Published in final edited form as:
Int J Parasitol. 2017 February 01; 47(2-3): 99–104. doi:10.1016/j.ijpara.2016.06.002.

Recent insights into humoral immunity targeting *Plasmodium falciparum* and *Plasmodium vivax* malaria

Michelle J. Boyle[a,b,*], **Linda Reiling**[a], **Faith H. Osier**[c], **Freya J.I. Fowkes**[a,d,e,f]

[a]Burnet Institute for Medical Research and Public Health, Melbourne, Victoria 3004, Australia

[b]Menzies School of Medical Research, Darwin, Northern Territory 0810, Australia

[c]KEMRI Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

[d]Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria 3010, Australia

[e]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria 3004, Australia

[f]Department of Infectious Diseases, Monash University, Melbourne, Victoria 3004, Australia

Abstract

Recent efforts in malaria control have led to marked reductions in malaria incidence. However, new strategies are needed to sustain malaria elimination and eradication and achieve the World Health Organization goal of a malaria-free world. The development of highly effective vaccines would contribute to this goal and would be facilitated by a comprehensive understanding of humoral immune responses targeting *Plasmodium falciparum* and *Plasmodium vivax* malaria. New tools are required to facilitate the identification of vaccine candidates and the development of vaccines that induce functional and protective immunity. Here we discuss recent published findings, and unpublished work presented at the 2016 Molecular Approaches to Malaria conference, that highlight advancements in understanding humoral immune responses in the context of vaccine development. Highlights include the increased application of ‘omics’ and ‘Big data’ platforms to identify vaccine candidates, and the identification of novel functions of antibody responses that mediate protection. The application of these strategies and a global approach will increase the likelihood of rapid development of highly efficacious vaccines.

Keywords

Malaria; Vaccine; Immunity; *Plasmodium falciparum*; *Plasmodium vivax*; Microarray; Complement; Phagocytosis

[*]Corresponding author at: Burnet Institute for Medical Research and Public Health, Melbourne, Victoria 3004, Australia. mboyle@burnet.edu.au (M.J. Boyle).

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

1 Introduction

Since 2000 there have been unparalleled increases in malaria control activities and re-invigorated goals of malaria elimination; there have been substantial increases in bed net usage, indoor residual spraying, chemoprophylaxis and the utilisation of highly efficacious artemisinin derivatives for the treatment of clinical malaria (World Health Organization, 2015). Consequently, infection prevalence has halved and the incidence of clinical disease and malaria mortality has dramatically reduced by more than 40% (Bhatt et al., 2015; World Health Organization, 2015). While the largest reductions have been primarily seen in areas of high stable transmission in Africa, substantial reductions have also been seen in areas of relatively low transmission in Asia. Despite these gains, malaria, caused predominantly by *Plasmodium falciparum* and *Plasmodium vivax,* remains a significant global public health problem causing approximately 200 million clinical cases and half a million deaths in 2015 (World Health Organization, 2015).

Reductions in malaria transmission are accompanied by changes in the epidemiology of malaria. In areas of stable medium–high transmission, the frequency of mild and severe malaria is highest in young children less than 5 years of age (reviewed in (Marsh and Kinyanjui, 2006; Carneiro et al., 2010)), whereas in areas with low transmission, severe malaria continues to occur in older children and adults (Snow et al., 1997; Carneiro et al., 2010). Decreases in transmission are often accompanied by a shift in the peak incidence of mild and severe malaria to later in childhood or adulthood, or rebounds of malaria in previously eliminated areas (Ceesay et al., 2010; Brasseur et al., 2011; Trape et al., 2011; Griffin et al., 2014). These observations have been attributed to declining naturally acquired immunity to malaria, which develops after repeated exposure to malaria in an age-dependent manner (Marsh and Kinyanjui, 2006). Anti-malarial antibody levels have reflected declines in malaria transmission in longitudinal studies spanning less than 5 years (Migot et al., 1993; Ceesay et al., 2010) and in serial cross-sectional studies 10 years apart (Diop et al., 2014). Recent longitudinal sero-epidemiological studies spanning decades have investigated how immunity to malaria changes in areas experiencing substantial reductions in malaria transmission. Recent studies have demonstrated considerable reductions in anti-merozoite immunity over a 10 year period in an area transitioning from low to very low transmission (Ataíde, R. and Fowkes, F., Burnet Institute, Australia, personal communication). In Kenya, which has transitioned from high to low transmission over the past 14 years, studies have demonstrated that in 2000 the magnitude and functional activity of antibodies against merozoite antigens, as quantified by the capacity of antibodies to fix complement to merozoites antigens, or to mediate opsonic phagocytosis, were associated with protection against clinical malaria. However by 2014, after a significant decline in malaria transmission and an increase in the median age of clinical presentation, anti-merozoite immunity had declined to below protective thresholds (Osier, F. and Marsh, K., KEMRI-Centre for Geographic Medicine Research-Coast, Kenya, personal communication). These studies highlight the importance of understanding how immunity to malaria is acquired and maintained over time in populations transitioning from high to low to no malaria transmission. The changes in sero-epidemiology with changing transmission emphasise the need to identify new targets of protective immunity and to understand functional

