from glucose (in blood forms) to proline (in procyclic forms), before migrating to the salivary glands, where they progress in to infective metecyclic forms by regaining their VSG coat and are then ready to be inoculated in to a new hosts during the next blood meal (Delespaux*et al*., 2008). *Trypanosome brucei* species migrate from the gut to the proventrculus, to the pharynx and eventually to the salivary glands; the cycle for *T. congolense*stops at the hypopharynx, and the salivary glands are not invaded, they entire cycle for *T. vivix* occurs in the proboscis. The animal infective form in the tsetse salivary glands is referred to as the metacyclic form (Vickerman, 1985).

Once inoculated in a new host trypanosomes quickly lose their surface coat transform in to the long cylinder trypomastigotes and proliferate by binary fission at the site of the bite after a few days, leading to an inflammatory chancre (Morrison *et al*., 2009). The parasites then spread to the draining lymph nodes and blood stream, through which they reach other organs such as the spleen, liver, heart and endocrine system (Delespaux*et al*., 2008).

2.3. Course of Infection

Clinical signs and the severity of the disease following infection vary depending on the virulence of the trypanosomes susceptibility of the host. The length of the preptent periods depends on many factors such as the number of infecting parasite, the route of inoculation and the genetic make-up of the host. Parasitemia becomes apparent within 1-2 weeks following natural infections and may persist for months, occurring in waves, until the host dies. Acute infection of *T.congolense* in ruminant is associated with intermittent fever, depression, anemia, subcutaneous edema of the mandible and prominent jugular pulse (Losos, 1986). The apatite is decreases and there is rapid weight loss. Often, death is related to sever anemia and circulatory collapse. Chronic syndromes often results in extreme emaciation and anemia. Lymphadenopathy is commonly seen in infection with other species of trypanosome but not common in *T. congolense*in cattle (Biryomunmaisho and Katunguka-Rwakishaya, 2007).

2.4. Trypanosomal Antigens

The trypanosomal antigens can be divided in to two groups; namely invariant or common antigens and variant antigens, based on the immunological specificity (Seed and Sechelski, 1999).

2.4.1. Trypanosomal Common Antigens

Invariant antigens of the trypanosomes do not change from one invariant type to another type during the course of infection. These include enzymes, trypanosomal membranes, structural and nucular proteins and some receptors such as those for transferrin and low density lipoproteins, high density lipoproteins, receptor for TNF-α and receptor for IFN- γ (Olsso*let al*., 1993). Some enzymes such phospholipase C and peptidases common to all species of trypanosomes (Knowles *et al*., 1989). The carbohydrate determinants in the C-terminal portion amino acid sequence of the variant surface antigen is also considered as the common antigen and show a high degree of homology among the members of a species (Rice- Ficht*et*

al., 1982). Flagelar pockets of African trypanosomes are not covered by the VSG and are invariant among members of species (Mkunza*et al*., 1995).

2.4.2. Variant Surface Glycoprotein (VSG)

The plasma member of trypanosomes is covered by a homogenous dense coat called variant surface glycoprotein, consists of millions of glycoprotein of a single molecular species (Vickerman, 1985). The genome of African trypanosomes contains about 1000diffrent VSG genes. Only one VSG gene is expressed at a time in a given blood stream parasite under normal conditions. The unexpressed VSG genes are scattered among the different chromosomes (cross *et al*., 1998). VSG genes need to be located in a specialized telomeric environment, which is known as a blood stream telomere linked VSG gene expression site (BES) in order to be transcribed. At any time only one BES is active and only one VSG gene is transcribed (EL-Sayed*et al*., 2000).

In the insect, VSG is only expressed in infective metacyclic form and it has been proposed that the VSG prevents the lysis of metacyclic forms by the host's serum factors. They form a 12-15 mm thick coat which functions as a barrier to lytic serum components but allows nutrients such as glucose to reach transporter in the membrane of the flagelar pocket. VSG constisute about 10% of the total protein of trypanosomes and is synthesized at a high rate. VSG is very immunogenic and therefore the target of the very potent immune response (Vanhamme*et al*., 2001).

