THE EFFECTS OF SCHISTOMIASIS ON THE PROTECTIVE POTENTIAL OF CANDIDATE HIV SAAVI DNA-MVA VACCINES IN *PAPIO ANUBIS*

WOODVINE OTIENO ODHIAMBO

MASTER OF SCIENCE

(Immunology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2016

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Woodvine Otieno Odhiambo

Thesis submitted in Partial Fulfillment for the degree of Master of Science in Immunology in the Jomo Kenyatta University of Agriculture and Technology

2016

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Sign…….….Date……..

Woodvine Otieno

This thesis has been submitted for examination with our approval as University supervisors.

Sign …….Date…….

Prof. Rebecca Waihenya

JKUAT, Kenya

Sign……..Date……..…..

Dr. Gerald Chege

University of Cape Town, South Africa

Sign…….Date……..

Dr. Lucy Ochola

Institute of Primate Research, Nairobi, Kenya

Sign…….Date……..

Dr. Thomas Kariuki

Institute of Primate Research, Nairobi, Kenya.

DEDICATION

To my beloved late maternal and paternal grandparents who saw the ability in me much earlier in life and nurtured it all through, thank you. To my Almighty Father in Heaven who gave me the patience, determination and capability:

"…being confident of this, that he who began a good work in you will surely carry it on to completion until the day of Christ Jesus." (Philippians 1:6)

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisors Dr. Gerald Chege, Prof Rebecca Waihenya, Dr. Tom Kariuki and Dr Lucy Ochola for their expert counsel in the design, execution and write-up of this work. To my mentor Dr, Gerald Chege for the expert guidance during the training for the Laboratory assays, Plates reading, statistical analysis and thesis development. I remember vividly the thousands of emails I sent you all of which you responded to promptly and positively. Thank you. To Dr. Lucy Ochola, thank you for always fitting into my shoes, for encouraging me at times of despair, for ensuring that I could access the laboratory at very odd hours and holidays and for encouraging me beyond the academic life to social and economic life. You became a sister to me.

To Dr. Tom Kariuki, thank you for believing in me entirely and letting me gain confidence in the work. To Prof Rebecca Waihenya, you are a mother, a real academician and Christian. You evangelized to me through academics.

I am grateful to the members of Virology and Schistosomiasis laboratory and Animals Science Department at the Institute of primate Research, who were of importance during the entire study period.

My deepest gratitude to the National Commission of Science and Technology- Kenya (NACOSTI) and the National Research Foundation- South Africa for entirely funding this crucial work.

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ABREVIATIONS AND ACRONYMS

AICD	Antigen-triggered activation-induced cell death
BSA	Bovine Serum Antigen
CCR5	Cystein-Cystein Linked Chemokine receptor type 5(β-Chemokine receptor
CD4	Cluster of Differentiation antigen number 4
CD4+	CD 4 positive effector T lymphocytes
CD8+	CD 8 positive effector T lymphocytes
CRF	Circulating Recombinant Forms
CTL	Cytotoxic T-Lymphocytes
CTLA	Cytotoxic T-Lymphocytes Associated Antigens
CXCR	Chemokine receptor
DCs	Dendritic cells
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
ELISPOT	Enzyme linked immunosorbent spot
Env	HIV envelope protein
FBS	Fetal Bovine Serum
FGS	Female Genital Schistosomiasis

Gag	HIV group antigen Specific
Gp 160	A sugar containing glycoprotein of approximately 160 Daltons
HIV-1	Human Immunodeficiency Virus type 1
HRP	Horseradish Peroxidase
IFN-γ	Interferon-γ
IL	Interleukins
IRC	Institutional Review Committee
ISERC	Institutional Scientific and Ethical Review Committee
Mab	Monoclonal antibody
MHC	Major Histocompatibility Complex
MVA	Modified Vaccinia Ankara
NAbs	Neutralizing antibodies
Nef	HIV negative infectivity factor
NHP	Non-Human Primate
NKT	Natural Killer T cells
NTDs	Neglected Tropical Diseases
OD	Optical Density
Pan	*Papio anubis*
PBMCs	Peripheral Blood Mononuclear Cells

PBS	Phosphate Buffer Saline
Pol	HIV polymerase gene
SAAVI	South African Aids Vaccine initiative
SCID	Severe Combined Immunodeficiency
SEA	Soluble Egg Antigen
SIV	Simian Immunodeficiency virus
SSA	Sub Saharan Africa
SWA	Soluble Worm antigen
Tat	HIV transcriptional transactivators protein
TGF-β	Tumour growth factor-beta
TH1	T Helper 1
TH2	T Helper 2
TMB	Tetramethylbenzidine
TNF	Tumour necrosis factor
UCT	University of Cape Town
UNAIDS	United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
VL	Visceral leishmaniasis

ABSTRACT

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) have no cure and HIV vaccine remains the only remedy. The effect of chronic Schistosomiasis on the efficacy of vaccines currently in clinical use and future HIV vaccines is not known. Several candidate HIV vaccines have been tested or are in ongoing clinical trials, but it is not clear how they might perform in the presence of ongoing helminthic infections. The overall objective of this study was to investigate whether Schistosomiasis compromises the protective potential of candidate HIV vaccines. Four naïve baboons were infected with *Schistosoma mansoni* cercariae and allowed to develop chronic infections before vaccination with HIV vaccines (SAAVI-DNA and MVA vaccines). A control group of Schistome-free four baboons were vaccinated in a similar manner. The protective potential of the HIV vaccines was assessed by measuring HIV-specific cellular immune responses by IFN-y˙Elispot assay and ELISA techniques. Immunogenicity data between Schistosome–free/infected vaccinated baboons was compared to show whether Schistosomiasis impacts on the protective potential of HIV vaccination. Graph pad prism software was used for data analysis. Peak HIV responses were elevated in all DNA vaccinated animals one week after boosting with MVA. The mean cumulative HIV responses were higher, though not significant, at P=0.2 in Schistosome-infected baboons (2242±874sfu/10^6 PBMC) compared to the control group (665±203 sfu/10^6 PBMC). Responses to individual HIV peptides were present in all animals for Gag whilst only one animal had a response to Tat. The highest magnitude of mean response was directed to Pol in both groups but there was no statistical difference between them. Similarly, there was no statistical difference in the mean responses to any of the other 3 vaccine Immunogens. The antibody response to both Schistosome crude antigens (SEA and SWAP) and HIV Gag peptides was highly elicited in Schistosomiasis. These data suggest that Schistosomiasis positively impacts on the protective potential of HIV vaccination thus elimination of Schistosomiasis is not necessary before administration of the HIV vaccine.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background information

HIV/AIDS infection is a great problem worldwide. As at 2013, approximately 32.2-38.8 million people were reported to be living with the disease, with 2.5 million new infections registered annually (UNAIDS, 2013). Sub-Saharan Africa carries the greatest disease burden globally at 68% (WHO/UNAIDS/UNICEF, 2011). It is estimated that HIV/AIDS kills between 1.4-1.9 million people, comprising about 70% of all new occurrences of the disease world over (UNAIDS, 2013). The statistics of South Africa reported 5.6 million HIV-infected people, (UNAIDS, 2013).

Schistosomiasis is among the leading parasitic infections worldwide. It is a water-borne helminth illness, endemic in Africa (Engelsa *et al.*, 2002). Approximately 779 million people are at risk and around 207 million are suffering, 93% of which are in the Sub Saharan Africa, (Mazigo *et al.*, 2012). In Africa, Urogenital and intestinal schistosomiasis (caused by S. *intercalatum* and *S. mansoni*) are of high frequency (Steinmann *et al.*, 2006). Disability-adjusted life years lost due to schistosomiasis is approximated at 4.5 million (Conlon, 2005). Worldwide prevailing schistosomiasis rate may still rise due to the effects of increasing human activities like for agricultural irrigation schemes, build-up of dams and man-made lakes for hydroelectric power (Steinmann *et al.*, 2006).

There is an extensive geographic overlap between the prevalence of helminthic infections and HIV/AIDS in Sub-Saharan Africa (SSA). These overlaps occur in high endemic areas (Fontanet *et al.*, 2000; Borkow *et al.*, 2004). It is hypothesized that infections with helminths in Sub-Saharan Africa are linked with a heightened transmission of HIV (Fontanet *et al.*, 2000, Borkow *et al.*, 2004) and a faster advancement of HIV to AIDS (Kallestrup *et al.*, 2005). In the presence of HIV,

helminths cause general immunological activation and affect the cytokine secretion system (Fontanet *et al.*, 2000; Mwanakasale *et al.*, 2003). There is also modulation of the immune response against helminths (Secor *et al.*, 2004; Brown *et al.*, 2006), a reduced Schistosome egg excretion (Secor *et al.*, 2003; Kallestrup *et al.*, 2005) and raised HIV-1 viral loads following deworming with chemotherapeutics (Nokta *et al.*, 2001; Mwanakasale *et al.*, 2003).

High prevalence of helminthic infections of ascariasis, hookworm, trichuriasis and Schistosomiasis either promote susceptibility to the HIV virus or aggravate the clinical course and progression of AIDS. Visceral Leishmaniasis has however emerged as an important opportunistic infection and NTD of HIV/AIDS, (Figure 1).

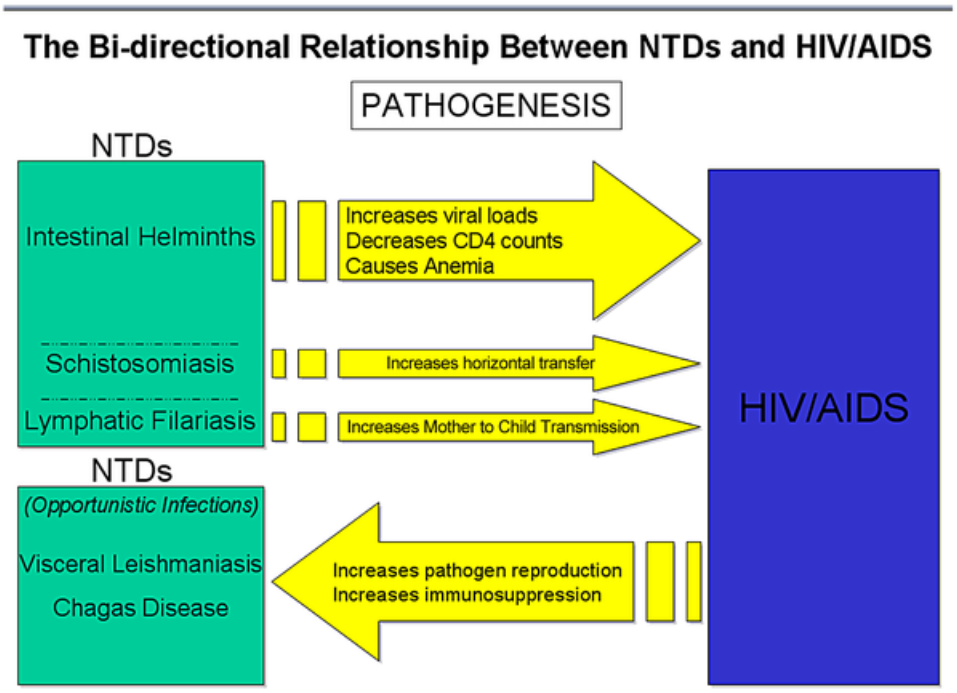

Figure 1.1: Immune interactions of NTDs and HIV (Noblick *et al.*, 2011**)**

Schistosome infections are chronic and are characterized immunologically by an early Th1 response that later switches to a Th2-dominated response after the onset of egg production, (Secor *et al.*, 2003). The most serious form of this infection is hepatosplenic

disease, usually accompanied by severe hepatic and periportal fibrosis **(**Noblick *et al.*, 2011**).** Although Th2 responses seem to have a crucial role in modulating the lethality of Schistosomiasis during its initial stages, its prolonged Th2 responses have been shown to contribute to the development of hepatic fibrosis and chronic morbidity. The main Th2 cytokine that is responsible for fibrosis is IL-13 (Mwanakasale *et al*., 2013)

1.2. Human Immunodeficiency Virus epidemiology

Two major types of HIV are type 1 and 2. HIV-1 is related to viruses found in chimpanzees and gorillas living in western Africa. HIV-2 on the other hand, is related to viruses found in sooty mangabeys (Cohen *et al*, 2008). HIV-1 viruses are further divided into four groups (M, N, O and P). The HIV-1 group M viruses predominate and are responsible for the AIDS pandemic. Group M is further sub classified into ten groups (A, B, C, D, F, G, K, J and CRFs) subtypes based on genetic sequence data. Based on group M, HIV-1 type C is the most common with largest occurrence in Eastern and Southern Africa (UNAIDS, 2001). HIV-1 is the most common pathogenic strain of the virus with more than 90% of cases worldwide. Contrastingly, HIV-2 viruses are less virulent and transmissible than HIV-1 M group viruses (WHO, 2007).

HIV/AIDS is a global pandemic (Cohen *et al*, 2008). Approximately between 32.2-38.8 million people worldwide are estimated to be HIV positive, with new cases being 2.5 million people (UNAIDS, 2013). As at 2013, global mortality rate remained between 1.4-1.9 million: 66% of global mortality still being in the sub Saharan Africa (UNAIDS, 2013). South and South East Asia has an estimated 12% of the global total infections (WHO, 2007). Kenya has a total of 1.6 million people infected with HIV (The Kenya AIDS Epidemic Update, 2012).

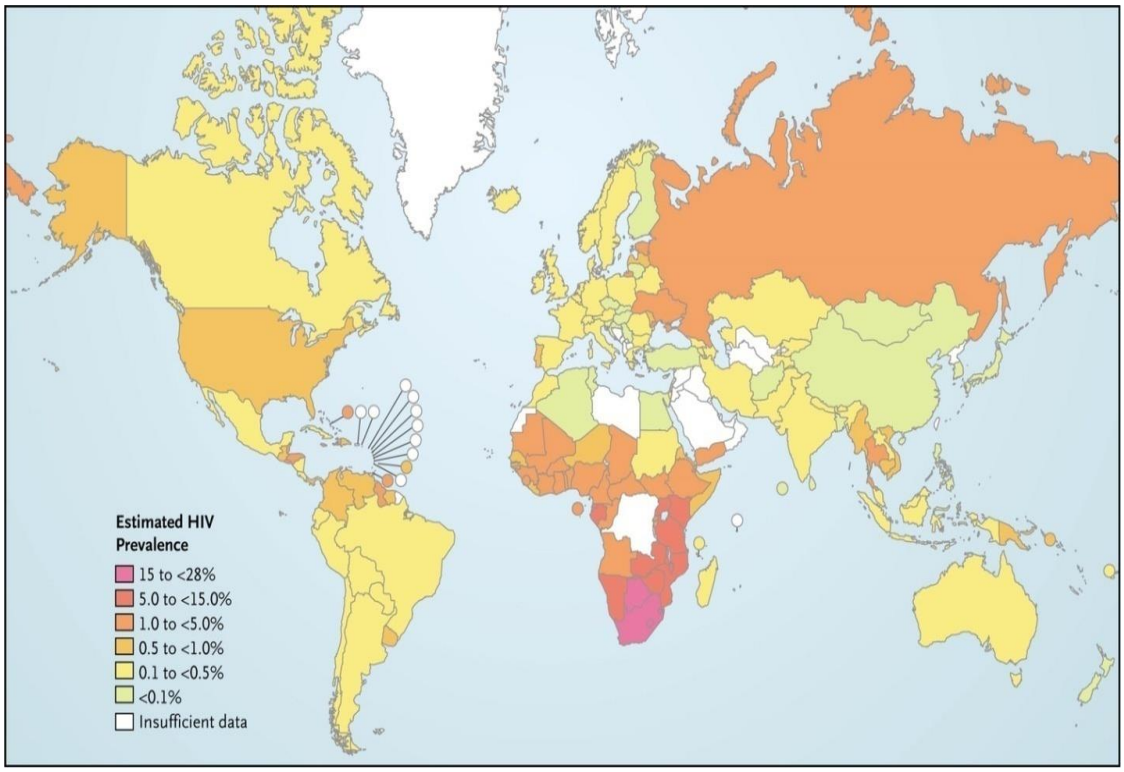

Figure 1.2: World Map of Prevalence of HIV Infection (Piot *et al.*, 2013)

1.3. Epidemiology of Schistosomiasis

Schistosomiasis is one of the most prevalent parasitic infections in the world, with more than 200 million cases (Chitsulo *et al.*, 2002). It causes more than 300,000 deaths annually (Norman *et al.*, 2010), with approximately 779 million people are at risk globally (Mazigo *et al.*, 2013). It is endemic in 76 countries, with emphasis in Asia, Africa, and South America. This makes it a global public health concern in the developing world (Norman *et al.*, 2010). Clinically, Schistosomiasis is a chronic insidious disease thus poorly recognized at initial stages. This becomes a threat to economic development as it disables men and women during their most productive years, (Mazigo *et al.*, 2013). It is mainly linked to agricultural and water development schemes which contain numerous freshwater snails, which may carry the parasite. This is associated to poor living conditions favouring transmission (Chitsulo *et al.*, 2004).

