THE ELEGANCE AND SUCCESS OF TRYPANASOMES AS PARASITES: IMMUNOLOGICAL PERSPECTIVE

BY

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Preamble

As a "Fag" in 1972 I used to trek along with fellow fags, every Saturday from Community Grammar School, Isi-Enu, through the Faculty of Agriculture Farm to St. Peter's Catholic Chaplaincy and down along Ikejiani Avenue to the University gate and finally to the Nsukka Urban Water Works bore holes to wash my clothes and fetch a bucket of water primarily for either my "School father" (Mr, Aloma Obuba) or my "School Mother" (Miss Onuabuchi Ugorji) but sometimes for myself depending on how charitable or "doting" they chose to become. Remarkable, was that one of my classmates, Miss Audrey Echezona (a day-student) whose father was the then Head of the Department of Music lived along Ikejiani Avenue, which was a most breath-taking street with beautiful and meticulously kept lush green lawns, trim flower hedges, kerbed and in some cases macadamized drive ways. As we passed, Audrey used to come out and make fun of us and in class would taunt me that I could never live in such a dream place as she did. Of course I did not then know who or what Audrey's father was or why and how they came to live there. Consequently I

enquired from my superiors about who lived in Ikejiani Avenue. I was informed that everyone that lived there was a doctor and that their job was to conduct research and teach university undergraduates. That was back in July 1972. I then decided that I must be one of those "doctors" and that I was going to conduct research and teach university undergraduates at the University of Nigeria, Nsukka and that I was going to live in Ikejiani Avenue. I then audaciously told Audrey that she lived there on account of her father but that I was going to live in the same avenue on my own account in my own right. Ten years later, exactly on July 16 1982 (4 days after my discharge from the N.Y.S.C. scheme) I assumed duty as a lecturer in the Department of Veterinary Parasitology and Entomology of the University of Nigeria Nsukka. Unfortunately, I could not live in Ikejiani Avenue (because there was no vacant houses), but even more unfortunate was that those beautiful lawns and flower hedges that attracted me most had all been converted to yam, cassava and corn fields!!! The soft green hills of UNN and the beautiful and scenic environment of the university community had become defaced, bastardised and ravaged by unabashed acts of poverty (mental poverty) and quest for gastronomic satisfaction!!! Mr. Vice-Chancellor Sir, I have told this childhood story to illustrate that my becoming a University of Nigeria Nsukka lecturer was not aberrant or fortuitous but

designed. It was a job I hoped for, worked for, lived for and have survived and prospered by.

In this journey, apart from the attraction of Ikejiani Avenue, a number of people shaped my character and prepared me for the job which made it possible for me to attain this rank. My late father Lawrence Onah (Ogbonnia) Onah, was my best friend, confidant, chief adviser, guardian, spiritual director and prayer warrior. He told me that hard work never kills a child that is well fed; rather such child grows healthier and stronger. My mother Marcillina Okike Onah (nee Nnamani), who taught me selflessness, patience and the power of love, forgiveness and alms-giving over the forces of evil and hatred. My uncle, Chief Innocent Nnamani Onah (Ochiliozuo, member, Igwe's Cabinet and retired Headmaster Special Class), who adopted and trained me from the time I was five years old until I became a lecturer of the University of Nigeria, Nsukka. He is the foundation and pillar of my academic career and success. Then my undergraduate project supervisor, Professor Samuel Nnagbo Chiejina, who moulded my research philosophy and taught me that negative research findings in live sciences are as important if not more important than positive findings and that I must always report what my findings are in any work irrespective of what already existed in literature. Late Professor Maximus Maduabuchi Ikeme (Ikemee mgbeodiyanma na Nimo, erstwhile Dean,

Faculty of Agriculture and Veterinary Medicine; Founder, Father, foundation and several times Head of Veterinary Parasitology and Entomology, and Deputy Vice-Chancellor, Academic, University of Nigeria Nsukka among innumerable other positions he served in during his career at UNN). This man loomed larger than life in my career development in this University. Apart from my parents and uncle, he played the single most important part in my academic career. He employed me, adopted me as a son, agreed and disagreed with me on issues based on principle and without malice, stood up for me and protected me from everything (even from myself!!), meticulously taught me the principles of administration and the importance of knowledge of the regulations guiding ones place or establishment of employment, the supremacy of the rule of law and more importantly, he introduced me to the world of the elegant parasites called **trypanosomes** and an emerging phenomenon in their host-parasite interaction called **"immunosuppression"**. I became fascinated and hooked in the scientific quest to better understand immune responses and immunosuppression in pathogenic African trypanosome infections, the results and output of which earned for me the rank of Professor of Veterinary Immunoparasitology of the University of Nigeria, Nsukka. Hence Mr. Vice-Chancellor, Sir, the title of my lecture: **"The elegance and success of trypanosomes as parasite: immunological perspective".**

