IMMUNOGLOBULIN G SUBCLASS RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS DURING A PERIOD OF LOW TO ABSENT MALARIA TRANSMISSION IN THE HIGHLANDS OF WESTERN KENYA

BY

WASSWA RONALD EPHRAIM

A thesis submitted in partial fulfillment of the requirements for the of Degree of Master of Science in Medical Immunology of Maseno University

DEPARTMENT OF BIOMEDICAL SCIENCE AND TECHNOLOGY

MASENO UNIVERSITY

ABSTRACT

Malaria control strategies have significantly reduced malaria transmission in many parts of Africa, including Kenya, yet the extent to which interruption of malaria transmission might affect immune correlates to malaria in areas of low and unstable malaria transmission is unknown which was the focus of this study. This thesis research was part of a prospective longitudinal cohort study undertaken in Nandi district, a highland area in western Kenya with a low and unstable malaria transmission. This area experienced a 13 months interruption of malaria transmission from April 2007 to May 2008, following high-level coverage (>70% of households) with indoor residual insecticide spraying, distribution of insecticide treated bed nets and introduction of artemisinin combination therapy in 2007. A cohort of 1000 participants being followed was stratified into 4 age groups (< 4 years, 4 ≥ age< 8 years, 8 ≥ age<17 years, and 17 ≥ years) and 50 participants were selected from each age group for the cross sectional study where IgG subclass antibody frequencies and levels to 11 *Plasmodium falciparum* antigens were assessed by cytometric bead assay or enzyme linked immunosorbent assay (ELISA) at two time points over the 13 months of interrupted malaria transmission. Data was analyzed using McNemar Chi-square test and Wilcoxon matched-pair rank test. Results showed that the frequencies of IgG1 antibodies against AMA-1, MSP-1_{19}, EBA-175, MSP-3, GLURP-R0 and LSA-1 decreased significantly pre and post intervention from 2007 to 2008 with a P=0.0151. IgG1 sero prevalence to MSP-1_{42} antigen remained stable pre to post intervention with 49.1% and 49% prevalence, respectively, and P=0.07963. IgG1 sero prevalence to TRAP, CSP and schizont extract increased pre to post intervention though not statistically significant. AMA-1 and MSP-1_{42} antigens had the highest prevalence of IgG1 antibodies pre and post intervention (58.5% to 49.5% and 49.1% to 49%, respectively). IgG2 sero prevalence decreased against MSP-1_{42}, AMA-1, MSP-3, GLURPR-0, GLURPR-2, LSA-NRC, TRAP and schizont extract and increased for EBA-175 and CSP while it remained stable for MSP-1_{19}. IgG3 sero prevalence decreased pre to post intervention for MSP-1_{42}, MSP-1_{19}, EBA-175, MSP-3, GLURPR-0, GLURPR-2, LSA-NRC, TRAP and schizont extract. AMA-1 and CSP sero prevalence increased pre to post intervention. IgG4 sero prevalence decreased for MSP-1_{42}, AMA-1, MSP-1_{19}, EBA-175, MSP-3, GLURPR-0, GLURPR-2, LSA-NRC, TRAP, while schizont extract remained stable and CSP increased significantly pre to post intervention. Across most of the malaria antigens tested, IgG subclass antibody frequencies and levels increased with age. IgG1 antibodies to all antigens were acquired earliest in life compared to the rest of the IgG subclass antibodies. In 2007 and 2008, an average of >70% of individuals of ≥17 years were IgG1 positive responders to blood stage malaria antigens compared to <50% for the pre-erythrocytic malaria antigens. These results show that successful malaria interruption may lead to substantial reductions in IgG subclass antibody levels and responses to *Plasmodium falciparum* in areas of unstable low transmission.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Enormous progress has been made in reducing malaria incidence and deaths, particularly in sub-Saharan Africa, through the use of insecticide-treated bed nets (ITN), indoor residual spraying (IRS) of insecticides, and artemisinin combination therapy (ACT) (Bhattarai *et al.*, 2007). Despite all these integrated malaria control strategies, malaria still remains one of the deadliest diseases in the world, killing almost one million people every year with 85% of them being children under the age of five (WHO, 2010). A malaria vaccine, like other vaccines that have eliminated or reduced disease burden for example small pox vaccine and polio vaccine, remains the most viable option for prevention of malaria. However, understanding how immune responses of children and adults change as transmission decreases is fundamental to malaria vaccine research as well as intervention strategies.

Natural immunity against malaria protects millions of people routinely exposed to *Plasmodium falciparum* infection from severe disease and death. Across sub-Saharan Africa where malaria is holoendemic, adults who have been continuously exposed to *Plasmodium falciparum* rarely experience overt disease despite the population of parasites in their blood, but infants and young children occasionally experience overt disease (Doolan *et al.*, 2009; Schwartz *et al.*, 2001). Interventions that reduce malaria exposure below a level capable of maintaining natural immunity risk the probability of a catastrophic rebound as experienced in the highlands of Madagascar in the 1980s where a

malaria epidemic occurred after an interruption in the malaria transmission (Romi *et al.*, 2002).

Highland areas (>1500 meters above sea level) in Africa experience unstable malaria transmission with low incidence of malaria in the dry season (Ernst *et al.*, 2006). These areas were targeted for attempting the interruption of malaria transmission. Starting 2005, the Ministry of Health of Kenya implemented malaria control interventions to reduce malaria transmission and improve malaria treatment in Kipsamoite and Kapsisiywa, which are in Nandi Hills district of Kenya, a highland area in western Kenya.

Demographic surveys in Nandi Hills district were started in April 2003 and conducted every 4 to 6 months revealing a malaria transmission of 5.9% in the dry season and 14.5% in the rainy season (John *et al.*, 2009). Following the successful malaria control interventions to reduce malaria transmission and improve malaria treatment from 2005 by Ministry of Health, Kenya, site wide malaria surveys revealed a possible interruption in malaria transmission between May 2007 and April 2008. The surveys revealed that <0.3% of persons were positive for asexual *Plasmodium falciparum* by microscopy or PCR at any time (John *et al.*, 2009).

Follow up studies in the area documented persistence of total IgG to multiple blood stage malaria antigens over the 13 months period of low to absent malaria transmission (<0.3% prevalence) with statistically significant differences observed against CSP and TRAP (pre-erythrocytic malaria antigens) over the same period (Ondigo *et al.*, 2010,

unpublished). This is consistent with previous studies, in particular a recent study in a highland population in Thailand that showed that antibodies to malaria antigens persist even in absence of infection (Wipasa *et al.*, 2010). It is important to know whether these differences in frequencies and levels were present or not in IgG subclasses to both blood stage and pre-erythrocytic malaria antigens as the levels of IgG subclasses are related to protection or susceptibility to clinical malaria (Chizzolini *et al.*, 1988; Astagneau *et al.*, 1995). This will inform the effects of malaria interventions on natural immunity against malaria and targets for vaccine design and development.

1.2 Statement of the Problem

Malaria control campaigns have reduced malaria transmission to low levels in many parts of Africa, yet the extent to which malaria interruption might affect immune correlates to malaria in areas of low unstable malaria transmission is unknown (Noland *et al.*, 2012). While recent studies appear to suggest that specific IgG responses to malaria antigens are stable (Wipasa *et al.*, 2010), a number of epidemiological studies suggest that protective immunity to malaria is lost in the absence of constant infection (Doolan *et al.*, 2009). A possible factor that can explain this loss is the balance between cytophilic and non-cytophilic antibody subclasses. Unfortunately, studies on the IgG subclasses remain inadequate. Immunoglobulin G (IgG) subclasses include IgG1, IgG2, IgG3, and IgG4 (Aribot *et al.*, 1996). The cytophilic IgG1 and IgG3 subclasses to blood stage malaria antigens have been associated with lower parasitemia and reduced risk for malaria pathology (Stanisic *et al.*, 2009). However, whether the distribution and levels of these subclasses are affected by reduced malaria transmission is unknown hence warrants

further studies. Similarly, other studies have also associated the protection of parasite-specific IgG subclasses with age and greater levels of protection are seen in adults due to high antibody levels (Tongren *et al.*, 2006). It would be interesting to know if a particular age group becomes vulnerable in areas with low to absent transmission.

1.3 Rationale of the Study

There have been efforts to design and develop malaria vaccines based on the understanding of human host immune responses against malaria parasites (Chauhan *et al.*, 2003). This study was designed to further shed light on immune correlates of IgG subclass responses to multiple *Plasmodium falciparum* antigens over time in a scenario where there was low to absent malaria transmission (<0.3%) in a traditionally unstable and low malaria transmission area. The scenario gave a unique opportunity to assess the impact of low to absent malaria transmission (<0.3%) on IgG subclass (important immune correlates) responses to *Plasmodium falciparum* antigens, which is important in understanding a population's epidemic risk after successful intervention and potential differences in vaccine immunogenicity and efficacy for malaria prevention strategies and vaccine design and development for areas with unstable and low malaria transmission.

1.4 Objectives of the Study

1.4.1 General objective

To examine IgG subclass responses to *Plasmodium falciparum* antigens after successful intervention strategies during a period of low to absent malaria transmission and among different ages in a prospective cohort study in a highland area of western Kenya.

1.4.2 Specific objectives

1. To determine the changes in the frequencies of IgG subclass antibody responses to selected malaria antigens over a 13 months period of interrupted malaria transmission.
2. To determine changes in levels of IgG subclass antibodies to *Plasmodium falciparum* antigens over a 13 months absence of persistent malaria transmission in the western highlands of Kenya (Nandi district).
3. To determine the effect of age on IgG subclass frequencies and levels to *Plasmodium falciparum* antigens in an area of low to absent malaria transmission in the western highlands of Kenya.

1.5 Alternative Hypotheses

1. IgG subclass frequencies to *Plasmodium falciparum* antigens will remain unchanged over the 13 months period of low to absent malaria transmission.
2. IgG subclass levels to *Plasmodium falciparum* antigens will remain stable over the 13 months period of low to absent malaria transmission.
3. IgG subclass frequencies and levels to *Plasmodium falciparum* antigens generally increase with age over time.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria and *Plasmodium falciparum* Life Cycle

Malaria is a parasitic infection causing morbidity and mortality in many parts of the world especially in sub-Saharan Africa. According to the 2010 World Health Organization (WHO) malaria report, an estimated 225 million people were affected by malaria worldwide and 78% of them were from sub-Saharan Africa (World Malaria Report, 2010). Malaria still kills over 780,000 people a year and about 85% of them are children less than 5 years of age and a significant number are pregnant women in sub-Saharan Africa (WHO, 2010). Even with the increased knowledge of the parasite biology and advanced technology in malaria research, there has been little success in eradicating malaria.

The vision of complete eradication of malaria has given way to more pragmatic measures of controlling malaria disease. Although malaria is a treatable and preventable disease, the widespread occurrence of drug-resistant parasites and the emergence of insecticide resistant mosquito vectors have remarkably hindered effective disease control (Guerin *et al.*, 2002). Therefore, an alternative strategy to reduce the burden of malaria is deployment of vaccination that has been successfully implemented for control and eradication of a number of infectious diseases (André, 2003). However, development of an effective malaria vaccine would require identification and incorporation of target immunogens derived from various stages of parasites and the need to circumvent antigenic polymorphism among major vaccine candidates (Moorthy *et al.*, 2004).

Malaria is caused by a unicellular protozoan parasite of the *Plasmodium* genus? (Perlmann *et al.*, 2000)?. There are five species of malaria affecting the human population: *Plasmodium ovale, Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax,* and *Plasmodium knowlesi* (WHO, 2010; Singh *et al.*, 2004). These *Plasmodium* species have a complex life cycle that is made up of a sexual stage that occurs in the stomach of the female *Anopheles* mosquitoes, and an asexual stage which takes place in the vertebrate host in this case humans (Klein *et al.*, 2013). Malaria is transmitted from an infected person to another through a bite from female *Anopheles* mosquito. Just after the female *Anopheles* mosquito has ingested infected blood from a person, male gametocytes fertilize female gametocytes to form ookinetes that burrow into the stomach walls developing into oocysts in the lining of the gut of the female *Anopheles* mosquito (Wipasa *et al.*, 2002). When the cysts rupture, they release sporozoites that enter the salivary glands. Within 60 minutes of the bite by the infected mosquito, the sporozoites migrate to the liver; invade the hepatocytes where they remain for 5-16 days depending on the species of the *Plasmodium* undergoing asexual multiplication (Wipasa *et al.*, 2002). The host remains asymptomatic during this stage.

The erythrocytic stage begins when the infected hepatocytes rupture releasing merozoites into the blood circulation. Within 1-2 minutes of rupture every merozoite attaches to specific receptors on the red blood cell (RBC) membrane using ligands on the merozoite surface (De Koning-Ward *et al.*, 2009). This leads to invagination of the RBC membrane so that the merozoite moves into the RBC forming the parasitophorous vacuole. In the parasitophorous vacuole the parasite undergoes maturation from the early ring stage trophozoite to the late trophozoite and after several mitotic divisions to the schizont stage

containing 6-32 merozoites depending on the species (Pouniotis *et al.*, 2004). The erythrocytic schizonts rupture and merozoites released circulate in the blood. Each merozoite will continue the life cycle by invading other RBCs. It is during the asexual part of the life cycle that the symptoms of malaria manifest. Some of the merozoites undergo meiosis and develop into male and female gametocytes, which can then be taken up by the female *Anopheles* mosquito during a blood meal to repeat the cycle Figure 1.0. shows the life cycle of *Plasmodium* parasite showing the various stages that can be targeted by host immune responses.

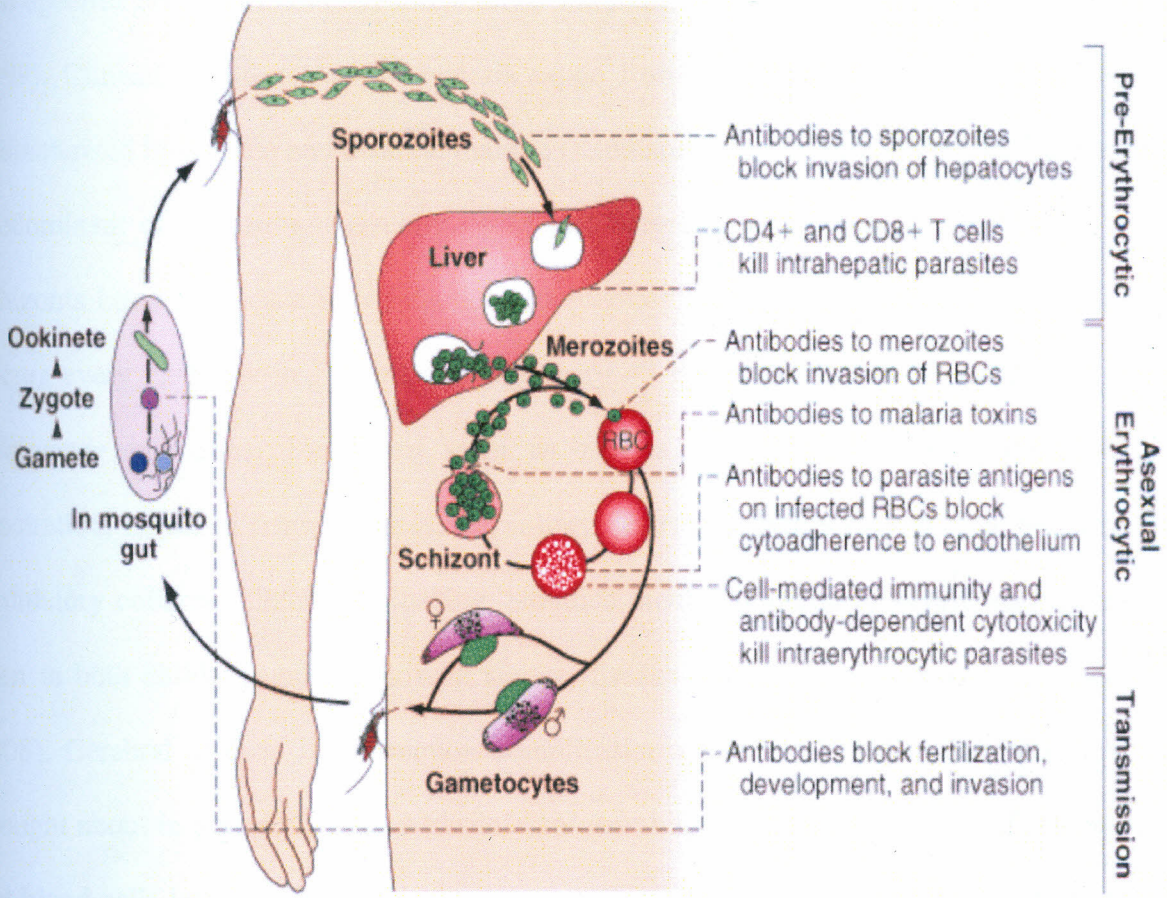


Fig 1.0 Life cycle of *Plasmodium* parasite showing the various stages that can be targeted by host immune responses. Adapted from Berman (2009).

Human antibodies can act on newly injected sporozoites, on merozoites and on parasite-derived proteins on the surface of infected erythrocytes. Immune cells and their related cytokines can act on infected hepatocytes and the infected erythrocytes. In the mosquito vector, antibodies and complement can act on the invasive ookinetes, and innate immunity can act on newly released sporozoites (Breman, 2009).

2.2 Clinical Manifestation of Malaria

The definition of clinical malaria is elusive because symptoms such as fever are often non-specific and asymptomatic infection is rampant in endemic areas (Bloland *et al.*, 1999). Clinical manifestations of malaria range from uncomplicated to severe disease characterized by anemia and cerebral malaria (Kremsner *et al.*, 1995). High fevers are the predominant of malaria and usually occur just before or at the time of RBC lysis as schizonts burst to release new infectious merozoites (Kwiatkowski *et al.*, 1989). This occurs every 72 hours in *P. malariae* and every 48 hours in *P. vivax, P. ovale* and *P. falciparum* infections. Symptoms such as nausea, headaches and muscular pain, prostration, impaired consciousness, respiratory distress or pulmonary edema, seizures, circulatory collapse, abnormal bleeding, jaundice, hemoglobinuria or severe anemia are seen in both children and adults with severe disease (WHO, 2000; Greenwood *et al.*, 2008). Cerebral malaria is a common complication of severe *P. falciparum* infection brought about in part due to the parasite's unique ability to alter the surface of infected red blood cells leading them to bind to the endothelial surfaces of blood vessels causing obstruction of cerebral blood flow (Dondorp *et al.*, 2000). In pregnancy, malaria is a

major contributor to premature deliveries, miscarriages, and low birth weight (Hay *et al.*, 2004). Manifestations of severe and uncomplicated malaria vary geographically but are usually associated with *P. falciparum* infection (John *et al.*, 2000).