Schistosomiasis is broadly divided into acute and chronic infection. It largely moves to the bladder but also occur in adjacent areas, such as the genital tract, causing ulcerative lesions around the vagina and cervix, resulting in Female Genital Schistosomiasis (FGS) (Kjetland *et al.*, 2005). A large proportion of the eggs become sequestered in the tissue of pelvic organs such as the urinary bladder, cervix, lower ureters, vagina, prostate gland, and seminal vesicles, where they cause chronic inflammation in the affected organs. These results in a number of symptoms and signs including pelvic pain, postcoital bleeding and an altered cervical epithelium in women (Poggensee *et al*, 2000; Kjetland *et al.*, 2005). Ejaculatory pain, hematospermia and leukocytospermia occur in men (Leutscher *et al.*, 2008).

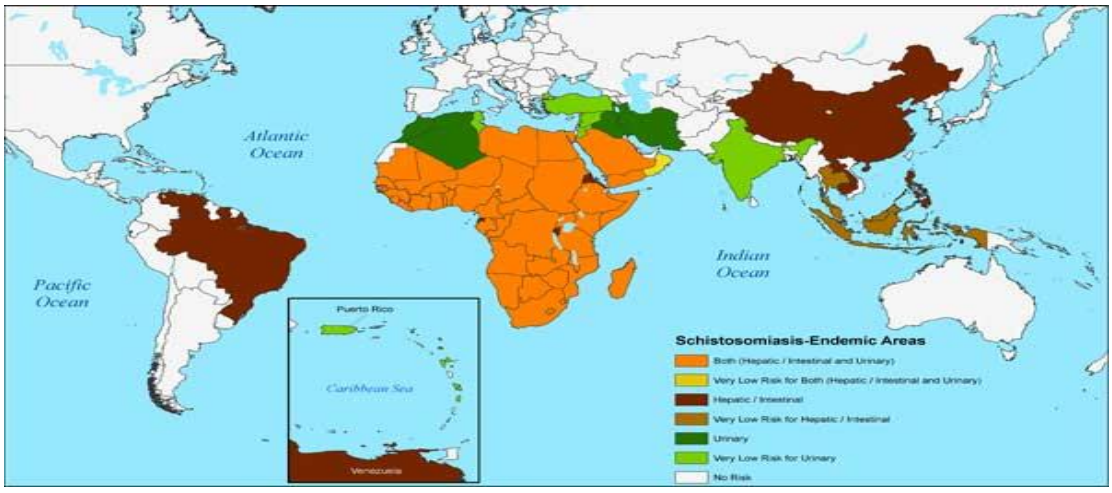

Figure 1.3: World map of Schistosomiasis Endemicity (Mazigo *et al.*, 2013)

1.3.1. Schistosome Life cycle

The stages in the snail include two generations of sporocysts and the production of cercariae. On release from the snail, the infective cercariae swim, enters the skin of the human host, and shed their forked tail, and becomes schistosomulae, which migrate through several tissues and stages into the veins. Adult worms in humans reside in the mesenteric venules in various locations. The females (7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The

eggs are moved progressively toward the lumen of the intestine and are eliminated with feces or urine, respectively. Eggs hatch and release miracidia that swim and penetrate snail intermediate hosts (Figure 1.4).

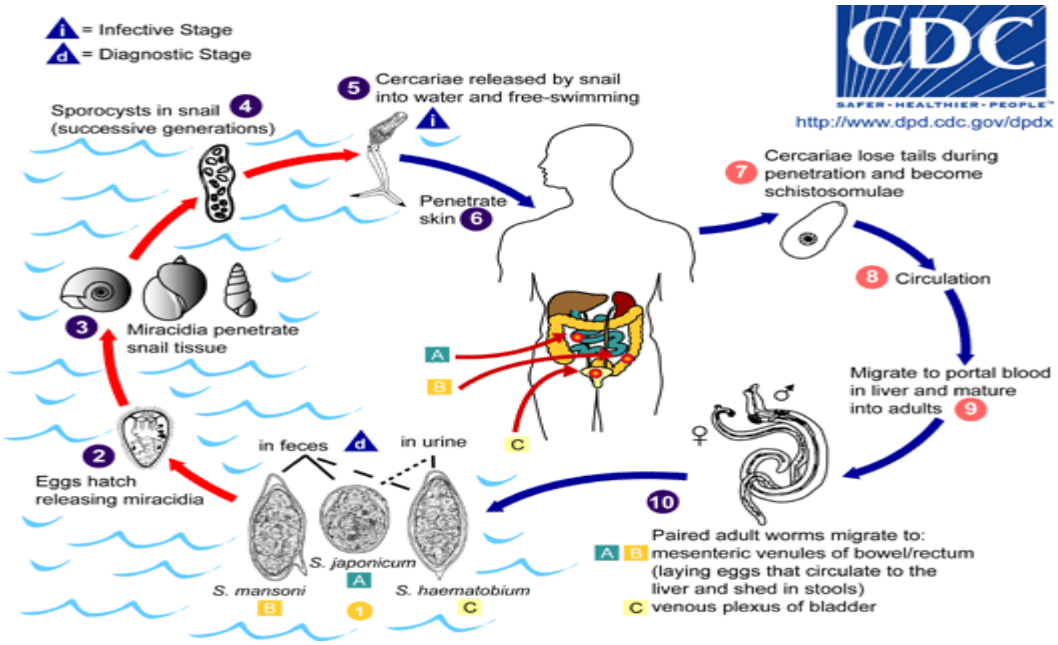

Figure 1.4: Life cycle of *Schistosoma mansoni*; http://www.dpd.cdc.gov/dpdx

1.3.2. Acute Schistosomiasis

Acute Schistosomiasis occurs in the first 3–5 weeks, during which the host is exposed to migrating immature parasites, the dominant response is T helper 1 (TH1)-like. Acute Schistosomiasis in humans is a debilitating febrile illness (Katayama fever) that can occur before the appearance of eggs in the stool and which is thought generally to peak between 6 and 8 weeks after infection (Rabello *et al.*, 1995). During acute illness, there is a raised level of tumour-necrosis factor in the plasma, and peripheral-blood mononuclear cells produce large quantities of TNF, interleukin-1 and IL-6 (Ellis *et al.*, 2008). This reflects a dominant T Helper 1, rather than TH2, response (Ellis *et al.*, 2008).

Abnormally, the febrile illness, associated with the initial stages of Schistosome infection is not common. Individuals living in Schistosomiasis-endemic areas don't experience febrile illness. It occurs, instead, in individuals who lack previous history of exposure who get infected after travelling into an endemic area (Ellis *et al.,* 2008). This is because individuals can become sensitized to Schistosome *in utero* as a result of maternal infection, which subsequently allows them to respond differently from 'naive' individuals when they themselves become infected. Data from analyses of cord-blood lymphocytes taken from the babies of infected and uninfected mothers support the view that in utero sensitization does occur and, more-over, indicate that the fetal response is phenotypically similar to the response of the mother (King *et al.,* 2003).

An examination of schistosome disease in mice has shown that an inability to develop a Th2 response to regulate the initial pro-inflammatory response that is associated with acute schistosomiasis is deadly. This was elucidated when C57BL/6 IL- 4−/− mice were infected with *S. mansoni*. Coincident with the onset of parasite egg production in mice, a condition resembling severe acute schistosomiasis in humans developed, characterized by cachexia and significant mortality (Fallon *et al*., 1999). These mice developed relatively normal hepatic granulomas but they lacked an eosinophil component.

1.3.3. Chronic Schistosomiasis

Chronic Schistosomiasis occurs after 6-8 weeks and it is due to prolonged TH2 response. It is the result of the ongoing host response to accumulating tissue-trapped eggs (Ellis *et al.,* 2008). In *S. mansoni* and *S. japonicum* infections, the liver is the principal site that is affected. A lot of the eggs are carried by the blood flow into this organ and they are trapped the since sinusoids are too small for the eggs to traverse. These eggs eventually die within this liver tissue (Ellis *et al.,* 2008).

Intestinal damage by traversing eggs can also be problematic. During *Schistosoma haematobium* infection, the passage of eggs across the bladder wall causes damage to this organ. The CD4+ T-cell response that is induced by egg antigens orchestrates the

development of granulomatous lesions — which are composed of collagen fibres and cells, including macrophages, eosinophils and CD4 +T cells — around the individual eggs (Raharisolo *et al.*, 2011).

As the eggs die, the granulomas resolve, leaving fibrotic plaques. Severe consequences of infection with *S. mansoni* and *S. japonicum* are the result of an increase in portal blood pressure as the liver becomes fibrotic, congested and harder to perfuse. Under these conditions, the diameter of the portal vein increases and the wall of the portal vein become fibrotic (Xu *et al.*, 2010). Associated with these changes is the development of ascites (the accumulation of serous fluid in the peritoneal cavity) and portal–systemic venous shunts (new blood vessels that bypass the liver), which can rupture, leading to life-threatening bleeding (Raharisolo *et al.*, 2011). The most serious effects of infection with *S. haematobium* are bladder cancer (Poggensee *et al.*, 2001) and genital schistosomiasis, a condition in which eggs pass through the cervix in women or into the testes in men (Ndhlovu *et al.*, 2005).

Paradoxically, granulomas might have an essential host-protective role. In mice that were tolerized against *S. mansoni* egg antigen, granuloma development did not occur during infection and the animals had severe hepatotoxic liver damage, which was evident as microvesicular lipid accumulations within hepatocytes (Xu, 2010). This is thought to be mediated by hepatotoxins that are secreted from eggs, and the granuloma, together with egg-antigen-specific antibodies (which might act in a neutralizing capacity), is envisaged as sequestering these toxins away from hepatocytes (Kjetland *et al*, 2005; Holmen *et al*, 2011). A central role for tumour-necrosis factor (TNF) in the development of the granuloma has been proposed on the basis of one finding that the injection of TNF into infected severe combined immunodeficient (SCID) mice is sufficient to allow the development of a focal lesion around parasite eggs (Jourdan *et al.*, 2011). The interleukin-13 (IL-13) is the major TH2 cytokine responsible for fibrosis. Thus schistosome-infected mice in which IL-13 is either absent (Fallon *et al.*, 2000),

ineffective or neutralized by treatment with soluble IL-13Rα2–Fc 14 fail to develop the severe hepatic fibrosis that normally occurs during infection.

1.3.4 TH1 and TH2 balance

The balance between Th1 and Th2 cell responses to an infectious agent can influence both pathogen growth and immunopathology. The schistosome parasites have evolved the capacity to induce Th2 responses in order to protect themselves against potentially toxic Th1-dependent antiparasitic effector mechanisms (Infante-Duarte, 1999; MacDonald *et al.*, 2002). Schistosomiasis is immunologically characterized by an early Th1 response that switches to a Th2-dominated response after the onset of parasite egg production (Wynn *et al.*, 2004).

Many factors influence the differentiation of Th1 and Th2 cells, including the antigen dose and form, the affinity between the peptide antigen and the T cell receptor (Rogers *et al.*, 1999), the nature and degree of co-stimulation (Balkhi *et al.*, 2004), the presence of antigen-presenting cells, (Gutcher *et al.*, 2007; Wiethe *et al.*, 2008) and the cytokine milieu surrounding the differentiating cells (Zhu *et al*, 2008). In addition, antigen-triggered activation-induced cell death (AICD), which is the primary form of apoptosis for clonally expanded T cells, can influence both pathogen growth and immunopathology. Antigen-triggered activation-induced cell death is considered the primary mechanism for deleting mature T cells in the periphery and it plays an important role during adaptive immune responses by ensuring that a defined number of specialized T cells remain in the organism (Baumann *et al.*, 2002; Krammer *et al.*, 2007).

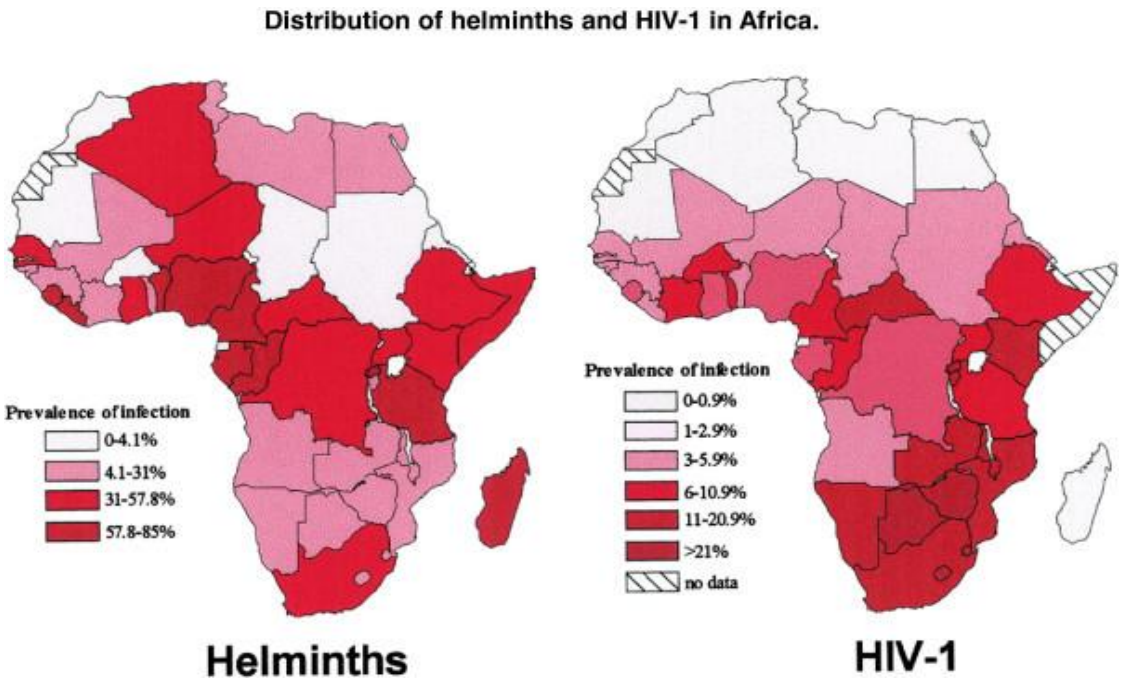

Figure 1.5: Distribution of Helminths and HIV-1 in Africa (Brown et al., 2006)

1.4. Immunological interactions of HIV and Schistosomiasis

The critical peak of HIV-1 includes the destruction or depletion of, both naïve and memory helper $CD4^+$ T-cells and a subsequent loss of immune competence (Stevenson, 2003; Borkow *et al.*, 2004). This raises susceptibility of the host to other diseases (Borkow *et al.*, 2004). An immune response shifts from $CD8^+$ T-lymphocytes to $CD4^+$ T-lymphocytes and subsequent production of its associated cytokines lead to quick disease progression and chronic activation of the immune responses (Maggi *et al.*, 1994; Lawn *et al.*, 2001). The proliferations of the Th2-$CD4^+$ is also related to the proliferations of other related $CD4^+$ regulatory subsets such as T regs which present Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) exerting a regulatory effect on T cell proliferation by producing immunosuppressive cytokines such as TGF-β and IL-10 (Elferafaei *et al.*, 2010).

The TGF-β regulates $CD4^+$ T-cell by inhibiting its proliferation and acquisition of the effectors function by the naïve T-cells (Gorelik *et al.*, 2002). Increased CTLA- 4 expression correlates with markers of HIV disease progression. The up-regulation of the CTLA- 4 also increases CCR5 expression on the surfaces of $CD4^+$ T-lymphocytes. This enhances the susceptibility of these cells to HIV-1 infections and cell to cell HIV transmission (Riley *et al.*, 2000). Destruction of the $CD4^+$ cell pool by HIV-1 infection increases susceptibility of the host to other infectious diseases (Borkow *et al.*, 2004). These immune HIV-1 and *S. mansoni* infections characteristics clearly lead to the potential for a number of immunological one-way and two-way modulating interactions between them in co-infected populations.

The $CD4^+$ T-helper lymphocytes responses are cardinal to the growth of immunopathology in *S. mansoni* infections (Cheever *et al.*, 1997). The chronic phase is characterized by the production of soluble egg antigens (SEA). The inflammatory cytokines response to SEA are significantly reduced and the Th2 response is characterized by up-regulation of IL-10 and TGF-β which down-regulates the production and effectors functions of the Th1 response (Cheever *et al.*, 2000). The release of these cytokines leads to the productions of the Ig G4, suppressed parasites-specific T cell proliferation, reduced level of Th2 and Th1 cytokines (McSorley *et al.*, 2012). At this stage only few *S. mansoni* infected individuals develop severe hepatosplenic disease characterized with development of granulomas The resulting T-dependent granulomas protect host tissues from egg-produced antigens (McSorley *et al.*, 2012).