Europe PMC Funders Author Manuscripts

mechanisms across diverse and changing transmission settings. Further, as studies have used only a few antigens which have not been comprehensively validated either as markers of exposure or as being associated with protection, more studies are needed to validate large numbers of antigens.

2 New strategies to identify targets of *P. falciparum* and *P. vivax* immunity

2.1 'Big data' – large screenings to identify vaccine candidates

Although antibodies have been known to be key components of acquired immunity against *P. falciparum* malaria for over 50 years (Cohen et al., 1961), it still remains unclear which of the thousands of parasite antigens presented to the human immune system induce protective antibodies and should thus be prioritised for malaria vaccine development. Prior to the completion of the genome of *P. falciparum*, a small number of antigens dominated studies aimed at identifying the targets of protective immunity (Fowkes et al., 2010). Now, more than 10 years into the post-genomic era, large panels of antigens and "omic" data sets are being applied to the same question (Davies et al., 2015), with the anticipation that these large-scale screening approaches will rapidly advance the development of highly efficacious malaria vaccines.

Two main strategies have been applied to the search for the targets of protective antibodies. The principle underlying the first approach is an unbiased proteome-wide analysis that has been applied to antigen discovery to guide vaccine development for multiple pathogens (Davies et al., 2005, 2015). A rapid and high-throughput *Escherichia coli* cell-free transcription/translation system is used to express proteins on a genome-wide scale. For malaria, given the size of the proteome, this has required a degree of down-selection based on a range of criteria including stage-specific protein expression and sub-cellular localisation (Doolan et al., 2008; Crompton et al., 2010a; Finney et al., 2014). In subsequent studies, the panels are further down-selected based on the sero-reactivity observed in previous studies (Nnedu et al., 2011; Dent et al., 2015; Helb et al., 2015). The second approach is more directly hypothesis driven, with careful selection of antigens that are either expressed on the merozoite surface or associated with it, or expressed in the apical organelles and secreted at the time of erythrocyte invasion. These platforms include antigens such as the parasite erythrocyte invasion ligands (erythrocyte binding antigens, EBAs) and the *P. falciparum* reticulocyte-binding homologues (PfRh) families of proteins, and are underpinned by the fact that these antigens are all exposed to the host immune system and thus biologically plausible candidates (Richards et al., 2013; Osier et al., 2014b). Individual recombinant proteins can be printed onto microarrays to increase throughput for analysis in sero-epidemiological studies. A bespoke protein microarray containing predominantly merozoite proteins has recently been developed (Tetteh, K. and Drakeley, C., London School of Hygiene and Tropical Medicine, UK, personal communication). In silico tools were used to identify key regions within these proteins that are then expressed in an *E. coli* system before printing onto microarrays. This approach resulted in the expression of regions most likely to be targets of acquired antibodies, avoids other regions such as trans-membrane domains and increases the solubility of expressed proteins. Early results have used this platform to identify a number of novel immune-reactive targets that may be priorities as

putative vaccine candidates (Tetteh, K. and Drakeley, C., London School of Hygiene and Tropical Medicine, UK, personal communication). Similarly, antigens on the surface of the infected erythrocyte are prime targets of protective immunity (Marsh and Howard, 1986; Bull and Marsh, 2002; Chan et al., 2012, 2016) and have been investigated using large scale screening approaches (Barry et al., 2011). In an extension of this approach, 543 *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) variants were analysed in a cohort of young children from Papua New Guinea and it was found that antibodies to specific variants were acquired early in life and were associated with functional immunity against clinical and severe malaria, providing strong candidate biomarkers for protection and possible vaccine candidates (Tessema, S. and Barry, A., Walter and Eliza Hall Institute, Australia, personal communication). While it is thought that many antibodies targeting surface antigens are strain-specific, recent published results indicate that broadly cross-reactive antibodies targeting RIFIN family proteins can be generated, demonstrating the existence of conserved epitopes that may be suitable candidates for malaria vaccine development (Tan et al., 2016).