2.3 Immunity to Malaria

Immunity against malaria is provided by both the innate and acquired immune mechanisms (Stevenson *et al.*, 2004). Naturally acquired immunity to malaria develops with repeated exposure as a person grows in a malaria endemic area, that is why individuals in malaria endemic areas frequently have parasitaemia and antibodies without symptoms (Baird, 1995). This protective immunity against malaria is not permanent, and in addition it is variant, stage, strain and species specific (Andrysiak *et al.*, 1986; Fandeur *et al.*, 1998; Rotman *et al.*, 1999). The acquired protective immunity involves both humoral and cell-mediated immunity.

Areas of the highlands of western Kenya that experience unstable and low malaria transmission are characterized by a persistent risk of clinical malaria in adults, older children and infants (John *et al.*, 2005). This is because natural protection against malaria is said to wane in absence of re-infection (Wipasa *et al.*, 2010).

2.3.1 Cell-mediated immunity

The contribution of T-cell subsets and their associated cytokines to the development of natural immunity to malaria is essential both in regulating antibody production and induction of antibody–independent protection (Winkler *et al.*, 1999). Pre-erythrocytic immunity to *P. falciparum* infection is partly mediated by T-lymphocytes acting against

the liver-stage parasite and they must recognize parasite-derived peptides on infected host cells in the context of major histocompatibility complex (MHC) antigens (Aidoo *et al.*, 2000). Although antibodies, $CD4^+$ and $CD8^+$ T cells have been implicated in pre-erythrocytic immunity, protection mainly depends on CD8+ T cells (Hafalla *et al.*, 2006).

$CD4^+$ T cells play an important role in protective immunity to blood stage malaria infection (Xu *et al.*, 2002). Therefore, the active immune response that develops after infection and is required to clear parasites is CD4+ T cell and B cell-dependent. CD4+ T cells are classified into two major subsets according to the type of cytokines they produce. Cytokines produced by T-helper 1 (Th1) cells include interleukin (IL)-2, interferon (IFN)-, and tumor necrosis factor (TNF)-, whereas those secreted by T-helper 2 (Th2) cells include IL-4, IL-5, IL-6 and IL-10 (Abbas *et al.*, 1996). T-helper 1 cells are responsible for cell-mediated immunity and activation of macrophages as well as other cells that produce mediators through the release of inflammatory cytokines, while T-helper 2 cells are responsible for regulating antibody dependent immunity by stimulating B cells to differentiate into plasma cells which produce antibodies including IgG subclasses (Abbas *et al.*, 1996). Th1 and Th2 cells contribute to protective immunity against blood stage malaria through the balance of cytokines produced by these two subsets, which is critical in determining the disease outcome (Abbas *et al.*, 1996). Activation of malaria-specific $CD4^+$ T cells is usually initiated when malaria antigen is presented in context of MHC-II molecules on antigen presenting cells (APC) such as dendritic cells which react with naïve $CD4^+$T cells (Bruña-Romero *et al.*, 2001). In addition, $CD4^+$ T cells are able to protect against malaria by themselves (Van der Heyde

et al., 1994). Nevertheless, studies show that immunity against blood stage malaria involves both $CD4^+$ T cells and antibodies (especially IgG) (Langhorne *et al.*, 1998).

2.3.2 Humoral immunity

B-lymphocytes and antibodies are known to play an important role in immunity against malaria. Antibodies may act in different ways, by preventing merozoite invasion of red blood cells (Dent *et al.*, 2008; Egan *et al.*, 1999), by attacking infected RBCs and facilitating phagocytosis, or by preventing cyto-adhesion of infected RBCs (Miller & Hoffman, 1998)). Murine studies show that mice that lack B cells are unable to clear *P. chabaudi* infection, which progressed to chronic parasitaemia (Von der Weid *et al.*, 1996). Studies have shown that antibodies in sera purified from African adults who are clinically immune to malaria and given by passive transfer to susceptible children show that immunoglobulin G is a main component in defense against the asexual blood stage of *P. falciparum* ((Druilhe & Pérignon, 1994). It has also been shown that naturally acquired immunity to malaria in people living in malaria endemic areas is majorly dependent on the acquisition of a high concentration of specific and protective antibodies directed against the polymorphic target antigen, *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) (Bull *et al.*, 1998). Studies have shown that the level of protective immunity in humans correlates with the level of antibodies against asexual blood stage malaria antigens and this is dependent on the antibody isotypes (Roussilhon *et al.*, 2007).

Host genetic factors and age of people living in malaria endemic areas contribute to the immunoglobulin G (IgG) subclass responses against ring-infected erythrocyte surface

antigen (RESA), merozoite surface antigens 1 and 2 (MSP-1 and MSP-2) and crude *P. falciparum* antigen (Aucan *et al.*, 2001).

Cytophilic antibodies of the IgG1 and IgG3 subclasses have been documented to be the most significant antibodies for protection against *P. falciparum*. They act in collaboration with effector cells such as monocytes and macrophages to mediate opsonization and antibody dependent cellular inhibition (ADCI) (Wipasa *et al.*, 2002). Sero-epidemiological studies have documented that increased levels of *P. falciparum* specific IgG1 and IgG3 in individuals living in endemic areas are associated with lower parasitaemia and reduced risk of malaria pathology (Aribot *et al.*, 1996). The protection attributed to parasite-specific IgG3 is age related with increased levels of protection seen in adults. High levels of IgG2 to RESA and MSP2 have been shown to be associated with resistance to *P. falciparum* at the end of the malaria transmission season where levels tend to be higher in older people who are better protected from infection and disease (Aucan *et al.*, 2001; Astagneau *et al.*, 1995). However, levels of IgG4 to schizont extract, RESA, MSP1 and MSP2 are highest in individuals with complicated malaria (Aucan *et al.*, 2000; Leoratti *et al.*, 2008). This suggests that IgG4 competes with IgG1 and IgG3 for antigen recognition thereby blocking antibody-mediated cytotoxicity by activated effector cells (Wipasa *et al.*, 2002). Selection of antigens capable of eliciting strong and harmless antibody responses is a prerequisite for the development of a malaria vaccine (Garraud *et al.*, 2003).

2.4 Antigens in Malaria Vaccine Development

The complex life cycle stages and estimated 5,000 genes of the *Plasmodium* parasite present a wide range of antigens, and it has been challenging to determine which of these antigens would make good vaccine targets (Genton *et al.*, 2007; Chauhan *et al.*, 2003).

2.4.1 Circumsporozoite protein (CSP)

Circumsporozoite protein (CSP) is a leading malaria vaccine candidate antigen, predominantly distributed on the surface of the sporozoites. Circumsporozoite protein forms a dense coat on the parasite surface and has been hypothesized to mediate many of the initial interactions between the sporozoite and its two hosts (female *Anopheles* mosquito and humans (Sinnis *et al.*, 2002). The structure of CSP is highly conserved among *Plasmodium* species infecting rodents, primates, and humans (Sinnis *et al.*, 2002). CSP is made up of a central repeat region that is diverse across *Plasmodium* species, and flanking the repeats are two conserved domains: region I, a 5-aa sequence at the N terminus of the repeats, and a known cell-adhesive motif C-terminal to the repeats termed the type1 thrombospondin repeat (TSR). Studies with recombinant CSP, peptides representing portions of CSP, and sporozoites expressing heterologous CSP, suggest that antibodies against this protein target sporozoites in both mosquito salivary glands and the mammalian liver (Frevert *et al.*, 1993; Sidjanski *et al.*, 1997; Pinzon-Ortiz *et al.*, 2001). CSP-specific antibodies (Potocnjak *et al.*, 1980), CD8+ and CD4+ T cells (Romero *et al.*, 1989; Rénia *et al.*, 1993) have been shown to elicit protective immunity in mouse models of malaria. In humans it seems clear that antibodies against CSP may be necessary but not entirely sufficient for the protection seen (Reece *et al.*, 2004). Indeed, CSP-specific

Th1 responses have also been suggested to correlate with protection in humans following vaccination or natural infection (Sun *et al.*, 2003; Reece *et al.*, 2004).

2.4.2 Thrombospondin-related anonymous protein (TRAP)

Thrombospondin related anonymous protein (TRAP) is a transmembrane protein present in the sporozoite secretory invasive organelles involved in sporozoite motility and infectivity of liver cells (Sultan *et al.*, 1997). *Plasmodium* TRAP is the most extensively studied transmembrane protein. TRAP is stored within the micronemes of sporozoites, released onto the cell surface at the anterior tip upon contact with a host cell and translocated to the posterior pole of the sporozoite along its surface during penetration. Its essential role is thought to link the actin–myosin motor through its cytoplasmic domain while binding to hepatocytes via its extracellular portion (Sultan *et al.*, 1997).

2.4.3 Liver stage antigen 1 (LSA-1)

Liver stage antigen 1 is a 230 kDa protein characterized by a central repeat region containing 86 repeats of the 17-amino-acid sequence EQQSDLEQERLAKEKLQ or minor variations thereof (Zhu *et al.*, 1991). Flanking these repeats are a non-repetitive 154 residue N-terminal region and a 280 residue C-terminal region, known to contain B cell and CD4+ and CD8+ T cell epitopes (Zhu *et al.*, 1991; Fidock *et al.*, 1994). The sequences of LSA-1 repeat and non-repeat regions are highly conserved across strains of *P. falciparum* (Fidock *et al.*, 1994) suggesting a crucial role during liver schizogony (Fidock *et al.*, 1994). LSA-1 is a surface protein, which is solely expressed by infected

hepatocytes and is believed to play a role in liver schizogony and the release of merozoites (Guerin-Marchand *et al.*, 1987; Hollingdale *et al.*, 1990).

Plasmodium falciparum liver stage antigen (PfLSA-1) induced specific humoral, cellular, and cytokine immune responses in infected individuals (Connelly *et al.*, 1997; Joshi *et al.*, 2000) has been considered a vaccine candidate for *P. falciparum*, due to their antigenic and protection-including immunogenic properties (Kurtis *et al.*, 2001; Taylor-Robinson, 2003). Three reasons support its development as a vaccine antigen. First, it is highly conserved suggesting that a candidate vaccine based on the 3D7 strain might elicit immune responses that cross-react with all other strains of *P. falciparum* (Fidock *et al.*, 1994; Yang *et al.*, 1995). Secondly, LSA-1 is abundantly expressed from early through late schizogony, presumably allowing time for both circulating and memory-recall effector cells to infiltrate the liver and exert their effector function (Cummings *et al.*, 2010). Third, it is possible that high titer antibody could act upon the cloud of flocculent liver stage antigen enveloping hepatic merozoites to impede the latter's emergence and subsequent invasion of erythrocytes (Hollingdale *et al.*, 1990). However, because LSA-1 appears to be unique to *P. falciparum*, there are no data from animal models of malaria to predict its suitability as a potential human vaccine antigen.

2.4.4 Apical membrane antigen 1 (AMA-1)

Apical membrane antigen 1 (AMA-1) is a 83kDa polymorphic membrane protein expressed in both sporozoites and merozoites. The protein is located in the micronemes organelles of merozoites (Crewther *et al.*, 1990) and is involved in the reorientation and

formation of tight-junction that is necessary for invasion of red blood cells by merozoites. Evidence suggests that AMA-1 is a potential vaccine candidate based on studies that observed that mice and monkeys are protected from parasitemia upon vaccination with recombinant AMA-1 from *P. falciparum* (Stowers *et al.*, 2002). Also protection has been shown to be acquired in rodents after immunization with recombinant AMA-1 against *P. chabaudi* and *P. yoelii* infections (Anders *et al.*, 1998). Studies in humans using antibodies to the full length AMA-1 have also been associated with protection prior to a malaria transmission season (Polley *et al.*, 2004). Further studies have also shown that polyclonal anti-AMA1 antibodies inhibit *in-vitro* merozoite invasion and in this way interfere with the processing of the antigen (Dutta *et al.*, 2005; Dutta *et al.*, 2003; Narum *et al.*, 2000).

2.4.5 Merozoite surface protein 1 (MSP-1)

Merozoite surface protein 1 (MSP-1) is synthesized as a high molecular weight (195 kDa), precursor membrane, which undergoes proteolytic processing to yield fragments of several sizes: 83, 42, 36, 28 - 30, and 19 kDa (Wipasa *et al.*, 2002; Lyon *et al.*, 1986). Among the fragments generated is MSP-1_{42} which undergoes secondary proteolytic processing resulting in a final conserved C-terminal membrane-anchored moiety, MSP-1_{19} which consists of two structurally constrained epidermal growth factor (EGF)–like domains (Han *et al.*, 2004; Ferreira *et al.*, 2003). MSP-1 is the most abundant surface component of the merozoite stage of the parasite life cycle, totaling up to 40% of the glycosylphosphatidylinositol (GPI)-anchored merozoite surface protein coat (Sanders *et al.*, 2007; Sanders *et al.*, 2005; Le Roch *et al.*, 2003).

Merozoite surface protein 1 (MSP-1) plays a role in the binding to and invasion of erythrocytes by merozoites (Cowman *et al.*, 2000). Secondary proteolytic processing of MSP-1_{42} results in a final conserved C-terminal membrane-anchored moiety MSP-1_{19}. Accessibility of the merozoites to the host immune system occurs between schizont rapture and the invasion of a new red cell making this stage to be a possible target for protective immunity (McBride *et al.*, 1987). Immune responses to the 83 kDa and 42 kDa fragments have been associated with protection against natural infections in West African children (Tolle *et al.*, 1993; Früh *et al.*, 1991).

Antibodies to MSP-1_{19} have been found abundantly in naturally exposed people from endemic areas (Cavanagh *et al.*, 2004; John *et al.*, 2004). *In vitro*, antibodies against MSP-1_{19} fragment have been associated with inhibition of merozoite invasion of red cells (Egan *et al.*, 1999). Sero-epidemiological studies indicate that levels of anti-MSP-1_{19} antibodies are strongly correlated with protection against clinical malaria among Sierra Leonean (Egan *et al.*, 1996), and Gambian children (Shai *et al.*, 1995) and also among Kenyan children and pregnant women (Branch *et al.*, 1998).

2.4.6 Merozoite surface protein 3 (MSP-3)

Merozoite surface protein 3 (MSP-3) is a 48-kDa protein (Oeuvray *et al.*, 1994). It is a polymorphic parasite antigen that may have a role in parasite invasion. This is evidenced by the finding that truncation of the MSP-3 gene reduces parasite invasion of erythrocytes (Mills *et al.*, 2002). Antibodies to the conserved portion of MSP-3 have been

shown to mediate antibody dependent cellular inhibition (ADCI) of parasite growth in cooperation with monocytes *in vitro* (Oeuvray *et al.*, 1994) and in an immunodeficient *in vivo* mouse model (Badell *et al.*, 2000; Singh *et al.*, 2004). In immunization/challenge studies with MSP-3, *Saimiri sciureus* (Carvalho *et al.*, 2005) and *Aotus nancymai* (Hisaeda *et al.*, 2002) monkeys were protected from lethal challenge with malaria. In longitudinal studies in endemic populations, antibodies to both conserved (Singh *et al.*, 2004; Meraldi *et al.*, 2004; Soe *et al.*, 2004) and allele-specific (Polley *et al.*, 2007) epitopes of MSP-3 have been associated with reduced risk of malaria.

2.4.7 Erythrocyte-binding antigen 175 (EBA-175)

Erythrocyte-binding antigen (EBA) is a 175 kDa merozoite expressed protein located in the micronemes which mediates sialic acid-dependent invasion of red blood cells (RBC) (Sim *et al.*, 1990; Camus & Hadley, 1985). It has also been shown to elicit potentially protective antibody response (John *et al.*, 2005; Okenu *et al.*, 2000)).

Erythrocyte-binding antigen 175 (EBA-175) was the first member of the erythrocyte-binding ligand (EBL) family characterized and shown to bind to the major glycoprotein found on human erythrocytes, glycophorin A (GPA), during invasion (Pandey *et al.*, 2002; Narum *et al.*, 2000). Recombinant fragments of EBA-175 are recognized by human sera from malaria-endemic areas (McCarra *et al.*, 2011; Daugherty *et al.*, 1997). Antibodies raised in rabbits against EBA-peptide 4 blocked binding of native EBA-175 to human erythrocytes and inhibited merozoite invasion *in vitro* (Orlandi *et al.*, 1990; Sim *et*

al., 1990). Additionally, IgG1 antibodies to EBA-175 peptide 4 are associated with protection against clinical malaria (Touré *et al.*, 2006).

2.4.8 Glutamate-rich protein (GLURP)

Glutamate rich protein (GLURP) is a 220-kDa protein expressed in the pre-erythrocytic stage, in schizonts, and on the surface of newly released merozoites (Borre *et al.*, 1991). The antigen contains an amino-terminal non-repeat region R0 (GLURP 94 - 489), a central repeat region (GLURP 489 - 705) (R1) and a carboxy-terminal (GLURP 705 - 1178) (R2) repeat region (Dodoo *et al.*, 2000). GLURP is an antigen expressed in all stages of the parasite life cycle in humans (Borre *et al.*, 1991).

Antibodies against GLURP were found to react with the asexual, hepatic, and gametocyte stages of the parasite (Borre *et al.*, 1991), suggesting that GLURP is synthesized throughout the entire life cycle of *P. falciparum* in the vertebrate host. Levels of GLURP-specific antibodies have been associated with protection against high levels of parasitemia (Hogh *et al.*, 1992) and with protection against clinical disease (Dziegiel *et al.*, 1993). Previous immune-epidemiologic studies performed in high transmission areas have shown a high prevalence of antibodies against GLURP in adults (Dziegiel *et al.*, 1993; Boudin *et al.*, 1993; Dziegiel *et al.*, 1991) as well as a significant association of high levels of GLURP-specific antibodies with low parasite densities (Høgh *et al.*, 1993; Hogh *et al.*, 1992) and protection against clinical malaria (Soe *et al.*, 2004; Dodoo *et al.*, 2000; Dziegiel *et al.*, 1993). In addition, there has been evidence that cytophilic antibody responses to GLURP play a primary role in protection against *P. falciparum* malaria by effector mechanisms such as antibody-dependent cellular inhibition (ADCI) (Theisen *et*

al., 1998). Studies performed in areas highly endemic for malaria have demonstrated a high prevalence of antibodies against two well-defined regions within *P. falciparum* GLURP, the relatively conserved N-terminal non-repeat region (R0) and the immuno-dominant repeat region (R2) (Theisen *et al.*, 1998).