1.5. Immunological consequences of HIV-1 on *Schistosoma mansoni*

When the helper $CD4^+$ T-lymphocytes are destroyed by the HIV-1 virus in co-infected individuals, granuloma formation could be affected thus altering egg excretion efficiency. Studies which have been done on immuno-suppressed animal models showed that the excretion of *S. mansoni* eggs is dependent on immunity, and that T-cells, most

specifically the response of anti-egg Th 2 (Fallon *et al.*, 1999) is vital to transpose and excrete eggs from the host blood stream into the intestinal lumen (Mwinzi *et al.*, 2001) as well as to develop granuloma (N'Zoukoudi-N'Doundou *et al.*, 1995).

HIV infected people have a reduced eggs excretion of *S. mansoni,* and a subsequent decrease in $CD4^+$ T lymphocytes counts (Karanja *et al.*, 1997). Same observations on the decreased eggs excretion were documented in HIV-1 positive individuals co-infected with *S. mansoni* and *S. haematobium* in Ethiopia, Zambia and Congo (Karanja *et al.*, 1997; Mkhize-Kwitshana *et al.*, 2011). Study arm in rural Zimbabwe was limited by low infection intensities of *S. mansoni*. No association was however shown between the HIV-1 status / $CD4^+$ T lymphocytes counts and the efficiency of eggs excretion (Kallestrup *et al.*, 2005).

The potential for HIV to affect *S. mansoni* egg excretion not only potentially affects parasitic diagnosis of infection, but also co-infection studies. This makes the detection of circulating schistosome antigens released by in situ worms particularly important in detecting and, to some extent quantifying, these infections. Circulating Cathodic Antigens (CCA) and Circulating Anodic Antigen (CAA) are *S. mansoni* related gut antigens which are regurgitated by the adult and juveniles stages with the by-products of host red blood cell digestion. CCA can now be detected by antibody-based rapid diagnostic tests in urine samples (Mwanakasale *et al.*, 2003; Van Dam *et al.*, 2004). Such antigen-detection tests have many advantages, including demonstration of active infections of *S. mansoni* in the absence of detectable egg excretion, the effects of treatment and in term of diagnosis it has high specificity (Mwanakasale *et al.*, 2003).

When CAA/CCA positivity has been employed as a diagnostic criterion for *S. mansoni* infection after praziquantel treatment in HIV-1 and *S. mansoni* co-infected individuals compared with those with only *S. mansoni* infections. The result showed a lower clearance rate of the adult worms in treated HIV-1 positive individuals co-infected with *S. mansoni* (Kallestrup *et al.*, 2006). This observation was inconsistent with the results

of Karanja *et al.,* (1998). Equally decreased levels of CCA was observed following praziquantel therapy in HIV and *S. mansoni* coinfected individuals as compared to individuals with *S. mansoni* infection only (Kallestrup *et al.,* 2006). It was argued that the difference in *S. mansoni* intensity of infection between the two study populations and the dominance of *S. haematobium* in the study of Kallestrup *et al.,* (2006) could have contributed to the discordance between those studies' results. The discrepancy observed between these studies calls for further studies to elucidate efficacy of praziquantel in HIV-1 infected individuals co-infected with *S. mansoni*.

Granuloma formation in *S. mansoni* infection is a $CD4^+$ dependent process. Earlier studies have hypothesized that the destruction of helper $CD4^+$ T-lymphocytes (Th 2) by HIV-1 may lead to a decreased ability of the Th2 arm to produce pro-inflammatory cytokines such as TNF-α, IL-6, IL-1, and IL-13. These cytokines stimulate the inflammatory cells responsible for fibrogenesis, and hence lead to severe hepatic morbidity (Maggi *et al.,* 1994; Mwinzi *et al.,* 2001). Immunological studies have demonstrated that, T-cells from the peripheral blood of HIV-1 positive individuals co-infected with *S. mansoni* responded to egg antigens by producing less IL-4 and IL-10 and a lower amount of IFN-γ as compared to those from individuals infected with *S. mansoni* alone, indicating immune skewing from Th2 to Th1 (Mwinzi *et al.,* 2001).

Supportive evidence from animal models indicates that, granuloma formation is severely restricted in immuno-deficiency mouse models (Actor *et al.,* 1993; N'Zoukoudi-N'Doundou *et al.,* 1995). It is possible that granuloma formation may help to contain hepatotoxins (omega-1-ribonuclease and IPSE/alpha-1) that are released from *S. mansoni* eggs trapped in the liver (Fitzsimmons *et al.,* 2005; Everts *et al.,* 2009). These hepatotoxins may increase the risk of liver parenchyma damage and cause severe necrosis (Doenhoff *et al.,* 1986; Doenhoff *et al.,* 1997).

A community-based study in fishing communities in northwest Tanzania, showed that there were no differences in the distribution of *S. mansoni* related morbidities, as

detected by ultrasonography, between HIV co-infected individuals with *S. mansoni* and those with single infection of *S. mansoni* (Malenganisho *et al.*, 2005). This study though did not measure the $CD4^+$ counts of the HIV-1^+ positive study participants co-infected with *S. mansoni.* Similarly, in Kenya, there were no significant differences in the distribution of ultrasound-detectable pathology (hepatomegaly, splenomegaly, hepatic fibrosis, periportal fibrosis and gall bladder wall thickness) in HIV-1 positive individuals co-infected with *S. mansoni* as compared with HIV-1 negative individuals infected with *S. mansoni* (Mwinzi *et al.*, 2004). The study demonstrated that hepatic fibrosis, in the absence of severe hepatosplenomegaly, was associated with a significant decrease in $CD4^+$ T-lymphocytes in HIV-1 negative individuals infected with *S. mansoni,* and that the decrease correlated with increasing grade of liver fibrosis (Mwinzi *et al.*, 2004).

In HIV-1 positive individuals co-infected with *S. mansoni,* reduced $CD4^+$ T-cells counts levels does not necessarily imply the development of severe hepatic morbidities (Mwinzi *et al.*, 2004). In addition, there is no difference in the level of measurable fibrosis and the level of liver parenchyma damage as measured by the levels of circulating liver enzymes (glutamic oxaloacetic transaminase and aspartate aminotransferase) in individuals with HIV-1 co-infection as compared with HIV-1 negative individuals infected with *S. mansoni* (Mwinzi *et al.*, 2004). Importantly, there were no significant correlations between $CD4^+$ T cells count and circulating liver enzyme levels in HIV-1 positive individuals co-infected with *S. mansoni* or in HIV-1 negative individuals infected with *S. mansoni* (Mwinzi *et al.*, 2004). However, it is worth noting that the passive transfer of specific anti-omega 1 antibody is sufficient to completely prevent hepatocytes damage in *S. mansoni* infected immunosuppressed mice that have severely impaired anti-egg granuloma (Dunne *et al.*, 1999). Anti-omega 1 antibodies were not assayed in the human studies (Mwinzi *et al.*, 2004).

The observation that hepatic fibrosis is associated with reduced $CD4^+$ T-lymphocytes in HIV-1 positive and negative individuals implies that *S. mansoni* associated liver pathologies could speed up the progression of HIV to AIDS through the depletion of

$CD4^+$ T cells in co-infected individuals (Mwinzi *et al.*, 2004). It could also be speculated that, the hyporesponsiveness of the T-cells due to chronic activations of the immune system and differentiations of the T regs and released of TGF-β and IL-10 could in part explains the low $CD4^+$ counts observed in HIV-1 positive and HIV-1 negative individuals infected with *S. mansoni*.

1.6. Immunological consequences of *Schistosoma mansoni* on HIV-1

Schistosoma mansoni infections induce an immune modulation, changing from T-helper 1 to predominant Th 2 dominated cytokines (Pearce *et al.*, 2002). These Th2 associated cytokines down-regulate the cytotoxic effects of $CD8^+$ lymphocytes which are critical for the early control of viral replication (Pearce *et al.*, 2002).

In mice studies, co-infections with *S. mansoni* and vaccinia virus expressing the HIV envelope showed a shift towards a Th_2 response which thus reduced Th1 cytokines production and impaired the cytotoxic effects of $CD8^+$ on the virus (Karanja *et al.*,1997). Additionally, up regulation in viral replication and the alteration of T-cells subsets have been observed in Rhesus Macaque monkeys co-infected with *S. mansoni* (Ayash-Rashkovsky *et al.*, 2007). In human studies, HIV-1 positive individuals co-infected with *S. mansoni* in western Kenya demonstrated an alteration of the immune response to *S. mansoni* characterized by a low level of IL-4 and IL-10 production (Mwinzi *et al.*, 2001).

Studies done *In vitro* on human PBMCs from schistosomiasis infected individuals have shown a heightened susceptibility of the said cells to HIV-1 in comparison with those people who are helminth free (Secor *et al.*, 2003). Chemokine receptors CCR5 and CXCR4 expression on $CD4^+$ T-lymphocytes surface, which have been stimulated by Th_2 cytokines, make these cells more susceptible to HIV-1 infection (Nokta *et al.*, 2001; Secor *et al.*, 2003). In fact, these receptors serve as co-receptors for HIV-1 entry into the cells (Nokta *et al.*, 2011). In Kenya, individuals infected with schistosomiasis expressed

higher cell surface densities of these receptors as compared to individuals cured of the disease (Secor *et al.*, 2003). These observations imply that HIV-1 replication proceeds more rapidly in activated T cells, especially in those with Th2 or Th0 phenotypes (Nokta *et al.*, 2001)

Individuals co-infected with HIV-1 and *S. mansoni* may have reduced ability to mount potent protective immune responses against a number of viral infections. For instance, individuals co-infected with chronic Hepatitis C virus and *S. mansoni* demonstrated a decreased HCV- specific $CD4^+$ T cell proliferative response as compared with individuals with HCV alone (Kamal *et al.*, 2001). In Uganda, concomitant infections of *S. mansoni* and HIV-1 was associated with decreased Gag-specific cytolytic ($CD8^+$) responses, showing an alteration of the effectors functions of HIV infection attributed to Schistosomiasis (McElroy *et al.*, 2005). Moreover, the detection of Gag-specific positive $CD8^+$ T cells in co-infected individuals shows that *S. mansoni* may be responsible for the modulation of the cellular immune response to HIV (McElroy *et al.*, 2005).

T-regulatory cells are an important component of regulation of T cell activation. It has been reported that an expansion in T-regulatory cells occurs during the chronic phase of HIV infection. There is some debate as to whether the expansion of T-reg numbers is detrimental, due to suppression of cellular mediated immunity, or beneficial, due to limiting cellular activation, and therefore co-receptor expression and targets for HIV-1 infection (Moreno-Fernandez *et al.*, 2012). *S mansonii* infection was found to expand the proportion of circulating CD25hi $CD4^+$ cells, a significant proportion of which are likely to be $FoxP3^+$ve T-reg cells, amongst sand-harvesters in Kisumu, Kenya (Watanabe *et al.*, 2007). However, no significant difference in the proportion of $CD4^+$CD25 Thi cells was observed between individuals who were sero-positive and negative for HIV-1 (Watanabe *et al.*, 2007).

Evidence suggests that the expression of the co-receptors on activated $CD4^+$ T lymphocytes increases the susceptibility of these cells to HIV infection in HIV-

uninfected populations, and may also speed up the progression of HIV to AIDS by increasing plasma viral loads and decreasing $CD4^+$ T-lymphocytes in co-infected individuals (Nokta *et al.*, 2002; Secor *et al.*, 2003). Increased HIV plasma viral loads determine disease progression and risk for HIV transmission in between partners (Mellors *et al.*, 1996). However, most cross-sectional and other observational studies have failed to provide evidence that decreased $CD4^+$ T cells and increased HIV-1 viral loads is associated with heavy helminth infections (Elliot *et al.*, 2003: Brown *et al.*, 2006,). Previous authors have suggested that helminth infection intensity could in part contribute to decreased $CD4^+$ T cell counts or speed up the progression of HIV-1 to AIDS, meaning that HIV-1 positive individuals with higher *S. mansoni* infection intensity could have reduced $CD4^+$ T cells counts and higher HIV-1 viral loads. Similar findings on the other helminth species have been reported (Mkhize-Kwitshana *et al.*, 2011).

1.7. SAAVI DNA and MVA Prototype vaccines

HIV SAAVI HIV DNA and MVA vaccines are prototypes T-cell vaccines which contain the major HIV peptides like Gag, Tat, Nef Pol and Env which are together aimed at inducing TH_1 immunity. The vaccines have genes which are codon-optimized to improve their expression in humans. These HIV genes are also modified to increase immunogenicity, stability and safety and their safety and efficacy in preclinical use has been proven.

1.8. HIV Prophylactic vaccines

The most tremendous achievement in HIV research up to date is the development of drug-based anti-retroviral therapy (ART) (Piot & Quin, 2013). This has slowed, or even halted, the progression of AIDS though it cannot cure the disease. For most cases, HIV-infected individuals left untreated rarely survive; hence ART intervention remains crucial in a patient's lifetime. This lifelong dependence on anti-retroviral drug therapy

raises important concerns on their sustainability and affordability and presents daunting global economic and health problems (Piot & Quin, 2013).

A HIV vaccine, whether prophylactic or curative, remains the best solution for the control of HIV (Hanke, 2014). A safe and most efficacious vaccine has been widely agreed as the most cost effective means to stop the spread of AIDs epidemic, (Yang *et al.*, 2014). Vaccine research is a long process that begins with basic research to identify potential immune correlates of protection, designs of appropriate Immunogens, or vaccine concepts, and obtains safety, immunogenicity, and protection data in animals. The proceeding step is to translate promising vaccine concepts into candidate vaccines that are appropriate for clinical evaluation in human volunteers (Esparza *et al.*, 2000).

The research of HIV vaccine has been a long excruciating process that has lasted for over three decades (Esparza, 2013). This is because the paradigm that has been formulated to develop most existing viral vaccines, which is based on the recreation of the protective immunity that develops after natural infection, does not work in the case of HIV. In HIV infection, virus-induced immune responses are not capable of preventing re-infection or are very inefficient in slowing progression to disease (Esparza, 2013). Other challenges which have limited the production of efficacious HIV vaccines include lack of small experimental animal models of vaccine protection and largely unavailability of a safe controlled challenge for vaccinated human volunteers, establishment of latent infection, huge pathogens genetic plasticity leading to variant strains circulating in the population and sudden escape from assembled immune responses. The HIV vaccine research has been widely categorised into induction of broad neutralizing antibodies and cell mediated immune response (Esparza 2013).

1.8.1. Induction of neutralizing antibodies

This was the initial HIV vaccine target research that was done from 1986 to 2003. It was thought to be the most effective way of conferring protection (Plotkin *et al.*, 2008). This was based on the concept that neutralizing antibodies (nAbs) is sufficient in protection

against HIV infection. It however came clear later that HIV, an enveloped retrovirus, presents challenges for conventional neutralizing antibody-based strategies of vaccines. It quickly mutates to alter its surface structure, uses host-derived non-immunogenic glycans to block its exposed surface, and hides its conserved and potentially susceptible regions, like the CD4 binding site in the oligomeric proteins interfaces (Zollar-Pazner *et al.*, 2010). The mechanism of neutralization by neutralizing antibodies lies in either blocking the interaction between viral envelopes with their receptors or inhibiting viruses for their further transport to cytoplasm (Yang *et al.*, 2014). Numerous HIV vaccines constructs were developed based on the envelope glycoproteins of the Human immunodeficiency virus principally gp120 and gp160, which are critical for the viral binding to the target cells. The said envelope glycoproteins are the principal targets for the neutralizing antibodies. In 1988, an initial HIV vaccine trial was conducted in the USA. It evaluated a recombinant gp160 produced in a baculovirus-insect cell system. It however showed no significant antibody protection against HIV (Myers *et al.*, 1992).

Several lines of research have been explored targeting HIV antibodies use of poxvirus vectors to prime the antibody responses, identification of different genetic subtypes of the virus, R5 and X4 virus phenotypes classification (Myers *et al.*, 1992). The first efficacy trial of neutralizing antibody vaccine in 1998 was the phase III vax 003 vaccine study trial done in Thailand which was composed of two rgp120 HIV-1 envelope antigens from subtype B and E. The result of this vaccine on elicitation of neutralizing antibody was disappointing (Buchbinder *et al.*, 2009).

A related study was a phase III trial of vax004 was done in 1999 in North America and Denmark. The study vaccine also contained 2 rgp120 HIV-1 envelope antigens AIDSVAX B/B that had been derived from two different HIV -1 subtype B strains and that were adsorbed onto 600 mg of alum. The rgp 120 protein contained the CCR5 phenotype. This vaccine candidate showed a 6 % result in the induction of neutralizing antibodies against HIV (Pitisuttithum *et al.*, 2006). Generally, in most vaccine trials, close to 20% of chronically HIV-infected people have generated nAbs and 2%–4% of

them have broadly neutralizing antibodies able to neutralize most tested HIV strains (Simek *et al.*, 2009), these antibodies are only produced after months to years of virus infection.