2.2 Identifying vaccine candidates for *P. vivax* malaria

For *P. vivax*, fewer studies have been done and the data in support of any given target of protective immunity needs strengthening (Cutts et al., 2014; Finney et al., 2014; Hostetler et al., 2015). Additional studies on *P. vivax* immunity were therefore timely, given the burden of disease particularly in central and southeastern Asia, and the more recent appreciation that infection with this species of *Plasmodium* can also result in severe clinical syndromes (Anstey et al., 2012). Studies on responses to a relatively small panel of *P. vivax* antigens but against a large number of patient samples in a low transmission setting in Thailand utilising multiplexed bead-based approaches have been undertaken. Antibodies to pre-erythrocytic antigens *P. vivax* circumsporozoite protein (CSP), thrombospondin-related anonymous protein (TRAP) and cell-traversal protein for ookinetes and sporozoites (CelTOS) were detected and moreover maintained over 1 year in the apparent absence of new *P. vivax* infections (Longley, R. and Sattabongkot, J., Walter and Eliza Hall Institute, Australia and Mahidol University, Thailand, personal communication), confirming previous findings in smaller sero-epidemiological studies within the same geographical region (Longley et al., 2015). Reticulocyte binding proteins in *P. vivax* have been implicated in invasion and are plausible biological targets. Antibodies against this family of proteins were found to be mainly of the IgG1 and IgG3 subclasses, and were associated with a reduced risk of symptomatic malaria as well as high density *P. vivax* infections, identifying these as promising vaccine candidates (He, W.Q. and Tham, W.H., Walter and Eliza Hall Institute, Australia, personal communication). However, a recent large unbiased screening study of 34 exodomain *P. vivax* merozoite proteins identified that a previously un-studied novel hypothetical protein was strongly associated with reduced risk of clinical malaria (França et al., 2016), reiterating the need to investigate a large number of proteins, including those outside the ‘usual suspects’ which have dominated previous work.

2.3 Working together – ‘Big data’ and the future of research

With multiple groups using ‘Big data’ and ‘omics’ approaches to address the central problem of identifying appropriate vaccine candidates against both *P. falciparum* and *P. vivax* malaria, it is essential that analysis and data sharing platforms develop in line with

data generation. One example of this is the recent launch of an additional platform on PlasmoDB (*Plasmodium* genomic resource, http://plasmodb.org/plasmo/.org), to share antibody response data generated via micro-array platforms from multiple US National Institutes of Health (NIH) funded International Centers of Excellence for Malaria Research studies. Here, data from Amazonia, Malawi, Uganda, South Pacific, southeastern Asia and southern African field sites can be analysed based on a variety of epidemiological, environmental and clinical parameters by the wider research community. Spanning multiple transmission settings, data sharing such as this is an important step forward in identifying vaccine candidates that are valid in settings of changing transmission intensities. These platforms have implications for not only vaccine candidate identification, but also tracking changes in transmission and evaluating control strategies (King et al., 2015).