2.4.2.1. Effects of VSG on the Immune System of the Hosts

Since the trypanosomes are extracellular parasite and they release vast amounts of sVSG in to the circulation, the immune system of the infected mammalian hosts is continuously exposed to the sVSG (Magez*et al*., 2002). There is evidence that blood stream forms of *T. congolense* evade complement lysis by shedding their immune complexes and endocytosis of immune complexes. VSG of *T. brucei*causes consumption of complement proteins, which may occur via the massive amounts of immune complexes generated during antibody mediated clearance of each wave of the parasitemia (Engstler*et al*., 2007). Immunostimulatory and regulatory activity of protozoan derived GPI anchors has been documented. It has been suggested that soluble VSG which carries the carbohydrate core (GPI- VSG), once released from the parasite surface, is affecting the functions of macrophages, including the induction of cytokine synthesis. It has been shown that NO is trypanoststic for *T.congolense, T.*

musculi, *T. gambiense*and *T. brucei* in vitro. Soluble VSG could inhibit IFN- γ induced nitric oxide production by macrophages (Coller*et al*., 2003).

2.5. Antigenic Variation

Antigenic variation is the immune evasion strategy that has evolved in African trypanosomes. Trypanosomes have made a huge investment on antigenic variations. Trypanosomes persistence in the mammal is due to antigenic variations, which involves change in the identity of the variant surface glycoprotein (VSG) that form a dense cell surface coat to shield invariant surface antigens from immune recognition (Wickstead*et al*., 2004).antibodies against the VSG kill the trypanosomes, but the population survives due to trypanosome switching and expressing a different VSG gene, hence enabling an entirely different surface coat to be produced. Antibodies previously mounted by an immune response will not be able to recognize this newly expressed VSG gene and thus it is this special functioning coat, which helps trypanosomes evade the immune system and maintain their survival within hosts (Morrison *et al*., 2009).

The undulating wave of parasiteamia in infected animals is a result of interactions between the parasite and the host's immune factors. It has been shown that antibodies are not necessary to induce antigenic variation because antigenic variation has been observed in vitro (Doyle *et al*., 1980). Even though the precise molecular events lead to the antigenic variations are still not understood, it is clear that antigenic variations is advantageous to the parasite in its evasion of the hosts immune defense because the hosts is always a step behind the switching trypanosomes (Marcello and Barry, 2007).

3. IMMUNE RESPONSES OF HOSTS AGAINST AFRICAN TRYPANOSOMES

African trypanosomes are exposed to the host immune system from the time of infection. Since a single trypanosome is a package of about 10 million copies of a single VSG and thousands of invariant antigens (Vickerman, 1985), the immune system of the host is continuously assaulted by excessive amounts of invariant and variant antigens.

3.1. Innate Resistance

Antigen non -specific defense mechanisms that are designed to recognize highly conserved structure present in many different microorganisms and called pathogen associated molecular patterns, are considered to be a form of innate immunity (Aderen and Ulevitch, 2000). The presence of invading microbes and resulting tissue damage is detected by sentinel cells. One

of the sentinel cells is macrophages; not only involved in sense the presence of the invading parasite but involved in triggering the acquired and innate immune response (Mwangi*et al.*, 1996). Following inoculation of trypanosomes in to mammalian hosts, by the tsetse fly, a local response in the skin (chancre) is induced by trypanosome proliferation and appears a few days after inoculation. In efferent lymphatic vessels, trypanosomes have been detected in lymph 1-2 days before the chancre. Their number declined during development of the chancre and later increased. They are detected in the blood 5 days after inoculation. Neutrophils predominate in the early days and then T and B lymphocytes infiltrate the chancre (Vanhamme and Pays, 2004).

3.2. Adaptive Immune Response

Adaptive immunity or acquired immunity is an antigen specific defense mechanism consists of two major categories; humoral immunity and cell mediated immunity (Reinitz and Mansfield, 1990).

3.2.1. Humoral Immune Responses

Hummoral immunity is mediated by antibody molecules mediated by B lymphocytes is response to antigens. Specific B cell response against VSG surface epitopes represents composite T cell dependent and T cell independent process. The T cell independent B cell responses are associated with temporary immunity to the variant antigenic types of trypanosomes arising during acute and chronic infections (Reinitz and Mansfield, 1990).

