Expression pattern of immunoglobulin G subclasses in response to UB05 antigen in a *Plasmodium falciparum* endemic area in Cameroon

The aim of this study was to show that UB05 a newly identified malaria antigen elicits protective antibody immune responses mainly of IgG1 and IgG3 subclasses. The study population comprised 240 randomly selected adults and children, febrile and none febrile, grouped into various age groups who consulted at the Health Post in Bolifamba. Serum from each individual was analyzed by Enzyme linked immunosorbent assay (ELISA) for the presence of antibodies to UB05 of the IgG subclasses. The levels of all IgG subclasses to UB05 increased with age and were significantly higher in adults than in infants \((P<0.05)\). Cytophilic antibodies, IgG1 and IgG3 were significantly higher in adults than in children \((P<0.05)\). IgG3 reactivity was generally higher when compared with IgG1 levels for all age groups and the difference was statistically significant \((P<0.0001)\). There was a negative correlation between IgG3 antibodies and parasite density. Individuals with fever had lower levels of IgG3 when compared with those without fever and this was most obvious in children. IgG2 and IgG4 responses to UB05 were generally higher in infected subjects although IgG4 responses were lowest across all age groups. We conclude that UB05 elicits protective immunity against malaria.

Key words: UB05, malaria antigen, immune response, IgG subclasses

INTRODUCTION

Malaria in Sub-Saharan Africa is a top parasitic disease accounting for 90% of all malaria deaths globally (WHO, 2014). Several malaria control intervention strategies have been put in place ranging from the use of impregnated mosquito bed nets to the use of potent anti-malarial drug combinations. However, some difficulties still exist with these intervention strategies. The use of vaccines remain the most effective strategy in the eradication or control of infectious diseases (Plotkin and Plotkin, 2004) hence the need to develop a vaccine against malaria which still remains a major health threat in endemic areas.

Several candidate vaccines are presently at different stages of development but so far, none of them either singly or in combination is able to confer full immune protection (Schwartz et al., 2012; Palacpac et al., 2015). A possible reason for this among other factors is that not all the antigens necessary for protective immunity have been identified. Against this background, Titanji et al. (2009), identified a new malaria antigen, UB05 through differential immunoscreening of a cDNA library using sera from semi-immune and susceptible subjects. UB05 (GenBank Accession Number DQ235690) was previously listed as a hypothetical protein (PlasmoDB PF10_0372) of unknown function on chromosome 10 of the *P. falciparum* genome. UB05 has been shown to be expressed on the surface of schizonts and dispersed merozoites and is preferentially recognized by total IgG antibodies from semi-immune adults (Titanji et al., 2009).

Several studies on antigens that are presently considered as potential vaccine candidates have shown that protection...
against the asexual stages of malaria seems to rely largely on cytophilic antibodies (Duah et al., 2010; Palacpaca et al., 2015). In this study, we show that total antibodies elicited against UB05 can be protective and are mainly cytophilic IgG1 and IgG3 antibodies.

MATERIALS AND METHODS

The study was carried out in Bolifamba, a rural village 530 m above sea level situated on the eastern slope of Mount Cameroon in the South West region of Cameroon. Bolifamba has a temperature range of 18-28 °C, an annual rainfall of 4096 mm and humidity above 80%. Malaria parasite transmission is perennial and peaks during the rainy season (Fru-Cho et al., 2014). The prevalence of P. falciparum infection among the villagers usually exceeds 90% at any time (Akenji et al., 2005).

The population of Bolifamba, estimated to be approximately 10976, is multi ethnic in nature with farming and small scale trading as the major occupation (Nyasa et al., 2015). Transmission of the parasites causing malaria is mainly by members of the Anopheles gambiae complex. The peak period of transmission is the rainy season from March to October (Akenji et al., 2005) although the pattern has been changing in recent times. Ethical clearance was obtained from the South West regional delegation of public health.