Gene therapy approach was also explored as an alternative to generate Vaccine-Like Protection by delivering genes which encode nAbs into non-hematopoietic tissues, like muscles, a method referred to as Vectored ImmunoProphylaxis (VIP) (Berkhout *et al.*, 2012; Balaz & West, 2013). This was because a passive immunization by the discussed antibody, which, although conferred some immunity, requires a long term repeated treatment which is too expensive, making its broad implementation in humans a great challenge. The VIP method involves a single injection of Adenovirus Associated Antibody vectors for in vivo delivery of genes encoding broad neutralizing antibodies. Some studies in monkey models have shown that VIP is feasible against HIV. This gives a clear picture of what can be done in human trials.

1.8. 2. Induction of Cytotoxic T-Lymphocytes responses

The step started with the recognition that importance of CD8+ T-cell responses in the control of HIV infection, (McMichael and Hanke, 2002). This new prototype led to the development and refinement of live recombinant viral vectors, especially poxvirus and adenovirus vectors, as well as of DNA vaccines. A phase IIb double-blind, randomized test-of-concept study in Phambili trial/v520-027 was done in South Africa, a region with the highest prevalence of HIV in Africa at 5.7 million people (Grey *et al.*, 2011). This was an MRKAd5 HIV-1 gag/pol/nef vaccine, which consisted of equal mixture of three separate replication-defective Adenovirus serotype 5 vectors, one each expressing the gag gene from the HIV-1 strain CAM-1, the pol gene from HIV-1 strain IIIB and the *nef* gene from HIV-1 strain JR-FL. This vaccine was targeting a CTL response but unfortunately it was stopped in 2007 due to its failure to induce the said response.

A related study of the MRKAd5 HIV-1 gag/pol/nef vaccine, named as the STEP trial was done in HIV-1 clade B endemic regions in North America, South America,

Caribbean and Australia in 1999. It's make up was as described in the Phambili trial (Grey *et al.,* 2011) and was administered in three doses at various time points. The result of this CTL induction was insignificant too. (Buchbinder *et al.,* 2009).

In Kenya, the Kenya Aids Vaccine Initiative conducted three of the first phase and a phase 2Aclinical trials of HIV vaccine which included a clade of HIV DNA plasmid vaccine, a recombinant vaccine which is vectored with MVA (MVA.HIVA), the VRC recombinant adenovirus which is defective of replication and has a subtype 5-vectored multi-clade vaccine. The phase 2A tested a plasmid DNA.HIVA in a prime boost which was followed by MVA.HIVA boost. The study reported that over 61 percent of the included participants were due to the abnormalities of the laboratory (Omosa-Manyonyi *et al*., 2011).

1.8.3. Combinations of Cytotoxic T-Lymphocytes and Neutralizing antibody Immune Responses

This is an approach that was taken after the failure of the STEP trial (Esparza 2013). It was proposed that a successful vaccine to prevent HIV infection would likely require both the elicitation of antibody and cell-mediated responses (Johnson *et al.,* 2013). This approach has used various non-replicating viral vectors as canarypox virus, adenovirus, fowl pox virus, vaccinia vectors and DNA plasmid vectors. Some of the viral-vectored vaccine approaches have reached advanced testing – with two vaccine strategies reaching clinical efficacy trials– canarypox virus(ALVAC) and adenovirus serotype 5 (Ad5) modified vaccinia Ankara (MVA) and highly attenuated vaccinia virus (NYVAC) (Johnson *et al.*, 2013).

In the study of Canarypox vectored HIV vaccines, ALVAC-based vaccines studies in adult have modestly and effectively induced HIV antibodies, but with the suboptimal cellular immune responses in early trials. In HIV Vaccine Trials Network (HVTN 203), ALVAC vCP1452 boosted with recombinant gp120 (MN-GNE8 rgp120) yielded interferon gama (IFN-gama) enzyme-linked immunosorbent spot (ELISPOT) reactions

in 16% of the vaccinated participants (Russell *et al.*, 2007). This prime–boost regimen failed to produce predetermined significant CD8 þ CTL responses in the phase II study, leading some in the field to argue to reconsider any further development of this vaccine approach against HIV. However, a prime–boost regimen with ALVAC and recombinant gp120 boost was advanced to a phase III efficacy trial (RV144) and gave hope in the vaccine field research (Russell *et al.*, 2007).

RV 144 phase III trial in Thailand was done in two years after the STEP trial failure. This is an example of a canarypox vectored vaccine which reached a clinical trial. It was a community-based, randomized, multicenter, double-blind, placebo-controlled efficacy trial. The study was aimed at evaluating four priming injections of a recombinant canarypox vector vaccine (ALVAC-HIV [vCP1521]) with additional two boosting injections of a recombinant glycoprotein 120 subunit vaccine (AIDSVAX B/E). The said recombinant canarypox was genetically modified. Consequentially, it expressed HIV-1 Gag and Pro (subtype B) and CRF01_AE (subtype E) HIV-1 gp120 linked to the transmembrane 3 anchoring portion of gp41. So far, in the history of HIV vaccine research, this trial gave the most promising results and 31.2% of the participants showed an elicited $CD^{+}8$, CD+ 4 T cells and binding antibodies to Env in most vaccine recipients (Rerks-Ngarm *et al.*, 2009). The post-trial investigations of the immune correlates of protection in RV144 are ongoing (Johnson *et al.*, 2013).

In vaccine field researches involving adenovirus vectors, a phase one trial of recombinant Adenovirus 5 vaccine was carried out in volunteer participants in the USA. This vaccine regimen was administered as a four prime vaccinations with rAd5 vectored vaccines containing HIV-1 subtype B gag-pol fusion peptides and env from HIV -1 clade A,B and C in the 3:1:1:1 ratio respectively. A single injection induced HIV-1 antigen–specific CD4+ T cell, CD8+ T cell, and antibody responses in the majority of vaccine recipients.

A similar study was also done in the USA from 2009-2013 and was referred to as HVTN 505. This was a randomized, double-blind, placebo-controlled trial of the VRC's

DNA/rAd5 HIV-1 vaccine. The DNA prime was made of six closed circular plasmids (in a 1:1:1:1:1:1 ratio) designed to individually express HIV-1 clade B Gag, Pol, and Nef and Env proteins from clades A, B, and C. The rAd5 boost consisted of four rAd5 vectors (in a 3:1:1:1 ratio) expressing an HIV-1 clade B Gag-Pol fusion protein and Env glycoproteins from clades A, B, and C. The DNA and rAd5 placebo was phosphate-buffered saline. The DNA/rAd5 vaccine regimen did not reduce either the rate of HIV-1 acquisition or the viral-load set point in the population studied and was halted in 2013 (Hammer *et al.*, 2013).

Studies by Chege *et al.*, (2008) of a DNA vaccine expressed HIV type 1 southern African subtype C Gag (pTH Gag) and a recombinant baculovirus Pr55gag virus-like particle. The recombinant particle was obtained using a subtype C Pr55gag protein (Gag VLP). When tested in a prime–boost inoculation regimen in Chacma baboons, the DNA vaccine candidate showed an elicited cellular and humoral response.

Further studies in South Africa whereby a DNA vaccine (SAAVI DNA-C) was prime-boosted with an MVA (SAAVI-MVA-C) vaccine confirmed a heightened cellular and humoral immune response. TH-1 cytokine IFN-Ɣ level was increased 20-folds and TNF-α was increased 8-fold. The combination also induced high titers of antibody to gp 120 (Enid *et al.*, 2008). These studies confirm that HIV-1 DNA vaccines are immunogenic and their efficacy can be improved further in a prime-boost. In relationship to Schistosomiasis worm infection, the cellular and the humoral responses as the arm of immunity may be interesting to study as a correlate of protection in the performance of the HIV DNA vaccine. The type of response that may be elicited by an HIV candidate vaccine in the presence of Schistosomiasis worm may be determined by the stage of the worm infection: that is, if the worm infection is just starting (acute) or it is already advanced (chronic). The cellular immune response remains by far the most potent weapon as a correlate of protection in the RV 144 Thai trial (Pittisutithum *et al.*, 2009). South African SAAVI DNA-C vaccine alone (Burgers *et al*, 2006), SAAVI DNA –C with an MVA prime boost (Shephard *et al.*, 2008), Recombinant Modified Vaccinia

Ankara- rMVA expressing gag, env-pol genes (Earl *et al.,* 2009), ALVAC-HIV priming and AIDSVAX B/E boosting (Rerks-Ngarm *et al.*, 2009), where in most cases, the response to $CD^{+}8$ T cells cytokine was often higher. Depending on the stage of the worm infection, the presence of $CD4^{+}$T CD 25^{+} T cells can be detrimental to the performance of HIV vaccines (Wang *et al.,* 2013). $CD4^{+}$ $CD25^{+}$ T cells are T regulatory cells which are meant to regulate immune reactions following an increased immune activation. These cells can be vaccine induced or naturally occurring. At the advanced stage of Schistosomiasis infection, in the presence of HIV infection there could an imbalance between the T regs, evidenced by the higher presence of IL-10, and TH1 cytokines-mostly IFN-Ɣ (Wang *et al*., 2014). The continuous presence of IL-10 in a co-infection can negate the performance of the candidate HIV vaccine and vice versa.

A TH2 IL-13, cytokine responsible for hepatic fibrosis can also be linked to HIV DNA vaccines performance. This cytokine occurs on a prolonged TH2 response following an initial switch from TH1 on the chronic Schistosomiasis infection. Studies have shown that Th1 responses associated mediators, such as IFN-γ, IL-12, TNF and NO can prevent IL-13-mediated fibrosis. HIV DNA vaccines have been shown to induce Th1 responses, such as IFN-γ, IL-2 and IL-4 (Wang *et al*, 2014).

1.9. Statement of the Problem

The ability to evoke an effective immune response after vaccinations may be severely compromised in a host with concurrent helminthic infections. The efficacy of HIV vaccines in established Schistosomiasis infection has not been scientifically verified. The HIV DNA and MVA vaccines under study have the potential of eliciting a cell mediated immunity because of the HIV peptides as their components. The efficacy of HIV-1 DNA vaccine in ongoing chronic Schistosomiasis infection in a host could severely be compromised or enhanced, however, this has not been scientifically proven. It is on this scope that the study aims at linking the gap of cellular immune correlate of

HIV candidate vaccine in an initiated *Schistosoma mansonii* worm infection to the protective potential of a developed candidate HIV vaccine.

1.10. Justification

Parasitic worm infections such as Schistosomiasis, filariasis or a variety of hookworms are extremely prevalent in many parts of Africa. Such acute infection and/or re-infection have been shown to down-modulate immune responses against several viral pathogens *in vivo.* It has been shown to seriously impact not only ongoing viral pathogen infections but also has the capacity to markedly reduce the effectiveness of vaccination campaigns against pathogens such as HIV-1 in areas of high endemicity. It is thus of importance to find out if Schistosomiasis could have an impact on the protective potential of the HIV vaccine. Baboons are the best models which have been used to test the safety and immunogenicity of various candidate human vaccines, including HIV vaccines. Although macaques are commonly used as vaccine immunogenicity models, several factors make vaccine studies more attractive in baboons. Importantly there is a high sequence homology between the components of the human immune system and that of the baboon, with the baboon having four subclasses of immunoglobulin analogous to humans, unlike the macaque, which has only three subclasses.

HIV SAAVI HIV DNA and MVA vaccines are prototypes T-cell vaccines which contain the major HIV peptides like Gag, Tat, Nef Pol and Env which are together aimed at inducing TH_1 immunity. The vaccines have genes which are codon-optimized to improve their expression in humans. These HIV genes are also modified to increase immunogenicity, stability and safety and their safety and efficacy in preclinical use has been proven.

1.11. Hypothesis

Schistosomiasis does not negatively affect the protective immune responses induced by vaccination against HIV infection.

1.12. Objectives

1.12.1. General Objective

To investigate the effects of Schistosomiasis on the protective potential of candidate HIV SAAVI DNA-MVA vaccines in *Papio anubis*.

1.12.2. Specific objectives

1. To determine the response of Immunoglobulin G Antibody to crude Schistosomiasis antigens (SEA and SWAP) and HIV peptides in *Papio anubis*
2. To determine the cytokine response (Interleukin 4) in Schistosomiasis infected and vaccinated *Papio anubis*
3. To determine the influence of Schistosome infections on the production of interferon gamma in *Papio anubis.*
4. To determine IFN-γ responses to individual vaccine Immunogens in Schistosome worm-infected and uninfected *Papio anubis.*
5. To determine the response of Immunoglobulin G Antibody against HIV gag protein.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study Site

This study was carried out at the Institute of Primate Research (IPR) in Karen, Nairobi, Kenya. IPR is a fully fledged research institute of the National Museums of Kenya. Experimental animals were kept and cared for by staff of the Animal Science Department. All laboratory analyses were performed in the Schistosomiasis and Virology laboratories under the Department of Tropical Infectious Diseases. The study animals were obtained from the colony at the IPR in Karen and were screened for any type of infection before being included in the study.

2.2. Experimental Design

The study used the simple random sampling technique to sample the experimental baboons. In this technique, a group of subjects or a sample for a given study is selected from a larger population. The technique was employed since every baboon can be chosen randomly entirely by chance and each baboon in the entire population had an equal chance of inclusion in the sample. Every possible sample of a given size had the same chance of selection. The total sample taken was 8 baboons (Chege *et al*., 2013). The selection was based on the health of a baboons. The study animals therefore included healthy, adult baboons which weighed between 10 to 13 kilograms. The study baboons included both male and female animals. This study comprised of the experimental baboons (Group A) and the control baboons (Group B) groups. Each of the group had 4 baboons ((n=4) per group). The experimental baboons were infected with *S. mansoni* by exposing them to the infective stage 3 (L3) larvae (500 cercariae per animal) as described in above (Section 2.2.5(b)). Animals in Group B were left uninfected to serve as the control.

Fifteen weeks later, Group A animals were immunized with two DNA vaccines four weeks apart during the chronic stage. A final boost with MVA was done 12 weeks later. The study animals were terminated and perfused 8 weeks later. For animals in Group A, faecal samples for determination of schistosomal egg shedding were collected once per week, between Weeks 5 and 21post infection. Blood was obtained from both the experimental group 15 weeks after cercariae infection (the same time when the 1st DNA vaccine was administered), 4 weeks later (2nd DNA vaccination), 6th, 8th 12th Week (MVA boost), 13th 15th 17th and the 19th week (time of termination and perfusion) (Figure 2.1).

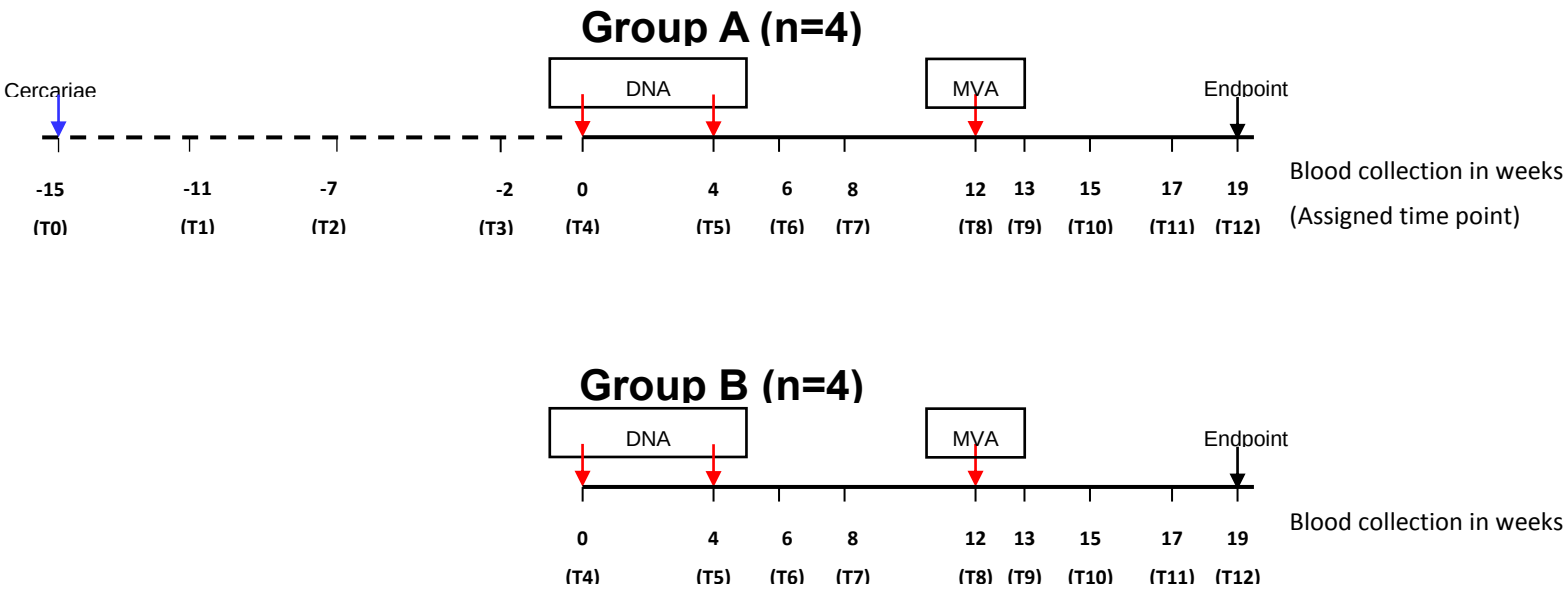

Figure 2.1: Schematic diagram of experimental design.