Introduction

Trypanosomes are obligate protozoan (haemoflagellate) parasites found in all classes of the phylum vertebrata, but which assume immense medical and veterinary importance only in mammals. The first pathogenic trypanosome (*Trypanosoma evansi*) was discovered by a British military officer and veterinarian, Captain Griffith Evans, in India in horses suffering from a disease known locally as **surra** (Evans, 1880; 1881-1882). Other important trypanosome species include: *T. brucei*, T. *congolense* and *T. vivax*, which cause the disease known as nagana in animals and, *T. gambiense* and *T. rhodesiense* that respectively cause the human disease known as West and East African sleeping sickness. All except *T. evansi* are transmitted cyclically by haematophagus arthropod vectors that belong to the genus *Glossina*. In partnership with these vectors, trypanosomes have caused the death of tens of thousands of people and hundreds of thousands of domestic livestock and, in so doing, have played a prime role in limiting the pace and extent of human development in tropical Africa

(Nash, 1969; Jordan, 1986; Onah *et al*., 2001). Even now, trypanosomes still deny vast areas of land to all domestic livestock in Africa. An estimated 45 million people, 147 million cattle, 125 million goats and over 103 million sheep are under threat of the disease (FAO/WHO/OIE, 1982; UNDP/WORLD BANK/WHO, 1983; Dwinger, 1985).

The rationale that the eradication of trypanosomosis would directly improve human development in Africa caused various control measures which fall into three broad categories to be instituted in various parts of Africa. These included measures against the parasite such as chemotherapy and chemoprophylaxis; those against the vectors such as insecticide application, trapping, bush clearing, sterile male release, destruction of reservoir hosts etc. and finally, measures involving the host such as restricted movement in and out of enzootic areas and breeding and introduction of trypanotolerant animals (Murray *et al*., 1981). Despite these measures, which over the years have recorded various levels of success, the trypanosome scourge in Africa remains.

The irony is that trypanosomes induce strong immune responses in the host, which ordinarily should ablate the infection and ensure the host's survival. This rarely happens and this elegant parasite somehow establishes itself in the host producing various forms of the disease which ultimately kills the host unless treated. In this

sense trypanosomes could be said to be a group of unsuccessful parasites because a dead host can hardly be said to be of any use to a parasite. The question therefore is, given its pathogenicity and the challenge of strong immune responses in the host, how has the trypanosome parasite succeeded as an etiologic agent of disease? The simple answer is that although it produces a disease that may be sub-acute, acute or chronic in nature, in most cases the chronic form, which guarantees an extended longevity of the host and therefore the survival of the parasite, subsists. But it is not as simple as that because; how then is the parasite shielded from the hostile environment of the host's immune responses? The answer lies in the fact that parasites and/or many other disease causing agents may survive destructive host's immune responses by either **mimicry** of host proteins, **sequestration** to host's cells and body sanctuaries, **immunosuppression** which renders immune attacks futile or by **antigenic variation** which helps in the exhaustion of the host's immune potentials (Borst, 1991).

Antigenic variation and immunosuppression are two of the most effective of these survival strategies and no other parasite has developed and perfected them to the same high degree of sophistication as the pathogenic African trypanosomes (Onah, 1999). Consequently, antigenic variation and immunosuppression are the secrets of the elegance and success of trypanosomes as

human and livestock parasites. The former is the business of structural and molecular biologists, which I am not; while the latter concerns immunologists and immunoparasitologists of which I am one and therefore competent to and will briefly talk to you about in this lecture.

Immunosuppression in African trypanosomes

Immunosuppression is a phenomenon in which the ability of a host to activate normal body defence systems and generate protective or sterilising immune responses (immunity) against an invading pathogen is compromised or suppressed. The earliest indication that trypanosomes are associated with this phenomenon came from observations that cattle suffering from *T. congolense* infection became more susceptible to concurrent infections (Parkin and Hornby, 1930; Fiennes, 1954). This was proved by numerous experiments in rodent and livestock animals using various antigens and/or disease causing pathogens (parasites, bacteria and viruses), in studies that spanned a decade from the 70s to the 80s (reviewed in Onah, 1992). These studies also showed that the suppression was generalised, affecting both the cellular and humoral arms of immunity against homologous (inducing) and heterologous (unrelated) antigens or pathogens. Once established, it became clear that trypanosome-induced immunosuppression possessed serious practical implications in vaccine-

based disease control programs in livestock animals especially in sub-Saharan Africa where such diseases abound. For instance *T. congolense* and *T. vivax* infections were shown to cause depression in serum antibody responses induced in cattle vaccinated with clostridial, contagious bovine pleuropneumonia, *Brucella abortus*, *Leptospira biflexia*, louping ill and rinderpest vaccines (Onah, 1992). It was therefore necessary to understand the mechanism(s) of the phenomenon in order to stem its effects. This quest engaged the attention of scientists across the globe for the next decade from the 80s to the 90s consuming hundreds of millions of US dollars in research funds in mostly rodent-based model studies. These studies resulted in significant understanding of some of the events underlying the immunosuppression as briefly outlined below.

