1 Comparative analysis of IgG Responses to recombinant Q β phage displayed MSP3 and
2 UB05 in Dual HIV-malaria infected adults living in areas differing in Malaria transmission
3 intensities.

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Abstract

Immunoglobulin G specific responses against *Plasmodium falciparum* merozoite antigens such as the merozoite surface protein 3 (MSP3) and UB05 are known to play critical roles in parasitemia control and protection from symptomatic illness. However when there is intense perennial malaria transmission coupled with concurrent infection with the human immunodeficiency virus type 1 (HIV), knowledge of IgG antibody response profiles is limited.

In this study we assessed the impact of dual HIV-Malaria infections on IgG subclass responses to MSP3 (QβMSP3) and UB05 (QβUB05) in individuals living in two areas of Cameroon differing in transmission intensity. We observed differences in antigen specific IgG and IgG subclass responses which was dependent upon the antigen type, malaria transmission intensity, HIV infection, malaria infection and dual HIV-malaria infections. Individuals living in high malaria transmission areas irrespective of HIV or malaria status had significantly higher IgG responses to both antigens (P=0.0001 for QβMSP3, P=0.0001 for QβUB05) than their counterpart from low transmission areas. When dual HIV-Malaria infection is considered significantly higher QβMSP3 specific IgG1 (P=0.0001) and IgG3 (P=0.04) responses in double negative individuals was associated with protection against malaria in low transmission areas. Superior QβUBO5 specific IgG1 responses (P=0.0001) in double negative individuals were associated with protection in high transmission areas in contrast to significantly higher IgG3 responses to

QβUB05 (P=0.0001) which were more relevant to protection in low malaria transmission areas in the same population. Thus, understanding immune responses to QβUB05 and QβMSP3 could facilitate the development of immunotherapeutic strategies suitable for areas differing in malaria transmission intensity.

Key words: Dual HIV-Malaria, QβUB05, QβMSP3, IgG subclass, antibodies, natural immunity

Introduction

In Cameroon like in most sub Saharan African countries people living in low or high malaria transmission areas are exposed to different frequencies of *Plasmodium falciparum*. Within such regions long term inhabitants suffer repeated exposure to varying strains of malaria parasite during the course of several years eventually developing protection from infection and symptomatic illness irrespective of malaria transmission intensity (1). A critical component of this naturally acquired immunity is *Plasmodium falciparum* induced IgG and IgG subclass antibody responses targeting a number of parasite derived antigens. However little is known about the IgG antibody subclass profile that mediates protective immunity to malaria in both low and high malaria transmission areas. Also, when there is concurrent infection with the human Immunodeficiency virus type 1(HIV), knowledge of the precise nature of parasite antigen directed IgG subclass antibody responses functional in the afflicted individuals is limited. Given that HIV infection depletes the immune system there is need to understand the role of promising malaria target antigens and the profile of IgG subclass responses driving protective immunity to malaria in both low and high transmission areas.

Antibodies to several asexual blood stage antigens including apical membrane antigen 1 (AMA-1), erythrocyte binding antigen (EBA-175), the merozoite surface proteins (MSPs), reticulocyte-binding protein homologue (Rh5), Glutamate-rich protein (GLURP), UB05 and

circumsporozoite protein (CSP) have been demonstrated to be an essential component of naturally acquired immunity reducing parasite multiplication thereby preventing infection and clinical disease in long term inhabitants of endemic regions (2-8). In this regards high levels of IgG antibody subclass responses and diversity of the target antigens have been associated with naturally acquired immunity to malaria (6-9). However, due to inherent polymorphism in the asexual blood stage antigens (10) some elements of antibody-mediated immunity to *P. falciparum* have been reported to be strain specific (11, 12) thereby limiting their utility as global malaria vaccine candidates.

In low and high transmission areas the attainment of clinical immunity against malaria or protection from infection is largely dependent upon continuous exposure to multiple parasite variants (13) leading to an accumulation of a broad range of antibody specificities responsible for the naturally acquired immunity. In areas differing in transmission intensities it is uncertain which IgG subclass respond profiles are relevant to naturally acquired immunity. The scenario becomes even more challenging when there is attendant co-infection with HIV which dysregulates antibody responses and depletes the immune system.

In this study we have determined in low and high malaria transmission areas the impact of dual HIV-malaria infection on IgG subclass responses to two conserved *P. falciparum* derived asexual blood stage antigens displayed separately upon a recombinant RNA coliphage Q β as previously described by our group (14, 15). The recombinant phage Q β MSP3 displays the conserved C-terminal 88 aa of the merozoite surface protein 3 (16, 17) whilst Q β UB05 bears the previously described malaria antigen UBO5 (18). Surface display upon the recombinant RNA coliphage Q β as previously demonstrated by our group improves the antigenicity of inserted antigens (14, 15).

Antibodies specific to *Plasmodium falciparum* MSP3 are known to mediate parasite killing in association with monocytes in a process referred to as (19-21) antibody-dependent cellular inhibition (ADCI). MSP3 specific antibodies therefore contribute in preventing symptomatic disease through the inhibition of blood parasite invasion cycles ultimately leading to a reduction in parasite burden and episodes of malaria (22, 23). A number of malaria vaccine candidates incorporating this highly conserved C-terminal end of MSP3 have been assessed in clinical trials with promising outcomes (19, 24-26). On other hand UB05 specific antibodies have also been associated with protection in exposed populations (18). We compared between dual HIV-malaria infected and double negative individuals the IgG subclass responses specific to the malaria vaccine antigens in both low and high malaria transmission area of Cameroon. Our study can facilitate the identification of surrogate markers of malaria immunity useful in the design of novel highly efficacious vaccines and the development of immunotherapeutic strategies to enhance immunity to malaria in people living in areas differing in transmission intensity.