3 New assays to identify functional antibodies and protective mechanisms

3.1 Complement fixing antibodies targeting blood stages and beyond

Together with difficulties in vaccine candidate identification, a key issue that hampers the development of an effective antimalarial vaccine is the lack of knowledge about mechanisms of acquired immunity. In order to progress, it is necessary to characterise potential effector mechanisms of antibodies that are associated with protection from malaria, so that vaccines can be developed that induce a functional and protective antibody response. Little is known about how antibodies mediate protection from malaria, but it is thought they act by limiting blood stage replication to avoid high-density parasitaemia (reviewed in Marsh and Kinyanjui, 2006; Mueller et al., 2013; Chan et al., 2014; Beeson et al., 2016). Functional antibody studies to date have focused on a limited number of assays, with the most widely used being growth inhibition in classical growth inhibition assays (Persson et al., 2006; Wilson et al., 2010). These assays quantify antibodies that inhibit parasite erythrocyte invasion by direct disruption of protein–ligand interactions. For example, data by Katherine Wright and colleagues from the University of Oxford, UK shows that antibodies that directly disrupt the binding of the essential parasite invasion ligand PfRh5 (Crosnier et al., 2011) to its erythrocyte receptor have strong growth inhibitory activity (Douglas et al., 2014; Wright et al., 2014). High-throughput assays to measure directly blocking antibodies have also been developed, which are suitable for measuring receptor-ligand binding inhibitory antibodies, in large cohort studies on both *P. falciparum* (Irani et al., 2015) and *P. vivax* malaria (King et al., 2008). However, while affinity-purified antibodies directed against merozoite antigens (Egan et al., 1999; Reiling et al., 2012; Tran et al., 2014) as well as whole sera samples (Nkuo and Deas, 1988; Shi et al., 1999; Wilson et al., 2011) have been shown to limit parasite growth in vitro, the use of serum samples from cohort studies has failed to show consistent or strong protective associations (Marsh et al., 1989; Dent et al., 2008; McCallum et al., 2008; Crompton et al., 2010b). The presence of multiple and redundant invasion pathways, and the parasite's ability to switch between these and evade invasion inhibitory antibodies have further limited the use of these assays (Persson et al., 2008, 2013; Beeson et al., 2016). The recent development of an invasion inhibition assay using purified merozoites offers the possibility of measuring true invasion inhibition as opposed to growth inhibition (Boyle et al., 2010, 2013). Recently, this method has been used to investigate the function of

naturally acquired and vaccine-induced antibodies in inhibiting invasion and showed that many antibodies targeting the merozoite required complement to inhibit invasion (Boyle et al., 2015). Antibodies fixed the first factor of the classical complement cascade, C1q, on the surface of merozoites, leading to lysis and invasion inhibition (Boyle et al., 2015). This finding is consistent with protective IgG responses against *P. falciparum* antigens being predominantly of the cytophilic IgG1 and IgG3 subtypes (Taylor et al., 1998; Polley et al., 2006; Roussilhon et al., 2007; Stanisic et al., 2009; Reiling et al., 2010; Richards et al., 2010), both of which have high complement fixation capacity.

Following the observation that antibodies bound C1q to inhibit invasion, an ELISA based C1q deposition assay was developed, allowing the examination of cohort sera samples for antibody-dependent fixation of C1q to the merozoite surface, and showed very strong associations with protection from clinical malaria (Boyle et al., 2015). The same ELISA-based assay now allows the dissection of this protective effect and the assessment of protective associations with individual merozoite antigens. Follow-up studies investigated the capacity of individual sera from a longitudinal cohort study to bind C1q on a variety of merozoite surface antigens; results suggest that the ability of acquired antibodies to fix C1q to a broad range of merozoite surface antibodies is strongly associated with protection (Reiling, L. and Beeson, J., Burnet Institute, Australia, personal communication). Encouragingly, antibodies induced by vaccination of human volunteers with recombinant merozoite surface protein 2 (MSP2) (McCarthy et al., 2011) generated antibodies that inhibited invasion in a complement-dependent manner in vitro (Boyle et al., 2015), and these antibodies were shown to specifically fix C1q on MSP2. The role of antibody-complement interactions might also be important in immunity against other parasite stages. For example, antibodies acquired by pregnant women promoted the deposition of complement components on the surface of placental-binding infected red blood cells and the fixation of complement resulted in greatly enhanced phagocytosis by monocytes. (Opi, D.H. and Beeson, J., Burnet Institute, Australia, personal communication). It also appears that naturally acquired antibodies and those generated by vaccination of animals with the major sporozoite antigen, CSP, fix complement to recombinant protein and sporozoites, suggesting a potential role for antibody-complement fixation in immunity against pre-erythrocytic stages (Kurtovic, L. and Beeson, J., Burnet Institute, Australia, personal communication).