Mechanisms of trypanosome-induced immunosuppression

1. ***Polyconal activation of B lymphocytes:*** Many studies showed that trypanosomes induce massive activation and proliferation of lymphocytes in infected rodents, which results in clonal exhaustion of the potential of B cells to function normally (Oka *et al.*, 1984). This activation is non-specific resulting in massive production of high levels of IgM, heterophile and auto-antibodies, which are ineffectual in the

ablation of the trypanosome parasites (reviewed in Onah, 1992).

2. ***Generation of suppressor macrophages and T cells:*** Spleen cells taken from trypanosome-infected mice fail to respond when stimulated *in vitro* with the T or B cell mitogen Concanavalin A (Con A) and bacterial Lipopolysaccharide (LPS) respectively (reviewed in Onah, 1992). When macrophages were removed by glass-adherence and the T cells by treatment with anti-Thy 1 serum and complement, the responses to both mitogens were fully restored. It was then hypothesized that suppressor T cells directly stimulated by trypanosomes release factors that have affinity for macrophages, which are in turn activated to become suppressive for T and B cell responses (Eardley and Jayawardena, 1977; Roelants and Pinder, 1984). However, Alcina and Fresno (1985) suggested that trypanosome-induced immunosuppression is a result of complex immunoregulatory circuit in which macrophages, after interaction with the parasites, release suppressor molecules that act preferentially on immune T cells. This inhibits the proliferation of helper T cells by either defective interleukin 2 (IL-2) production or inhibition of IL-2 action or both. Several reports as reviewed in Onah (1992) as well as recent

finding in both laboratory and livestock animals have supported Alcina and Fresno's proposition. In addition, it was shown that lymphocytes from trypanosome-infected mice fail to express interleukin 2 receptor (IL-2R). IL-2R binds IL-2 with high affinity and is responsible for IL-2 internalisation and signal transduction required for activated lymphocytes to progress through their full normal cell division cycles (Kierszenbaum and Sztein, 1990).

3. ***Secretory Products of Macrophages and T cells:*** There is elevated secretion of trypanocidal proinflammatory molecules such as reactive oxygen and nitrogen intermediates, tumor necrosis factor (TNF) and prostaglandins by macrophages, and gamma-interferon (IFN-γ) in trypanosome-infected rodents. IFN-γ is produced primarily by activated Th_1 cells, which, in turn, induce macrophages to release the trypanocidal molecules. These molecules play a central role in resistance to trypanosomosis but the irony is that they also play significant role in immunosuppression and other trypanosome-induced immunopathology (Sternberg *et al*., 1996; Onah and Wakelin, 1999; Hertz and Mansfield, 1999; Shi *et al*., 2003).

Despite the large body of literature from rodent studies, the conflicting roles of macrophages, T cells and their secretory molecules in immune protection made it difficult to clearly define in a sequential manner the mechanism(s) of trypanosome-induced immunosuppression. It was our view then that this difficulty lay in the fact that the majority of the studies concentrated on the "immunity" to the parasite during the course of infection and largely ignored the "immunology" of the infectious process. Immunology of the infectious process means the general alterations in the host's immune effector cells orchestrated by the parasites. In order words, we wanted to examine what happens to the soldiers at war rather than concentrating on the outcome of each battle that the soldiers fought.

Studies in Sheep

Hybridoma technology and the development of monoclonal antibodies offered us an excellent opportunity to address this issue in one of the natural hosts of trypanosomes, the sheep as against rodent studies. Thus, using a panel of monoclonal antibodies against sheep lymphocytes in indirect immunofluorescence staining and flow cytometry we dissected the immunological events in effector lymphocyte demographics in sheep during experimental trypanosome infections. Our studies revealed that infection of sheep with *Trypanosoma evansi* caused major alterations in the expression of

different effector phenotypes in blood and efferent lymph lymphocytes, which underlie the immunological paradigms seen during the infection. Specifically, the observations made are briefly itemised bellow.

In the blood:

1. *T. evansi* infection suppressed immunological responses to *Pasteurella Haemolytica* vaccine antigen including inflammation at the site of vaccine administration, post vaccination systemic (peripheral) neutrophilia and serum IgG_1 antibody responses (Onah *et al*., 1998a).