The study animals were divided into Group A and group B of 4 animals each. Group A was infected with 500 cercarie and allowed to progress to chronic infection then vaccinate at various time points shown. Group B was not infected with Schistosomiasis but was vaccinated at the same time points. Blood collection was done at various stated time points.

2.3. Study Animals

Eight olive baboons were included in the study. The eight baboons were divided into two groups of four baboons each. The healthy baboons used were weighing between 10-13 kilogram. The baboons were screened for Schistosomiasis and their negative infection status was confirmed by the absence of Schistosome antibodies (by using the enzyme-linked immunosorbent assay (ELISA), and faecal eggs (by using a Kato-Katz smear). SIV screening was also done by antibody ELISA to ensure they were negative. The individuals selected for the study were then assigned to the experimental and control groups through simple random sampling technique.

2.4. *Schistosoma mansoni* parasite maintenance and cercariae production

Adult *Biomphalaria pffeiferi* snails were picked from paddy rice fields at Mwea, Muranga County in Kenya and brought to the IPR laboratory and they were screened after one week by exposing the snails to an electric light of 100 watts for ten to twenty minutes to ensure that the snails were free from parasites. The adult snails are kept in water at a room temperature and fed on soft lettuce twice a week. The parasite-free snails were then infected with myracidia which then developed to cercariae after one week. The obtained cercariae were percutaneously introduced into the baboon via the skin. In the baboons, the cercariae losed tails and became schistosomules which entered into circulation, migrated into the portal blood in the liver and matured into adults. The adults migrated into the rectum and laid eggs which circulated into the liver and shed in the stool. The stool sample was collected from the infected baboon, washed three times

in a petri dish and exposed to 100 watts of light for 30 minutes to 1 hour after which the eggs in the faeces hatched thus developed to myracidia (Farah *et al*., 1999). The snails were then infected with the myracidia and the cycle continued.

2.5. HIV Vaccines

Two vaccines were used in the study.

2.5.1. HIV-1 DNA vaccine

This is a prototype T-cell HIV vaccine candidate developed at the University of Cape Town (UCT). The vaccine contains the major HIV peptides Gag, Tat, Nef Pol and Env which are together aimed at inducing TH_1 immunity. The vaccine code –named SAAVI DNA-C, comprising of two DNA plasmids as equivalent molar mixture. These plasmids named pTHr.grttnC and pTHr.gp150CT plasmids are expressing a polyprotein derived from *gag, reverse transcriptase*, *tat* and *nef* and a truncated *env* respectively (Burgers *et al*., 2006). These genes which were included in the vaccine were obtained from isolates within 3 months of HIV infection. All these genes were optimized in terms of codons to improve their expression in humans. Also, the said HIV genes were modified to increase immunogenicity, stability and safety as described (Burgers *et al.*, 2006). This included inactivation of *tat* by gene fragments shuffling, mutating, *reverse Transcriptase* and truncation of gp160 by removing 124 amino acid from its cytoplasmic tail. Myristylation sites of *nef* and *gag* were also inactivated.

2.5.2. HIV-1 MVA vaccine

This is also prototype T-cell HIV booster vaccine candidate developed at UCT. It is intended as a booster vaccine after an HIV -1 DNA vaccination. It is made of the same five genes of HIV-1 subtype C, that is, *gag, reverse transcriptase*, *tat nef* and *env* (*grttn*) as a booster vaccination. All these genes are expressed as a polyprotein and a truncated *env* (gp 150). The genes used were human codon-optimized to improve genetic stability.

The grttn and gp 150 were inserted into two various sites in a stable Modified Vaccinia Ankara to yield a double recombinant code named SAAVI MVA-C, (Burgers *et al.*, 2008).

2.6. Infection and Vaccination

2.6.1. Animal Restrain and Handling

The experimental animals were kept in individual cages with proper medication and feeding for the entire research period. Any procedure like bleeding, Cercarial infection, vaccination, and treatment, was done under the supervision and guidance of the veterinary doctor in charge. During perfusion, a mixture of 10 mg/kg of ketamine and 0.5 mg/kg of xylazine was used as an anaesthesia and it was given intramuscularly.

2.6.2. Infection of the baboons with *S. mansoni* cercariae

Infection with cercariae was done under a controlled environment in the procedure room. The skin of the study animals were shaven below the belly. 500 cercariae were exposed to the shaven skin percutaneously for thirty minutes to mimic the natural exposure to the skin. The baboons were then allowed to progress to chronic phase of schistosomiasis.

2.6.3. Schistosome egg counts in Faeces by Kato-Katz technique

To determine the eggs in the faeces, a layer was made using a paper towel on the working bench in the fume chamber then a template with a hole placed in the centre of the microscope slide. A small amount of a freshly collected faecal sample was obtained, weighed, sieved then 50-mg of the sample placed on three templates per sample over the slide covering the whole template holes. The template was removed carefully leaving the small portion of the faecal sample on the slide. The faecal sample was then covered with a cellophane strip pre-soaked in a malachite green stain. The microscope slide was

inverted and the faecal sample firmly pressed against the cellophane strip on a smooth hard surface to obtain a very thin film. The slide left to air-dry in the hood then counting of the eggs was done using a coulter counter (Farah *et al*., 1999). This was done by observation of the schistosomal eggs under a light microscope.

Mean eggs per gram (epg) was estimated from the triplicate 50-mg fresh faecal samples and the results reported as mean eggs per gram.

2.6.4. Animal vaccination

Preparation of the injection site was done by bringing the vaccine from a condition of -80^0 C to the room temperature then the quadriceps muscles for both the two limbs were injected intramuscularly with I mL of DNA vaccine per limb. The dosage of DNA vaccine was 4 mg (two vials) per animal which was given bilaterally. For MVA boost eight weeks later, the standard preparation for injection was also followed. 0.5 mL of the MVA vaccine was injected into each muscle of the hind limbs. The dosage was $1x10^9$ pfu MVA (2 x 0.5 mL) per animal which was also given on both limbs (Appendix 2). Four baboons in control group B, served as the *Schistosoma* naïve. They were vaccinated at time zero with HIV SAAVI DNA vaccine and boosted with SAAVI MVA vaccine 8 weeks later (Chege *et al*., 2013). Blood was also obtained from the control group at the same time point though there was no termination in this group.

2.7. Ethical Clearance

The study was approved by the Ethical Review Committee. The approval number for this research work was IRC/08/10.

2.8. Blood Processing for PBMCs

Plasma and peripheral blood mononuclear cells (PBMC) were obtained from blood using established standard operating procedures (SOPs). Briefly, heparinised whole blood obtained from animals was collected in BD Vacutainer heparin tubes then poured directly into Leucosep Separating Tubes (Greiner bio-one) which were prefilled with

ficoll and centrifuged for 1 minute at 800 X. The blood was then centrifuged for 15 minutes at 1000 X g. the gradient centrifugation technique was used to separate PBMCs where packed red blood cells and granulocytes settled at the bottom followed by Ficoll layer, a clear PBMC band and plasma which settled on top. The plasma on top was removed using a 5 ml disposable pipette and aliquoted into cryovials (Greiner bio-one) and stored at -20^0C fridge for future use. The PBMC layer was then transferred to a 50 ml centrifuge tubes then mixed with 1% Fetal Bovine Serum in Phosphate Buffer Saline wash solution. The cells were centrifuged again at 250 X g, supernatant decanted then cells resuspended by gentle finger-tapping. 40 ml of the was solution were added again, well mixed after which 10 µl of the cell suspension were taken into a 96-well plate for counting by adding equal amount of trypan blue solution.

2.8.1. Cell counting

Counting of PBMCs was done using a Trypan blue dye exclusion technique as per established SOPs in the laboratory. This technique is based on the principle that live cells, unlike dead ones, are capable of actively pumping out trypan blue dye, thus excluding it from their cytoplasm. This results in the ability to easily distinguish between viable and nonviable cells, because the viable(live) cells will not take in the dye and are thus not stained and have rounded edges while the dead (non-viable) cells are stained with wrinkled edges. Briefly, 10µl of 0.4% w/v trypan blue was mixed with 10µl of cell suspension in a culture plate. 10µl of the mixture was added to the chamber on a haemocytometer and cells counted under a light microscope.

Calculations of Cell counts:

Total # viable cells = # viable cells per square x 10 x 10,000 x total volume cell suspension (in ml) (10ml) (Pohla, 2007).

36 x10, 000 x 10x10=36x10^6 PBMCs

The percentage viable cells was calculated as follows:

$$\% \text{ viable cells} = \frac{\text{No of viable cells counted}}{\text{No. of total cells counted}} \times 100$$

2.9. Immune analysis

Cell-mediated responses measured included T cell proliferation in response to SEA, SWAP, HIV Gag, Env, Pol, Nef and Tat peptides. Cytokine assays included measurement of intracellular IFN-γ cytokine by ELISPOT.

2.9.1. IFN-γ ELISPOT Assay

This technique has been used to detect IFN-γ-secreting cells in an ELISPOT assay using baboon PBMC (Chege *et al.*, 2008). Briefly, 50 ul containing 5µg/ml of purified Anti-human IFN-γ monoclonal coating antibody (clone 1-DIK Mabtech) was added to each well of 96-well plate (Multi Screen-IP Millipore). It was incubated overnight at 4^0C to immobilize the capture antibody on the surface of the plate. The plates were washed and non-specific sites blocked by using RPMI medium with 10% FBS.

PBMC were incubated for 24 h at 37°C in triplicate at 200,000 cells per well with pools of overlapping peptides of Gag, Pol, Nef Tat and Env as the stimulants (4 µg/mL). Triplicate wells with cells and PHA-P (4 µg/mL) and culture medium alone, served as positive and background stimulation controls respectively. After washing the wells, anti-IFN-γ biotinylated secondary antibody (Mabtech, clone-7-B6-1) was added (at 2 µg/mL) and the plates incubated further at 37°C for 2 hours. After further washing, a Streptavidin-HRP Antibody [BD Biosciences)-diluted to 2 µg/ml was added, and plate incubated for I hour. Finally, Tetramethylbenzidine substrate (Mabtech) was added, and incubated until dark spots emerge. The colour development was finally stopped by washing in tap water and the plate left to dry. The number of spots was finally counted using automated CTL immunospot analyser.

The average of triplicate counts of IFN-γ spot forming cells (SFC) was calculated for each stimulant and normalized to 10^6 PBMC to give IFN-γ SFC/10^6 PBMC. The results were reported as net IFN-γ SFC/10^6 PBMC after subtracting the background SFC/10^6 PBMC obtained in the absence of any stimulant (PBMC plus culture medium alone).

To determine whether a response was positive, a cut-off value was set at 50 SFU based on previous studies by Chege *et al*, (2008).

2.9.2. Cell Stimulation

Approximately 200, 000 of Peripheral blood mononuclear cells suspension in a culture media were plated in each well of a culture plate and each well stimulated with Schistosomiasis egg antigen (SEA) (2 mg/ml), schistosomiasis adult worm antigen protein (SWAP) (5 µg/ml), con A (5 µg /ml), PHA-P (300 µl/ml) and RPMI media under sterilized condition in the biosafety cabinet. The plate was then cultured for 72 hours in a CO_2 incubator after which the supernatants of SEA, SWAP, Con A and PHA-P were harvested and placed into cryotubes and kept at -80^{0}C for antibody Elisa assays (Farah *et al*., 1999).

2.9.3. Enzyme Linked Immunosorbent Assays

Three ELISAs were conducted in the study.

2.9.3 (a) Schistosomiasis antibody ELISA

The ELISA plates (Nunc) were coated with various antigens at a concentration of 5 µg/ml for SWAP and 2 µg/ml for SEA then the plates incubated overnight at 4^{0}C. The plates were then blocked with 100 µl of 3% of BSA in PBS-Tween (0.5%) (Sigma-Aldrich) per well then incubated for 2 hours at 37^{0}C. Serum samples were diluted at 1:1000 for SEA and 1:100 for SWAP with PBS-Tween then 50 µl added per well and incubated overnight at 4^0 C. plates were then washed five times with PBS-Tween using an automatic plate washer then flipped. 50 µl of Goat anti-human Ig. G diluted to 1:2000 with PBS-Tween was added per well to the plates then incubated at 37^0 C for 1 hour.

Washing followed again 5 times with PBS-Tween in the washer, flipped, then 50µl of TMB substrate added per well. The plates were then incubated for 15 minutes at room temperature in the dark then the absorbance was read at 450 nm after 10, 20 and 30 minutes. A cut-off value was established by multiplying the average Optical density at week 0 by 2. Any value above this was considered positive. The result was presented as a graph of the trend of optical densities of the experimental group.

2.9.3 (b). HIV Gag ELISA

The ELISA plates (Nunc TM, Denmark) were coated with 100µl/well of recombinant HIV-1 BH10 Pr gag protein diluted to 1 µg/mL then sealed and incubated overnight at 4^0 C. The plates were then washed three times with PBS-Tween 20 (Sigma –Aldrich, USA) using ELISA washer. Samples diluted with PBS (Sigma Aldrich, USA) containing 2% non-fat skim milk at 1:100 were then added to the flicked plates at 100 µl/well in duplicates. The plate was sealed and again incubated overnight at 4^0C. The plates were washed five times again with PBS-Tween then 100 µl of diluted rabbit anti-monkey Ig G Horseradish Peroxidase-Conjugated Antibodies polyclonal Antibody (sigma-Aldrich, USA) in PBS with 2% milk was added to the plates then incubated for one hour at 37^0 C.

The wells were again washed five times with PBS Tween-20 then the plates tapped on paper towels. 100 µl of Tetramethylbenzidine microwell Peroxidase substrate (KPL) was immediately dispensed then incubated at a room temperature in the dark for 5 minutes until colour developed. The Optical Density was immediately read at 450 nm after the reaction was stopped with 4N sulphuric acid. The result was presented as an optical density trend in the experimental group.

2.9.3 (c) Interleukin 4 ELISA

The ELISA plates (Nunc TM, Denmark) were coated with 100 µl per well of mAb IL-I (Mabtech) diluted to 2 µg/ml in PBS and incubated overnight at 4^0 C. The plates were then washed twice with PBS, blocked with 200 µl of PBS with 0.05% tween-20 and 1% Bovine Serum Antigen then incubated for 1 hour at room temperature. Washing was

repeated 5 times then IL -4 standards (Mabtech) prepared according to kit manufacturers guideline. 100µl of samples or standards dilute in PBST with 0.1% Bovine Serum Antigen were added to each well and incubated again for two hours. 50µl per well of mAb IL-II biotin was added then incubated for 1 hour at a room temperature. It was washed, 50µl per well of streptavidin- Horseradish Peroxidase-Conjugated antibody added, incubated for 1 hour, washed and Tetramethylbenzidine added. The Optical Density was measured at 630nm after 30 minutes. The averages of the Optical Densities obtained was calculated and the results together with the standard curve obtained extrapolated to determine the concentration in pg/ml. the results were then reported graphically as the trend of the concentration in pg/ml.

2.10. Statistical analysis

Raw data of IFN-γ ELISPOT counts, antibody, cytokine ELISAs and schistosomal egg counts were entered in a laboratory book and a soft copy in a computer as an excel sheet. Analysis of *s mansoni* eggs per gram of faeces, Spot Forming Unit and Optical Densities were done. Results were presented in terms of bar graphs, line graphs, box plots and charts. The egg shedding result was presented in a graph in terms of the trend of the total egg counts. The antibody ELISA data was presented in terms of the Optical Densities of the experimental group. IFN-γ ELISPOT results were analysed by Graph pad prism 5 software. Net cumulative responses to peptide pools, cumulative median responses and mean individual responses to the peptide pools between the experimental and the control groups were compared. The student's t test was used to test for differences in the cumulative means of quantitative variables. All tests were two-tailed and a P-value < 0.05 was considered significant.

CHAPTER THREE

RESULTS

3.1. Antibody Response

3.1.1. Antibody response to Schistosome Worm Adult Protein

Further confirmation of Schistosomiasis was done by evaluating the presence of anti-schisto antibodies to the adult worm in the sera of exposed animals (Group A). This was achieved by measuring the humoral response to crude Schistosome adult worm protein (SWAP) as determined by antibody ELISA as previously described in section 2.4.2 (a). As shown in Figure 3.1, increased Optical Densities above 0.5 was evident from time point T2 (week 8 after Schistosomiasis infection) all through to time pointed T9 (1 week after MVA vaccination). This showed that there was high antibody response as the experiment progressed. The low Optical Density of below 0.5 at the beginning of the experiment depicted that there was no circulating antibody against the Schistome adult worm protein while the optical densities above 0.5 from 8 weeks post infection showed the presence of antibodies against the developed schistosome adult worm protein. These data confirmed progressive schistosomiasis in these animals suggesting an attainment of the chronic phase of schistosomiasis.