3.2 Antibodies that interact with cells to mediate protection

Together with interacting with complement, cytophilic antibodies also interact with Fc Receptors (FcR) on effector cells. In the antibody-dependent cellular inhibition assay (ADCI), antibodies against parasite antigens have been shown to interact with monocytes via FccRII, resulting in the parasite's uptake and the release of soluble factors that inhibit parasite growth (Bouharoun-Tayoun et al., 1995). This assay has been employed to assess ADCI activity in post vaccination samples, and found vaccine induced antibodies were able to trigger ADCI (McCarthy et al., 2011; Jepsen et al., 2013). Similarly, the uptake of opsonised parasites by neutrophils has been described which results in the respiratory burst that inhibits parasite replication (antibody-dependent respiratory burst, ADRB) (Kumaratilake et al., 1992). In a high-throughput assay, ADRB was found to be associated with protection from clinical malaria (Joos et al., 2010), and ADRB antibodies can be

induced by vaccination (Joos et al., 2015). Interestingly, both ADCI and ADRB were shown to be significantly more efficient after the uptake of opsonised merozoites compared with parasitised red blood cells (Khusmith et al., 1982; Joos et al., 2010). Optimised assays for measuring phagocytosis of both merozoites and parasitised RBCs have been reported (Ataide et al., 2010; Hill et al., 2012). Importantly, protective associations for monocytemediated, opsonic phagocytosis induced by antibodies against the merozoite surface were recently employed in assessing antibody function in longitudinal cohort studies from Papua New Guinea (Hill et al., 2013) and Kenya (Osier et al., 2014a). These studies used a flow-cytometry based assay developed to assess the magnitude of phagocytosis mediated by naturally acquired antibodies against merozoites. It has been suggested that MSP2 vaccineinduced antibodies from rabbits, as well as from human vaccination trials (McCarthy et al., 2011), is revealing how opsonic phagocytosis of merozoites is mediated through interacting with specific Fcc-receptors on phagocytic cells. Blocking the inhibitory FcR with blocking antibodies enhanced phagocytosis, while mutation of the Fc-region of human IgG inhibited phagocytosis (Feng, G. and Beeson, J., Burnet Institute, Australia, personal communication). These approaches to explore the opsonic activity of merozoite are now being applied to large cohort studies, in a diversity of transmission settings to demonstrate how functional opsonic activity varies according to transmission. Preliminary studies indicate that functional antibody responses vary significantly across transmission settings and set a base line measure to understand how magnitudes of functional antibodies change with decreasing transmission and what that might mean for acquired protective immunity (O'Flaherty, K. and Fowkes, F., Burnet Institute, Australia, personal communication).

3.3 Applying new assays to understand protection and for vaccine development

It is exciting to see the number of tools and assays to assess antibody-mediated functional immunity growing. From data gathered to date it seems that antibodies against different antigens may act by very different modes of action, and that protective immunity may well arise from the complex interplay of different effector mechanisms. It is therefore important to comprehensively assess correlates of protection for each antigen individually, in order to avoid missing important information. For example, MSP2 proved to be an effective part in the combination B vaccine trial, but antibodies failed to consistently show any growth inhibition in traditional assays (Genton et al., 2002; Flück et al., 2004). Recently developed approaches such as the C1q complement deposition assay (Reiling, L. and Beeson, J., Burnet Institute, Australia, personal communication), complement-dependent invasion inhibition assay (Boyle et al., 2015) and the opsonic phagocytosis assays ((Osier et al., 2014a); Feng, G. and Beeson, J., Burnet Institute, Australia, personal communication) are revealing the role of MSP2 vaccine-induced antibodies in mediating immunity. Further, antibodies with strong growth inhibition properties need to be tested in additional assays as growth inhibition per se has traditionally failed to consistently show protective associations. In the future, functional assays need to be standardised in order to produce reliable and comparable results.

4 Conclusions and future directions

While the most advanced anti-malarial vaccine, RTS,S, has recently completed Phase 3 clinical trials (RTS,S Clinical Trials Partnership, 2015), the low efficacy, safety concerns and feasibility of implementation of this vaccine has led to the World Health Organization concluding that it is not appropriate for widespread usage in its current form without further assessment (World Health Organization Secretariat, 2015). As the research community strives towards the development of improved and highly effective vaccines, the use of multiple approaches and collaborative research across centres becomes paramount. With the continued development of 'omics' and 'Big data' approaches, increased data sharing improves the chances of timely identification of the most likely vaccine candidates suitable across diverse transmission settings. To ensure that vaccine candidates are assessed appropriately, it is of utmost importance that our understanding of the key mechanisms of antibody function against specific antigens continues to improve, and that assays are developed for the screening of functional antibodies in cohort studies and for vaccine evaluation. In combination, it is hoped that these approaches will lead to the development of vaccine approaches to add to the current strategies for malaria control, and contribute to the current World Health Organization goal of a malaria-free world.