2.4.9 Schizont crude extract

Schizont crude extract is a mixture of numerous malaria antigens harvested from culturing erythrocytic stages of *Plasmodium falciparum*. The crude extract is obtained using centrifugation methods. Previous studies have shown that responses to schizont crude extract are less sensitive to genetic polymorphisms compared to characterized antigens (Takala et al., 2009).

2.5 Malaria Transmission and Interruption

Malaria transmission is affected by climate and geography and defined by malaria endemicity. Malaria endemicity has been defined in terms of rate of parasitemia or palpable spleen rates in children 2 to 9 years of age as; hypoendemic (<10%), mesoendemic (11 to 50%), hyperendemic (51 to 75%), and holoendemic (>75%) (Malaria Conference in Equatorial Africa 1950: Kampala, Organization, & Sahara, 1951). The intensity of malaria transmission is best expressed as the entomologic inoculation rate (EIR), which is defined as the number of infectious (sporozoite carrying) female anopheline bites per year (Macdonald, 1957). EIR of <10 bites per year is known as a low transmission area, 10 to 49 bites per year is an intermediate transmission area, and >50 bites per year is a high transmission area (Macdonald, 1957; WHO, 2005; Kelly-Hope *et*

al., 2009; Smith *et al.*, 2006). EIR directly reflects the exposure of humans to *Plasmodium* parasites (Beier *et al.*, 1999).

In Kenya, a wide range of malaria transmission intensities have been mapped, ranging from areas with low malaria risk to holoendemic areas with continuous exposure to malaria throughout the year (John *et al.*, 2000). In addition, the highlands of Kenya are prone to unpredictable epidemics of malaria due to the unstable and low malaria transmission they experience throughout the year (Ernst *et al.*, 2006).

The Ministry of Health of Kenya between 2005 and 2006 undertook malaria control interventions in Kenya including the highland villages of Kipsamoite and Kapsisiywa in Nandi district of western Kenya to reduce malaria transmission. These included: indoor residual spraying (IRS) of houses, distribution of insecticide treated bed nets (ITNs) to pregnant women and children <5 years, and the use of artemether/lumefantrine as first-line treatment of uncomplicated malaria (Bhattarai *et al.*, 2007). As a result, the highland area experienced interruption of malaria transmission between April 2007 and March 2008 where a malaria prevalence of <0.3% was reported compared to 5.9% to 14.5% prevalence before interruption (John *et al.*, 2009). The wide spread use of ITNs in Kenya resulted in a 44% reduction in infant (<5 years) mortality over a two year period (Fegan *et al.*, 2007).

CHAPTER THREE

3.0 STUDY DESIGN AND METHODS

3.1 Study Area and Design

The study was conducted in Nandi district (Kapsisiywa and Kipsamoite sites) in the western Kenya highlands. Kapsisiywa is characterized by a seasonal *P. falciparum* malaria transmission pattern while Kipsamoite has always had fewer cases of malaria compared to Kapsisiywa (Noland *et al.*, 2008a). The average population of each of the study sites is about 3,700 individuals. Both study sites are adjacent to each other, lie between $0^0$16'55. 64^0 N to 0^0 21'52. 42^0 N latitude and 34^0 59'7. 17"E to 35^0 5'19. 90"E longitude located in Nandi district, a highland area prone to malaria epidemics, with an estimated entomological inoculation rate of <1 infectious bite per person per year (Noland *et al.*, 2008a), and most of the people, are of Kalenjin tribe. There are usually two rainy seasons, short rains in October and long rains beginning in March to May mostly followed by a peak in malaria in June or July. In this area, *Anopheles gambiae s.l* is the most abundant vector (97.5%) and *Anopheles funestus* (2.5%) (Ernst *et al.*, 2006).

As already mentioned in Chapter 2 (2.5), previously, the Ministry of Health in Kenya undertook public health interventions to control malaria and these sites were targeted for indoor residual spraying (IRS) of houses, distribution of insecticide treated bed nets (ITNs) to pregnant women and children <5 years, and introduction of artemether/lumefantrine therapy as first-line treatment of uncomplicated malaria from the year 2005. As a result of these malaria control strategies, malaria transmission in these sites was interrupted. This was noted through surveillance surveys undertaken as part of the prospective longitudinal cohort study between May 2007 and July 2008

revealing a malaria prevalence of <0.3% (John *et al.*, 2009). Because of these surveys blood samples of the same participants was available from surveys before malaria interruption and after the interruption. This gave me an opportunity to perform a cross sectional study on the same participants before and after interruption.

This was a cross sectional study conducted on samples collected in May 2007 and July 2008 from people who were permanent residents of Kipsamoite and Kapsisiywa study sites that were part of a site wide cohort malaria study by Kenya Medical Research Institute and University of Minnesota (KEMRI/UMN). Ethical approval for the study was obtained from Ethical Review Committee at Kenya Medical Research Institute and Institutional Review Board at the University of Minnesota. Written informed consents were also obtained from study subjects or from guardians.

3.2 Sample Size

The sample size for this cross sectional study was determined from a cohort of 1000 participants who were earlier tested for total IgG responses against the 11 selected malaria antigens from the cohort study (Ondigo *et al.*, 2010, unpublished). For each of the 11 malaria antigens tested, I estimated sero-deconversion fraction as a function of age, giving an average sero-conversion rate for each age, averaging over antigens. The 1000 participants from the cohort were sorted by age from the youngest to the oldest. Using the sero-deconversion probability (p-i) where p= probability, i= person, it was expected that sum (p-i) people will sero-deconvert. Four age groups were formed from the cohort of 1000 participants (<4 years, 4≤ age<8 years, 8≤age<17 years, and ≥17 years).

Fifty participants were then randomly selected from each age group making a total of 200 participants. Also taking into account total IgG antibodies prevalence to EBA-175 of 54% from the previous study (Noland *et al.*, 2008b), this number was capable of giving the study a power of >80% capable of detecting a 40% decrease in IgG isotypes in participants within the cross-sectional surveys. Those selected must have had their blood samples collected in both surveys.

3.3 Samples Collection and Processing

Blood samples for this study were collected during two site wide surveys from Kapsisiywa and Kipsamoite (Nandi district) in May 2007 and July 2008. Blood samples were transported to the University of Minnesota laboratory at the Center for Global Health Research (CGHR), Kenya Medical Research Institute (KEMRI), Kisumu. Each participant sample was given a unique study identification number, which included information on site of residence within the study area, village of residency, household and member status of the participant. Data was double entered and verified using File Maker Pro database software (File maker Inc). Blood was collected by vein-puncture into heparinized tubes. The plasma was separated and stored at −80 °C for later batch analysis. Stored plasma samples from 200 randomly selected individuals from the two study sites were tested for antibodies subclasses to a panel of malaria antigens in the current study.

3.4 *Plasmodium falciparum* Recombinant and Peptide Antigens

Recombinant AMA-1 and LSA-1 had been expressed in *E. coli* and were provided by David Lanar, Walter Reed Army Institute for Research. Recombinant MSP-1_{42} and MSP-3 had been expressed in *E. coli*, and recombinant EBA-175 had been expressed in *Pichia pastoris*, and provided by David Narum, National Institutes of Health. Recombinant GLURP had been expressed in *E. coli* and was provided by Michael Theisen, Statens Seruminstitut, and Copenhagen, Denmark. Recombinant MSP-1_{19} had been expressed in *Saccharomyces cerivisiae*. It was provided by the Malaria Research and Reference Reagent Resource Center (Manassas, VA), and originally deposited there by David Kaslow.

3.5 Cytometric Bead Assay

The bioplex^{100} system can simultaneously quantitate up to 100 different proteins, peptides, DNA fragments and RNA fragments from a single drop of sample in a well of a microtiter plate (Giavedoni, 2005). The multiplex assay is a bead format assay in which each bead set is internally color coded with different ratio of red to infrared dyes that, and results in a unique bead set that can be classified separately by the BioPlex machine. The beads in multiplex assay anchor the antigens. The BioPlex machine has two lasers; one laser beam excites the internal colored dyes for classification of the bead sets while the other laser excites the reporter fluorochrome phycoerythrin (PE) (Cham *et al.*, 2008). Through classification of the bead set, various bead sets are distinguished which correspond to up to 100 different analytes that the machine could quantitate, while the amount of analyte present in the plasma, serum or supernatant is quantified by excitation of the reporter fluorochrome (Giavedoni, 2005).

3.5.1 Coupling of recombinant antigens to magnetic carboxylated microspheres for the cytometric bead assay (CBA)

Microspheres (BioPlex carboxylated beads) were purchased from Luminex Corporation (Austin, Texas, USA). Each malaria antigen (MSP-1_{42} FVO, AMA-1FVO, MSP-1_{19}, EBA-175, MSP-3 FVO, LSA-1, GLURPR-0, GLURPR-2, TRAP) preparation was chemically cross-linked to the microspheres using the BioPlex Amine Coupling kit. The bead stock was resuspended by gentle inversion for 1 minute. An aliquot of 612,500 beads was removed and centrifuged at 16,000g for 3 min. The supernatant was removed and 100µl of distilled water added and centrifuged at 16,000g for 3min. Beads were resuspended in 80µl of activation buffer (100mM monobasic sodium phosphate; pH 6.2) by vortexing and sonication for 20 sec. To activate the beads for cross-linking to proteins, 10µl of 50mg/ml sulfo-N-hydroxysulfosuccinamide was added and the beads were mixed by vortexing for 10 sec. Next, 10µl of 50mg/ml 1-ethyl-3- [3-dimethylaminopropyl] carbodiimide (EDC) were added, and the beads mixed again by vortexing for 10 sec. All incubations of beads were performed in the dark (covered with foil). The bead mixture was rotated on a rotary shaker at room temperature for 20 min and vortexed for 10 sec at 10 min and at 20 min. Beads were pelleted by centrifugation at 13,500g for 3min. Beads were then washed twice with 250µl of 100mM morpholineethanesulfonic acid (pH 6.0) (MES) buffer. Beads were pelleted again by centrifugation at 16,000g for 3 min. To coat the beads with antigens, pelleted beads were resuspended in the relevant antigen and the volume adjusted to 500µl per reaction by addition of coupling buffer (100mM MES, pH

6.0). The antigen and activated beads mixture were then incubated by rotating on a rotary shaker for 2 hr at room temperature in the dark for coupling to occur. After being coated with proteins, beads were centrifuged at 16,000g for 3 min and washed twice with 250µl of PBS-TBN (PBS, 0.1 % BSA, 0.02% Tween, 0.05% sodium azide) and resuspended in 200µl of PBS-TBN. To determine the percentage recovery after the coupling procedure, coupled beads were counted on a hemocytometer under a light microscope.

3.5.2 Quantification of IgG subclasses specific to malaria antigens

The volume of working solution (50µl/ well) was calculated together with the number of beads that would result in 1000 beads/region/well. Bead stocks were then added together in a 15 ml amber conical tube and diluted with PBNT (0.1% BSA, 0.05%, Tween 20, 0.05% sodium azide in PBS) to result in 100 microspheres/µl. Plasma samples were thawed at room temperature, mixed and centrifuged at 16,000g for 3 minutes then diluted to 1:100 in PBNT (1xPBS, 1% BSA, 0.05% Tween 20, 0.05% sodium azide, 0.5% polyvinyl alcohol, and 0.8% polyvinylpyrrolidone). 50µl of working bead solution was transferred using a pipette to each well of the 96 well microtiter plate. 50µl of the diluted plasma was then added to each well of the microtiter plate and the plates were incubated in the dark (covered by aluminum foil) on a microplate shaker at 600 rpm for thirty seconds followed by 300 rpm for thirty minutes.

Plates were washed using 100 µl/well of PBNT in the BioPlex pro II wash station with the protocol MAG3 (wash plate X3 times with PBNT wash buffer). The beads in the well of the plate were then resuspended in 50µl PBNT. Then 50µl of the secondary antibody

(biotin mouse anti human IgG1, biotin mouse anti human IgG2, biotin mouse anti human IgG3 and biotin mouse anti human IgG4) diluted at 1:1000 in PBNT was added to each well and then incubated in the dark with shaking at 600 rpm for thirty seconds followed by 300 rpm for thirty minutes. Plates were washed using 100 µl/well of PBNT in the BioPlex pro II wash station with the protocol MAG3. The beads in the well of the plate were then resuspended in 50µl PBNT 50 µl of Streptavidin-R-Phycoerythrin diluted at 1:2000 in PBNT and added to each well and then incubated in the dark with shaking at 600 rpm for thirty seconds followed by 300 rpm for thirty minutes. Plates were washed using 100 µl/well of PBNT in the BioPlex pro II wash station with the protocol MAG3. The beads in the well were then resuspended in 100 µl PBNT by mixing and analyzed on BioPlex machine. The reader was set to read a minimum of 100 beads of unique fluorescent signature/region and the results were expressed as median fluorescence intensity (MFI).

3.6 ELISA for IgG Subclasses Against CSP (NANP) 5 Peptide and Schizont Extract Protein.

Immunoglobulin G (IgG) subclass antibody levels was determined by an indirect enzyme-linked immunosorbent assay (ELISA) using plasma samples as described by Chelimo *et al.* (2005) and John *et al.* (2005). Circumsporozoite protein (CSP) peptide and schizont extract (an impure *P. falciparum* protein extract) were dissolved in 0.01M phosphate-buffered saline (PBS) to a concentration of 10 µg/ml. 50µl of antigen solution was added to Immulon-4 plates (Dynex Technologies, Chantilly, VA). Thereafter plates were incubated overnight at 4°C, washed with PBS 0.05% Tween 20, and blocked with

50 µl/well 3% (wt/vol) bovine serum albumin (BSA) in PBS. 50µl of plasma diluted at 1:100 in 1% BSA was added to the wells and incubated for 2 hours at room temperature. After washing with PBS 0.05% Tween 20, 50 µl of biotinylated secondary antibody (mouse antihuman IgG1, IgG2, IgG3 or IgG4) diluted 1:1000 in 1% BSA was added and incubated for 45 min at room temperature. The plates were then washed x3 in wash buffer. For each washing step, the plate filled with washing buffer was left undisturbed for 1 minute before it was emptied. 50µl /well of Streptavidin conjugated Alkaline Phosphatase diluted to 1: 2000 in diluent buffer was added to each well and incubated at room temperature for 30min. The plate was washed x3 with wash buffer, then x3 with 1x PBS only. 50µl/ well Alkaline Phosphatase substrate-developing solution was added and allowed to develop (15 – 20 min) in the dark. The plate was gently tapped to mix solution or turned on mixing feature in template settings of plate reader. Optical densities of the samples were read at 405 nm using a plate reader (Molecular Devices, Sunnyvale, CA). The conversion of absorbance values into AU was performed by Microsoft-Excel worksheet.

3.7 Statistical Analysis

For each of the 11-malaria antigens investigated sero-deconversion fraction was estimated as a function of age. Differences in the frequencies of paired samples of individuals with antibody subclasses to various *P. falciparum* antigens were evaluated using McNemar Chi-square test. To determine how malaria transmission interruption affected IgG subclass levels over time, antibody levels of paired samples were evaluated by Wilcoxon matched-pair signed rank test was used. Antibody subclass levels were

expressed in arbitrary units (AU), which were calculated by dividing the OD and MFI generated by the test sample by the mean OD and MFI plus 3 SD generated by samples from nine North Americans never exposed to malaria. Study participants with values >1.0 AU were considered positive responders. P values <0.05 were considered as statistically significant.

CHAPTER FOUR

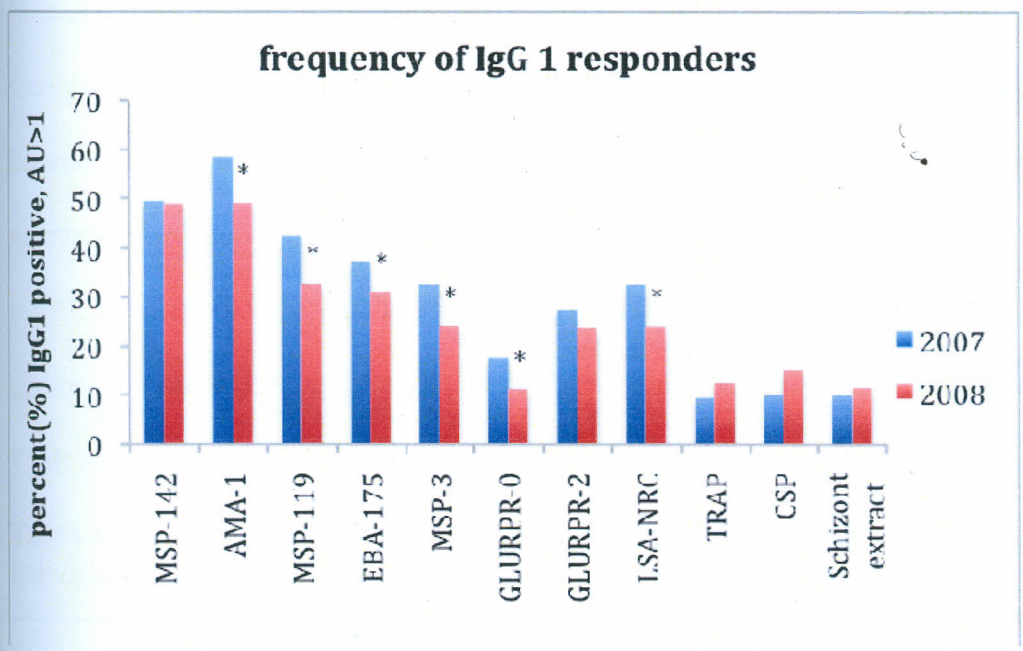
4.0 RESULTS

4.1 Changes in IgG Subclass Frequencies to Eleven *Plasmodium falciparum* Antigens During the Period of Malaria Transmission Interruption.