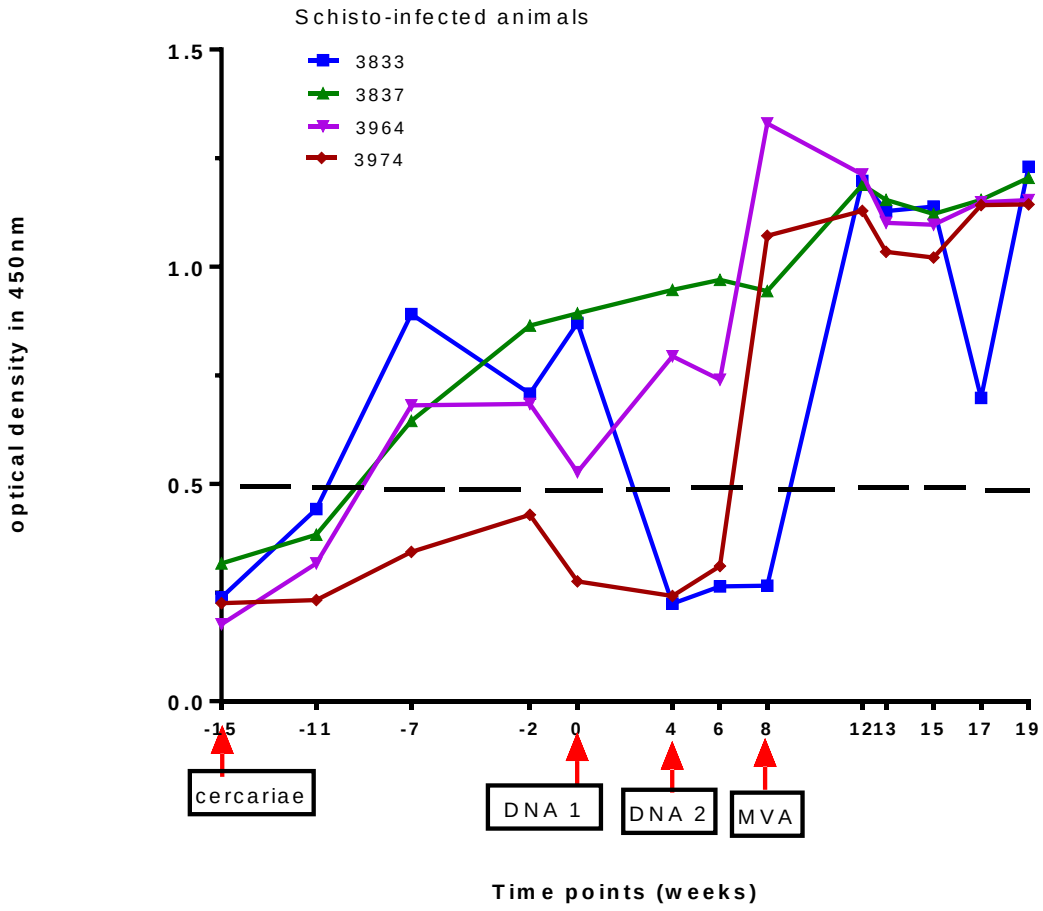

Figure 3.1: Schistosoma adult worm protein response in the sera of infected animals. Four baboons (Group A) were infected with *S. mansoni* cercariae 15 weeks before initial HIV DNA vaccination. Samples of sera from fresh blood were collected at all the time points shown. The line graph shows the trend of antibody response to schistosome adult worm proteins over a period of 35 weeks (15 weeks pre-infection and 19 weeks post infection). The line indicates a cut-off Optical Density value at 0.5.

3.1.2. Antibody Response to Schistosomiasis Egg Antigen (SEA)

Evaluation of antibodies elicited by schistosome egg produced by adult worms was performed by detecting antibody responses to crude schistosomiasis Egg antigen (SEA) by ELISA as previously described in section 2.5.3(a).This was done in sera obtained from group A animals, which were infected with schistosome cercariae. Baseline OD at the time of infection (T0) was<0.5. As shown in Fig 3.2, increased Optical Densities above 0.5 was evident from time point T2 (8 weeks after exposure to schistosomiasis), depicting the presence of antibodies against schistomiasis Egg antigen. The Optical Density below 0.5 at the initial time point was due to the absence of antibodies while the high Optical densities as the experiment progressed was due to the presence of antibodies towards schistosomiasis egg antigen. The trend of SEA response showed an average rising response in the schistosomiasis infected animals as the time advanced as shown in Figure 3.2

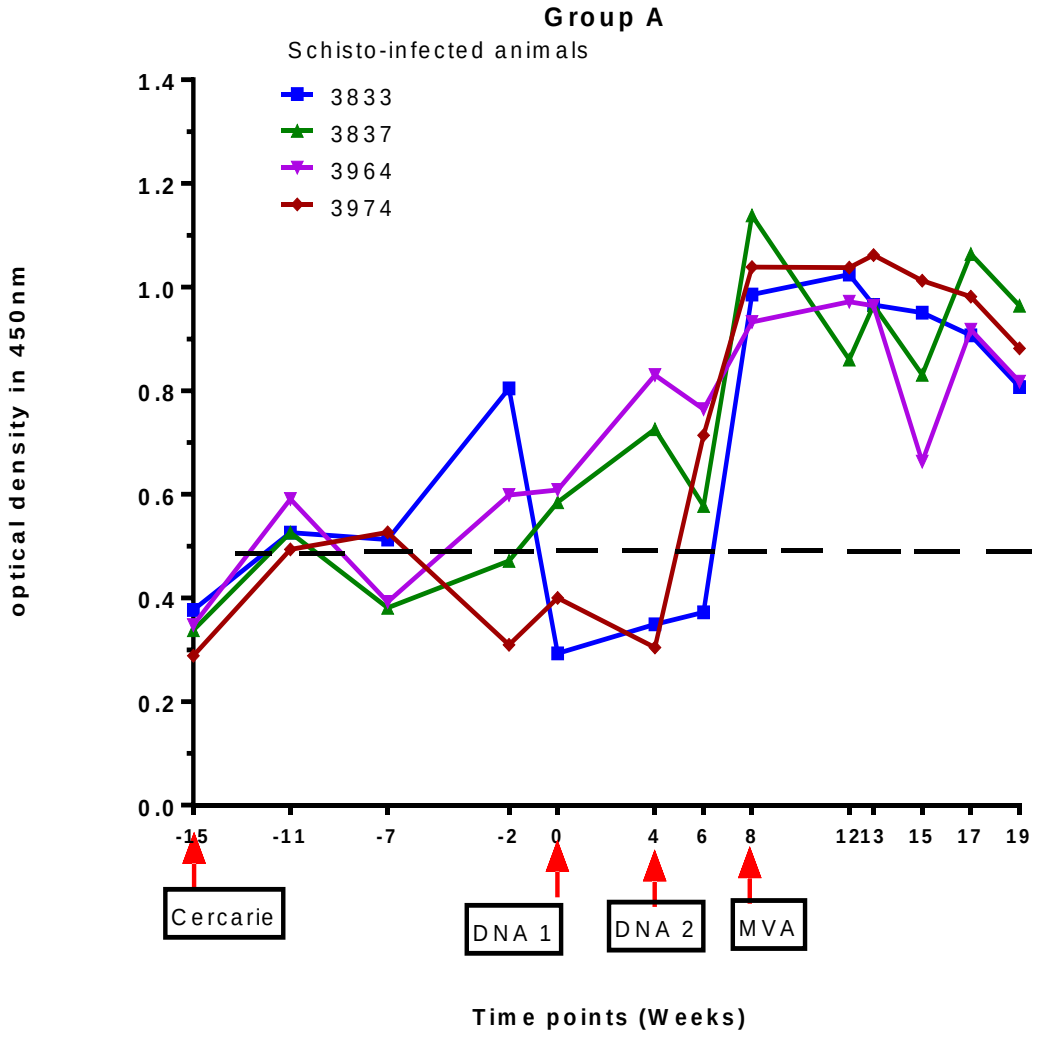

Figure 3.2: Schistosoma Egg Antigen response in the sera of infected animals. Four baboons (Group A) were infected with S. mansoni cercariae 15 weeks before initial HIV-DNA vaccination. Samples of sera from fresh blood were collected at various times as shown. The line graph shows the trend of antibody response to schistosome Egg Antigen over a period of 35 weeks (15 weeks pre-infection and 19 weeks post infection). The line indicate a cut-off Optical Density value at 0.5.

3.2. Responses to Interleukin 4

Experimental group A which was infected with schistosomiasis showed the presence of interleukin 4. Supernatants stimulated by Schistosomiasis Egg Antigen showed the highest presence of interleukin 4 which rose after the 8th week of infection with schistosomiasis to the highest peak during the 16th week, the time in which the first DNA vaccine was introduced. The presence of IL-4 cytokine diminished after vaccination.

The trend of IL-4 cytokine in supernatants stimulated by Schistosomiasis Worm Antigen also showed IL-4 after 8 weeks post infection with schistosomiasis to the 16th week. The IL-4 cytokine diminished after 2nd DNA vaccination. Stimulation of the supernatants with Concanavalin A generally gave a high IL-4 results all through with an increasing peak from week 29th all through to the end of the experiment. Stimulation of the supernatants with PHA-P and media alone generally showed a low stimulated level of Interleukin 4 as depicted in figure 3.3

An observation in the control group B baboons which were not infected with schistosomiasis but supernatants stimulated by SEA, SWA, Con-A, PHA-P and media alone generally showed no presence of Interleukin 4. The levels of interleukin 4 in these supernatants remained at a below limit level of positivity all through the experiment as shown in figure 3.3. The infected animals generally had elevated IL-4 secretions compared to uninfected animals.

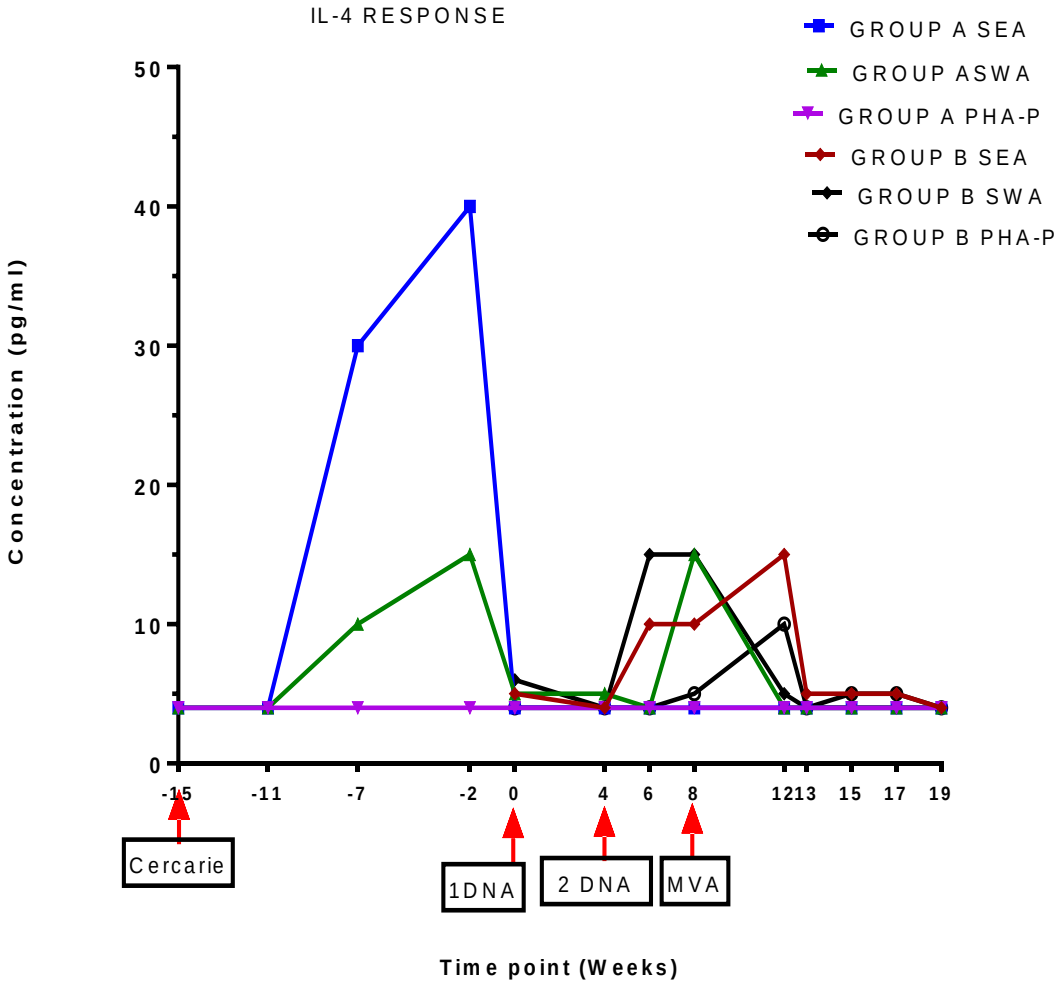

Figure 3.3: IL-4 response in the sera of study animals. Samples of sera from blood of Group A and B animals were collected at various times from T0 time line for Group A and from T4 for Group B. The IL-4 antibody response was determined by ELISA. The line graph shows the trend of antibody response to IL-4 over a period of 13 time points (35 weeks).

3.3. IFN-γ Enzyme Linked Immunosorbent SPOT cumulative responses

The responses in the schistosomiasis positive + DNA –MVA vaccinated group differed markedly in magnitude compared with those of the schistosomiasis negative + DNA–MVA group as shown in Figure 3.5.

The high response to HIV peptides in Schistosomiasis infected group was maintained further 6 weeks after vaccination with MVA as shown in figure 3.6. The median response in group A at six weeks after MVA was 1502 sfu in comparison to the Schistosomiasis free group B which had a lower response of 164.5 sfu, (Figure 3.6).

Cumulatively, Schistosomiasis infected animals responded strongly to the Immunogens under study with strongest responders reaching up to 3788 sfu/10^6 PBMC and 3700 sfu/10^6 PBMCs. The magnitudes were uniform to the various immunogens with pol and gag being the highest in the strong responders. Schistosomiasis uninfected baboons showed the weakest response with the weakest responders reaching as low as 63 sfu/10^6 PBMCs. A notable though insignificant difference was observed between the median magnitude of responses in schistosomiasis positive + DNA–MVA responders and schistosomiasis negative + DNA-MVA animals (2242±874sfu/10^6 PBMC) versus (665±203 sfu/10^6 PBMC), (Figure 3.9). The results were however not statistically significant.

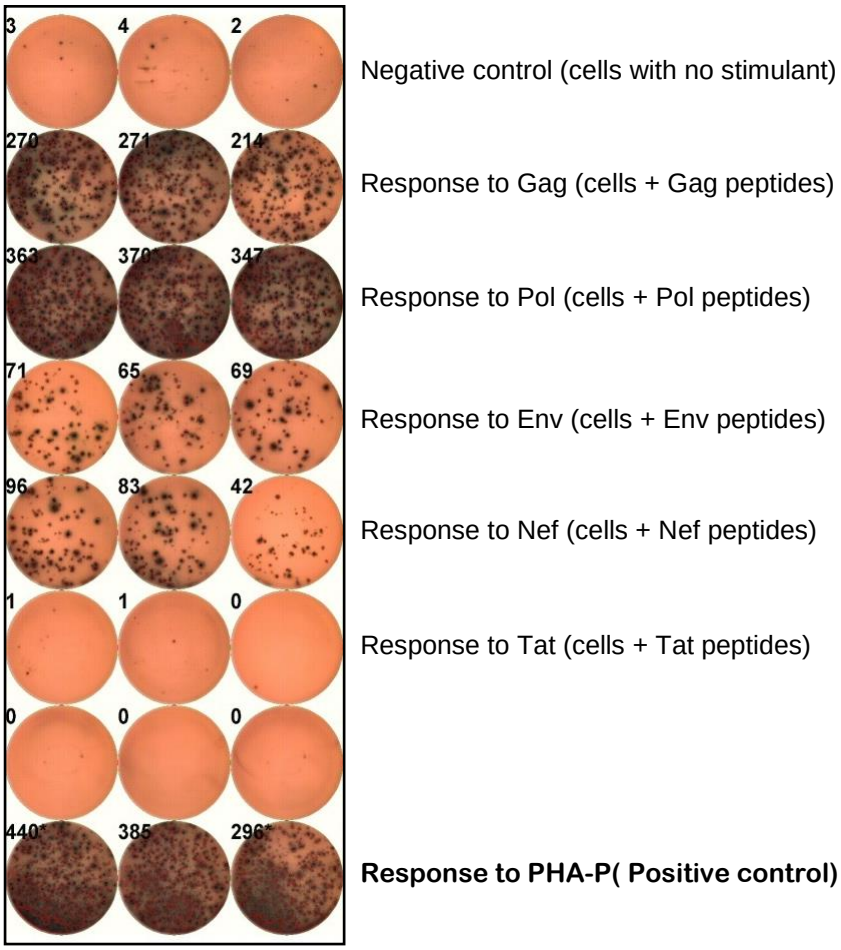

Figure 3.4: A representative IFN-γ ELISPOT assay.