The B cell specific responses to VSG result in elimination of trypanosomes expressing the target surface antigen and control parasiteamia. In the presence of specific antibody, trypanosomes are rapidly eliminated from the circulation (Shi *et al*., 2004). The primary immune response to VSG consists of both IgM and IgG classes of antibodies and reaches the maximum in 7-14 days following challenge. During the initial parasitemic wave, IgM was the only detectable class of antibody (Radwanska*et al*., 2008).

The production of antibodies against various predominant VSGs provides protective immunity in infected animals. VSG specific antibodies mediated compliment mediated lysis and increase the uptake of trypanosomes by macrophages. The clearance of the parasites is an immune mediated mechanism and needs VSG specific antibodies. Antibodies against non-variant antigens may neutralize toxic or pathogenic effects of certain trypanosomal molecules and may prevent anemia after repeated infections (Shi *et al*., 2004).

3.2.2. Cellular Immune Responses

T cells are central to the regulation and activation of immune responses. The T cell which cooperate with B cells, helping them to respond to the antigens, resulting in the differentiation of B cells in to antibody secreting plasma cells are termed helper T cells (Shi *et al*., 2006a). Distinct sub populations of T helper cells preferentially promote T cell immunity (TH1) or stimulate antibody production (TH2) based on the cytokines they produce. Some T cells are able to suppress immune responses and therefore are designated suppressor T cells. Some other T cells are able to kill the expressing foreign determinants on their surfaces and to kill virus infected cells, and are named cytotoxic T cells (Aderem and Ulevitch, 2000). The role of T cells in protection against African trypanosomiasis is poorly investigated. The VSG surface epitope specific B cell responses in mice infected with trypanosomes represent composite T cell dependent and T cell independent processes, and a significantly stronger response is made in the presence of T cells (Shi *et al*., 2006a).

3.3. Immunomodulation

3.3.1. Polyclonal B-Cell Activation

Generally B-cell activation was noted in trypanosomiasis (hypergammaglobulinemia and a large increase of B cells in the spleen, as well as the presence of numerous mott cells in cerebral spinal fluids and plasma cells in perivascular infiltrate), whereas specific antibody response to trypanosome antigens were reduced (Maudlin, 2006).

Hypergammaglobulinemia with mainly IgM antibody and marked B cell expansion is consistently found in the spleen and lymph nodes in trypanosome infections. The increased level of immunoglobulins consist of antibodies against both trypanosomes related and unrelated antigens, including auto-antibodies (Hudson *et al*., 1996). It has been reported that the purified soluble VSG molecules are mitogenic for B cells. Mice immunized with purified VSG showed marked enlargement of B cell compartments in the spleen and an increase in serum IgG levels mostly due to production of polyclonal antibodies. The mechanism of the polyclonal B cell activation is unknown, however, it is known that the binding of an antigen by the B cell receptor and cross linking of the compliment receptor 2 (CR2) simultaneously has a synergistic effect on B cell activation (Tedder*et al*., 1997). The membranes of insect stages of trypanosomes which do not possess VSG do not cause polyclonal activation suggesting that bloodstream forms of trypanosomes may induce polyclonal B cell activation as an evasion mechanism. Because affinity maturation does not occur during polyclonal B

cell activation, selective proliferation and the production of high affinity antibodies against the trypanosomes might be prevented (Roitt*etal*., 2001).

3.3.2. Immune Suppression

Immunosuppression is remarkable feature of trypanosomiasis in cattle, mice, and human. B and T cells responses to trypanosomes and non-trypanosome antigens have been suppressed in most hosts; with the exception of trypanoresistant wildlife (Barry and MeCuloch, 2001). It was proposed that the major causes of increased susceptibility of trypanosome bearing individuals to opportunistic infections is generalized immunosuppression observed in patients. Immunosuppresstion was also observed and investigated in experimental trypanosomiasis and trypanosome infected cattle (Ilemobade*et al*., 1982)infections of cattle with T. congolense and *T. vivix* cause suppression of antibody response too some vaccines. Suppressed antibody response to brucellaabortus was observed in sheep infected with *T. congolense*. In dogs, infections with *T. congolense*have been shown to suppress antibody response to brucellaabortus vaccine. A progressive depletion or exhaustion of antigen reactive B cells due to polyclonal activation could later results in immunosuppression (Raper*et al.,* 1999).