4.1.0 IgG 1 frequency

Immunoglobulin G1 (IgG1) antibodies to the eleven malaria antigens tested were present in 30% of the individuals tested in 2007 and 25% in 2008 except for TRAP, CSP and Schizont extract (Figure 4.1).

Frequencies of IgG 1 antibodies against AMA-1, MSP-1_{19}, EBA-175, MSP-3, GLURP-R0 and LSA-1 decreased significantly in Nandi district from 2007 to 2008 (Figure 4.1). Frequencies of IgG 1 antibodies in 2007 vs. 2008 decreased significantly for AMA-1 (58.5% vs. 49.1; p = 0.0253), MSP-1_{19} (42.5% vs. 32.7%; p= 0.0028), EBA-175 (37.4% vs. 31.0%; p= 0.0325), MSP-3 (32.7% vs. 24.1%; p= 0.0027), GLURPR-0 (17.6% vs. 11.2%; p= 0.0076), LSA-1 (32.7% vs. 24%; p= 0.0196). AMA-1 and MSP-1_{42} malaria antigens recorded the highest percentage of IgG1 positive responders (58.5% and 49.5%, respectively, in 2007, and 49.1% and 49%, respectively, in 2008) with P-values = 0.0253 and 0.7963, respectively (Figure 4.1). The frequencies of IgG1 positive responders over the 13 months to MSP-1_{42} and GLURPR-2 decreased though not significantly while IgG1 positive responders to TRAP, CSP and schizont extract increased over the same period of interruption though not significantly (Figure 4.1).



NB: * P< 0.05 (McNemar's Chi- square test)

Figure 4.1. IgG-1 antibody frequencies against malaria antigens from western Kenya highlands in 2007 and 2008.

Across all the malaria antigens tested, IgG1 antibody positive responders increased with age as shown in Table 4.1. In 2007 and 2008 an average of >70% of individuals of ≥ 17 years (age group 4) were IgG 1 positive responders to blood stage malaria antigens compared to <50% for the pre-erythrocytic malaria antigens as shown in Table 4.1. Among age group 1 (<4 years) MSP-3 had statistically significant reduction in the number of positive responders between 2007 and 2008 while the rest of the malaria antigens did not have significant decreases in IgG 1 frequencies. Group 2 (4≤ age< 8 years) did not register any statistically significant decrease in the number of positive responders across all the malaria antigens tested. Age group 3 (8≤ age <17 years) registered statistically significant decreases in IgG 1 frequencies between 2007 and 2008 against AMA-1, MSP-1_{19}, GLURP-R0 and LSA-1 as shown in Table 4.1.

In individuals below four years (age group 1), merozoite surface antigen -3 (MSP-3) recorded the highest percentage of positive responders (11.8%,) while in individuals between 4 and 8 years (age group 2) and 8 years to 17 years (age group 3) apical membrane antigen-1 (AMA-1) recorded the highest percentage of positive responders (34%). In adults 17 years and above (age group 4) AMA-1 and MSP-1_{42} had the highest number of positive responders (92% and 92%), respectively, in 2007 survey as shown in Table 4.1.

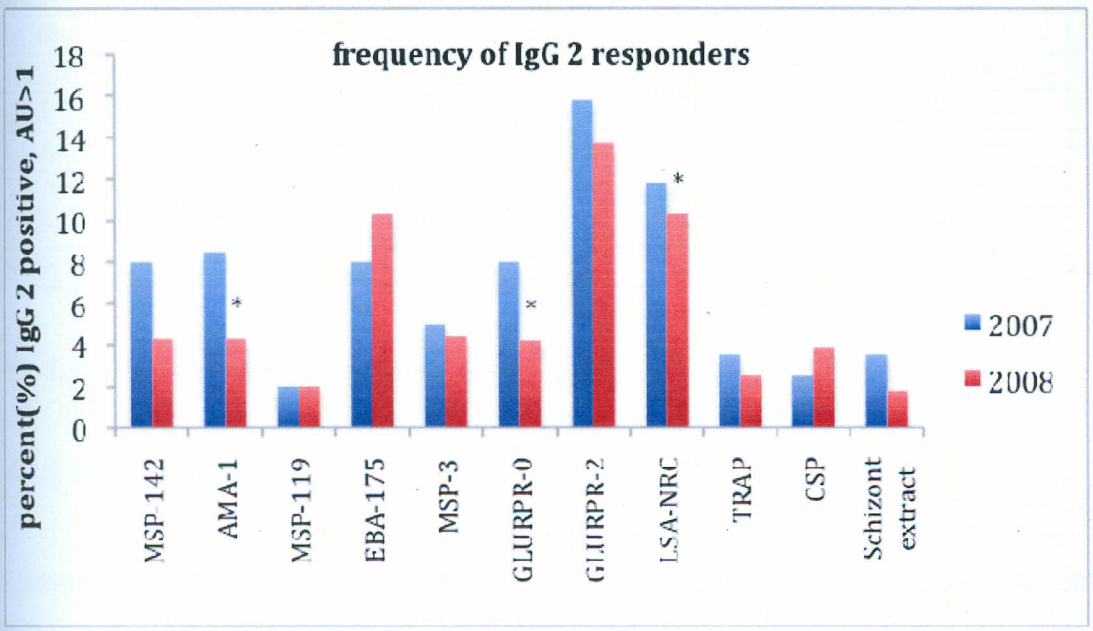
Table 4.1. IgG1 positive responders against malaria antigens over a 13-month period in individuals across ages.

	Percentage of positive responders (AU>1)							
IgG-1	< 4 years n = 51		4 ≤ age <8 years n=50		8 ≤ age < 17 years n=50		≥ 17 years n = 49	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	5.9%	6.5%	28.0%	16%	74.0%	65.5%	92.0%	96.0%
AMA-1	11.8%	3.2%	34.0%	21%	96.0%	*80.0%	92.0%	92.0%
MSP-1_{19}	7.8%	0.0%	16.0%	6.5%	62.0%	*45.5%	84.0%	78.9%
EBA-175	7.8%	0.0%	10.0%	3.2%	52.0%	40.0%	79.6%	80.8%
MSP-3	17.4%	*6.5%	8.0%	4.8%	36.0%	23.6%	69.0%	*61.5%
GLURP-R0	5.9%	0.0%	8.0%	3.2%	24.0%	*12.7%	32.6%	28.9%
GLURP-R2	2.0%	3.2%	10%	3.2%	38.0%	32.7%	59.0%	53.9%
LSA-1	7.8%	3.2%	18.0%	17.7%	50.0%	*30.9%	55.1%	*44.2%
TRAP	7.8%	6.5%	8.0%	17.7%	12.0%	10.9%	10.2%	15.4%
CSP	11.8%	16.1%	8.0%	12.9%	18.0%	16.4%	4.1%	15.4%
SCH	3.9%	9.7%	8.0%	8.1%	12.0%	12.7%	16.3%	15.4%

*P<0.05 (McNemar's Chi-square)

4.1.1 IgG 2 frequencies

Frequencies of IgG 2 positive responders generally decreased over the 13-month except for EBA-175 and CSP (Figure 4.2). Frequencies of IgG 2 antibodies in 2007 vs. 2008 decreased significantly for AMA-1 (8.5% vs. 4.3%; P = 0.0325), GLURPR-0 (8.0% vs. 4.2%; P= 0.0196), LSA-1 (11.8% vs. 10.3%; P= 0.0039). GLURPR-2 malaria antigen had the highest IgG 2 sero prevalence of 15.8% in 2007 and 13.7% in 2008 though there was no statistical difference over the period of malaria transmission interruption.



NB: * P< 0.05 (McNemar's Chi- square test)

Figure 4.2. IgG-2 antibody frequencies against malaria antigens from western Kenya highlands in 2007 and 2008.

Immunoglobulin G 2 (IgG2) sero-prevalence increased with age as shown in Table 4.2 with the highest frequencies in age group 4 of ≥17 years. For all the malaria antigens tested, the changes in the IgG 2 sero prevalence between 2007 and 2008 were not

significant. GLURPR-2 antigen had the highest sero prevalence of the antigens tested across all the age groups.

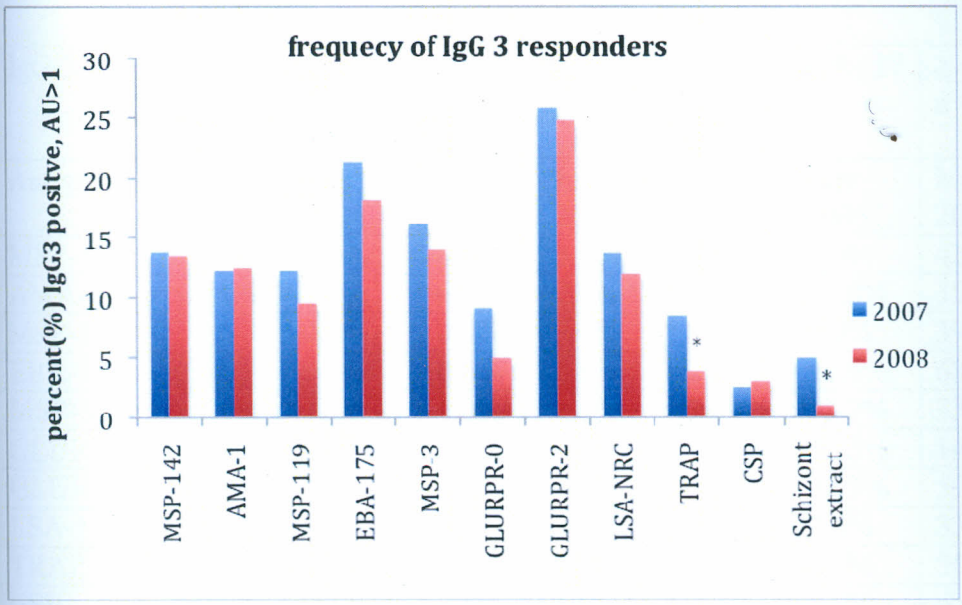
Table 4.2. IgG2 positive responders against malaria antigens over a 13-month period in individuals across ages.

	Percentage of positive responders (AU>1)							
IgG-2	< 4 years n = 51		4 ≤ age < 8 years n=50		8 ≤ age < 17 years n=50		≥ 17 years n = 49	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	4%	0.0%	4%	0.0%	4.0%	2.0%	20.0%	15.0%
AMA-1	4.0%	0.0%	0.0%	0.0%	12.0%	7.0%	18.0%	10.0%
MSP-1_{19}	0.0%	0.0%	2.0%	0.0%	2.0%	0.0%	4.0%	8.0%
EBA-175	2.0%	3.0%	4.0%	6.0%	6.0%	7.0%	20.0%	25.0%
MSP-3	0.0%	0.0%	0.0%	1.6%	4.0%	4.0%	16.0%	12%
GLURPR-0	3.9%	0.0%	4.0%	1.6%	12.0%	5.0%	12.0%	10.0%
GLURPR-2	4.0%	3.2%	8.0%	1.6%	20.0%	15.0%	31.0%	35.0%
LSA-1	2.0%	0.0%	2.0%	3.2%	16.0%	7.3%	23.0%	30.8%
TRAP	0.0%	0.0%	0.0%	2.0%	6.0%	0.0%	8.0%	8.0%
CSP	0.0%	6.0%	0.0%	6.0%	6.0%	4.0%	6.0%	2.0%
SCH	0.0%	0.0%	2.0%	0.0%	10.0%	5.0%	2.0%	2.0%

*P<0.05 (McNemar's Chi-square)

4.1.2 IgG 3 frequencies

Immunoglobulin G3 (IgG3) sero prevalence generally decreased for most antigens between 2007 and 2008 though not significantly. Frequencies of IgG 3 antibodies in 2007 vs. 2008 decreased significantly for TRAP (8.5% vs. 3.8%; P= 0.0126) and schizont extract (5.0% vs. 1%; P= 0.0047). On average, 12.8% of individuals in 2007 compared to 10.9% in 2008 were IgG 3 sero positive for all the malaria antigens tested (P= 0.3461) with GLURPR-2 having the highest number of positive 25% in 2007 and 24% in 2008 (Figure 4.3).



NB: * P< 0.05 (McNemar's Chi- square test)

Figure 4.3. IgG-3 antibody frequencies against malaria antigens from western Kenya highlands in 2007 and 2008.

Immunoglobulin G3 (IgG3) sero-prevalence increased with age as shown in Table 4.3 with the highest frequencies recorded in age group4 (≥17 years). EBA-175 and GLURPR-2 had highest frequencies in adult group with the decrease in frequencies between 2007 and 2008 not being statistically significant. The changes in sero prevalence for all antigens for age groups 1 and 2 over the 15 months were not statistically significant while in age group 3 (GLURPR-0 and schizont extract) and age group 4 (TRAP and schizont extract) IgG 3 frequency changes were statistically significant as shown in Table 4.3.

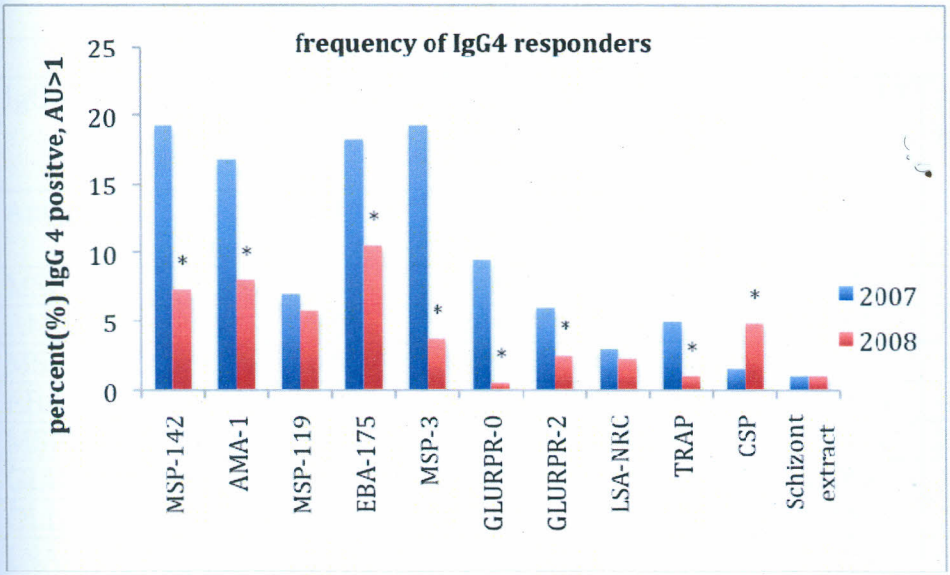
Table 4.3. IgG3 positive responders against malaria antigens over a 13-month period in individuals across ages.

	Percentage of positive responders (AU>1)							
IgG-3	< 4 years n = 51		4 ≤ age < 8 years n=50		8 ≤ age < 17 years n=50		≥ 17 years n = 49	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	0.0%	0.0%	0.0%	3.0%	12.0%	7.0%	43.0%	44.0%
AMA-1	0.0%	0.0%	0.0%	2.0%	14.0%	11.0%	35.0%	37.0%
MSP-1_{19}	0.0%	0.0%	0.0%	2.0%	10.0%	7.0%	39.0%	29.0%
EBA-175	2.0%	0.0%	0.0%	1.6%	20.0%	11.0%	63.3%	59.6%
MSP-3	3.9%	12.9%	4.0%	1.6%	24.0%	12.7%	33.0%	28.9%
GLURPR-0	3.9%	0.0%	0.0%	4.8%	18.0%	*7.3%	14.3%	7.7%
GLURPR-2	2.0%	3.2%	0.0%	1.6%	42.0%	32.7%	59.0%	61.5%
LSA-1	3.9%	6.5%	4.0%	3.2%	14.0%	7.3%	32.7%	30.8%
TRAP	2.0%	0.0%	4.0%	3.0%	8.0%	4.0%	20.0%	*8.0%
CSP	2.0%	0.0%	0.0%	6.0%	2.0%	6.0%	6.0%	2.0%
SCH	0.0%	0.0%	4.0%	0.0%	8.0%	*2.0%	8.0%	*2.0%

*P<0.05 (McNemar's Chi-square)

4.1.3 IgG 4 frequencies

Immunoglobulin G4 (IgG4) sero prevalence decreased significantly between 2007 and 2008 against most of the malaria antigens tested (Figure 4.4). Frequencies of IgG 4 antibodies in 2007 vs. 2008 decreased significantly for MSP-1_{42} (19.3% vs. 7.3%; P= 0.0001), AMA-1 (16.8% vs. 8%; P= 0.0006), EBA-175 (18.3% vs. 10.5%; P= 0.0002), MSP-3 (19.3% vs. 3.7%; P= 0.0001), GLURPR-0 (9.5% vs. 0.5%; P= 0.0001), GLURPR-2 (6% vs. 2.5%; P= 0.0082), TRAP (5% vs. 1%; P= 0.0114) and increased significantly for CSP (1.5% vs. 4.8%; P= 0.0348). IgG 4 frequencies against MSP-1_{19}; LSA-1 and schizont extract did not decrease significantly over the two time points (Figure 4.4).



NB: * P< 0.05 (McNemar's Chi- square test)

Figure 4.4. IgG-4 antibody frequencies against malaria antigens from western Kenya highlands in 2007 and 2008.

There was a general increase in IgG 4 sero prevalence against all the tested malaria antigens with age as shown in Table 4.4. IgG 4 frequencies reduced for all malaria antigens over the two time points across the four age groups with age group 4 having the highest number of antigens where IgG 4 positive responders decreased significantly. EBA-175 antigens had the highest IgG 4 sero prevalence in age group 4 (49.0%) as shown in Table 4.4.

Table 4.4. IgG4 positive responders against malaria antigens over a 15-month period in individuals across ages.