Freshly isolated PBMC from PAN 3837 at T9 time point (1 week post SAAVI MVA-C boost) were cultured in triplicate wells of a 96-well ELISPOT plate for 22-24 hours with RPMI medium alone (Negative control) or RPMI medium containing pools of HIV-1 peptides (Gag, Pol, Env, Nef or Tat) and Phytohaematoglutinin-P (PHA-P, Positive control). The numbers of spots were counted using a Cellular Technology Ltd ELISPOT analyzer. The net response to any peptide pool was considered positive if it was equal or

greater than a cut-off value of 50 SFU/10^6 PBMC. Values below this cut-off value were set to zero when calculating the cumulative net response (sum of responses to the five peptide pools). A positive control with a value of >500 SFU/10^6 PBMC was used to validate the assay.

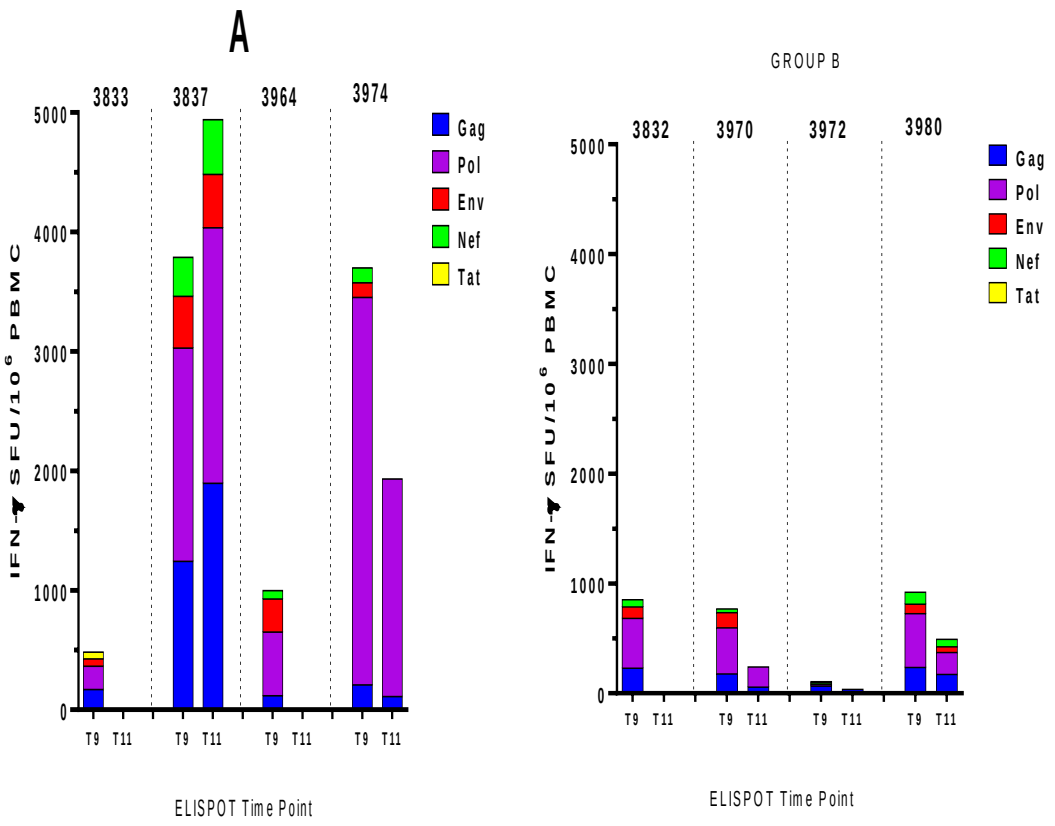

Figure 3.5: Net cumulative response to Gag, Pol, Env, Nef and Tat pools at T9 (I week post MVA vaccination and T 11(16 weeks post MVA vaccination) in Group A and B animals as determined by IFN-γ ELISPOT. Results are reported as net sfu per 10^6 PBMC at peak time points.

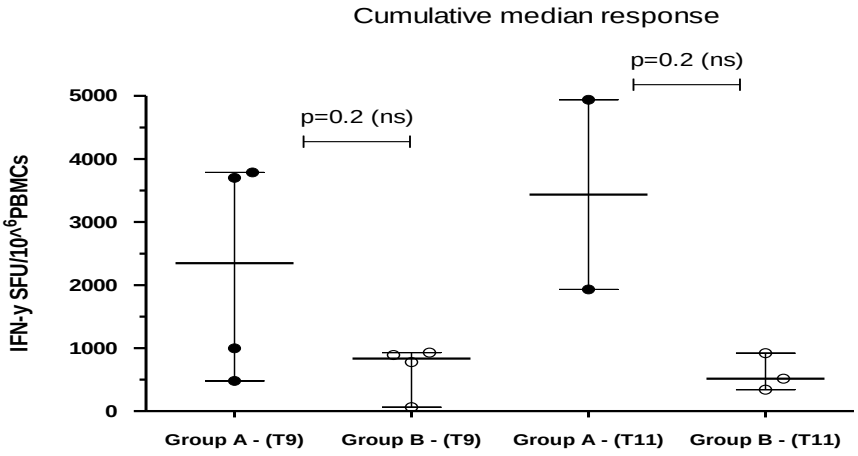

Figure 3.6; Graph showing cumulative median response.

Cumulative IFN-γ ELISPOT responses to peptide pools spanning Gag, Pol, Nef, Tat and Env are shown. Medians are represented for schistosomiasis infected-DNA–MVA-vaccinated animals (Group A), and schistosomiasis-free, DNA-MVA vaccinated animals (Group B). Results are reported as net s.f.u. per 106 PBMC at peak time points at a P value of 0.2.

3.4. IFN-γ ELISPOT response to individual vaccine Immunogens

Considerable response to individual peptides was noted in T9 time point, a week after MVA prime boost and T11 (6 weeks after MVA vaccination). In the schistosomiasis infected + vaccinated group, there was a notable response to all the Immunogens of the vaccine, (Figure 3.7 and 3.8). At T 9, A representation of pol response only showed that schistosomiasis infected group responded highly with the highest responders reaching up to 3245 sfu/10^6 PBMC and 1785 sfu/10^6 PBMC. All group A animals responded to Pol with the least responder reaching as high as 193 sfu/$10^{\wedge 6}$ PBMC. The schistosomiasis uninfected group responded weakly to Pol with the weakest responder reaching as low as 452 sfu/10^6 PBMC. Three group B animals showed a positive response to pol peptide. In terms of mean, mean response to pol alone was 1440 ±

692.3 sfu/10^6 PBMC in the schistosomiasis group and 352.3 ± 117.7 sfu/10^6 PBMC in the schistosomiasis uninfected group as shown in Figure 3.8.

At T11, two group A animals still showed elevated Pol response at 2140sfu/10^6 PBMCs and 1822 sfu/10^6 PBMCs, the response to Pol in group B was still maintained by 2 animals though at a much lower level. In response towards Gag peptide at T9, the schistosomiasis infected animals reacted strongly with the strongest responders reaching up to 1243sfu/10^6 PBMC. All the group A animals responded highly to Gag. The schistosomiasis uninfected group reacted weakly in comparison to the schistosomiasis infected group with the weakest responders reaching as low as 63 sfu/10^6 PBMC. All the group B animals under study also responded to gag peptide. Mean response towards gag alone was 434.0 ± 270.3 sfu/10^6 PBMC in the schistosomiasis positive and 183.5 ± 43.93 sfu/10^6 PBMC in the schistosomiasis uninfected animals. At T11 only one animals still had an elevated response to Gag in group A at 116 sfu/10^6 PBMCs.

In the Env responses at T9, all the schistosomiasis positive animals also responded strongly. The highest responders had 276 sfu/10^6 PBMC. Three Schistosomiasis negative animals responded though weakly to the same peptide with the weakest responder forming only 87 sfu/10^6 PBMC. One schistosomiasis negative animal did not respond to the Env peptide. The mean response for Env in the schistosomiasis infected group was 223.8 ± 82.92 sfu/10^6 PBMC while for schistosomiasis negative group was 84.25 ± 30.11 sfu/10^6. At T 11, the response had ceased drastically and only one animal in group still responded to the Env.

Schistosomiasis positive animals also responded strongly to nef. The highest responders had 327sfu/10^6 PBMC. One animal in the schistosomiasis positive group did not respond. The control animals responded weakly with as low as 70 sfu/10^6 PBMC. The mean response towards *Nef* in the schistosomiasis infected group was 130.8±70.25 sfu/10^6 PBMC while for Schistosomiasis negative group was 45.50 ± 27.63 sfu/10^6PBMC. The response however reduced at T11 with only one animal in Group A showing a response to Nef (Figure 3.7 and 3.8).

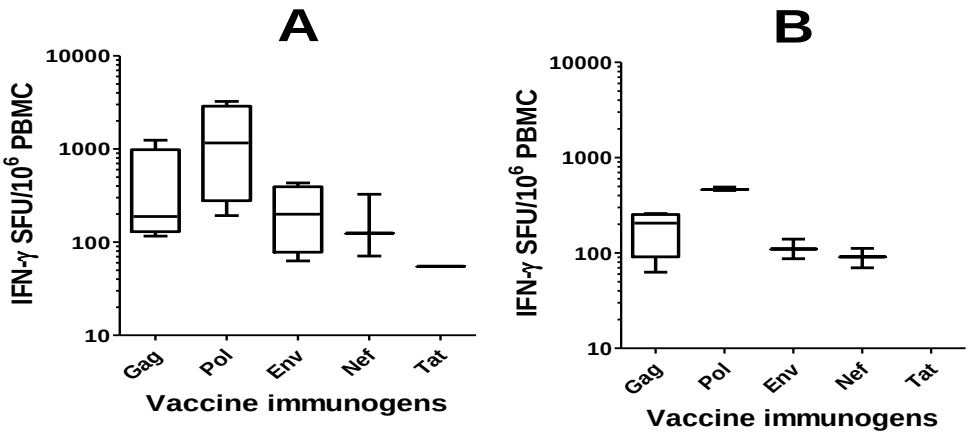

Figure 3.7: cumulative response to individual vaccine immunogens

Cumulative IFN-γ ELISPOT responses to individual peptide pools of Gag, Pol, Nef, Tat and Env on week 13 (1 week after post-MVA vaccination are shown. Medians, interquartile ranges and ranges are represented for schistosomiasis infected- DNA–MVA-vaccinated animals (group A) and schistosomiasis-free-DNA-MVA vaccinated animals (Group B). Results are reported as net s.f.u. per 10^6 PBMC.

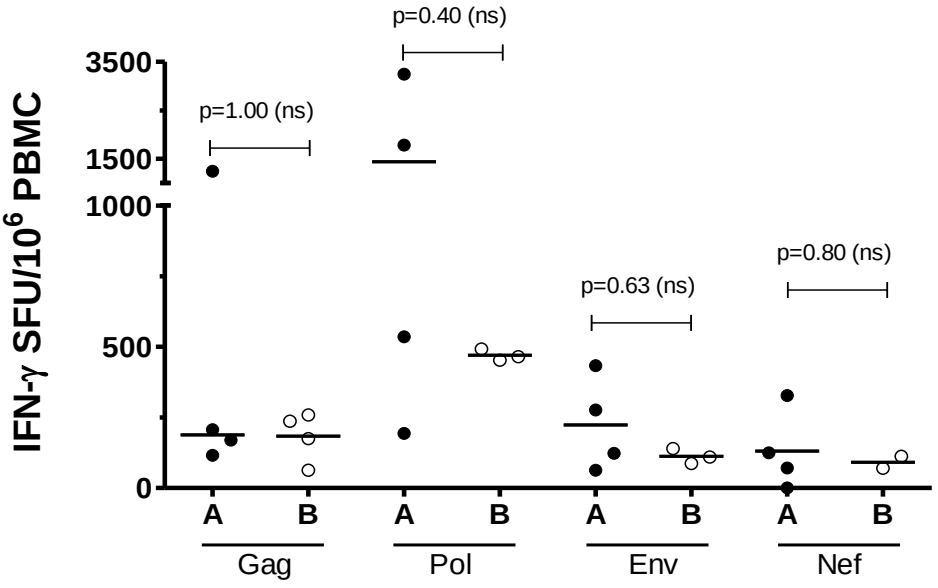

Figure 3.8 Cumulative response to individual vaccine immunogens

Comparisons of Individual response of the peptides was done for the schistosome infected animals (Group A) and schistosomiasis free animals (Group B) 1 week post-MVA vaccination using Mann Whitney t test. Results are reported as net s.f.u. per 10^6 PBMC.

3.5. Antibody Response to HIV-1 Gag

HIV antibody response to *gag* peptide was done by antibody ELISA from sera samples of Group A and Group B animals as previously described in section 2.4.2(b). In group A analysis, a low general trend was reported in schistosomiasis infected animals as evident by optical densities from time point zero when the animals were infected with schistosomiasis worms to the point of various vaccines introduction. The trend was due to a systemic progression of Schistosomiasis following infection and the various response of the body to control the infection. HIV gag antibody was detected from T6 (2 weeks after the second SAAVI DNA vaccination) which kept on increasing in quantity until the T11 as depicted in figure 3.9. An Optical Density between T0 and T5 was below the Cut-off threshold of 1.1 thus there was no Antibody to HIV Gag produced at these time points. Analysis of Group B schistosomiasis free animals indicated a low Optical density of below the Cut-off value of 1.1 confirming the absence of HIV Gag antibodies in Group B baboons.

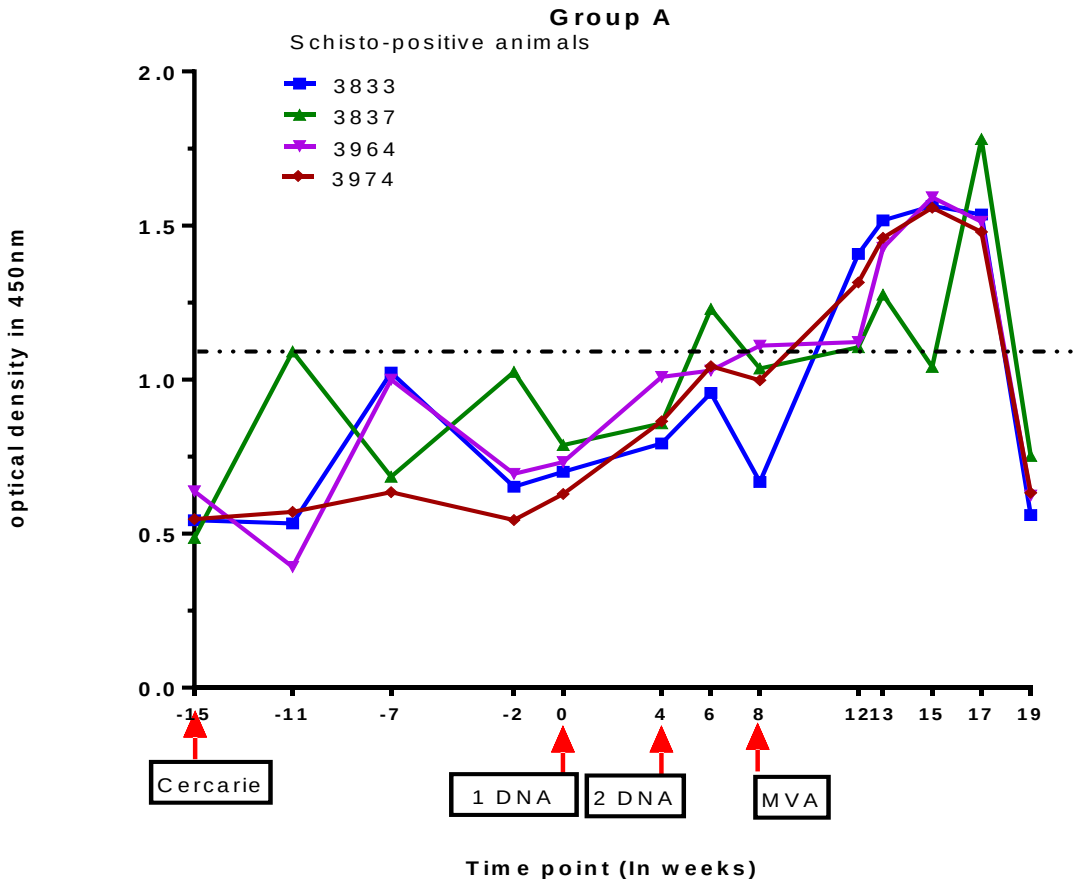

Fig 3.9: HIV Gag response in the sera of infected animals.