4. VACCINE DEVELOPMENT TRIALS AGAINST AFRICAN TRYPANOSOMES

Initially, vaccine trials against trypanosomiasis started targeting the surface coat of the parasite. In principle, of this surface protein would be an ideal vaccine candidate, if it were not for the antigenic variation strategy that the parasites have cunningly evolved (Cornelissen*et al*., 2005). However, it became obvious that such an approach would never succeed due to: the innumerable possible molecules that the parasite can generate through gene rearrangement and the fact that the main immunoglobulin response that they elicit is that of the IgMisotype, which is short lived (Murray and Urquhart, 1977).

The result is that many workers in trypanosomiasis research consider the possibility of vaccination to be remote (Murray *et al*., 1979). It should be borne in mind, however, that many of these conclusions have been drawn from work on laboratory animals, which invariably succumb to massive parasitaemia. There is evidence to show that under certain circumstance cattle can control parasiteamia and then clinical recover. While this is particularly true for trypanotolerant breeds such as the N'Dama, it can also occur in the more susceptible Zebu. The greater capability of the bovine to control parasiteamia creates a new perspective on the question of vaccination. Furthermore, advances in scientific knowledge

and technology have opened up several different avenues of research (Philippe and Bernard, 2006).

4.1. Vaccine Development Approaches Against African Trypanosomiasis

4.1.1. Variable Antigen Types (VATs)

The immune response against each variant, although rapid and highly effective in destroying any trypanosome that possesses that particular antigen, is invariably too late to affect that proportion of the population that has altered its antigenic identity. Thus, parasiteamia rises and falls in waves with each parasite population carrying different surface antigens (Philippe and Bernard, 2006).

VATs can be divided into early "predominant "types and other groups of VATs that occurs later (Capbern*et al*., 1977). The total number of VATs that a trypanosomes can express is known as its "VATs repertoire "the full extent of which is as yet unknown although there have been able to isolate 101 VATs from one clone of trypanosome equiperdum. Comparison of VATs repertoires from different clones has been initiated and has revealed a surprisingly high degree of similarity; in fact, some VATs have been found in every repertoires examined. In addition, indirect evidence from serological studies that during an infection certain VATs may recur, in some cases within a few weeks of one another (Murray and Urquhart, 1977).

As regards vaccination, a rational approach may be successful. Immunization against individual VATs is highly effective using such regimes as infection and treatment; irradiated organisms; killed organisms; crude emulsions containing released soluble antigens; formalized whole infected blood or plasma and purified variable antigen glycoprotein. A cocktail vaccine based on predominant VATs is likely to be effective against with that repertoire. Investigation of the feasibility of such an approach requires complete analyses of the number of VATs, both predominant and others, and within a repertoire, or between repertoires the extent of cross reaction (Vercruysse*et al*., 2007).

4.1.2. Metacyclic Antigens

Following ingestion by the tsetse fly, trypanosomes loses its surface coat, which contains the variable antigen. It eventually regains the coat in the fly's salivary gland in becoming the mammalian infective metacyclic stage. Vaccination against such type would obviously be of importance. A drawback to the potential use of metacyclic populations for vaccination is that they are antigenicaly unstable, preventing mass production of antigen and mRNA for potential vaccine preparation (Barry and Vickerman, 1979).

However, these difficulties may be overcome by a recently devised protocol whereby antigenically more stable mammalian bloodstream forms with the same VAT as metacyclic can be identified and cloned giving rise to population suitable for bulk preparative procedures (Vercruysse*et al*., 2007). This approach could be pursued to define the VAT complement of metacyclic populations with a view to vaccination against trypanosomes of that VAT repertoire. Furthermore, it is essential to determine the degree of cross reaction between metecyclic of different repertoires (Barry *et al*., 1979b).

4.1.3. In *Vivo* and In *Vitro* Attenuation

At a later stage of infection, after expression of predominant VATs, it appears that trypanosomes are in some way biologically altered as evidenced by their decreased infectivity and virulence in fresh hosts. The basis of this and whether it is linked to VAT or some other characteristic of the parasite remain to be investigated (Herbert, 1975).