Materials and Methods

Study site

The study was carried out in two areas of Cameroon (Yaounde and Bikop) differing in malaria transmission intensity. As the Capital city of Cameroon Yaounde (3°52'N11°31'E) is a multi-ethnic city situated at an average elevation of 750 m. Bikop on the other hand is a remote rural area located 48 KM away from Yaounde with year round intense malaria transmission. Both Yaoundé and Bikop are holendemic for malaria however with differing transmission intensity. This is mainly because unlike Yaounde, Bikop is located in the heart of the rain forest with a large number of mosquito breedings sites and poorly constructed houses favoring sustained high malaria transmission. The temperature in both areas is around 23.7°C with a similar average

annual rain fall of 1643 mm. There also have similar rainy (March to June, September to November) and dry seasons (December to February, July–August) (27).

Ethical clearance

This study received ethical approval from the Cameroon National Ethics Committee for Human Health Research (Reference numbers 2015/03/561/CE/CNERSH/SP and 2018/01969/CE/CNERSH/SP) and the CIRCB institutional review board (protocol number 14-11). All participants provided written informed consent. Data were processed using specific identifiers for privacy and confidentiality purposes. Clinical data generated during the course of this study was provided free of charge to all participants.

Study design

This was a cross-sectional study which enrolled HIV-1 infected and non-infected people who were 21 years or older. Participants with other infection (including microfilaria, dengue, TB, and hepatitis B and C) and pregnant women were excluded from the study. All participants were members of the CIRCB AFRODEC cohort (28-30)

Study area

This study was carried out in two areas of Cameroon (Yaounde and Bikop) differing in malaria transmission intensity. As the Capital city of Cameroon Yaounde (3°52'N11°31'E) is a multi-ethnicity situated at an average elevation of 750 m. Bikop is malaria hotspot with a high incidence of malaria located 48 KM away from Yaounde with year round intense malaria transmission. Both Yaoundé and Bikop are holoendemic for malaria however with differing transmission intensities. This is mainly because unlike Yaounde, Bikop is located in the heart of the rain forest with a high density of mosquitoes and poorly constructed houses favoring sustained high malaria transmission. The temperature in both areas is around 23.7°C with similar

average annual rain fall of 1643 mm. There also have similar rainy (March to June, September to November) and dry seasons (December to February, July–August).

Study Population

A total of 124 participants were recruited for this study. All participants were adults participants of the CIRCB AFRODEC cohort 23-25. Participants were constituted in for groups consisting of dual HIV-malaria infected (HIV+/Mal+), HIV mono-infected (HIV+/Mal-), malaria mono-infected (HIV-/Mal+) and double negative (HIV-/Mal-) people.

Plasma sample collection and Processing

About 4 ml of blood was collected into plastic Vacuum blood spray-coated K2EDTA tubes called Vacutest (Vacutestkirma, Italy). Subsequently, samples were transported to the Vaccinology laboratory of Chantal BIYA International Reference Centre (CIRCB) for storage and analysis. All samples were stored at room temperature and processed within 4 hours of collection. To obtain plasma, samples were centrifuged at 2,000 rpm for 10 min at 4°C. The plasma fraction was harvested sterile under the hood, aliquoted in small single-use volumes and stored at -20°C until use. The plasma obtained from participants was heat inactivated for 30 minutes at 56°C prior to ELISA assay.

HIV infection and CD4 T cell Enumeration

Confirmation of HIV status was done as described for the CIRCB AFRODEC cohort using the Cameroon's national algorithm for the diagnosis of HIV infection as previously reported for the CIRCB AFRODEC cohort (31)

Absolute numbers of helper CD4+ T cells for HIV+ participants were determined in fresh whole blood using BD multitest CD3/CD8/CD45/CD4 and TruCount tubes (BD biosciences, USA) according to the manufacturer's instructions.

Malaria Diagnosis and microscopy

A malaria rapid diagnostic test was done on the blood samples according to the manufacturer's instructions (SD Bioline, USA). In addition, thick peripheral blood films were stained with Giemsa and examined using a microscope following standard quality-controlled procedures, for the presence of malaria parasites.

Study antigens

The antigens consisted of recombinant Q β displaying Plasmodium falciparum 3D7 strain sequence derived C-terminal part of MSP3 (Q β MSP3) and UB05 (Q β UB05) generated in our group as previously described (14, 15).