Study design

The study population comprised 240 adult inhabitants of Bolifamba and their children (whether febrile or not) who came to the local health post in response to a call from the local Chief, for free malaria screening and treatment of smear-positive cases of detected malaria. Informed consent was sought from all subjects or parents in the case of children participating in the study. Demographic and clinical data were collected from subjects, split into three age groups -1-5years (infants), 10-14 years (children) and ≥ 18years (adults) each having equal numbers of both P. falciparum positive and negative individuals.

Clinical examination and sample collection

A physician carried out clinical examination of individuals and used an electronic thermometer to measure body temperature. Axillary temperature was measured except for infants younger than 2 years for whom rectal measurements were preferred. Fever was defined as temperature ≥37.5 °C. Clinical examination included palpation of the spleen, graded according to the classification of Hackett (Gilles, 1993). Diagnosed cases of malaria were treated using quinine sulphate or amodiaquine as recommended by national guidelines at the time.

About 1-3 mL of venous blood was collected by a Physician or nurse following standard procedures into sterile EDTA tubes. The samples were transported on ice to the University of Buea laboratory for analysis. A portion of the blood was used to prepare a thick film. Blood smears were stained with giemsa solution (Merck, Darmstadt, Germany) and checked for malarial parasites following a previously described standard procedure (Akenji et al., 2005).

Determination of anti-UB05 IgG subtypes by ELISA

Serum samples from 240 individuals were analyzed for IgG subclasses. ELISAs were done as previously described (Ghogomu et al., 2002). UB05 antigen isolated and expressed by Titanji et al. (2009) was used. The concentration of UB05 antigen was determined by checkerboard titration.

Antibodies to UB05 antigen of the subclasses 1-4 in sera were determined as follows: The microtitre plates (Immunolon 4; Dynatech labs, Chantilly, VA) were coated with 100 µL of 2ug/ml of UB05 diluted in 50 mM carbonate per well and incubated overnight at 4 °C. After removal of excess antigens by washing thrice for 5 minutes each with 0.05% Tween 20 in phosphate buffered saline (PBS-Tween-20), unbound sites were blocked for 2 hours at room temperature with 100 µL of 5% bovine serum albumin (BSA). The plates were washed again as before and bound antigens were probed after this wash with 100 µL of sera per well diluted 1:100 for the detection of IgG subtypes. The plates were incubated for 1 hour at 37 °C after which 100 µL of mouse anti-human IgG, IgG1, IgG2, IgG3, IgG4 were added to the plates and incubated for 1 hour at 37 °C. The unbound antibodies were again washed and as above and 100 µL of 1:2000 dilution of goat anti-mouse IgG conjugated to alkaline phosphatase was added and incubated for 2 hours at room temperature. Plates were washed as above and developed with 1mg/mL pNPP prepared in substrate buffer. The plates were allowed to develop at 37 °C for 30 minutes. The optical densities were read at 405 nm in a microplate reader (MULTISKAN EX ELISA reader (Thermolabsystems).

Data analysis

The data were keyed into Microsoft Excel 2007 and then transferred into SPSS version 17.0 statistical package for statistical comparisons. Group means were compared using either analysis of variance (ANOVA) for two or more groups, Mann-Whitney U test for two groups that are not normally distributed or the Student's t-test for two group that are approximately normally distributed.

RESULTS

Relationship between UB 05 IgG Subclasses and Age

All IgG subclass levels of UB05 increased with age and were significantly higher in adults than in infants (p<0.05; Figure 1). Cytophilic antibodies IgG1 and IgG3 were significantly
higher in adults than in children (p<0.05). IgG3 reactivity was generally higher when compared with IgG1 levels for all age groups and the difference was statistically significant (p<0.0001).