Four baboons (Group A) were infected with S. mansoni cercariae at 15 weeks pre-vaccination as described in section 2.3(b). Samples of sera from fresh blood were collected at various times as shown. The antibody response was determined by ELISAs. The line graph shows the trend of antibody response to HIV Gag over a period of 13 time points (35 weeks). The line indicate a cut-off Optical Density value at 1.1 which is twice the O.D at time the initial time point. The arrows indicate the time points when these animals were infected with cercariae, vaccinated with 1st and 2nd SAAVI DNA vaccine and a boost with SAAVI MVA respectively.

CHAPTER FOUR

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1. Antibody Response

4.1.1. Immunoglobulin G Antibody responses to Schistosomiasis antigens

A heightened response of Ig G to SEA was observed in three of the four animals from the 8th week after infection with cercariae. This trend was heightened further after the introduction of 2nd DNA and MVA- HIV vaccines in all the study animals which lasted all through to perfusion. These results are supported by the same previous related studies which reported an increased SEA level at the acute stage of Schistosomiasis between weeks 6-9 (Farah *et al.*, 1999). The response to Schistosomiasis Adult Worm Antigen in Schistosomiasis infected group also reported an increased level of immunoglobulin G to SWAP in all the study animals from week eight of Cercarial infection, and a further increase with the introduction of HIV DNA and MVA vaccines. The response to Schistosomiasis is in line with the findings of the previous study of Farah *et al*., confirming that the protein is initially produced during the acute infection stage.

4.1.2. Cytokine response (Interleukin 4) in schistosomiasis infected and vaccinated ***Papio anubis***

IL- 4 is as a TH2 cytokine is produced on prolonged chronic schistosomiasis infection. On an advanced schistosomiasis infection, TH2 cytokines like IL-4 and IL-13 are highly expressed and later, regulatory cytokines like IL-10, CD4+CD25+ T regs and Tumour Growth Factor beta.

The IL-4 data in this study reported a higher level of SEA-induced IL-4 response at the acute stage of infection, which progressed further at the chronic stage. The production of the IL-4 confirmed the establishment and the progression of Schistosomiasis as supported by studies by Coelho *et al*, (1996), which reported an increased production of

IL-4 with the progression to the chronic stage. This SEA-induced IL-4 cytokine however dropped and remained to almost undetectable level with the introduction of HIV vaccines till the time of perfusion. The drop was contrasting to the studies of Coelho et al, (1996) which did not handle any component of a vaccine or a target of TH1 enhancement. The SWA-induced IL-4 response data also showed the same trend however, it was slightly lower than the SEA-induced IL-4 response. An increased SEA-induced IL-4 response in the acute stage which dropped in the chronic stage of infection (Farah *et al.*, 1999). In the study which investigated the cytokine control of the granulomatous response in Schistosomiasis infected baboons and the function of exposure and the treatment showed a high SEA-induced IL-4 with multiple infection with the parasite of Schistosome at the chronic reinfection stage compared to the acute primary stage. The results reported are in line with the findings reported in this HIV SAAVI DNA and MVA vaccines study which observed a drop in IL-4 level after the introduction of HIV DNA and MVA vaccines. The drop depicted an immunological shift from TH2 to TH1 immune response. During the shift to TH1, the related pro-inflammatory cytokines like IL-1, IL-6 and the IFN-gamma are in circulation while the anti-inflammatory cytokines like the IL-4 cease.

4.1.3. The influence of Schistosome infections on the production of interferon gamma in Papio anubis

The findings in this research showed that the experimental group A had a high response of 2349 SFU/10^6 PBMCs. The response was still high six weeks post vaccination with a median of 1502±203 sfu per $10^{\wedge 6}$ PBMCs In a related study, of baboons which were vaccinated with DNA-MVA HIV vaccines showed a cumulative median response of 4103 net sfu per $10^{\wedge 6}$ PBMCs, (Burgers *et al*, 2009) which was much higher than the findings in this research. The in this findings in the experimental group confirmed that the vaccines was immunogenic. The finding was in agreement with the previous related study of the HIV DNA and MVA vaccines (Burgers et al., 2009). However, the low response here in terms of median response in comparison with the previous related

studies could have been due to the long storage period of the vaccines which would have reduced immunogenicity.

The experimental group showed a much enhanced response to individual immunogens one week after MVA boosting with mean response to Pol pool alone at 1440 ± 692.3 sfu/10^6, Gag pool at 434.0 ± 270.3 sfu/10^6 PBMC, Env pool at 223.8 ± 82.92 sfu/10^6 PBMC, nef pool at 130.8±70.25 sfu/10^6 PBMCs. This is what almost resembled the findings of Burgers *et al* (2009). This enhanced response was still maintained 6 weeks after the MVA boost whereby the highest individual immunogen response was pol at 2140sfu/10^6 PBMCs for the highest responder. The maintenance of high response confirmed that the efficacy of the vaccines are long lasting hence can ensure longevity.

4.1.4. Structural Protein Peptides stimulate the highest response in Schistosomiasis

The study reports that structural proteins are highly immunogenic as was demonstrated by **Pol**, **Gag** and **Env** in the Schistosomiasis infected responders. **Gag** is a major component of the HIV DNA and MVA vaccines. The **Gag** response was high in the experiment since structurally, the Gag peptide is long with 55KDa in length. The **Pol** is also a major component of the vaccine the response to **Pol** was quite high in the experimental animals. The response to **pol** was much heightened since it is a long protein fused with **Gag** hence forming a precursor of **Gag** and **Pol** fusion protein. Based on the size, **Gag** and **pol** precursor is 340. 4 kDa. **Nef** peptide stimulation was quite low in the experiment. The low response to **Nef** is due to its small size which is only 27kDa in length and is an accessory protein. **Tat** protein was the lowest in stimulation amongst all the peptides of HIV. This is because **Tat** is the smallest in size with which is only 72 amino acids in length.

4.1.5. Schistosomiasis infection Elicit high antibody Production against Gag protein

The HIV DNA-C with an HIV MVA-C prime boost has an *Env* peptide (gp 160) and because of this, it is effective in Antibody mediated immunity besides being efficate in

providing cell mediated immunity. The observation in the Schistosomiasis positive animals (Group A) which showed a similarity to the findings of Burgers whereby the presence of binding antibodies to gp120 was seen and it was indeed boosted after DNA immunization. A similar study reported the presence of weak binding antibodies to gp120 which were boosted twelve folds after the DNA immunization (Burgers *et al*., 2009). The much increased response after the boost with MVA vaccine was also in agreement with the same related studies (Burgers *et al.*, 2009).

Several studies have reported that Schistosomiasis infection shifts immunity to TH2 response (Farah et al., 1999; Cheever et al., 2000). As a result TH1 cytokines as IFN-γ would be expected to be down regulated in the case of Schistosomiasis infection, (Cheever *et al.*, 2000). This study reports otherwise, that IFN-γ response is otherwise enhanced. This study also reports that the protection enhanced by the DNA and MVA is of high longevity which is still evident a while after MVA boosting thus it can confer a long-term protection.

In the presence of Schistosomiasis, CD8+ T cells, Natural Killer cells, Macrophages, NKT and myeloid cells were all activated as a response mechanism to the disease. All these cells in turn produced IFN-γ. On vaccination of the Schistosomiasis infected animals, the immune state which is at TH2 shifted to TH1 because of the cellular immune response caused by the efficacy of the candidate HIV DNA and MVA vaccines. The TH1 cells secrete IFN-γ which in turn causes more undifferentiated CD4+ T cells to differentiate into more TH1 cells in a positive feedback loop mechanism while suppressing other TH2 differentiation. Another explanation for the high IFN-γ in the Schistosomiasis infected group is that a shift during chronic Schistosomiasis leads to TH2 immunity which initiates the production of B cells. The recent studies have shown that B cell also produces IFN-γ (Bao *et al.*, 2014). The limitation of the study was that Pan 3833 experimental baboon fell sick at some point during the experiment and was treated. The animal however lost weight and this would have contributed to its low response to various kinetics of Interferon gamma.

4.2. Conclusions

The study concludes that:

1. There is Ig G antibody responses to crude Schistosomiasis SEA and SWAP antigens.
2. SAAVI DNA and MVA vaccines lower the production of IL-4 in Schistosomiasis infection.
3. Schistosomiasis infection elevates the production of IFN-γ.
4. Structural Protein Peptides stimulate the highest Immune response in Schistosomiasis infection. This was as shown by *Pol*, *gag* and *env* structural protein peptides being highly immunogenic based on their size in comparison to the regulatory proteins as tat and accessory proteins as *nef*.
5. Schistosomiasis infection Elicit high antibody Production against Gag protein as shown by the high level of the optical densities in the Schistosomiasis positive than the negative group.
6. Schistosomiasis does not affect the immunogenicity of SAAVI DNA-C and MVA-C vaccines.

4.3. Recommendations

Based on this, it is recommended that;

1. HIV vaccines be administered without the treatment or elimination of Schistosomiasis in situations of co-infections in baboons
2. A further investigations be done to elucidate how the vaccine can perform if administered on the acute Schistosomiasis infection stage.

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LIST OF APPENDICES

APPENDIX 1: SAAVI DNA**-C PREPARATION**
Vaccine:

Presentation:

- Each vaccine dose is contained in a glass vial as was provided by the manufacturer
- Each vial contains 2 mg DNA as an "equal-molar" mixture of two plasmids DNA in a volume of about 1 mL (perhaps 1.2 mL), one containing the GRtTN (Gag, RT, Tat & Nef) DNA sequences and the other containing the Env (gp150, truncated gp160)
- They were delivered frozen and have been kept frozen below -20 ˚C

Preparation:

- The required number of vials should be transferred from -70 ˚C freezer to -20 ˚C freezer a day before vaccination. [two vials are required for each animal]
- On the morning of vaccination, the vaccine should be removed from the freezer thawed on wet ice (~30-60min)
- Once the contents have thawed, the vials should be left on ice until the time of animal vaccinations
- When ready to administer the vaccine to animals, each vial should be vortexed well to mix the contents
- Then each vial is warmed up by massaging between palms of the hands
- Remove the aluminum covering the rubber portion of the cap and wipe the cap well with 70% ethanol
- Withdraw all the contents of the vial using a sterile Gauge 23 hypodermic needle (3/4 inch long) and a syringe (preferably a 2-mL syringe). Carefully re-cap the needle.

APPENDIX 2: DNA ANIMAL VACCINATION

- Injection is done in the quadriceps muscles (or the biceps if the hind limbs cannot be used for some reason)
- Prepare the injection site in the standard way (spraying 70% ethanol & wiping with gauze)
- Shaving of the injection site is for the discretion of the Veterinarian in charge. However, care must be taken to prevent contamination of the needle by the animal hairs or fur
- Inject 1 mL vaccine deep into the muscle (taking the normal precaution of avoiding to inject into a blood vessel)
- Give a deep massage to the muscles for a few seconds (to spread out the vaccine) while holding the needle penetration site firmly with the thumb after withdrawing the needle.
- Inject the second dose into the quadriceps of the other limb in the same way.
- [Dosage = 4 mg DNA (2 vials) per animal, given bilaterally

APPENDIX 3: SAAVI MVA-C PREPARATION

Vaccine:

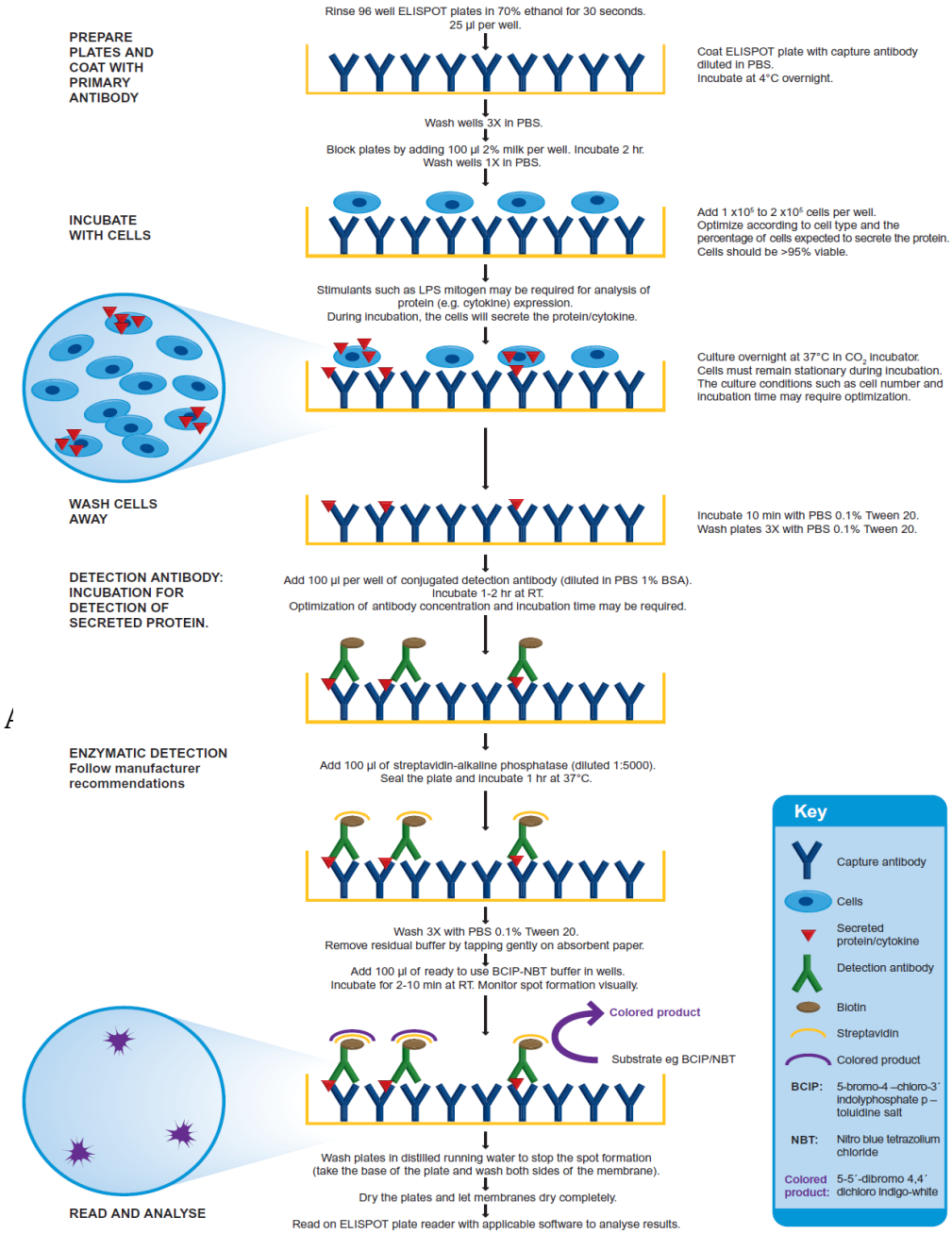
Preparation:

- On the morning of vaccination, prepare 1 mM Tris aseptically by diluting the stock from Sigma with deionised sterile water
- Defrost one vial of MVA stock on wet ice [15-30 min]
- Vortex the vial on a high setting for at least 30 seconds
- You need 13.2 µL per animal to give a dosage of $1x10^9$ pfu. However, always allow an extra 2 doses to cater for dead volume and losses incurred when aspirated into syringes. For example, use 92 µL of MVA stock to dilute to 7 mL with 1mM Tris pH9 to prepare dosages for 5 animals (with 2 extra mL for dead volumes & losses)
- Mix well by vortexing after diluting and aspirate 1-mL volumes into 2-mL syringes using Gauge 23 hypodermic needles.
- Leave them at room temperature if they will be used within 30 min otherwise leave them on wet ice and remove them 15 min before use (keep at room temperature during this time).
- For remainder of vaccine stock, aliquot into 50 µL volumes and re-freeze at -70 ˚C for future use. If used within 2 years, the titre will be assumed to have reduced to $3.6x10^9$ pfu/mL MVA

APPENDIX 4: MVA ANIMAL VACCINATION

- Injection is done in the quadriceps muscles (or the biceps if the hind limbs cannot be used for some reason)
- Prepare the injection site in the standard way (spraying 70% ethanol & wiping with gauze)
- Shaving of the injection site is for the discretion of the Veterinarian in charge. However, care must be taken to prevent contamination of the needle by the animal hairs or fur
- Inject 0.5 mL vaccine into the muscle (taking the normal precaution of avoiding to inject into a blood vessel)
- Give a gentle massage to the muscles for a few seconds (to spread out the vaccine) while holding the needle penetration site firmly with the thumb after withdrawing the needle.
- Inject the other 0.5 mL vaccine into the quadriceps of the other limb in the same way.
- [Dosage = $1x10^9$ pfu MVA (2 x 0.5 mL) per animal, given bilaterally]

APPENDIX 5: ELISPOT PRINCIPLE



APPENDIX 6: EGG SHEDDING DATA