	Percent positive (AU>1)							
IgG-4	< 4 years n = 51		4 ≤ age < 8 years n=50		8 ≤ age < 17 years n=50		≥ 17 years n = 49	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	2.0%	0.0%	10.0%	3.0%	26.0%	*7.3%	39.0%	*19.0%
AMA-1	0.0%	0.0%	8.0%	2.0%	20.0%	7.0%	39.0%	*23.0%
MSP-1_{19}	0.0%	0.0%	4.0%	5.0%	8.0%	4.0%	16.0%	14.0%
EBA-175	0.0%	0.0%	4.0%	0.0%	20.0%	13.0%	49.0%	*29.0%
MSP-3	14.0%	*3.0%	12.0%	1.6%	24.0%	*2.0%	27.0%	*8.0%
GLURPR-0	6.0%	0.0%	6.0%	0.0%	8.0%	0.0%	18%	*2.0%
GLURPR-2	0.0%	0.0%	0.0%	0.0%	4.0%	0.0%	20.0%	*10.0%
LSA-1	2.0%	0.0%	2.0%	3.2%	2.0%	0.0%	6.0%	6.0%
TRAP	6.0%	0.0%	4.0%	0.0%	0.0%	0.0%	10.0%	4.0%
CSP	0.0%	3.0%	0.0%	6.0%	0.0%	2.0%	6.0%	8.0%
SCH	0.0%	0.0%	0.0%	2.0%	2.0%	0.0%	2.0%	2.0%

*P<0.05 (McNemar's Chi-square)

4.2 Changes in IgG Subclass Antibody Levels Against Malaria Vaccine Candidate Antigens During the Period of Malaria Transmission Interruption.

4.2.0 IgG 1 levels

Across the two time points, IgG-1 antibody levels changed significantly ($P < 0.0001$) against ten of the malaria antigens tested except TRAP as shown in Table 4.5a and Table 4.5b. AMA-1 recorded the highest levels with a median IgG 1 antibody level of AU=1.62 as shown in Table 4.5a.

Table 4.5a. Antibody levels in AU (median and 25th and 75th percentile) for the year 2007 and 2008 against malaria antigens in Nandi district, western Kenya highlands.

	IgG-1		IgG-2		IgG-3		IgG-4	
Antigen	**May 2007**	**July 2008**	**May 2007**	**July 2008**	**May 2007**	**July 2008**	**May 2007**	**July 2008**
MSP-1_{42}	1.0(0.6 -21.8)	0.9(0.6 -15.0)*	0.6(0.6 -0.7)	0.7(0.6 -0.7)*	0.6(0.6 -0.8)	0.7(0.6 -.0.9)	0.8(0.6 -0.9)	0.6(0.6 -0.8)*
AMA-1	1.6(0.8 -51.1)	1.2(0.7 -32.5)*	0.7(0.6 -0.8)	0.6(0.6 -0.8)*	0.6(0.5 -0.7)	0.5(0.5 -0.7)	0.8(0.6 -1.0)	0.7(0.6 -0.7)*
MSP-1_{19}	0.9(0.7 -2.8)	0.8(0.7 -2.0)*	0.6(0.6 -0.7)	0.7(0.7 -0.8)*	0.7(0.7 -0.8)	0.7(0.7 -0.9)*	0.7(0.6 -0.8)	0.7(0.6 -0.7)*
EBA-175	0.8(0.7 -16.1)	0.8(0.7 -11.8)*	0.8(0.2 -0.6)	0.6(0.5 -0.7)*	0.7(0.6 -0.8)	0.7(0.6 -0.9)	0.8(0.7 -1.0)	0.6(0.6 -0.7)*
MSP-3	0.7(0.6 -1.2)	0.7(0.6 -1.0)*	0.7(0.5 -0.7)	0.6(0.5 -0.7)*	0.7(0.6 -0.8)	0.7(0.6 -0.8)*	0.8(0.6 -1.0)	0.8(0.6 -0.8)*
GLURPR -0	0.8(0.6 -0.8)	0.8(0.6 -0.8)*	0.6(0.6 -0.6)	0.6(0.6 -7)	0.7(0.7 -0.9)	0.6(0.6 -0.7)*	0.7(0.6 -1.0)	0.7(0.5 -0.7)*
GLURPR -2	0.8(0.5 -1.3)	0.7(0.5 -1.0)*	0.6(0.5 -0.8)	0.6(0.5 -0.8)*	0.7(0.5 -1.2)	0.7(0.6 -1.1)	0.6(0.5 -0.8)	0.6(0.5 -7)*
LSA-NRC	0.8(0.8 -1.1)	0.9(0.8 -1.0)*	0.8(0.8 -0.9)	0.8(0.7 -0.8)*	0.8(0.7 -0.9)	0.8(0.7 -0.9)	0.8(0.7 -0.9)	0.8(0.7 -0.8)*
TRAP	0.7(0.6 -0.8)	0.7(0.6 -0.8)	0.7(0.7 -0.8)	0.8(0.7 -8)*	0.7(0.7 -0.8)	0.7(0.6 -0.7)*	0.7(0.7 -0.9)	0.7(0.7 -0.8)

NB: * P< 0.05 (Wilcoxon matched-pair signed rank test). Results generated by cytometric bead assay (CBA)

Table 4.5b. Antibody levels in AU (median and 25th and 75th percentile) for the year 2007 and 2008 against malaria antigens in Nandi district, western Kenya highlands.

	IgG-1		IgG-2		IgG-3		IgG-4	
Antigen	**May 2007**	**July 2008**	**May 2007**	**July 2008**	**May 2007**	**July 2008**	**May 2007**	**July 2008**
CSP	0.8(0.6-1.0)	0.9(0.8-1.1)*	0.6(0.5-0.7)	0.7(0.6-0.7)*	0.6(0.6-0.8)	0.8(0.7-0.9)*	0.6(0.5-0.6)	0.7(0.6-0.8)*
SCH	1.0(0.9-1.2)	1.1(0.9-1.3)*	0.8(0.7-1.0)	0.7(0.6-0.9)	0.9(0.8-1.0)	0.8(0.7-0.9)*	0.6(0.2-0.7)	0.7(0.6-0.8)*

NB: * P< 0.05 (Wilcoxon matched-pair signed rank test). Results generated from Enzyme linked immunosobent assay (ELISA)

IgG1 antibody levels increased across the age groups as shown in Table 4.6. Age group 3 (8≤ age <17 years) recorded the highest number malaria antigens (MSP-1_{42}, AMA-1, MSP-1_{19}, EBA-175, MSP-3, GLUPRP-R0, GLUPRP-R2, and LSA-1) where IgG1 antibody levels decreased significantly due to malaria transmission interruption (May 2007 and July 2008) followed by age group 4 (≥ 17 years) (AMA-1, MSP-1_{19}, EBA-175, MSP-3, GLUPRP-0, GLUPRP-2, and LSA-NRC) as shown Table 4.6. Age groups 1 (< 4 years) and 2 (4 ≤age <8 years) had the least number of malaria antigens (AMA-1, EBA-175, GLUPRP-0, schizont extract and AMA-1, EBA-175, GLUPRP-0, respectively) where the decrease in IgG1 levels was statistically significant over the 15 months. IgG 1 levels against AMA-1 decreased significantly between 2007 and 2008 across all the age groups compared to the rest of the malaria antigens tested. TRAP is the only malaria antigen that did not record statistically significant changes in the IgG1 levels across all the four age groups between 2007 and 2008.

Table 4.6. IgG 1-antibody levels in AU (median and 25th and 75th percentile) for the year 2007 and 2008 for the antigens in Nandi district according to age.

IgG-1	**< 4 years n = 51**		**4 ≤ age < 8 years n=50**		**8 ≤ age < 17 years n=50**		**≥ 17 years n = 49**	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	0.7(0.6-0.8)	0.6(0.6-0.6)	0.7(0.6-1.3)	0.6(0.6-0.8)	7.4(0.9-32.4)	1.9(0.7-20.1)*	23.9(6.7-145.0)	30.7(6.0-119.2)
AMA-1	0.8(0.6-0.8)	0.7(0.7-0.8)*	0.8(0.7-2.7)	0.8(0.7-0.9)*	18.0(2.8-116.9)	8.6(1.2-57.8)*	69.5(11.6-193.8)	72.7(9.5-185.7)*
MSP-1_{19}	0.7(0.6-0.8)	0.7(0.7-0.7)	0.7(0.6-0.9)	0.7(0.7-0.8)	1.4(0.8-4.8)	0.9(0.7-3.3)*	3.7(1.2-29.3)	4.0(1.2-20.5)*
EBA-175	0.7(0.6-0.8)	0.7(0.6-0.7)*	0.7(0.6-0.8)	0.7(0.7-0.8)	1.4(0.7-53.4)	0.8(0.7-15.2)*	97.0(5.2-197.3)	68.4(4.6-174.3)*
MSP-3	0.6(0.6-0.9)	0.7(0.5-0.7)	0.6(0.5-0.7)	0.7(0.5-0.7)	0.7(0.6-1.9)	0.8(0.5-1.0)*	2.1(0.8-6.8)	1.6(0.7-4.5)*
GLURP R-0	0.6(0.6-0.8)	0.6(0.6.-0.7)*	0.6(0.6-0.8)	0.7(0.6-0.8)	0.8(0.6-0.8)	0.7(0.6-0.9)*	0.8(0.6-1.4)	0.8(0.7-1.1)*
GLURP R-2	0.7(0.5-0.8)	0.5(0.5-0.7)	0.7(0.5-0.8)	0.6(0.5-0.7)	0.8(0.6-2.7)	0.7(0.5-2.0)*	2.2(0.7-5.4)	1.4(0.7-1.3)*
LSA-1	0.8(0.7-0.8)	0.8(0.7-0.8)	0.8(0.7-0.9)	0.8(0.7-0.9)	1.0(0.8-1.9)	0.9(0.8-1.3)*	1.0(0.8-1.8)	1.0(0.8-1.8)*
TRAP	0.7(0.6-0.7)	0.7(0.6-1.6)	0.7(0.6-0.8)	0.7(0.6-2.2)	0.7(0.6-0.8)	0.7(0.5-2.9)	0.7(0.7-0.9)	0.8(0.6-3.5)
CSP	1.0(0.6-1.2)	0.9(0.8-1.7)	0.8(0.6-1.0)	0.9(0.9-1.2)*	0.8(0.6-1.0)	0.9(0.8-1.1)	0.8(0.6-0.9)	0.9(0.8-1.0)
SCH	1.0(0.8-1.0)	1.4(1.1-1.5)*	1.0(0.9-1.0)	1.0(0.9-1.2)	1.0(0.8-1.3)	1.1(0.8-1.2)	1.2(1.0-1.2)	1.1(1.0-1.3)

*P<0.05 (Wilcoxon matched-pair signed rank test

4.2.1 IgG 2 levels

Immunoglobulin G2 (IgG2) levels changed significantly across nine of the malaria antigens tested over the two time points. MSP-1_{42}, MSP-1_{19}, TRAP and CSP antigens recorded significantly increased IgG2 levels over the period of transmission interruption

and GLURPR-0 and schizont extract did not change significantly as shown in Table 4.5a and 4.5b.

Across the age groups, IgG2 antibody levels generally remained the same as shown in Table 4.7. IgG 2 levels against MSP-3, GLURPR-0, CSP, and schizont extract malaria antigens did not change significantly over the two time points across the ages as shown in Table 4.7.

Table 4.7. IgG 2-antibody levels in AU (median and 25th and 75th percentile range) for the year 2007 and 2008 for the antigens in Nandi district according to age.

IgG-2	**< 4 years n = 51**		**4 ≤ age < 8 years n=50**		**8 ≤ age < 17 years n=50**		**≥ 17 years n = 49**	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	0.6(0.6 -0.7)	0.7(0.6 -0.7)	0.6(0.5 -0.7)	0.7(0.6 -0.7)	0.7(0.6 -0.8)	0.7(0.6 -0.7)	0.7(0.6 -0.8)	0.7(0.7 -0.9)*
AMA-1	0.7(0.6 -0.8)	0.6(0.6 -0.6)*	0.7(0.6 -0.8)	0.6(0.6 -0.7)*	0.7(0.6 -0.8)	0.6(0.6 -0.8)*	0.8(0.6 -0.9)	0.7(0.6 -0.8)*
MSP-1_{19}	0.6(0.6 -0.7)	0.7(0.6 -0.8)	0.6(0.6 -0.7)	0.7(0.7 -0.8)*	0.7(0.6 -0.7)	0.7(0.7 -0.8)	0.7(0.6 -0.8)	0.7(0.7 -0.8)
EBA-175	0.5(0.2 -0.6)	0.5(0.5 -0.6)	0.5(0.2 -0.5)	0.6(0.5 -0.6)*	0.5(0.2 -0.6)	0.6(0.5 -0.7)*	0.6(0.3 -0.8)	0.7(0.6 -1.0)*
MSP-3	0.6(0.6 -0.7)	0.6(0.5 -0.7)	0.6(0.5 -0.7)	0.6(0.5 -0.7)	0.7(0.5 -0.7)	0.6(0.5 -0.7)	0.7(0.7 -27.6)	0.7(0.6 -0.7)
GLURPR -0	0.6(0.6 -0.6)	0.6(0.6 -0.7)	0.6(0.6 -0.6)	0.6(0.6 -0.7)	0.6(0.6 -0.8)	0.6(0.6 -0.7)	0.6(0.6 -0.8)	0.6(0.6 -0.7)
GLURPR -2	0.6(0.5 -0.7)	0.5(0.5 -0.6)*	0.6(0.5 -0.8)	0.5(0.5 -0.6)	0.7(0.5 -0.9)	0.7(0.6 -0.9)	0.8(0.5 -1.5)	0.8(0.5 -1.6)
LSA-1	0.8(0.7 -0.8)	0.8(0.7 -0.8)	0.8(0.8 -0.8)	0.8(0.7 -0.8)	0.8(0.8 -1.0)	0.8(0.7 -0.8)	0.8(0.8 -1.0)	0.8(0.8 -0.9)
TRAP	0.7(0.7 -0.8)	0.7(0.6 -0.8)	0.7(0.7 -0.8)	0.8(0.7 -0.8)	0.7(0.7 -0.8)	0.7(0.6 -0.8)*	0.7(0.7 -0.8)	0.8(0.7 -0.8)
CSP	0.6(0.5 -0.8)	0.6(0.5 -0.9)	0.6(0.5 -0.7)	0.6(0.5 -0.7)	0.6(0.5 -0.8)	0.6(0.6 -0.7)	0.6(0.5 -0.7)	0.7(0.6 -0.8)
SCH	0.7(0.6 -0.7)	0.6(0.6 -0.7)	0.8(0.7 -0.8)	0.6(0.5 -0.8)	1.0(0.7 -1.1)	0.7(0.5 -1.2)	0.9(0.7 -1.0)	0.8(0.7 -1.0)

*P<0.05 (Wilcoxon matched-pair signed rank test).

4.2.2 IgG3 levels

Levels of IgG3 antibodies in 2007 vs. 2008 changed significantly for AMA-1 (P= 0.0001, MSP-1_{19} EKNG (P= 0.0010), MSP-3 (P= 0.0006), GLURPR-0 (P= 0.0001, TRAP (P= 0.0001), CSP (P= 0.0035) and schizont extract (P= 0.0035) as shown Table 4.5. IgG 3 antibody levels increased with age where Individuals of ≥17 years (age group 4) had the highest antibody levels as shown in Table 4.8. IgG 3 antibody levels against EBA-175 and LSA-NRC did not change significantly across all the age groups as shown in Table 4.8.

Table 4.8. IgG 3-antibody levels in AU (median and 25th and 75th percentile range) for the year 2007 and 2008 for the antigens in Nandi district according to age.

IgG-3	**< 4 years n = 51**		**4 ≤ age < 8 years n=50**		**8 ≤ age < 17 years n=50**		**≥ 17 years n = 49**	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	0.6(0.5-0.7)	0.6(0.6-0.7)	0.6(0.6-0.7)	0.6(0.6-0.7)	0.7(0.6-0.8)	0.6(0.6-0.8)*	1.0(0.6-1.7)	1.0(0.7-1.4)
AMA-1	0.5(0.4-0.6)	0.5(0.5-0.5)*	0.5(0.5-0.6)	0.5(0.5-0.6)*	0.7(0.5-0.8)	0.6(0.5-0.7)*	0.7(0.5-1.5)	0.7(0.5-1.6)*
MSP-1_{19}	0.7(0.7-0.7)	0.7(0.7-0.7)	0.7(0.7-0.7)	0.7(0.7-0.8)*	0.7(0.7-0.9)	0.7(0.7-0.9)	0.7(0.7-1.2)	0.9(0.7-1.1)
EBA-175	0.7(0.6-0.7)	0.6(0.6-0.7)	0.7(0.6-0.7)	0.6(0.6-0.7)	0.7(0.7-0.9)	0.7(0.6-0.8)	1.4(0.8-9.9)	1.2(0.7-8.6)
MSP-3	0.6(0.6-0.7)	0.7(0.6-0.8)	0.6(0.6-0.8)	0.6(0.6-0.8)*	0.8(0.6-1.0)	0.7(0.6-0.8)*	0.8(0.6-1.2)	0.8(0.7-1.0)*
GLURPR-0	0.7(0.7-0.8)	0.6(0.6-0.7)*	0.7(0.7-0.9)	0.6(0.6-0.7)*	0.7(0.7-0.9)	0.6(0.6-0.8)*	0.7(0.7-1.0)	0.6(0.6-0.8)*
GLURPR-2	0.5(0.5-0.7)	0.6(0.5-0.7)	0.5(0.5-0.7)	0.6(0.5-0.7)*	0.8(0.5-8.2)	0.7(0.6-2.1)*	3.4(0.8-26.1)	1.9(0.7-19.5)*
LSA-1	0.7(0.7-0.8)	0.8(0.7-0.8)	0.7(0.7-0.8)	0.8(0.7-0.9)	0.8(0.7-0.9)	0.8(0.7-0.8)	0.9(0.8-1.1)	0.9(0.8-1.1)
TRAP	0.7(0.6-0.8)	0.7(0.6-0.7)*	0.7(0.6-0.8)	0.7(0.6-0.7)	0.8(0.7-0.8)	0.7(0.6-0.7)*	0.8(0.7-1.0)	0.7(0.6-0.8)*
CSP	0.7(0.6-0.9)	0.8(0.7-0.8)	0.6(0.6-0.7)	0.8(0.7-0.9)*	0.7(0.6-0.9)	0.8(0.7-0.9)	0.6(0.6-0.8)	0.8(0.7-0.8)

SCH	0.7(0.6-0.9)	0.8(0.7-0.9)	0.9(0.8-1.0)	0.7(0.6-0.9)	0.9(0.8-1.0)	0.8(0.7-0.9)*	1.0(0.9-1.1)	0.8(0.7-0.9)

*P<0.05 (Wilcoxon matched-pair signed rank test).