Acknowledgments

This work is funded by the National Health and Medical Research Council, Australia (https://www.nhmrc.gov.au/) Early-Career Fellowship (MJB), and the Australian Research Council (future fellowship to FJIF), Burnet Institute, Australia is supported by the National Health and Medical Research Council Australia Infrastructure for Research Institutes Support Scheme and by the Victorian State Government Operational Infrastructure Support, Australia. FHO is supported by the Wellcome Trust (089833) and an African Research Leader (ARL) Award that is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement, and is also part of the EDCTP2 program supported by the European Union. The funders had no role in preparation of the manuscript. This review is published with permission from the Director of the Kenyan Medical Research Institute (KEMRI).

References

Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. *Plasmodium vivax*: clinical spectrum, risk factors and pathogenesis. Adv Parasitol. 2012; 80:151–201. DOI: 10.1016/B978-0-12-397900-1.00003-7 [PubMed: 23199488]

Ataide R, Hasang W, Wilson DW, Beeson JG, Mwapasa V, Molyneux ME, Meshnick SR, Rogerson SJ. Using an improved phagocytosis assay to evaluate the effect of HIV on specific antibodies to pregnancy-associated malaria. PLoS One. 2010; 5:e10807.doi: 10.1371/journal.pone.0010807 [PubMed: 20520838]

Barry AE, Trieu A, Fowkes FJI, Pablo J, Kalantari-Dehaghi M, Jasinskas A, Tan X, Kayala MA, Tavul L, Siba PM, Day KP, et al. The stability and complexity of antibody responses to the major surface antigen of *Plasmodium falciparum* are associated with age in a malaria endemic area. Mol Cell Proteomics. 2011; 10(M111)doi: 10.1074/mcp.M111.008326

Beeson JG, Drew DR, Boyle MJ, Feng G, Fowkes FJI, Richards JS. Merozoite surface proteins in red blood cell invasion, immunity and vaccines against malaria. FEMS Microbiol Rev. 2016; 40:343–372. DOI: 10.1093/femsre/fuw001 [PubMed: 26833236]

Bhatt S, Weiss DJ, Mappin B, Dalrymple U, Cameron E, Bisanzio D, Smith DL, Moyes CL, Tatem AJ, Lynch M, Fergus CA, et al. Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017. Elife. 2015; 4doi: 10.7554/eLife.09672

Bouharoun-Tayoun H, Oeuvray C, Lunel F, Druilhe P. Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. J Exp Med. 1995; 182:409–418. [PubMed: 7629503]

Boyle MJ, Wilson DW, Richards JS, Riglar DT, Tetteh KKA, Conway DJ, Ralph SA, Baum J, Beeson JG. Isolation of viable *Plasmodium falciparum* merozoites to define erythrocyte invasion events and advance vaccine and drug development. Proc Natl Acad Sci USA. 2010; 107:14378–14383. DOI: 10.1073/pnas.1009198107 [PubMed: 20660744]

Boyle MJ, Wilson DW, Beeson JG. New approaches to studying *Plasmodium falciparum* merozoite invasion and insights into invasion biology. Int J Parasitol. 2013; 43:1–10. DOI: 10.1016/j.ijpara.2012.11.002 [PubMed: 23220090]

Boyle MJ, Reiling L, Feng G, Langer C, Osier FH, Aspeling-Jones H, Cheng YS, Stubbs J, Tetteh KKA, Conway DJ, McCarthy JS, et al. Human antibodies fix complement to inhibit *Plasmodium falciparum* invasion of erythrocytes and are associated with protection against malaria. Immunity. 2015; 42:580–590. DOI: 10.1016/j.immuni.2015.02.012 [PubMed: 25786180]

Brasseur P, Badiane M, Cisse M, Agnamey P, Vaillant MT, Olliaro PL. Changing patterns of malaria during 1996–2010 in an area of moderate transmission in southern Senegal. Malar J. 2011; 10:203.doi: 10.1186/1475-2875-10-203 [PubMed: 21787420]