Determination of IgG and IgG subclasses antibody responses specific to Q β UB05 and Q β MSP3

The plasma levels of antibodies specific to the malaria antigens Q β UB05 and Q β MSP3 were determined through ELISA assay. Briefly high binding ELISA plates were coated with 10^7 particles/well of each recombinant phage and incubated overnight at 4°C. The following day, plates were washed 3x with PBST (PBS containing 0.05% Tween 20) and blocked with 3% BSA in PBS for one hour at 37 °C. Heat inactivated plasma samples were diluted in PBS at 1:300 (for IgG detection) or 1:100 (for IgG subclasses detection), then 100 μ l/well added in triplicate and incubated for two hours at 37 °C. The plates were washed four times with PBST after which the bound antibody was probed with the peroxidase-conjugated mouse anti-human IgG and IgG subclasses (IgG1, IgG2, IgG3 and IgG4) diluted 1:4000 in 1X PBS. Bound conjugate was detected using ABTS substrate and stop solution according to the manufacturer's protocol (southern biotech, Birmingham USA). The colorimetric signal was measured at 405 nm using a multiscan FC microplate reader (Thermo Fisher Scientific, USA).

Statistical analysis

Data analysis was performed with Graphpad Prism Software version 6.1. The data were expressed as median (25th percentile-75th percentile). Comparisons of medians among two groups were performed by the U-Mann-Whitney test. Statistical significance was confirmed when $P < 0.05$.

Results

High malaria transmission intensity is associated with superior Q β MSP3 and Q β UB05 specific IgG antibodies

Individuals in areas of high perennial malaria transmission intensity developed significantly higher ($P=0.0001$) levels of Q β MSP3 and Q β UB05 specific IgG responses than those living in low transmission zones (compare Fig 1A with B). In high malaria transmission regions Q β MSP-3 specific IgG responses were comparatively higher but not significantly different than responses specific to Q β UB05 in both positive ($P=0.09$) and negative ($P=0.08$) individuals (Fig. S1). In addition, no significant difference is observed in dual HIV-Malaria infected people in their plasma reactivity with the two recombinant antigens both in low and high transmission areas (Fig 1A&C). Similarly, when people negative for both malaria and HIV (double negative individuals) were considered, no significant difference is observed in the IgG responses specific to the two malaria vaccine antigens. The effect of HIV-1 infection was a significant reduction in IgG responses specific to the two antigens in both low ($P=0.0001$ for Q β MSP3, $P=0.04$ for Q β UB05) and high ($P=0.0001$ for Q β MSP3) malaria transmission areas. Surprisingly, there was no difference in these values for UB05 in the high malaria transmission region (Fig. 1 C&D).

On the other hand, the overall effect of *Plasmodium falciparum* infection in both low and high malaria transmission regions was a significant increase in IgG responses specific to both antigens

irrespective of dual HIV-malaria infection (Fig. 1E&F). Thus whereas HIV infection resulted to a significant a reduction in antigen specific IgG antibody levels *Plasmodium falciparum* infection resulted into a significant increase in the IgG antibody levels.

IgG1 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

Whereas double negative individuals in low malaria transmission areas showed significantly higher ($P=0.0001$) IgG1 responses specific to Q β MSP3 than to Q β UB05; in high malaria transmission areas the converse was true (Fig. 2B) with IgG1 responses specific to Q β UB05 being superior ($P=0.0001$). The effect of dual HIV-malaria infection in low transmission area was a significant reduction in the Q β MSP3 specific IgG1 responses ($P=0.0001$) in contrast to individuals living in high malaria transmission areas where Q β MSP3 specific IgG1 responses were similar to those of the double negative participants. On the other hand IgG1 responses specific to Q β UB05 in high transmission areas remain comparatively higher than those to Q β MSP3 in both dual HIV-malaria positive ($P=0.004$) and double negative ($P=0.0001$) individuals (Fig. 2A&B). This probably indicates the relevance of Q β UB05 specific IgG1 in predicting malaria immunity in high transmission areas even under the challenging circumstances of HIV infection. The impact of dual HIV-malaria infection in low transmission areas was therefore a significant reduction ($P=0.0001$) in IgG1 responses specific to Q β MSP3 (Fig 1C) in contrast to Q β UB05 where IgG1 responses in this group remain higher than the double negative participants ($P=0.0001$). Again in a high transmission area the IgG1 responses to the two antigens were comparatively higher than values for the low transmission area. In addition IgG1 responses to Q β UB05 in double negative individuals were also significantly higher ($P=0.004$) than dual HIV-malaria infected people. Thus in addition to malaria

transmission intensity differences in IgG1 subclass antibody levels might also be dependent upon co-infection with HIV-1 and the antigen of choice.

Thus in a low malaria transmission area, significantly high IgG1 responses specific to Q β MSP3 is associated with resistance to malaria in contrast to superior Q β UB05 specific IgG1 antibodies which were instead relevant to protection in high a transmission area.

IgG2 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

In low malaria transmission areas significantly higher IgG2 subclass responses (P=0.0001 for Q β MSP3 and P=0.0001 for Q β UB05) were observed in double negative individuals relative to dual HIV-malaria positive participants (Fig. 3A&B). The converse was the case for high malaria transmission areas where dual HIV-malaria infection resulted to significantly higher IgG2 responses to both Q β MSP3 (P=0.0001) and Q β UB05 (P=0.0001). Overall the IgG2 responses specific to both antigen was comparatively superior in low relative to high transmission areas (Fig. 3A&B). The effect of an infection with HIV was a general increase in IgG2 responses specific to both antigens in low and high transmission areas (Fig. 3A&B). Several studies from other endemic areas of Africa have indicated that high circulating levels of malaria parasite antigen specific IgG2 could be a marker of severity of malaria infection (32-34). Except for MSP3 specific IgG2 an infection with plasmodium falciparum was associated with an increased in IgG2 responses to both antigens in all transmission areas. In a low malaria transmission area the impact of dual HIV-malaria infection was therefore a significant reduction in IgG2 responses to both antigens. In contrast in a high malaria transmission area dual HIV-malaria infection resulted to superior IgG2 responses to both Q β MSP3 (P=0.0001) and Q β UB05 (p=0.0001)

relative to the double negative participants. Thus with respect to both antigens there is a differential IgG2 response between high and low malaria transmission areas.