4.2.3 IgG 4 levels

IgG 4 levels changed significantly across 10 of the 11 tested malaria antigens over the two time points. MSP-1_{42} and AMA-1 had the highest median levels of IgG 4 as shown in Table 4.5a and b. Apart from CSP and schizont extract that recorded an increase in IgG4 levels during interruption, the rest of the tested antigens recorded a decrease in IgG 4 between 2007 and 2008. Generally the IgG 4 levels did not change across the ages as shown in Table 4.9.

Table 4.9. IgG 4-antibody levels in AU (median and 25th and 75th percentile range) for the year 2007 and 2008 for the antigens in Nandi district according to age.

IgG-4	**< 4 years n = 51**		**4 ≤ age < 8 years n=50**		**8 ≤ age < 17 years n=50**		**≥ 17 years n = 49**	
Antigen	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008	May 2007	July 2008
MSP-1_{42}	0.7(0.6-0.9)	0.6(0.6-0.6)*	0.7(0.6-0.9)	0.6(0.6-0.8)	0.8(0.6-1.1)	0.6(0.6-0.8)*	0.9(0.6-1.5)	0.6(0.6-1.0)*
AMA-1	0.7(0.6-0.9)	0.7(0.6-0.7)*	0.7(0.5-0.9)	0.7(0.6-0.7)*	0.9(0.6-1.0)	0.7(0.6-0.7)*	1.0(0.8-1.3)	0.8(0.7-1.0)*
MSP-1_{19}	0.7(0.6-0.8)	0.7(0.6-0.8)	0.7(0.6-0.8)	0.7(0.6-0.7)	0.7(0.6-0.8)	0.7(0.6-0.7)*	0.8(0.7-1.0)	0.7(0.6-0.8)*
EBA-175	0.7(0.7-0.9)	0.6(0.6-0.6)*	0.7(0.6-0.8)	0.6(0.6-0.7)	0.8(0.7-1.0)	0.6(0.6-0.7)*	1.0(0.9-1.7)	0.7(0.6-1.0)*
MSP-3	0.8(0.6-1.0)	0.8(0.6-0.8)*	0.6(0.6-1.0)	0.7(0.6-0.8)	0.8(0.6-1.0)	0.7(0.6-0.8)*	0.8(0.6-1.0)	0.8(0.6-0.8)*
GLURPR-0	0.7(0.6-1.0)	0.7(0.5-0.7)*	0.7(0.6-0.9)	0.6(0.5-0.7)*	0.7(0.6-1.0)	0.6(0.5-0.7)*	0.8(0.7-1.0)	0.7(0.6-0.7)*
GLURPR-2	0.6(0.5-0.8)	0.5(0.5-0.7)*	0.6(0.5-0.7)	0.5(0.5-0.7)	0.6(0.5-0.8)	0.6(0.5-0.7)	0.7(0.5-1.0)	0.7(0.5-0.7)*
LSA-1	0.8(0.7-0.9)	0.8(0.7-0.8)*	0.8(0.7-0.9)	0.7(0.7-0.8)	0.8(0.7-0.9)	0.7(0.7-0.8)*	0.8(0.7-0.9)	0.8(0.7-0.8)*

TRAP	0.7(0.7 -0.9)	0.8(0.7 -0.8)	0.7(0.7 -0.8)	0.7(0.7 -0.8)	0.7(0.6 -0.9)	0.7(0.7 -0.8)	0.8(0.7 -0.9)	0.7(0.7 -0.8)
CSP	0.5(0.4 -0.6)	0.7(0.6 -0.8)*	0.6(0.5 -0.7)	0.7(0.6 -0.9)*	0.6(0.5 -0.6)	0.7(0.6 -0.7)*	0.6(0.5 -0.7)	0.7(0.6 -0.9)*
SCH	0.5(0.2 -0.5)	0.7(0.6 -0.8)*	0.4(0.2 -0.7)	0.6(0.6 -0.7)	0.6(0.6 -0.7)	0.7(0.6 -0.8)	0.6(0.5 -0.7)	0.7(0.6 -0.8)

*P<0.05 (Wilcoxon matched-pair signed rank test)

CHAPTER FIVE

5.0 DISCUSSIONS

This study has used a panel of immunologically well-characterized recombinant proteins and peptides to measure naturally acquired IgG subclass antibody responses to *P. falciparum* antigens in asymptomatic individuals living in Nandi district, a highland area in western Kenya where malaria incidence was recently reported as interrupted (John *et al.*, 2009). This study illustrates the importance of identifying malaria antigens that induce long lasting antibodies when transmission is absent. Determining the dynamics of antibody responses to various malaria antigens during periods of very low/absent malaria transmission is critical in vaccine studies, since such studies are likely to distinguish between antibody responses that are long lasting and those that are short lived. These would provide knowledge on longevity of antibody responses of the suitable vaccine candidates and when these responses need to be initiated and when best to administer boosting responses. Understanding these correlates of immunity is critical before vaccine trials are initiated in areas of unstable seasonal malaria transmission (Moorman *et al.*, 2006).

The results of the present study suggest that low to absent malaria transmission and age of the individual are the main factors influencing the levels and frequencies of IgG subclass antibodies to malaria vaccine candidate antigens tested. This study reported that there was a general decrease in frequencies and levels of both cytophilic and non-cytophilic antibodies to most of the malaria antigens tested during the period of low to absent malaria transmission (May 2007 - July 2008). The frequencies and levels of cytophilic antibodies (IgG1; AMA-1FVO, MSP-1_{19} EKNG, EBA-175, MSP-3 FVO,

GLURP-R0, LSA-1 and IgG3; TRAP and heterogeneous schizont crude protein extract) and non-cytophilic antibodies (IgG2; AMA-1 FVO, LSA-1 and IgG4; $MSP1_{42}$ FVO, AMA-1 FVO, EBA-175, MSP-3FVO, GLURP-R0, GLURP-R2, CSP) decreased significantly between May 2007 and July 2008 in an area with unstable seasonal malaria transmission. This probably suggests that the these malaria antigens do not induce long term antibodies in this population hence not as immunogenic enough to be developed as malaria vaccines for areas that experience unstable and low malaria transmission. This trend of loss of antibodies due to low to absent malaria transmission has been noted by other studies suggesting that immunity against malaria in unstable malaria transmission areas is short lived (Wipasa *et al.*, 2010). This fast rate of decline in the antibodies especially the cytophilic antibodies has implications on the vaccine trials in areas that experience unstable malaria since these cytophilic antibodies have been implicated in protection against parasitemia and clinical malaria (Wipasa *et al.*, 2002; Groux *et al* ., 1990).

The data presented here also shows that blood stage antigens induced better immune responses across the IgG subclasses compared to the pre-erythrocytic antigens. This finding is important in light of the fact that antibodies to these antigens have been associated with protection against clinical disease (Gray *et al.*, 2007). The data presented here further shows that IgG3 antibody frequencies to all malaria vaccine antigens tested except TRAP and schizont extract did not decrease significantly suggesting that although IgG3 levels and frequencies were lower than IgG1 levels, IgG3 seems to be a more stable cytophilic antibody. The data also shows that blood stage antigens especially $MSP\text{-}1_{42}$ and AMA-1 for IgG1, GLURP-R2 for IgG2, GLURP-R2 for IgG3 and MSP-3 for IgG4

recorded the highest mean levels and frequencies suggesting that they are the most immunogenic malaria antigens in the area. This is probably because this highland area of Kenya is prone to unpredictable epidemics of malaria due to unstable low malaria transmission throughout the year which has led the population to select for blood stage malaria antigens better since antibodies against blood stage antigens have been shown to be protective against clinical malaria (Gray *et al.*, 2007).

Importantly, this data suggests that IgG1 antibodies are the most frequent against malaria antigens followed by IgG3 antibodies in the population, which is in agreement with other studies that show that cytophilic antibodies are implicated in protection against clinical malaria in malaria prone areas (Soe *et al.*, 2004). The results also revealed that IgG antibody frequencies and levels to CSP increased though not significantly at the end of the low to absent malaria transmission period. This may be associated with recent infection as suggested by other studies (Wipasa *et al.*, 2010; Cobelens *et al.*, 1998; Cobelens *et al.*, 1998).

Furthermore, the data suggests that children under the age of four years had higher IgG1 responses to CSP compared to infants (4≤ age <8 years), teens (8≤ age <17 years) and adults (≥17 years), suggesting that CSP antigen may be eliciting immune responses earliest in children below four years in areas with unstable malaria transmission. Clinical trials of *P. falciparum* CSP-based vaccines have shown that it induces both humoral and cellular immune responses (Hutchings *et al.*, 2007) and as the most advanced malaria vaccine candidate, it has entered phase III clinical trial (Casares *et al.*, 2010). The

generation of relevant epidemiologic and immunologic data for CSP is of crucial significance.

Antibody frequencies and levels against most malaria antigens increased with age for the cytophilic antibodies and not non-cytophilic antibodies. This suggests that IgG2 and IgG4 antibodies do not change significantly as one grows in this population. The data further suggests that IgG1 antibodies were acquired earliest in life than IgG3, IgG2 or IgG4. This is probably because IgG1 is an important subset of antibodies that naturally occurs in higher proportions and is also important in protection against infections including malaria especially in malaria endemic areas like Nandi district. This age wise increase in especially cytophilic antibody levels and frequencies to both blood stage and pre-erythrocytic malaria antigens suggests that exposure to repeated infections is needed to attain a level of protection from clinical malaria which is in agreement with other findings (Roussilhon *et al.*, 2007; John *et al.*, 2005; John *et al.*, 2004).

A limitation of our findings is that the type of study design we adopted did not have the capability of individual longitudinal follow-up for a prolonged period to determine whether the pattern of antibodies decreases to what levels. Also, the study population was majorly monoethnic (Kalenjin - Nandi sub tribe), and this population may have restricted MHC alleles frequencies and distributions significantly different from those of mixed populations (John et al., 2009). Further, the study used a CSP peptide which is known to have restricted number of epitopes as compared to using a CSP recombinant antigen which has T cell epitopes (Rénia *et al.*, 1993), so it is likely that whatever antibody

responses we got from the CSP peptide are likely to be of a lower magnitude and frequency compared to responses when a recombinant antigen would be used.

CHAPTER SIX

6.0 CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHUR RESEARCH

6.1. Conclusions

This study reports that:

i) The 13 months of interruption in malaria transmission from average 5.9% to 14.5% pre intervention to <0.3% post intervention led to significant changes in IgG subclass responses to the selected malaria antigens tested. IgG 1 frequencies and levels to IgG1 (AMA-1FVO, MSP-1_{19} EKNG, EBA-175, MSP-3 FVO, GLURP-R0, and LSA-1), IgG3 (TRAP and heterogeneous schizont crude protein extract), IgG2 (AMA-1FVO and LSA-1) and IgG4 (MSP1_{42} FVO, AMA-1 FVO, EBA-175, MSP-3FVO, GLURP-R0, GLURP-R2, CSP) decrease significantly in unstable, seasonal areas during periods of low to absent malaria transmission.

IgG subclass frequencies remained stable after transmission interruption for IgG1 to MSP-1_{42} and GLURP-R2; IgG2 to MSP-1_{19}EKNG, MSP-3 and GLURP-R2; IgG3 to MSP-1_{42}, AMA-1, GLURP-R2 and LSA-1; and IgG4 to Schizont extract and LSA-1.

Antibody frequencies against some malaria antigens increased 13 months after intervention, i.e., IgG1 frequencies against TRAP, CSP and schizont extract, IgG2 frequencies against EBA-175 and CSP, IgG3 frequencies against AMA-1 and CSP, and IgG4 frequencies against CSP.

ii) Immunoglobulin G subclass levels to IgG1 (AMA-1FVO, MSP-1_{19} EKNG, EBA-175, MSP-3 FVO, GLURP-R0, and LSA-1); IgG3 (TRAP and heterogeneous schizont crude protein extract), IgG2 (AMA-1FVO and LSA-1) and IgG4 ($MSP1_{42}$ FVO, AMA-1 FVO, EBA-175, MSP-3FVO, GLURP-R0, GLURP-R2, CSP) decreased significantly during the period of malaria transmission interruption.

iii) The frequency of IgG1 antibodies against all the tested malaria vaccine candidate antigens increased with age from as low as 2% prevalence at an age of <4 years to a prevalence of 92% at an age of >17 years. Whereas in the case of IgG3, the study revealed that for some malaria antigens the first sero-response occurred at about 8 years and increased to about 60% prevalence in adults.

The study showed that merozoite surface antigen-3 (MSP-3) had the highest number of IgG1 sero-responses at ages below 4 years, 17.4%, followed by AMA-1 and CSP at 11.8%, respectively. The study further showed that IgG1 sero-responses continued to increase as age increased for most malaria antigens tested except for CSP whereas in adults, the sero-prevalence of IgG1 reduced to about 4% from 18% in teenagers. Also, the study revealed that adults had an IgG1 sero-prevalence greater that 80% against blood stage malaria antigens compared to a less than 50% IgG3 sero-prevalence against the same.

6.2. Recommendations

In developing malaria vaccines and conducting malaria research in areas with seasonal unstable malaria transmissions, MSP-1_{42}, GLURP-R2, MSP-1_{19}EKNG, MSP-3, AMA-1, LSA-1, CSP and schizont extract malaria antigens should be considered as malaria vaccine candidates since the present study shows that these malaria antigens have the highest IgG subclass positive responses levels and IgG subclass levels, and responses are elicited early in life.

6.3. Suggestions for Further Research

Future studies should be designed for longer period of follow-up to be able to assess better the impact of low to absent malaria transmission to immune correlates and natural acquisition of antibody responses in unstable seasonal transmission areas as this will generate more accurate information needed by the malaria research community and the malaria vaccine development program.

REFERENCES

Abbas, A. K., Murphy, K. M., & Sher, A. (1996). Functional diversity of helper T lymphocytes. *Nature, 383*(6603), 787–793. doi:10.1038/383787a0

Aidoo, M., Lalvani, A., Gilbert, S. C., Hu, J. T., Daubersies, P., Hurt, N., ... Hill, A. V. (2000). Cytotoxic T-lymphocyte epitopes for HLA-B53 and other HLA types in the malaria vaccine candidate liver-stage antigen 3. *Infection and Immunity, 68*(1), 227–232.

Anders, R. F., Crewther, P. E., Edwards, S., Margetts, M., Matthew, M. L., Pollock, B., & Pye, D. (1998). Immunisation with recombinant AMA-1 protects mice against infection with *Plasmodium chabaudi. Vaccine, 16*(2-3), 240–247.

André, F. E. (2003). Vaccinology: past achievements, present roadblocks and future promises. *Vaccine, 21*(7-8), 593–595.

Andrysiak, P. M., Collins, W. E., & Campbell, G. H. (1986). Stage-specific and species-specific antigens of *Plasmodium vivax* and *Plasmodium ovale* defined by monoclonal antibodies. *Infection and Immunity, 54*(3), 609–612.

Aribot, G., Rogier, C., Sarthou, J. L., Trape, J. F., Balde, A. T., Druilhe, P., & Roussilhon, C. (1996). Pattern of immunoglobulin isotype response to *Plasmodium falciparum* blood-stage antigens in individuals living in a holoendemic area of Senegal (Dielmo, west Africa). *The American Journal of Tropical Medicine and Hygiene, 54*(5), 449–457.

Astagneau, P., Roberts, J. M., Steketee, R. W., Wirima, J. J., Lepers, J. P., & Deloron, P. (1995). Antibodies to a *Plasmodium falciparum* blood-stage antigen as a tool

for predicting the protection levels of two malaria-exposed populations. *The American Journal of Tropical Medicine and Hygiene, 53*(1), 23–28.

Aucan, C., Traoré, Y., Fumoux, F., & Rihet, P. (2001). Familial correlation of immunoglobulin G subclass responses to *Plasmodium falciparum* antigens in Burkina Faso. *Infection and Immunity, 69*(2), 996–1001. doi:10.1128/IAI.69.2.996-1001.2001

Aucan, C., Traoré, Y., Tall, F., Nacro, B., Traoré-Leroux, T., Fumoux, F., & Rihet, P. (2000). High immunoglobulin G2 (IgG2) and low IgG4 levels are associated with human resistance to *Plasmodium falciparum* malaria. *Infection and Immunity, 68*(3), 1252–1258.

Badell, E., Oeuvray, C., Moreno, A., Soe, S., Van Rooijen, N., Bouzidi, A., & Druilhe, P. (2000). Human malaria in immunocompromised mice: an in vivo model to study defense mechanisms against Plasmodium falciparum. *The Journal of experimental medicine, 192*(11), 1653–1660.

Baird, J. K. (1995). Host age as a determinant of naturally acquired immunity to Plasmodium falciparum. *Parasitology Today (Personal Ed.), 11*(3), 105–111.

Beier, J. C., Killeen, G. F., & Githure, J. I. (1999). Short report: entomologic inoculation rates and Plasmodium falciparum malaria prevalence in Africa. *The American Journal of Tropical Medicine and Hygiene, 61*(1), 109–113.

Bhattarai, A., Ali, A. S., Kachur, S. P., Mårtensson, A., Abbas, A. K., Khatib, R., ... Björkman, A. (2007). Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS medicine, 4*(11), e309. doi:10.1371/journal.pmed.0040309

Bloland, P. B., Boriga, D. A., Ruebush, T. K., McCormick, J. B., Roberts, J. M., Oloo, A. J., ... Campbell, C. C. (1999). Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. *The American Journal of Tropical Medicine and Hygiene, 60*(4), 641–648.

Borre, M. B., Dziegiel, M., Høgh, B., Petersen, E., Rieneck, K., Riley, E., ... Harada, M. (1991). Primary structure and localization of a conserved immunogenic Plasmodium falciparum glutamate rich protein (GLURP) expressed in both the preerythrocytic and erythrocytic stages of the vertebrate life cycle. *Molecular and Biochemical Parasitology, 49*(1), 119–131.

Boudin, C., Chumpitazi, B., Dziegiel, M., Peyron, F., Picot, S., Hogh, B., & Ambroise-Thomas, P. (1993). Possible role of specific immunoglobulin M antibodies to Plasmodium falciparum antigens in immunoprotection of humans living in a hyperendemic area, Burkina Faso. *Journal of clinical microbiology, 31*(3), 636–641.