2. This was associated with large increases in both proportion and numbers of circulating B lymphocytes many of which expressed the pan-T cell antigen CD5 i.e. there was a large increase in $CD5^+$B cells (Onah *et al*., 1998a).

3. Consequent reduction in T cell subsets including $CD5^+$, $CD4^+$, $CD8^+$ and $\gamma\delta$ TcR^+ T cells (Onah *et al*., 1998a).

4. These increases and alterations in B cell phenotypes and decreases in T cell subsets are related to depression in serum antibodies. This is because, in animals that selfcured, **a).** There was significantly less increase in B cell numbers and no increase in the expression and output of

$CD5^+$B cells and; **b).** There was an increase in the CD4:CD8 ratio arising from a greater reduction in $CD8^+$ than $CD4^+$ T cells (Onah, 1992).

In the lymph the effects of *T. evansi* on cellular immunology of infected sheep was evaluated by cannulation of the efferent duct of peripheral lymph nodes draining the site of *P. haemolytica* vaccine administration, collecting lymph daily and subjecting the isolated efferent lymphocytes to indirect single- and dual-colour immuno-fluorescence and flow cytometry. As with peripheral blood lymphocytes, the following principal observations were made:

1. Large increases in the number of $CD5^+$B cells (Onah *et al*., 1998b).

2. Decreases in the numbers of helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells (Onah *et al*., 1998b).

3. Appearance and increase in the number of $CD4^+CD8^+$ (double positive) lymphocytes, which disappear terminally accompanied by terminal depletion of the major T cell subsets as well as terminal decreases in efferent B cell output (Onah *et al*., 1998b).

Apart from FACS analysis of the kinetics of immune cells during the infection, we utilized both cell depletion and separation techniques in *in vitro* functional assays to assess the involvement of suppressor T cells and cells of the monocyte/macrophage lineage in trypanosome-induced immunosuppression.

$CD8^+$ T cells were depleted from peripheral blood leucocytes by antibody-mediated complement lysis using anti-CD8 monoclonal antibody and rabbit complement while monocytes were removed by plastic adherence upon incubation. The two techniques were combined to remove both cell populations. The depleted cell populations were then tested for functional activity by stimulation *in vitro* with either Con A, *Pasteurella* antigen or *T. evansi* antigen and thymidine incorporation determined using beta liquid scintillation spectrometry. The following observations were made:

1. *T. evansi* infection significantly depressed proliferative responses of peripheral blood leucocytes (PBLs) to *in vitro* stimulation with Con A and *Pasteurella* antigen, which were restored after drug treatment (Onah *et al*., 1998c).

2. PBL proliferative responses to stimulation with homologous live or soluble trypanosome antigen were completely suppressed during the period of active infection but fully restored following drug treatment (Onah *et al*., 1998c).

3. Depletion of $CD8^+$ T cells from PBLs failed to restore their proliferative responses to stimulation with Con A, *Pasteurella* and homologous trypanosomal antigens (Onah *et al*., 2000).

4. Depletion of monocytes restored proliferative responses to Con A stimulation (Onah *et al*., 2000).

5. Depletion of both monocytes and $CD8^+$ T cells significantly restored and enhanced PBL proliferative responses to Con A stimulation (Onah *et al*., 2000).

Conclusion

These observations clearly indicate that trypanosomes survive partly by the induction of a series of complex immunoregulatory circuits originally intended to ablate the infection but which eventually work in favour of the parasite. The early appearance of $CD5^+$B cells in the course of the infection if probably directed towards the production of IgM autoantibodies, which usually

act as the first line of defence in infectious processes by enhancing phagocytosis and complement-mediated lysis of the parasites. However, aberrations in the expression of CD4, CD8 and B cell antigens undermine effective immunity and these aberrations offered us the opportunity to formulate a working hypothesis for trypanosome-induced immunosuppression as follows. Trypanosomes interact with monocytes, which are triggered to increased production of nitric oxide (which enhances parasite killing), and prostaglandins E_2 (PGE_2) and interleukin-1 (IL-1) (which are suppressive). IL-1 potentiates further activation of macrophages thereby exacerbating the reaction and in addition, induces the TH1 subset of $CD4^+$ T cells and $CD8^+$ T cells to increased production of IFN-γ, which in turn inhibits the expression of interleukin-2 receptor (IL-2R) on $CD4^+$ and $CD8^+$ T cells. In addition, PGE_2 directly inhibits the secretion of IL-2 by $CD4^+$ T cells. The inhibition of IL-2R expression prevents the binding and internalisation by T cells of the dwindling amount of IL-2 available, thus there is a failure in the signal transduction required for activated lymphocytes to progress through their full and normal proliferation and differentiation cycles for effective generation of protective immunity.

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