IgG3 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

In low malaria transmission areas the IgG3 subclass antibody responses were significantly higher in double negative ($p=0.0001$ for Q β MSP3 and $p=0.0001$ for Q β UB05) compared to dual HIV-malaria infected participants. On the other hand in high malaria transmission areas no difference is observed between dual HIV-malaria infected and double negative individuals with respect to IgG3 subclass responses to both antigens. The effect of HIV infection was a significant reduction of IgG3 responses specific to Q β MSP3 in both low ($p=0.0003$) and high ($p=0.0001$) malaria transmission areas. Similarly IgG3 specific responses to Q β UB05 were significantly reduced in low ($P=0.01$) and high ($P=0.0001$) transmission areas after HIV infection. In low transmission areas *Plasmodium falciparum* infection resulted to a significant increase in IgG3 responses specific to both Q β MSP3 ($P=0.0003$) and Q β UB05 ($P=0.0004$) respectively. In contrast in high malaria transmission we observed significantly lower IgG3 responses specific to Q β MSP3 ($P=0.0001$) and Q β UB05 ($P=0.0001$) after *Plasmodium falciparum* infection. Thus there is differential effect of HIV and *Plasmodium falciparum* infections on IgG3 specific responses to both antigens which was also associated with the malaria transmission intensity.

IgG4 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

There was a differential expression of antigen specific IgG4 subclass responses between low and high malaria transmission areas (Fig. 5A&B). Overall for both antigens in low compared to high transmission areas dual HIV-malaria positive and double negative individuals showed

significantly higher IgG4 responses. However in low transmission areas whereas Q β MSP3 specific IgG4 responses in dual HIV-malaria infected individuals were superior to those of double negative individual (P=0.0001); for Q β UB05 IgG4 responses the converse was true. On the other hand in high transmission areas no difference was observed between dual HIV-malaria infected and double negative individuals with respect to antigen specific IgG4 responses. The effect of HIV infection in all the malaria transmission areas was a significant reduction in IgG4 responses specific to both antigens (Fig.5 C&D). *Plasmodium falciparum* infection in low transmission areas resulted to a significant increase in IgG4 responses specific to Q β MSP3 (P=0.0001) and Q β UB05 (P=0.0001) respectively (Fig.5E&F). In contrast in high transmission areas the converse is true as IgG4 responses specific to both Q β MSP3 (P=0.0001) and Q β UB05 (P=0.0001) decreased significantly after *Plasmodium falciparum* infection (compare Fig. 5E with F). Thus variation in IgG4 specific responses was dependent upon several factors including transmission intensity, malaria parasite antigen, HIV infection, *Plasmodium falciparum* infection and dual HIV-malaria infection.

Discussion

In this population based cross sectional study we profiled IgG and IgG subclass immune responses to Q β UB05 and Q β MSP3 in dual HIV-malaria infected people living in two areas of Cameroon differing in malaria transmission intensity. MSP3 and UB05 are asexual blood stage antigens known to be associated with naturally acquired immunity in individuals living in malaria endemic regions (18, 21, 23). However in populations living in malaria endemic regions parasite prevalence rate differ significantly between areas of low and high transmission (35). Since naturally acquired immunity to malaria is dependent upon the cumulative exposure in endemic regions low malaria transmission could limit exposure to parasites which has been

linked to a waning antimalarial immunity and an increase in clinical episodes of malaria in adults (36-38). This in effect can modulate the profiles of target antigen specific IgG and IgG subclass responses that can be achieved especially when the individuals are co-infected with HIV which depletes the immune system. The impact of a high malaria transmission intensity in both dual HIV-malaria infected and double negative individuals was a significant increase in antigen specific IgG irrespective of the targeted antigen type. This might be in line with previous reports suggesting that high levels of antibodies to several blood stage antigens (4-7, 36) were a necessary component of protective immunity to malaria. Whereas this is probably correct for IgG antibody levels specific to Q β UB05 and Q β MSP3 in high malaria transmission areas the comparatively lower antibody levels in low transmission areas indicates that partial immunity to malaria could be waning in these areas due to a reduction in parasite antigen challenge (39) HIV infection resulted to a significant reduction in IgG antibody responses specific to Q β MSP3 in both low (P=0.0001) and high (P=0.0001) malaria transmission areas. In contrast, IgG antibody responses specific to Q β UB05 were not significantly affected by HIV infection (compares S1A&B). Infection with *Plasmodium falciparum* was associated with a significant increase in IgG antibodies specific for both antigens in the two transmission areas. This is especially true when dual HIV-malaria infected individuals are compared with participants infected with HIV alone. Here we demonstrated that even when there was HIV infection the impact of *Plasmodium falciparum* infection was a significant increase in IgG antibodies specific to MSP3 in both low (P=0.0001) and high (P=0.0001) malaria transmission areas (S1E&F). This indicates that asexual blood stage antigens are capable of modulating parasite antigen specific IgG antibody levels in both low and high malaria transmission areas. Such antibody levels are influenced by other factors including malaria and HIV infection.