Branch, O. H., Udhayakumar, V., Hightower, A. W., Oloo, A. J., Hawley, W. A., Nahlen, B. L., ... Lal, A. A. (1998). A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kiloDalton domain of Plasmodium falciparum in pregnant women and infants: associations with febrile illness, parasitemia, and anemia. *The American journal of tropical medicine and hygiene, 58*(2), 211–219.

Breman, J. G. (2009). Eradicating malaria. *Science progress, 92*(Pt 1), 1–38.

Bruña-Romero, O., & Rodriguez, A. (2001). Dendritic cells can initiate protective immune responses against malaria. *Infection and Immunity, 69*(8), 5173–5176. doi:10.1128/IAI.69.8.5173-5176.2001

Bull, P. C., Lowe, B. S., Kortok, M., Molyneux, C. S., Newbold, C. I., & Marsh, K. (1998). Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nature Medicine, 4*(3), 358–360.

Camus, D., & Hadley, T. J. (1985). A Plasmodium falciparum antigen that binds to host erythrocytes and merozoites. *Science (New York, N.Y.), 230*(4725), 553–556.

Carvalho, L. J. M., Alves, F. A., Bianco, C., Jr, Oliveira, S. G., Zanini, G. M., Soe, S., ... Daniel-Ribeiro, C. T. (2005). Immunization of Saimiri sciureus monkeys with a recombinant hybrid protein derived from the Plasmodium falciparum antigen glutamate-rich protein and merozoite surface protein 3 can induce partial protection with Freund and Montanide ISA720 adjuvants. *Clinical and diagnostic laboratory immunology, 12*(2), 242–248. doi:10.1128/CDLI.12.2.242-248.2005

Casares, S., Brumeanu, T.-D., & Richie, T. L. (2010). The RTS,S malaria vaccine. *Vaccine, 28*(31), 4880–4894. doi:10.1016/j.vaccine.2010.05.033

Cavanagh, D. R., Dodoo, D., Hviid, L., Kurtzhals, J. A. L., Theander, T. G., Akanmori, B. D., ... McBride, J. S. (2004). Antibodies to the N-terminal block 2 of Plasmodium falciparum merozoite surface protein 1 are associated with protection against clinical malaria. *Infection and immunity, 72*(11), 6492–6502. doi:10.1128/IAI.72.11.6492-6502.2004

Cham, G. K. K., Kurtis, J., Lusingu, J., Theander, T. G., Jensen, A. T. R., & Turner, L. (2008). A semi-automated multiplex high-throughput assay for measuring IgG antibodies against Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) domains in small volumes of plasma. *Malaria Journal, 7*, 108. doi:10.1186/1475-2875-7-108

Chauhan, V. S., & Bhardwaj, D. (2003). Current status of malaria vaccine development. *Advances in Biochemical Engineering/Biotechnology, 84*, 143–182.

Chelimo, K., Ofulla, A. V., Narum, D. L., Kazura, J. W., Lanar, D. E., & John, C. C. (2005). Antibodies to Plasmodium falciparum antigens vary by age and antigen in children in a malaria-holoendemic area of Kenya. *The Pediatric Infectious Disease Journal, 24*(8), 680–684.

Cobelens, F. G., Verhave, J. P., Leentvaar-Kuijpers, A., & Kager, P. A. (1998). Testing for anti-circumsporozoite and anti-blood-stage antibodies for epidemiologic assessment of Plasmodium falciparum infection in travelers. *The American journal of tropical medicine and hygiene, 58*(1), 75–80.

Connelly, M., King, C. L., Bucci, K., Walters, S., Genton, B., Alpers, M. P., ... Kazura, J. W. (1997). T-cell immunity to peptide epitopes of liver-stage antigen 1 in an area of Papua New Guinea in which malaria is holoendemic. *Infection and immunity, 65*(12), 5082–5087.

Cowman, A. F., Baldi, D. L., Healer, J., Mills, K. E., O'Donnell, R. A., Reed, M. B., ... Crabb, B. S. (2000). Functional analysis of proteins involved in Plasmodium

falciparum merozoite invasion of red blood cells. *FEBS letters, 476*(1-2), 84–88.

Crewther, P. E., Culvenor, J. G., Silva, A., Cooper, J. A., & Anders, R. F. (1990). Plasmodium falciparum: two antigens of similar size are located in different compartments of the rhoptry. *Experimental parasitology, 70*(2), 193–206.

Cummings, J. F., Spring, M. D., Schwenk, R. J., Ockenhouse, C. F., Kester, K. E., Polhemus, M. E., ... Heppner, D. G., Jr. (2010). Recombinant Liver Stage Antigen-1 (LSA-1) formulated with AS01 or AS02 is safe, elicits high titer antibody and induces IFN-gamma/IL-2 CD4+ T cells but does not protect against experimental Plasmodium falciparum infection. *Vaccine, 28*(31), 5135–5144. doi:10.1016/j.vaccine.2009.08.046

Daugherty, J. R., Murphy, C. I., Doros-Richert, L. A., Barbosa, A., Kashala, L. O., Ballou, W. R., ... Lanar, D. E. (1997). Baculovirus-mediated expression of Plasmodium falciparum erythrocyte binding antigen 175 polypeptides and their recognition by human antibodies. *Infection and immunity, 65*(9), 3631–3637.

De Koning-Ward, T. F., Gilson, P. R., Boddey, J. A., Rug, M., Smith, B. J., Papenfuss, A. T., ... Crabb, B. S. (2009). A newly discovered protein export machine in malaria parasites. *Nature, 459*(7249), 945–949. doi:10.1038/nature08104

Dent, A. E., Bergmann-Leitner, E. S., Wilson, D. W., Tisch, D. J., Kimmel, R., Vulule, J., ... Kazura, J. W. (2008). Antibody-mediated growth inhibition of Plasmodium falciparum: relationship to age and protection from parasitemia in Kenyan children and adults. *PloS one, 3*(10), e3557. doi:10.1371/journal.pone.0003557

Dodoo, D., Theisen, M., Kurtzhals, J. A., Akanmori, B. D., Koram, K. A., Jepsen, S., ... Hviid, L. (2000). Naturally acquired antibodies to the glutamate-rich protein are associated with protection against Plasmodium falciparum malaria. *The Journal of Infectious Diseases, 181*(3), 1202–1205. doi:10.1086/315341

Dondorp, A. M., Kager, P. A., Vreeken, J., & White, N. J. (2000). Abnormal blood flow and red blood cell deformability in severe malaria. *Parasitology Today (Personal Ed.), 16*(6), 228–232.

Doolan, D. L., Dobano, C., & Baird, J. K. (2009). Acquired Immunity to Malaria. *Clin. Microbiol. Rev., 22*(1), 13–36. doi: 10.1128/CMR.00025-08

Druilhe, P., & Pérignon, J. L. (1994). Mechanisms of defense against P. falciparum asexual blood stages in humans. *Immunology letters, 41*(2-3), 115–120.

Dutta, S., Haynes, J. D., Barbosa, A., Ware, L. A., Snavely, J. D., Moch, J. K., ... Lanar, D. E. (2005). Mode of action of invasion-inhibitory antibodies directed against apical membrane antigen 1 of Plasmodium falciparum. *Infection and immunity, 73*(4), 2116–2122. doi:10.1128/IAI.73.4.2116-2122.2005

Dutta, S., Haynes, J. D., Moch, J. K., Barbosa, A., & Lanar, D. E. (2003). Invasion-inhibitory antibodies inhibit proteolytic processing of apical membrane antigen 1 of Plasmodium falciparum merozoites. *Proceedings of the National Academy of Sciences of the United States of America, 100*(21), 12295–12300. doi:10.1073/pnas.2032858100

Dziegiel, M., Borre, M. B., Jepsen, S., Hogh, B., Petersen, E., & Vuust, J. (1991). Recombinant Plasmodium falciparum glutamate rich protein; purification

and use in enzyme-linked immunosorbent assay. *The American journal of tropical medicine and hygiene, 44*(3), 306–313.

Dziegiel, M., Rowe, P., Bennett, S., Allen, S. J., Olerup, O., Gottschau, A., ... Riley, E. M. (1993). Immunoglobulin M and G antibody responses to Plasmodium falciparum glutamate-rich protein: correlation with clinical immunity in Gambian children. *Infection and Immunity, 61*(1), 103–108.

Egan, A. F., Burghaus, P., Druilhe, P., Holder, A. A., & Riley, E. M. (1999). Human antibodies to the 19kDa C-terminal fragment of Plasmodium falciparum merozoite surface protein 1 inhibit parasite growth in vitro. *Parasite immunology, 21*(3), 133–139.

Egan, A. F., Morris, J., Barnish, G., Allen, S., Greenwood, B. M., Kaslow, D. C., ... Riley, E. M. (1996). Clinical immunity to Plasmodium falciparum malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. *The Journal of infectious diseases, 173*(3), 765–769.

Ernst, K. C., Adoka, S. O., Kowuor, D. O., Wilson, M. L., & John, C. C. (2006). Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. *Malaria Journal, 5*, 78. doi:10.1186/1475-2875-5-78

Fandeur, T., & Chalvet, W. (1998). Variant- and strain-specific immunity in Saimiri infected with Plasmodium falciparum. *The American Journal of Tropical Medicine and Hygiene, 58*(2), 225–231.

Fegan, G. W., Noor, A. M., Akhwale, W. S., Cousens, S., & Snow, R. W. (2007). Effect of expanded insecticide-treated bednet coverage on child survival in rural

Kenya: a longitudinal study. *Lancet, 370*(9592), 1035–1039. doi:10.1016/S0140-6736(07)61477-9

Ferreira, M. U., Ribeiro, W. L., Tonon, A. P., Kawamoto, F., & Rich, S. M. (2003). Sequence diversity and evolution of the malaria vaccine candidate merozoite surface protein-1 (MSP-1) of *Plasmodium falciparum*. *Gene, 304*, 65–75.

Fidock, D. A., Gras-Masse, H., Lepers, J. P., Brahimi, K., Benmohamed, L., Mellouk, S., ... Meis, J. F. (1994). *Plasmodium falciparum* liver stage antigen-1 is well conserved and contains potent B and T cell determinants. *Journal of immunology (Baltimore, Md.: 1950), 153*(1), 190–204.

Frevert, U., Sinnis, P., Cerami, C., Shreffler, W., Takacs, B., & Nussenzweig, V. (1993). Malaria circumsporozoite protein binds to heparan sulfate proteoglycans associated with the surface membrane of hepatocytes. *The Journal of experimental medicine, 177*(5), 1287–1298.

Früh, K., Doumbo, O., Müller, H. M., Koita, O., McBride, J., Crisanti, A., ... Bujard, H. (1991). Human antibody response to the major merozoite surface antigen of Plasmodium falciparum is strain specific and short-lived. *Infection and immunity, 59*(4), 1319–1324.

Garraud, O., Mahanty, S., & Perraut, R. (2003). Malaria-specific antibody subclasses in immune individuals: a key source of information for vaccine design. *Trends in immunology, 24*(1), 30–35.

Genton, B., & Reed, Z. H. (2007). Asexual blood-stage malaria vaccine development: facing the challenges. *Current Opinion in Infectious Diseases, 20*(5), 467–475. doi:10.1097/QCO.0b013e3282dd7a29

Giavedoni, L. D. (2005). Simultaneous detection of multiple cytokines and chemokines from nonhuman primates using luminex technology. *Journal of Immunological Methods, 301*(1-2), 89–101. doi:10.1016/j.jim.2005.03.015

Gray, J. C., Corran, P. H., Mangia, E., Gaunt, M. W., Li, Q., Tetteh, K. K. A., ... Crisanti, A. (2007). Profiling the Antibody Immune Response against Blood Stage Malaria Vaccine Candidates. *Clinical Chemistry, 53*(7), 1244–1253. doi:10.1373/clinchem.2006.081695

Greenwood, B. M., Fidock, D. A., Kyle, D. E., Kappe, S. H. I., Alonso, P. L., Collins, F. H., & Duffy, P. E. (2008). Malaria: progress, perils, and prospects for eradication. *The Journal of Clinical Investigation, 118*(4), 1266–1276. doi:10.1172/JCI33996

Groux, H., & Gysin, J. (1990). Opsonization as an effector mechanism in human protection against asexual blood stages of Plasmodium falciparum: functional role of IgG subclasses. *Research in immunology, 141*(6), 529–542.

Guerin, P. J., Olliaro, P., Nosten, F., Druilhe, P., Laxminarayan, R., Binka, F., ... White, N. J. (2002). Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *The Lancet infectious diseases, 2*(9), 564–573.

Guerin-Marchand, C., Druilhe, P., Galey, B., Londono, A., Patarapotikul, J., Beaudoin, R. L., ... Langsley, G. (1987). A liver-stage-specific antigen of Plasmodium falciparum characterized by gene cloning. *Nature, 329*(6135), 164–167. doi:10.1038/329164a0

Hafalla, J. C. R., Cockburn, I. A., & Zavala, F. (2006). Protective and pathogenic roles of CD8+ T cells during malaria infection. *Parasite Immunology, 28*(1-2), 15–24. doi:10.1111/j.1365-3024.2006.00777.x

Han, H.-J., Park, S.-G., Kim, S.-H., Hwang, S.-Y., Han, J., Traicoff, J., ... Chung, J.-Y. (2004). Epidermal growth factor-like motifs 1 and 2 of Plasmodium vivax merozoite surface protein 1 are critical domains in erythrocyte invasion. *Biochemical and Biophysical Research Communications, 320*(2), 563–570. doi:10.1016/j.bbrc.2004.06.008

Hay, S. I., Guerra, C. A., Tatem, A. J., Noor, A. M., & Snow, R. W. (2004). The global distribution and population at risk of malaria: past, present, and future. *The Lancet Infectious Diseases, 4*(6), 327–336. doi:10.1016/S1473-3099(04)01043-6

Hisaeda, H., Saul, A., Reece, J. J., Kennedy, M. C., Long, C. A., Miller, L. H., & Stowers, A. W. (2002). Merozoite surface protein 3 and protection against malaria in Aotus nancymai monkeys. *The Journal of Infectious Diseases, 185*(5), 657–664. doi:10.1086/339187

Hogh, B., Petersen, E., Dziegiel, M., David, K., Hanson, A., Borre, M., ... Jepsen, S. (1992). Antibodies to a recombinant glutamate-rich Plasmodium falciparum protein: evidence for protection of individuals living in a holoendemic area of Liberia. *The American Journal of Tropical Medicine and Hygiene, 46*(3), 307–313.

Høgh, B., Thompson, R., Zakiuddin, I. S., Boudin, C., & Borre, M. (1993). Glutamate rich Plasmodium falciparum antigen (GLURP). *Parassitologia, 35 Suppl*, 47–50.

Hollingdale, M. R., Aikawa, M., Atkinson, C. T., Ballou, W. R., Chen, G. X., Li, J., ... Zhu, J. D. (1990). Non-CS pre-erythrocytic protective antigens. *Immunology letters, 25*(1-3), 71–76.

Hutchings, C. L., Birkett, A. J., Moore, A. C., & Hill, A. V. S. (2007). Combination of Protein and Viral Vaccines Induces Potent Cellular and Humoral Immune Responses and Enhanced Protection from Murine Malaria Challenge. *Infection and Immunity, 75*(12), 5819–5826. doi:10.1128/IAI.00828-07

John, C. C., Sumba, P. O., Ouma, J. H., Nahlen, B. L., King, C. L., & Kazura, J. W. (2000). Cytokine Responses to Plasmodium falciparum Liver-Stage Antigen 1 Vary in Rainy and Dry Seasons in Highland Kenya. *Infection and Immunity, 68*(9), 5198–5204.

John, Chandy C, Moormann, A. M., Pregibon, D. C., Sumba, P. O., McHugh, M. M., Narum, D. L., ... Kazura, J. W. (2005). Correlation of high levels of antibodies to multiple pre-erythrocytic Plasmodium falciparum antigens and protection from infection. *The American Journal of Tropical Medicine and Hygiene, 73*(1), 222–228.

John, Chandy C, O'Donnell, R. A., Sumba, P. O., Moormann, A. M., De Koning-Ward, T. F., King, C. L., ... Crabb, B. S. (2004). Evidence that invasion-inhibitory antibodies specific for the 19-kDa fragment of merozoite surface protein-1 (MSP-1 19) can play a protective role against blood-stage Plasmodium

falciparum infection in individuals in a malaria endemic area of Africa. *Journal of immunology (Baltimore, Md.: 1950), 173*(1), 666–672.

John, Chandy C., Riedesel, M. A., Magak, N. G., Lindblade, K. A., Menge, D. M., Hodges, J. S., ... Akhwale, W. (2009). Possible Interruption of Malaria Transmission, Highland Kenya, 2007–2008, *15*(12), 1917–1924. doi:10.3201/eid1512.090627

Joshi, S. K., Bharadwaj, A., Chatterjee, S., & Chauhan, V. S. (2000). Analysis of immune responses against T- and B-cell epitopes from Plasmodium falciparum liver-stage antigen 1 in rodent malaria models and malaria-exposed human subjects in India. *Infection and immunity, 68*(1), 141–150.

Kelly-Hope, L. A., & McKenzie, F. E. (2009). The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. *Malaria journal, 8*, 19. doi:10.1186/1475-2875-8-19

Klein, E. Y. (2013). Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread. *International Journal of Antimicrobial Agents, 41*(4), 311–317. doi:10.1016/j.ijantimicag.2012.12.007

Kremsner, P. G., Radloff, P., Metzger, W., Wildling, E., Mordmüller, B., Philipps, J., ... Bienzle, U. (1995). Quinine plus clindamycin improves chemotherapy of severe malaria in children. *Antimicrobial Agents and Chemotherapy, 39*(7), 1603–1605.

Kurtis, J. D., Hollingdale, M. R., Luty, A. J., Lanar, D. E., Krzych, U., & Duffy, P. E. (2001). Pre-erythrocytic immunity to Plasmodium falciparum: the case for an LSA-1 vaccine. *Trends in parasitology, 17*(5), 219–223.

Kwiatkowski, D., Cannon, J. G., Manogue, K. R., Cerami, A., Dinarello, C. A., & Greenwood, B. M. (1989). Tumour necrosis factor production in Falciparum malaria and its association with schizont rupture. *Clinical and Experimental Immunology, 77*(3), 361–366.