**Relationship between UB05 IgG subclasses and parasitaemia**

There was no significant difference between the mean antibody levels of IgG subclasses in infected and non-infected subjects (p>0.05). IgG2 and IgG4 reactivity to UB05 was higher in infected individuals than in non-infected individuals. IgG4 reactivity was low across all age groups compared with the other 3 subclass responses (Figure 2). Mean parasite density decreased with increase in mean IgG3 and IgG1 levels.

**Relationship between UB 05 IgG subclasses and fever**

Individuals with fever had lower IgG subclass immune reactivity when compared with those without fever (Figure 3). Individuals with fever had lower levels of IgG3 than
their non-febrile counterparts and this difference was statistically significant (P<0.0001). The highest rates of fever were in children.

**DISCUSSION**

Protection against the blood forms *P. falciparum* seems to rely largely on cytphilic antibodies of the IgG1 and/or IgG3 isotypes that targets the surface antigens expressed on the surface of merozoites and/or schizonts (Cavanagh et al., 2001). Hence it is important that any molecule that is a marker of protective immunity to malaria should preferentially illicit strong immune responses mainly of IgG1 or IgG3 antibody subclasses.

We have studied the pattern of IgG subclass responses in subjects in Bolifamba to the UB05 antigen. All IgG subclass responses were elicited by UB05 antigen and their levels increased with age. Cytophilic antibody responses, IgG1 and IgG3 were the most dominant and were significantly higher in adults than in children. Other studies with MSP1-19 and MSP2 antigens which are both vaccine candidates have shown similar responses with either IgG1 and/or IgG3 (Taylor et al., 1995; Shi et al., 1996; Fernandez-Becerra et al., 2010). Opsonising IgG1 and IgG3 antibodies which predominate in the sera of clinically immune individuals appear to be responsible for both inhibition of merozoite invasion of erythrocytes and killing of intra-erythrocyte parasites by monocytes (Tebo et al., 2001). Preliminary assessment of anti-parasite activity *in vitro*, in reinvasion/growth inhibition assays using rabbit anti-UB05 antibodies showed marginal levels of invasion inhibition of merozoites (Titanji et al., 2009). This suggests that antibodies to UB05 in subjects living in Bolifamba may be involved in limiting parasite invasion of erythrocytes. There was no significant difference in parasite positive and parasite negative individuals within each age group although adults and young children 10-14 generally had higher levels of all IgG antibody subclasses compared to children <5 years of age (Figure 2). Other studies with MSP1-19 antigen, a vaccine candidate, have shown age dependence of acquired immunity with increase in IgG1 and IgG3 antibodies with age (Riley et al., 1992; Shi et al., 1996). This trend which we also observed with UB05 signifies a role of UB05 in acquired immunity to malaria. Other studies with a UB05 homologue in cattle has shown high levels of UB05 specific antibodies in vaccinated cattle compared with non-vaccinated cattle (Dinga et al., 2015).

IgG2 and IgG4 responses were higher in subjects positive for malaria than in negative subjects and increased with age although IgG4 responses were generally low. Other studies have shown that in addition to IgG1 and IgG3 IgG2 may also be involved in protection. High levels of IgG2 to RESA and to MSP2 are associated with resistance to malaria at the end of the transmission season and levels tend to be higher in older individuals who are better protected against infection and disease (Aucan et al., 2000). Previous studies in Bolifamba (Titanji et al.,2002) also showed high levels of IgG 1-3 in adults compared with children residing in this area. These results show that IgG2 may have an important role in immunity to malaria in Bolifamba.

There was a significantly strong IgG3 response in parasitized individuals without fever compared to those with fever (Figure 3). This may be a pointer to the role of IgG3 in UB05 mediated immune protection against malaria in Bolifamba.

The present investigation suggests that UB05 may be capable of eliciting protective antibody responses mediated
by IgG1 and IgG3 subclass. UB05 preferential reactivity with sera from partially immune subjects may be compatible with a role in the development of protective immunity to malaria.

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Conflict of Interest: None

Ethical Standards: The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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