When responses are examined qualitatively with respect to IgG subclasses and dual HIV-malaria infections differential outcomes are observed. In comparing IgG1 responses to the two antigens Q β MSP3 specific IgG1 was associated with protection against malaria in low transmission areas in contrast to Q β UB05 specific IgG1 responses which were relevant to protection in high malaria transmission areas. In this regards IgG1 responses specific to Q β UB05 in high malaria transmission areas were significantly higher than responses to Q β MSP3 both for dual HIV-malaria infected (P=0.004) and double negative (P=0.0001) individuals. This is probably in line with previous reports which associated increase UB05 specific antibody responses with recovery from clinical malaria (18). Similarly significantly higher IgG2 and IgG3 responses specific to both antigens were associated with protection in low transmission areas. This is in line with previous findings indicating that high levels of malaria parasite antigen specific IgG3 and IgG2 were relevant to protection against all forms of malaria (23, 40). This probably indicates that in malaria endemic regions novel recombinant phages such as Q β UB05 could be more useful for detecting protective levels of antibodies implicated in the control of malaria in both low and high transmission areas. On the other hand the recombinant phage Q β MSP3 would be more relevant for profiling IgG subclass responses in low malaria transmission areas.

The effect of dual HIV-malaria infection was a significant reduction in Q β MSP3 specific IgG1 (P=0.0001), IgG2 (P=0.0001) and IgG3 (P=0.04) responses in low transmission areas. In low transmission areas this significant reduction of Q β MSP3 specific IgG2 together with the cytophilic antibodies IgG1 and IgG3 in dual HIV-malaria infected people could diminish protection from *Plasmodium falciparum* infection and symptomatic illness. In the case of IgG subclass antibodies specific to Q β UB05 such a reduction in dual HIV-malaria infected people might lead to increase morbidity and mortality to malaria in both low and high transmission

areas. This is mainly because parasite antigen specific IgG1 and IgG3 are critical in monocyte mediated antibody-dependent cellular inhibition (ADCI) of *Plasmodium falciparum* which is responsible for killing asexual blood stages. Other reports have also shown that dual HIV-malaria infections escalate episodes of symptomatic malaria (41) or severe disease in both children and adults (42, 43). In endemic regions such individuals due to persistent parasitaemia could serve as reservoirs of malaria parasite thereby sustaining infection in both low and high transmission areas.

In low transmission areas dual HIV-malaria infection resulted to significantly higher Q β MSP3 specific IgG4 responses which are probably the reason why IgG1 and IgG3 responses to Q β MSP3 were significantly lower in this group. On the other hand, IgG4 responses to Q β UB05 were significantly higher in double negative participants relative to dual HIV-malaria infected individuals. In high transmission areas IgG4 responses to both antigens were low which could explain why IgG antibody responses were comparatively high in this area. Since IgG4 is a noncytophilic IgG subclass which may block antibody mediated natural immunity to malaria (23, 32, 44-46) high IgG4 levels tended to be associated with lower IgG1 or IgG3 antibody levels. Overall the antibody responses to both antigens are relatively heterogeneous being certainly influenced by a number of factors including transmission area, ongoing *plasmodium falciparum* infection and coinfection with HIV. Never the less there was a clear indication that MSP3 specific IgG subclass responses were associated with protective immunity to malaria mainly in low transmission areas whilst similar responses to Q β UB05 were related to protection in both low and high malaria transmission areas. This implies that Q β MSP3 might not be suitable as a standalone vaccine in areas differing in transmission intensity. On the other hand antigenicity of UB05 most likely predicts immunity in both low and high transmission areas and could be used

either alone or in combination with other antigens for vaccine studies in areas differing in transmission intensities. Thus understanding immune responses to Q β UB05 and Q β MSP3 could enable the development of efficacious vaccines or commensurate immunotherapeutic strategies suitable for areas differing in malaria transmission intensity.

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References

1. Doolan DL, Dobano C, Baird JK. 2009. Acquired immunity to malaria. *Clinical microbiology reviews*, 22(1): 13-36, Table of Contents.
2. Chan JA, Howell KB, Reiling L, Ataide R, Mackintosh CL, Fowkes FJ, Petter M, Chesson JM, Langer C, Warimwe GM, Duffy MF, Rogerson SJ, Bull PC, Cowman AF, Marsh K, Beeson JG. 2012. Targets of antibodies against *Plasmodium falciparum*-infected erythrocytes in malaria immunity. *The Journal of clinical investigation*, 122(9): 3227-3238.
3. Fowkes FJ, Richards JS, Simpson JA, Beeson JG. 2010. The relationship between anti-merozoite antibodies and incidence of *Plasmodium falciparum* malaria: A systematic review and meta-analysis. *PLoS medicine*, 7(1): e1000218.
4. Richards JS, Stanisic DI, Fowkes FJ, Tavul L, Dabod E, Thompson JK, Kumar S, Chitnis CE, Narum DL, Michon P, Siba PM, Cowman AF, Mueller I, Beeson JG. 2010. Association between naturally acquired antibodies to erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and high-density parasitemia. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 51(8): e50-60.
5. McCarra MB, Ayodo G, Sumba PO, Kazura JW, Moormann AM, Narum DL, John CC. 2011. Antibodies to *Plasmodium falciparum* erythrocyte-binding antigen-175 are associated with protection from clinical malaria. *The Pediatric infectious disease journal*, 30(12): 1037-1042.
6. Daou M, Kouriba B, Ouedraogo N, Diarra I, Arama C, Keita Y, Sissoko S, Ouologuem B, Arama S, Bousema T, Doumbo OK, Sauerwein RW, Scholzen A. 2015. Protection of

Malian children from clinical malaria is associated with recognition of multiple antigens.
Malaria journal, 14: 56.