The possibility now exists of attenuating trypanosomes by continuously passing in culture. In preliminary studies, it has been found that mice infected with parasites maintained in vitro by serial sub cultivation over 12 months have shown alteration in pathogenicity when compared with non-cultured organisms or organisms that have been maintained in vitro for less than three months (Callow, 1997).

4.1.4. Molecular and Genetic Engineering

There is little doubt that the basis of understanding antigenic variation will come from investigations of the molecular biology of the trypanosome. In vitro cultivation technique and recently developed tools in biochemistry and genetic engineering have opened up new horizons. Thus the study on trypanosomal RNA will provide much essential information on trypanosome biology (Vercruysse*et al*., 2007). Re-annealing studies on the nucleic acid coding for the VAT repertoire should give an insight in to the size of the repertoire, the extent of similarity between different repertoire and the molecular nature of the gene involved. The genetic control of the expression of antigenic variation should be studied; artificial restriction of a trypanosome vaccination (Williams *et al*., 1978).

It is possible that in the near future many protein vaccines will be produced from large scale bacterial cultures that contain the gene sequences coding for the appropriate proteins. Many of the tissue culture cells were able not only to incorporate the DNA sequences in to their genome but also were able to produce the enzyme at apparently normal levels. It may be

possible, therefore, to modify certain tissues during a proliferative stage so as to yield a gene product to correct a genetic deficiency or possibly to produce a foreign protein for use in vaccination (Dimitriadis, 1978).

4.1.5. Immunogenicity of Sub Cellular Fraction

Modern biochemical technology has allowed the isolation, purification, and characterization of a whole range of trypanosomal sub cellular fractions. It is possible, however, that at some time in the trypanosomes complex lifecycle "weak spots" amenable to immunological control might be exposed. Thus, investigations have been made in to the purification of a range of sub cellular fractions of the trypanosome such as flagellum, membranes and kinetoplast (Kohler and Milstein, 1995). The biological characteristics and immunogenicity of these fractions have been investigated and compared with those of variable antigens. It has been found in studies on *T. brucei*in mouse is that flagellum and membrane fractions stimulate protection against homologous VAT challenge to the same degree as variable antigen (Shine *et al*., 1997). It is likely that this is the result of the presence of variable antigen in these sub cellular fractions although it is interesting that, per unit weight protein, flagellum is more effective than the purified variable antigen. No protection was achieved on challenge with a different VAT although with the membrane and kinetoplast preparations there was significant prolongation of survival accompanied by an alteration in the parasitaemic profile (Philippe and Bernard, 2006).

Using a sub cellular fractions of *T.brucei*or *T. rhodesiense* that probably contained a mixture of variable antigen, mitochondrion and kinetoplast to immunize mice, found increased survival times and reduced parasitaemia in mice challenged with *T. brucei*. Using *T. brucei* in mice and a similar fraction for immunization were able to stimulate protection only if trypanosomes of the same VAT were used for challenge (table 1). When another VAT was used for challenge, protection was not achieved although there was a significant increase in survival time (Murray *etal*., 1998).

Table1. Immunization with various sub cellular fractions of *Trypanosome brucei*

Fraction	**Challenge**	
	Same VAT	Different VAT
Variable antigen	Complete protection	No effect
Flagella	Complete protection	No effect
Membrane	Complete protection	Prolonged survival
Kinetoplast	Increased resistance	Prolonged survival

4.2. Major Impediment on Vaccine Development against African Trypanosomes

Despite all the anti-trypanosome trials reviewed above, not a single promising experimental result obtained in positive field trials. 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 host capacity to mount an efficient immune response and to maintain its immunological memory. The prior one is their ability to regularly switch their surface coat and hence evade immune destruction. This mechanism is known as antigenic variation (Pays *et al*., 2008).

African trypanosomes have developed a highly sophisticated and complex system of antigenic variation. In the mammalian host, the whole parasite is covered with a coat of about 10^7 identical molecules of a glycoprotein, the variant surface glycoprotein (VSG). When antigenic change occurs, the VSGs in the old coat are shed and replaced by an antigenically different VSG (Marcello and Barry, 2007). Analysis of this process indicates that, although, the trypanosomes possess about 1000 VSG genes, only one VSG genes is active at a time. Antigenic variation occurs as a result of replacing an active VSG gene with one from the silent VSG gene pool. The unlimited capacity for antigenic variation of the surface glycoproteins by the African trypanosomes is the major hurdle for producing a vaccine (Brun*et al*., 2010).