Langhorne, J., Cross, C., Seixas, E., Li, C., & Von der Weid, T. (1998). A role for B cells in the development of T cell helper function in a malaria infection in mice. *Proceedings of the National Academy of Sciences of the United States of America, 95*(4), 1730–1734.

Le Roch, K. G., Zhou, Y., Blair, P. L., Grainger, M., Moch, J. K., Haynes, J. D., ... Winzeler, E. A. (2003). Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science (New York, N.Y.), 301*(5639), 1503–1508. doi:10.1126/science.1087025

Leoratti, F. M. S., Durlacher, R. R., Lacerda, M. V. G., Alecrim, M. G., Ferreira, A. W., Sanchez, M. C. A., & Moraes, S. L. (2008). Pattern of humoral immune response to Plasmodium falciparum blood stages in individuals presenting different clinical expressions of malaria. *Malaria Journal, 7*, 186. doi:10.1186/1475-2875-7-186

Lyon, J. A., Geller, R. H., Haynes, J. D., Chulay, J. D., & Weber, J. L. (1986). Epitope map and processing scheme for the 195,000-dalton surface glycoprotein of Plasmodium falciparum merozoites deduced from cloned overlapping

segments of the gene. *Proceedings of the National Academy of Sciences of the United States of America, 83*(9), 2989–2993.

Malaria Conference in Equatorial Africa (1950: Kampala, U., Organization, W. H., & Sahara, C. for T. C. in A. S. of the. (1951). Report on the Malaria Conference in Equatorial Africa, held under the joint auspices of the World Health Organization and the Commission for Technical Co-operation in Africa South of the Sahara, Kampala, Uganda, 27 November-9 December 1950. Retrieved July 17, 2013, from http://apps.who.int/iris/handle/10665/40153

McBride, J. S., & Heidrich, H. G. (1987). Fragments of the polymorphic Mr 185,000 glycoprotein from the surface of isolated Plasmodium falciparum merozoites form an antigenic complex. *Molecular and biochemical parasitology, 23*(1), 71–84.

Meraldi, V., Nebié, I., Tiono, A. B., Diallo, D., Sanogo, E., Theisen, M., ... Sirima, B. S. (2004). Natural antibody response to Plasmodium falciparum Exp-1, MSP-3 and GLURP long synthetic peptides and association with protection. *Parasite immunology, 26*(6-7), 265–272. doi:10.1111/j.0141-9838.2004.00705.x

Miller, L. H., & Hoffman, S. L. (1998). Research toward vaccines against malaria. *Nature medicine, 4*(5 Suppl), 520–524.

Mills, K. E., Pearce, J. A., Crabb, B. S., & Cowman, A. F. (2002). Truncation of merozoite surface protein 3 disrupts its trafficking and that of acidic–basic repeat protein to the surface of Plasmodium falciparum merozoites. *Molecular Microbiology, 43*(6), 1401–1411. doi:10.1046/j.1365-2958.2002.02834.x

Moorthy, V. S., Good, M. F., & Hill, A. V. S. (2004). Malaria vaccine developments. *Lancet, 363*(9403), 150–156. doi:10.1016/S0140-6736(03)15267-1

Narum, D. L., Haynes, J. D., Fuhrmann, S., Moch, K., Liang, H., Hoffman, S. L., & Sim, B. K. (2000). Antibodies against the Plasmodium falciparum receptor binding domain of EBA-175 block invasion pathways that do not involve sialic acids. *Infection and immunity, 68*(4), 1964–1966.

Noland, G. S., Ayodo, G., Abuya, J., Hodges, J. S., Rolfes, M. A. R., & John, C. C. (2012). Decreased prevalence of anemia in highland areas of low malaria transmission after a 1-year interruption of transmission. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 54*(2), 178–184. doi:10.1093/cid/cir768

Noland, G. S., Hendel-Paterson, B., Min, X. M., Moormann, A. M., Vulule, J. M., Narum, D. L., ... John, C. C. (2008a). Low prevalence of antibodies to preerythrocytic but not blood-stage Plasmodium falciparum antigens in an area of unstable malaria transmission compared to prevalence in an area of stable malaria transmission. *Infection and Immunity, 76*(12), 5721–5728. doi:10.1128/IAI.00591-08

Noland, G. S., Hendel-Paterson, B., Min, X. M., Moormann, A. M., Vulule, J. M., Narum, D. L., ... John, C. C. (2008b). Low Prevalence of Antibodies to Preerythrocytic but Not Blood-Stage Plasmodium falciparum Antigens in an Area of Unstable Malaria Transmission Compared to Prevalence in an Area of Stable Malaria Transmission. *Infect. Immun., 76*(12), 5721–5728. doi:10.1128/IAI.00591-08

Oeuvray, C., Bouharoun-Tayoun, H., Gras-Masse, H., Bottius, E., Kaidoh, T., Aikawa, M., ... Druilhe, P. (1994). Merozoite surface protein-3: a malaria protein inducing antibodies that promote Plasmodium falciparum killing by cooperation with blood monocytes. *Blood, 84*(5), 1594–1602.

Okenu, D. M., Riley, E. M., Bickle, Q. D., Agomo, P. U., Barbosa, A., Daugherty, J. R., ... Conway, D. J. (2000). Analysis of human antibodies to erythrocyte binding antigen 175 of Plasmodium falciparum. *Infection and immunity, 68*(10), 5559–5566.

Orlandi, P. A., Sim, B. K., Chulay, J. D., & Haynes, J. D. (1990). Characterization of the 175-kilodalton erythrocyte binding antigen of Plasmodium falciparum. *Molecular and biochemical parasitology, 40*(2), 285–294.

Perlmann, P., & Troye-Blomberg, M. (2000). Malaria blood-stage infection and its control by the immune system. *Folia Biologica, 46*(6), 210–218.

Pinzon-Ortiz, C., Friedman, J., Esko, J., & Sinnis, P. (2001). The binding of the circumsporozoite protein to cell surface heparan sulfate proteoglycans is required for plasmodium sporozoite attachment to target cells. *The Journal of biological chemistry, 276*(29), 26784–26791. doi:10.1074/jbc.M104038200

Polley, S. D., Mwangi, T., Kocken, C. H. M., Thomas, A. W., Dutta, S., Lanar, D. E., ... Marsh, K. (2004). Human antibodies to recombinant protein constructs of Plasmodium falciparum Apical Membrane Antigen 1 (AMA1) and their associations with protection from malaria. *Vaccine, 23*(5), 718–728. doi:10.1016/j.vaccine.2004.05.031

Polley, S. D., Tetteh, K. K. A., Lloyd, J. M., Akpogheneta, O. J., Greenwood, B. M., Bojang, K. A., & Conway, D. J. (2007). Plasmodium falciparum merozoite surface protein 3 is a target of allele-specific immunity and alleles are maintained by natural selection. *The Journal of infectious diseases, 195*(2), 279–287. doi:10.1086/509806

Potocnjak, P., Yoshida, N., Nussenzweig, R. S., & Nussenzweig, V. (1980). Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *The Journal of experimental medicine, 151*(6), 1504–1513.

Pouniotis, D. S., Proudfoot, O., Minigo, G., Hanley, J. L., & Plebanski, M. (2004). Malaria parasite interactions with the human host. *Journal of Postgraduate Medicine, 50*(1), 30–34.

Reece, W. H. H., Pinder, M., Gothard, P. K., Milligan, P., Bojang, K., Doherty, T., ... Hill, A. V. S. (2004). A CD4(+) T-cell immune response to a conserved epitope in the circumsporozoite protein correlates with protection from natural Plasmodium falciparum infection and disease. *Nature medicine, 10*(4), 406–410. doi:10.1038/nm1009

Rénia, L., Grillot, D., Marussig, M., Corradin, G., Miltgen, F., Lambert, P. H., ... Del Giudice, G. (1993). Effector functions of circumsporozoite peptide-primed CD4+ T cell clones against Plasmodium yoelii liver stages. *Journal of immunology (Baltimore, Md.: 1950), 150*(4), 1471–1478.

Romero, P., Maryanski, J. L., Corradin, G., Nussenzweig, R. S., Nussenzweig, V., & Zavala, F. (1989). Cloned cytotoxic T cells recognize an epitope in the

circumsporozoite protein and protect against malaria. *Nature, 341*(6240), 323–326. doi:10.1038/341323a0

Romi, R., Razaiarimanga, M. C., Raharimanga, R., Rakotondraibe, E. M., Ranaivo, L. H., Pietra, V., ... Majori, G. (2002). Impact of the malaria control campaign (1993-1998) in the highlands of Madagascar: parasitological and entomological data. *The American journal of tropical medicine and hygiene, 66*(1), 2–6.

Rotman, H. L., Daly, T. M., & Long, C. A. (1999). Plasmodium: immunization with carboxyl-terminal regions of MSP-1 protects against homologous but not heterologous blood-stage parasite challenge. *Experimental Parasitology, 91*(1), 78–85. doi:10.1006/expr.1999.4357

Roussilhon, C., Oeuvray, C., Müller-Graf, C., Tall, A., Rogier, C., Trape, J.-F., ... Druilhe, P. (2007). Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. *PLoS Medicine, 4*(11), e320. doi:10.1371/journal.pmed.0040320

Sanders, P. R., Cantin, G. T., Greenbaum, D. C., Gilson, P. R., Nebl, T., Moritz, R. L., ... Crabb, B. S. (2007). Identification of protein complexes in detergent-resistant membranes of Plasmodium falciparum schizonts. *Molecular and biochemical parasitology, 154*(2), 148–157. doi:10.1016/j.molbiopara.2007.04.013

Sanders, P. R., Gilson, P. R., Cantin, G. T., Greenbaum, D. C., Nebl, T., Carucci, D. J., ... Crabb, B. S. (2005). Distinct protein classes including novel merozoite surface antigens in Raft-like membranes of Plasmodium falciparum. *The Journal of biological chemistry, 280*(48), 40169–40176. doi:10.1074/jbc.M509631200

Schwartz, E., Sadetzki, S., Murad, H., & Raveh, D. (2001). Age as a risk factor for severe Plasmodium falciparum malaria in nonimmune patients. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 33*(10), 1774–1777. doi:10.1086/322522

Shai, S., Blackman, M. J., & Holder, A. A. (1995). Epitopes in the 19kDa fragment of the Plasmodium falciparum major merozoite surface protein-1 (PfMSP-1(19)) recognized by human antibodies. *Parasite immunology, 17*(5), 269–275.

Sidjanski, S. P., Vanderberg, J. P., & Sinnis, P. (1997). Anopheles stephensi salivary glands bear receptors for region I of the circumsporozoite protein of Plasmodium falciparum. *Molecular and biochemical parasitology, 90*(1), 33–41.

Sim, B. K., Orlandi, P. A., Haynes, J. D., Klotz, F. W., Carter, J. M., Camus, D., ... Chulay, J. D. (1990). Primary structure of the 175K Plasmodium falciparum erythrocyte binding antigen and identification of a peptide which elicits antibodies that inhibit malaria merozoite invasion. *The Journal of cell biology, 111*(5 Pt 1), 1877–1884.

Singh, B., Sung, L. K., Matusop, A., Radhakrishnan, A., Shamsul, S. S., Cox-Singh, J., ... Conway, D. J. (2004). A large focus of naturally acquired Plasmodium knowlesi infections in human beings. *The Lancet, 363*, 1017–1024. doi:10.1016/S0140-6736(04)15836-4

Singh, S., Soe, S., Mejia, J.-P., Roussilhon, C., Theisen, M., Corradin, G., & Druilhe, P. (2004). Identification of a conserved region of Plasmodium falciparum MSP3

targeted by biologically active antibodies to improve vaccine design. *The Journal of infectious diseases, 190*(5), 1010–1018. doi:10.1086/423208

Sinnis, P., & Nardin, E. (2002). Sporozoite antigens: biology and immunology of the circumsporozoite protein and thrombospondin-related anonymous protein. *Chemical Immunology, 80*, 70–96.

Smith, T., Maire, N., Dietz, K., Killeen, G. F., Vounatsou, P., Molineaux, L., & Tanner, M. (2006). Relationship between the entomologic inoculation rate and the force of infection for Plasmodium falciparum malaria. *The American Journal of Tropical Medicine and Hygiene, 75*(2 Suppl), 11–18.

Soe, S., Theisen, M., Roussilhon, C., Aye, K.-S., & Druilhe, P. (2004). Association between protection against clinical malaria and antibodies to merozoite surface antigens in an area of hyperendemicity in Myanmar: complementarity between responses to merozoite surface protein 3 and the 220-kilodalton glutamate-rich protein. *Infection and Immunity, 72*(1), 247–252.

Stanisic, D. I., Richards, J. S., McCallum, F. J., Michon, P., King, C. L., Schoepflin, S., ... Beeson, J. G. (2009). Immunoglobulin G subclass-specific responses against Plasmodium falciparum merozoite antigens are associated with control of parasitemia and protection from symptomatic illness. *Infection and Immunity, 77*(3), 1165–1174. doi:10.1128/IAI.01129-08

Stevenson, M. M., & Riley, E. M. (2004). Innate immunity to malaria. *Nature Reviews. Immunology, 4*(3), 169–180. doi:10.1038/nri1311

Stowers, A. W., Kennedy, M. C., Keegan, B. P., Saul, A., Long, C. A., & Miller, L. H. (2002). Vaccination of monkeys with recombinant Plasmodium falciparum apical membrane antigen 1 confers protection against blood-stage malaria. *Infection and immunity, 70*(12), 6961–6967.

Sultan, A. A., Thathy, V., Frevert, U., Robson, K. J., Crisanti, A., Nussenzweig, V., ... Ménard, R. (1997). TRAP is necessary for gliding motility and infectivity of plasmodium sporozoites. *Cell, 90*(3), 511–522.

Sun, P., Schwenk, R., White, K., Stoute, J. A., Cohen, J., Ballou, W. R., ... Krzych, U. (2003). Protective immunity induced with malaria vaccine, RTS,S, is linked to Plasmodium falciparum circumsporozoite protein-specific CD4+ and CD8+ T cells producing IFN-gamma. *Journal of immunology (Baltimore, Md.: 1950), 171*(12), 6961–6967.

Takala, S. L., & Plowe, C. V. (2009). Genetic diversity and malaria vaccine design, testing and efficacy: preventing and overcoming "vaccine resistant malaria". *Parasite immunology, 31*(9), 560–573.

Taylor-Robinson, A. W. (2003). Immunity to liver stage malaria: considerations for vaccine design. *Immunologic research, 27*(1), 53–70. doi:10.1385/IR:27:1:53

Theisen, M., Soe, S., Oeuvray, C., Thomas, A. W., Vuust, J., Danielsen, S., ... Druilhe, P. (1998). The glutamate-rich protein (GLURP) of Plasmodium falciparum is a target for antibody-dependent monocyte-mediated inhibition of parasite growth in vitro. *Infection and immunity, 66*(1), 11–17.

Tolle, R., Früh, K., Doumbo, O., Koita, O., N'Diaye, M., Fischer, A., ... Bujard, H. (1993). A prospective study of the association between the human humoral immune

response to Plasmodium falciparum blood stage antigen gp190 and control of malarial infections. *Infection and immunity, 61*(1), 40–47.

Tongren, J. E., Drakeley, C. J., McDonald, S. L. R., Reyburn, H. G., Manjurano, A., Nkya, W. M. M., ... Riley, E. M. (2006). Target Antigen, Age, and Duration of Antigen Exposure Independently Regulate Immunoglobulin G Subclass Switching in Malaria. *Infection and Immunity, 74*(1), 257–264. doi:10.1128/IAI.74.1.257-264.2006

Touré, F. S., Deloron, P., & Migot-Nabias, F. (2006). Analysis of human antibodies to erythrocyte binding antigen 175 peptide 4 of Plasmodium falciparum. *Clinical medicine & research, 4*(1), 1–6.

Van der Heyde, H. C., Huszar, D., Woodhouse, C., Manning, D. D., & Weidanz, W. P. (1994). The resolution of acute malaria in a definitive model of B cell deficiency, the JHD mouse. *Journal of Immunology (Baltimore, Md.: 1950), 152*(9), 4557–4562.

Von der Weid, T., Honarvar, N., & Langhorne, J. (1996). Gene-targeted mice lacking B cells are unable to eliminate a blood stage malaria infection. *Journal of immunology (Baltimore, Md.: 1950), 156*(7), 2510–2516.

Winkler, S., Willheim, M., Baier, K., Schmid, D., Aichelburg, A., Graninger, W., & Kremsner, P. G. (1999). Frequency of cytokine-producing T cells in patients of different age groups with Plasmodium falciparum malaria. *The Journal of Infectious Diseases, 179*(1), 209–216. doi:10.1086/314571

Wipasa, J., Elliott, S., Xu, H., & Good, M. F. (2002). Immunity to asexual blood stage malaria and vaccine approaches. *Immunology and Cell Biology, 80*(5), 401–414. doi:10.1046/j.1440-1711.2002.01107.x

Wipasa, J., Suphavilai, C., Okell, L. C., Cook, J., Corran, P. H., Thaikla, K., ... Hafalla, J. C. R. (2010). Long-Lived Antibody and B Cell Memory Responses to the Human Malaria Parasites, Plasmodium falciparum and Plasmodium vivax. *PLoS Pathog, 6*(2), e1000770. doi:10.1371/journal.ppat.1000770

Xu, H., Wipasa, J., Yan, H., Zeng, M., Makobongo, M. O., Finkelman, F. D., ... Good, M. F. (2002). The mechanism and significance of deletion of parasite-specific CD4(+) T cells in malaria infection. *The Journal of Experimental Medicine, 195*(7), 881–892.

Yang, C., Shi, Y. P., Udhayakumar, V., Alpers, M. P., Povoa, M. M., Hawley, W. A., ... Lal, A. A. (1995). Sequence variations in the non-repetitive regions of the liver stage-specific antigen-1 (LSA-1) of Plasmodium falciparum from field isolates. *Molecular and biochemical parasitology, 71*(2), 291–294.

Zhu, J., & Hollingdale, M. R. (1991). Structure of *Plasmodium falciparum* liver stage antigen-1. *Molecular and Biochemical Parasitology, 48*(2), 223–226.