7. Trieu A, Kayala MA, Burk C, Molina DM, Freilich DA, Richie TL, Baldi P, Felgner PL, Doolan DL. 2011. Sterile protective immunity to malaria is associated with a panel of novel *P. falciparum* antigens. *Molecular and cellular proteomics : MCP*, 10(9): M111007948.
8. Gray JC, Corran PH, Mangia E, Gaunt MW, Li Q, Tetteh KK, Polley SD, Conway DJ, Holder AA, Bacarese-Hamilton T, Riley EM, Crisanti A. 2007. Profiling the antibody immune response against blood stage malaria vaccine candidates. *Clinical chemistry*, 53(7): 1244-1253.
9. Dent AE, Nakajima R, Liang L, Baum E, Moormann AM, Sumba PO, Vulule J, Babineau D, Randall A, Davies DH, Felgner PL, Kazura JW. 2015. Plasmodium falciparum Protein Microarray Antibody Profiles Correlate With Protection From Symptomatic Malaria in Kenya. *The Journal of infectious diseases*, 212(9): 1429-1438.
10. Bai T, Becker M, Gupta A, Strike P, Murphy VJ, Anders RF, Batchelor AH. 2005. Structure of AMA1 from Plasmodium falciparum reveals a clustering of polymorphisms that surround a conserved hydrophobic pocket. *Proceedings of the National Academy of Sciences of the United States of America*, 102(36): 12736-12741.
11. Hodder AN, Crewther PE, Anders RF. 2001. Specificity of the protective antibody response to apical membrane antigen 1. *Infection and immunity*, 69(5): 3286-3294.
12. Miura K, Zhou H, Muratova OV, Orcutt AC, Giersing B, Miller LH, Long CA. 2007. In immunization with Plasmodium falciparum apical membrane antigen 1, the specificity of

- antibodies depends on the species immunized. *Infection and immunity*, 75(12): 5827-5836.
13. Jones TR, Obaldia N, 3rd, Gramzinski RA, Hoffman SL. 2000. Repeated infection of Aotus monkeys with Plasmodium falciparum induces protection against subsequent challenge with homologous and heterologous strains of parasite. *The American journal of tropical medicine and hygiene*, 62(6): 675-680.
14. Skamel C, Aller SG, Bopda Waffo A. 2014. In vitro evolution and affinity-maturation with Coliphage qbeta display. *PloS one*, 9(11): e113069.
15. Waffo AB, Ngu LN, Singleton RL, Egbo T, Simo JL, Sanders CA, Ambada GE, Nji NN, Ngoh AA, Matthews QL, Robertson BK, Tchouangueu TF, Lissom A, Bawage S, Tchadji C, Okoli AS, Esimone CO, Chukwuanukwu R, Mbacham W, Kaptue L, Nchinda GW. 2017. Surface Engineering of Recombinant RNA Coliphage Q β to Display gp41 MembraneProximal External-Region Epitopes from HIV-1. *J Clin Exp Immunol*, 2(1): 1-15.
16. Singh S, Soe S, Mejia JP, 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.
17. Daher LJ, Demanga CG, Prieur E, Perignon JL, Bouharoun-Tayoun H, Druilhe P. 2010. Toward the rational design of a malaria vaccine construct using the MSP3 family as an example: contribution of immunogenicity studies in models. *Infection and immunity*, 78(1): 477-485.

18. Titanji VP, Amambua-Ngwa A, Anong DN, Mbandi SK, Tangie E, Tening I, Yengo R. 2009. Isolation and expression of UB05, a Plasmodium falciparum antigen recognised by antibodies from semi-immune adults in a high transmission endemic area of the Cameroonian rainforest. *Clinical chemistry and laboratory medicine*, 47(9): 1147-1158.
19. Jepsen MP, Jogdand PS, Singh SK, Esen M, Christiansen M, Issifou S, Hounkpatin AB, Ateba-Ngoa U, Kremsner PG, Dziegiel MH, Olesen-Larsen S, Jepsen S, Mordmuller B, Theisen M. 2013. The malaria vaccine candidate GMZ2 elicits functional antibodies in individuals from malaria endemic and non-endemic areas. *The Journal of infectious diseases*, 208(3): 479-488.
20. Bouharoun-Tayoun H, Attanath P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P. 1990. Antibodies that protect humans against Plasmodium falciparum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *The Journal of experimental medicine*, 172(6): 1633-1641.
21. Oeuvray C, Bouharoun-Tayoun H, Gras-Masse H, Bottius E, Kaidoh T, Aikawa M, Filgueira MC, Tartar A, 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.
22. Good MF, Doolan DL. 2010. Malaria vaccine design: immunological considerations. *Immunity*, 33(4): 555-566.
23. Roussillon C, Oeuvray C, Muller-Graf C, Tall A, Rogier C, Trape JF, Theisen M, Balde A, Perignon JL, 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.

24. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB, Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko MS, Niangaly A, Sissoko M, Takala-Harrison S, Lyke KE, Wu Y, Blackwelder WC, Godeaux O, Vekemans J, Dubois MC, Ballou WR, Cohen J, Thompson D, Dube T, Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner DG, Jr., Plowe CV. 2011. A field trial to assess a blood-stage malaria vaccine. *The New England journal of medicine*, 365(11): 1004-1013.
25. Ouattara A, Laurens MB. 2015. Vaccines against malaria. *Clinical infectious diseases* : an official publication of the Infectious Diseases Society of America, 60(6): 930-936.
26. Lusingu JP, Gesase S, Msham S, Francis F, Lemnge M, Seth M, Sembuche S, Rutta A, Minja D, Segeja MD, Bosomprah S, Cousens S, Noor R, Chilengi R, Druilhe P. 2009. Satisfactory safety and immunogenicity of MSP3 malaria vaccine candidate in Tanzanian children aged 12-24 months. *Malaria journal*, 8: 163.
27. Njunda AL, Njumkeng C, Nsagha SD, Assob JC, Kwenti TE. 2016. The prevalence of malaria in people living with HIV in Yaounde, Cameroon. *BMC public health*, 16: 964.
28. Njambe Priso GD, Lissom A, Ngu LN, Nji NN, Tchadji JC, Tchouangueu TF, Ambada GE, Ngane CSS, Dafeu BL, Djukouo L, Nyebe I, Magagoum S, Ngoh AA, Herve OF, Garcia R, Gutierrez A, Okoli AS, Esimone CO, Njiokou F, Park CG, Waffo AB, Nchinda GW. 2018. Filaria specific antibody response profiling in plasma from anti-retroviral naive Loa loa microfilaraemic HIV-1 infected people. *BMC infectious diseases*, 18(1): 160.
29. Ambada GN, Ntsama CE, Nji NN, Ngu LN, Sake CN, Lissom A, Tchouangueu FT, Tchadji J, Sosso M, Etoa FX, Nchinda GW. 2017. Phenotypic characterization of

regulatory T cells from antiretroviral-naïve HIV-1-infected people. *Immunology*, 151(4): 405-416.

30. Sake CS, Ngu L, Ambada G, Chedjou JP, Nji N, Tchadji JC, Lissom A, Tchouangueu TF, Djukouo L, Njambe G, Garcia R, Gutierrez A, Waffo AB, Park CG, Mbacham W, Etoa F-X, Nchinda GW. 2017. The Effect of Antiretroviral Naïve HIV-1 Infection on the Ability of Natural Killer Cells to Produce IFN- γ upon Exposure to *Plasmodium falciparum* -Infected Erythrocytes *Biomed Hub*, 2.
31. Domkam IK, Sonela N, Kamgaing N, Takam PS, Gwom L-C, Michel T, Betilene A, Fokam J, Billong SC, Moukam LV, Etounou TM, Minka CSM, Ndjolo A. 2018. Prevalence and risk factors to HIV-infection amongst health care workers within public and private health facilities in Cameroon. *Pan African Medical Journal*, 29(158).
32. Aucan C, Traore Y, Tall F, Nacro B, Traore-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.
33. Nasr A, Iriemenam NC, Troye-Blomberg M, Giha HA, Balogun HA, Osman OF, Montgomery SM, ElGhazali G, Berzins K. 2007. Fc gamma receptor IIa (CD32) polymorphism and antibody responses to asexual blood-stage antigens of *Plasmodium falciparum* malaria in Sudanese patients. *Scandinavian journal of immunology*, 66(1): 87-96.
34. Ntoumi F, Ekala MT, Makuwa M, Lekoulou F, Mercereau-Puijalon O, Deloron P. 2002. Sick cell trait carriage: imbalanced distribution of IgG subclass antibodies reactive to *Plasmodium falciparum* family-specific MSP2 peptides in serum samples from Gabonese children. *Immunology letters*, 84(1): 9-16.

35. Nkumama IN, O'Meara WP, Osier FHA. 2017. Changes in Malaria Epidemiology in Africa and New Challenges for Elimination. *Trends in parasitology*, 33(2): 128-140.
36. Murungi LM, Kamuyu G, Lowe B, Bejon P, Theisen M, Kinyanjui SM, Marsh K, Osier FH. 2013. A threshold concentration of anti-merozoite antibodies is required for protection from clinical episodes of malaria. *Vaccine*, 31(37): 3936-3942.
37. Bouyou-Akotet MK, Offouga CL, Mawili-Mboumba DP, Essola L, Madoungou B, Kombila M. 2014. Falciparum malaria as an emerging cause of fever in adults living in Gabon, Central Africa. *BioMed research international*, 2014: 351281.
38. Kipanga PN, Omondi D, Mireji PO, Sawa P, Masiga DK, Villinger J. 2014. High-resolution melting analysis reveals low Plasmodium parasitaemia infections among microscopically negative febrile patients in western Kenya. *Malaria journal*, 13: 429.
39. Pinkevych M, Petravic J, Chelimo K, Kazura JW, Moormann AM, Davenport MP. 2012. The dynamics of naturally acquired immunity to Plasmodium falciparum infection. *PLoS computational biology*, 8(10): e1002729.
40. Giha HA, Nasr A, Iriemenam NC, Balogun HA, Arnot D, Theander TG, Troye-Blomberg M, Berzins K, ElGhazali G. 2010. Age-dependent association between IgG2 and IgG3 subclasses to Pf332-C231 antigen and protection from malaria, and induction of protective antibodies by sub-patent malaria infections, in Daraweesh. *Vaccine*, 28(7): 1732-1739.
41. Kanya MR, Gasasira AF, Yeka A, Bakyaite N, Nsomba SL, Francis D, Rosenthal PJ, Dorsey G, Havlir D. 2006. Effect of HIV-1 infection on antimalarial treatment outcomes in Uganda: a population-based study. *The Journal of infectious diseases*, 193(1): 9-15.