If animal are infected with pathogenic trypanosomes*; T. vivax , T. congolense,* or *T. brucei* and their parasitemia measured at regular intervals, the number of circulating organisms are found to fluctuate greatly. Each period of high parasitemia corresponds to the expression of the population of trypanosomes with a new surface glycoprotein antigen. The elimination of this population by antibody leads to rapid fall in parasitemia (Magez*et al*., 2008). Among the survivors, however some parasites express a new surface glycoprotein and grow without hindrance. As a result, a fresh population arises to produce yet another period of high parasitemia. The cyclic fluctuation in parasite level with each peak reflecting the appearance of parasite with a new surface glycoprotein can continue for many months. Trypanosome grow in tissue culture also show spontaneous antigenic variation demonstrating that the change in surface VSGs is not induced by antibody (Barbour and Restrepo, 2000). 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 trypanosome have developed, i.e., the abrogation of B- cell homeostasis and the destruction of the hosts immunological memory (Vanhamme*etal*., 2001). Together, these immune dysfunctions result in the lack of buildup of anti-VSG memory, and hence allow the parasite to use over time very similar VSG molecules, or even re-use a surface coat protein that already has been encountered by the host. During experimental trypanosome infections in mice, exposure to 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 (Mageze*t al.*, 2008).

4.3. Future Prospect

Since discovery of more efficacious drug is slow and the development of resistance of the existing drug, vaccination is viewed as the most promising sustainable method of controlling African trypanosomiasis (Mageze*t al*., 2010). But, vaccine development for trypanosomiasis has a chequered history. ILRAD spent the best part of 30 years from the early 1970s in pursuit of a trypanosomiasis vaccine. The effort failed completely, and the work was finally shut down in the early 2000s after a thorough-going review (McKeever, 1995). From 1972 to about 2002, there was high class science, good work on immunology and so on, but the practical outputs were nil.

No effective vaccine currently exists, but development of vaccine is the subject of current research. The bill and Melinda gates foundation and united kingdom DFID have involved in funding research. And then along comes GALVmed; they are all new faces, a new generation of researchers. New laboratories have been developed in Mozambique, Ethiopia and Burkina faso and animal testing has been underway. They have been advances in immunology and it has been suggested that it may be more productive to generate an immune response against the sub-cellular fractions rather than the organisms itself (Giles, 2005). Events in the vector and host suffering from the disease suggest a possibility of immunity and these may form a basis for vaccine development. Since trypanosomes after their surface glycoprotein regularly, vaccine design strategies should focus on the invariant surface glycoprotein (ISGs), flagellar packet proteins, cysteine proteinases and intracellular antigens such as microtubule associated proteins (MAPs) and tubulin. As such several groups have explored the possibility that an

effective anti-trypanosome vaccine can be developed at least in trypanotolerant animals in the future (Rasooly and Balaban, 2002).

5. CONCLUSION

African trypanosomes are hemoprotozoa that cause disease in humans and livestock. Each trypanosome is covered by a single layer of about 10^7 identical molecules of surface glycoprotein. African trypanosomes are exposed to the host immune system from the time of infection and the immune system of the hosts is continuously attacked by excessive amounts of invariant and variant antigens. Antigen non-specific defense mechanism known as adaptive immunity or acquired immunity are both commenced to the invariant as well as variant surface glycoprotein of the trypanosome. Specific B cell responses against VSG surface epitopes represent composite T cell dependent and T cell independent processes. It is generally believed that that almost unlimited capacity for antigenic variation of the surface glycoprotein is the major impediment for developing vaccines against African trypanosomiasis. While a vaccine against African trypanosomiasis is not an immediate prospect, but there are several promising boulevards for immunological exploration, namely, trypanosomes attenuated in *in vitro* and *in vivo* culture systems, genetic engineering, cross-reacting subcellular fractions and metacyclic antigen. It is likely, if any one of these areas is rewarding, that the resulting vaccine will be more successfully exploited, at least initially, in trypanotolerant animals.

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