42. Saracino A, Nacarapa EA, da Costa Massinga EA, Martinelli D, Scacchetti M, de Oliveira C, Antonich A, Galloni D, Ferro JJ, Macome CA. 2012. Prevalence and clinical features of HIV and malaria co-infection in hospitalized adults in Beira, Mozambique. *Malaria journal*, 11: 241.
43. Tay SC, Badu K, Mensah AA, Gbedema SY. 2015. The prevalence of malaria among HIV seropositive individuals and the impact of the co- infection on their hemoglobin levels. *Annals of clinical microbiology and antimicrobials*, 14: 10.
44. Stanistic DI, Richards JS, McCallum FJ, Michon P, King CL, Schoepflin S, Gilson PR, Murphy VJ, Anders RF, Mueller I, Beeson JG. 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.
45. Mewono L, Matondo Maya DW, Matsiegui PB, Agnandji ST, Kendjo E, Barondi F, Issifou S, Kremsner PG, Mavoungou E. 2008. Interleukin-21 is associated with IgG1 and IgG3 antibodies to erythrocyte-binding antigen-175 peptide 4 of *Plasmodium falciparum* in Gabonese children with acute *falciparum* malaria. *European cytokine network*, 19(1): 30-36.
46. Bouharoun-Tayoun H, Oeuvaray C, Lunel F, Druilhe P. 1995. Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. *The Journal of experimental medicine*, 182(2): 409-418.

Figure Legends

Figure 1: specific IgG responses to recombinant phages Q β MSP3 and Q β UB05 with respect to dual HIV-Malaria infections in individuals living in low and high malaria transmission areas

Comparison of IgG antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG specific responses to the recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG responses in the two transmission areas. IgG responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals positive for *Plasmodium falciparum* in both transmission areas.

Figure 2: IgG1 specific response to recombinant phages Q β MSP3 and Q β UB05 with to dual HIV-Malaria infections in individuals living in low and high malaria transmission areas

Comparison of IgG1 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG1 responses specific to the recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG1 responses in the two transmission areas. IgG1 responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals positive for *Plasmodium falciparum* in both transmission areas.

Figure 3: IgG2 responses specific to the recombinant phages Q β MSP3 and Q β UB05 with respect to dual HIV-Malaria infection in individuals living in low and high malaria transmission areas

Comparison of IgG2 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG2 responses specific to the recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG2 responses in the two transmission areas. IgG2 responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals positive for *Plasmodium falciparum* in both transmission areas.

Figure 4: IgG3 specific response to the recombinant phages Q β MSP3 and Q β UB05 with respect to dual HIV-Malaria infection in individuals living in low and high malaria transmission areas

Comparison of IgG3 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG3 responses specific to the recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG3 responses in the two transmission areas. IgG3 responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals positive for *Plasmodium falciparum* in both transmission areas.

Figure 5: IgG4 response specific to the recombinant phages Q β MSP3 and Q β UB05 with respect to dual HIV-Malaria infection in individuals living in low and high malaria transmission areas

Comparison of IgG4 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG4 responses specific to the

651 recombinant phages QβMSP3 (A) and QβUB05 (B) in low and high transmission areas. Effect
652 of HIV infection on QβMSP3 (C) and QβUB05 (D) specific IgG4 responses in the two
653 transmission areas. IgG responses specific to QβMSP3 (E) and QβUB05 (F) in individuals
654 positive for *Plasmodium falciparum* in both transmission areas.

655 **Table 1: Study population characteristics**

Variables	HIV-/Mal- (n=62)		HIV-/Mal+ (n= 59)		HIV+/Mal- (n= 62)		HIV+/Mal+ (n= 62)	
Gender	Male	Female	Male	Female	Male	Female	Male	Female
Participants (%)	30 (48)	32 (52)	30 (51)	29 (49)	44 (71)	18 (29)	44 (71)	18 (29)
Median Age (IQR)	30 (22- 34.5)	35.5 (22.75-48.75)	32 (23–46.25)	29 (26-45)	46 (36.25-58.25)	36 (30-46.25)	38 (31 - 46)	42 (33-52)
Median CD4 count (cell/mm3)	N/A		N/A		480.6 (IQR : 270.5-635)		459.6 (IQR : 241.0–594.8)	

656 **N/A = Not Applicable, IQR = interquartile range, Mal+= malaria positive**

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Figures

Figure 1

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