Bull PC, Marsh K. The role of antibodies to *Plasmodium falciparum*-infected-erythrocyte surface antigens in naturally acquired immunity to malaria. Trends Microbiol. 2002; 10:55–58. [PubMed: 11827798]

Carneiro I, Roca-Feltrer A, Griffin JT, Smith L, Tanner M, Schellenberg JA, Greenwood B, Schellenberg D. Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: a systematic review and pooled analysis. PLoS One. 2010; 5:e8988.doi: 10.1371/journal.pone.0008988 [PubMed: 20126547]

Ceesay SJ, Casals-Pascual C, Nwakanma DC, Walther M, Gomez-Escobar N, Fulford AJC, Takem EN, Nogaro S, Bojang KA, Corrah T, Jaye MC, et al. Continued decline of malaria in The Gambia with implications for elimination. PLoS One. 2010; 5:e12242.doi: 10.1371/journal.pone.0012242 [PubMed: 20805878]

Chan J-A, Howell KB, Reiling L, Ataide R, Mackintosh CL, Fowkes FJI, Petter M, Chesson JM, Langer C, Warimwe GM, Duffy MF, et al. Targets of antibodies against *Plasmodium falciparum*-infected erythrocytes in malaria immunity. J Clin Invest. 2012; 122:3227–3238. DOI: 10.1172/JCI62182 [PubMed: 22850879]

Chan J-A, Fowkes FJI, Beeson JG. Surface antigens of *Plasmodium falciparum*-infected erythrocytes as immune targets and malaria vaccine candidates. Cell Mol Life Sci. 2014; doi: 10.1007/s00018-014-1614-3

Chan J-A, Howell KB, Langer C, Maier AG, Hasang W, Rogerson SJ, Petter M, Chesson J, Stanisic DI, Duffy MF, Cooke BM, et al. A single point in protein trafficking by *Plasmodium falciparum* determines the expression of major antigens on the surface of infected erythrocytes targeted by human antibodies. Cell Mol Life Sci. 2016; doi: 10.1007/s00018-016-2267-1

Cohen S, McGregor A, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature. 1961; 192:733–737. [PubMed: 13880318]

Crompton PD, Kayala MA, Traore B, Kayentao K, Ongoiba A, Weiss GE, Molina DM, Burk CR, Waisberg M, Jasinskas A, Tan X, et al. A prospective analysis of the Ab response to *Plasmodium falciparum* before and after a malaria season by protein microarray. Proc Natl Acad Sci USA. 2010a; 107:6958–6963. DOI: 10.1073/pnas.1001323107 [PubMed: 20351286]

Crompton PD, Miura K, Traore B, Kayentao K, Ongoiba A, Weiss G, Doumbo S, Doumtabe D, Kone Y, Huang C-Y, Doumbo OK, et al. In vitro growth-inhibitory activity and malaria risk in a cohort study in mali. Infect Immun. 2010b; 78:737–745. DOI: 10.1128/IAI.00960-09 [PubMed: 19917712]

Crosnier C, Bustamante LY, Bartholdson SJ, Bei AK, Theron M, Uchikawa M, Mboup S, Ndir O, Kwiatkowski DP, Duraisingh MT, Rayner JC, et al. Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. Nature. 2011; 480:534–537. DOI: 10.1038/nature10606 [PubMed: 22080952]

Cutts JC, Powell R, Agius PA, Beeson JG, Simpson JA, Fowkes FJI. Immunological markers of *Plasmodium vivax* exposure and immunity: a systematic review and meta-analysis. BMC Med. 2014; 12:150.doi: 10.1186/s12916-014-0150-1 [PubMed: 25199532]

Davies DH, Liang X, Hernandez JE, Randall A, Hirst S, Mu Y, Romero KM, Nguyen TT, Kalantari-Dehaghi M, Crotty S, Baldi P, et al. Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. Proc Natl Acad Sci USA. 2005; 102:547–552. DOI: 10.1073/pnas.0408782102 [PubMed: 15647345]

Davies DH, Duffy P, Bodmer J-L, Felgner PL, Doolan DL. Large screen approaches to identify novel malaria vaccine candidates. Vaccine. 2015; 33:7496–7505. DOI: 10.1016/j.vaccine.2015.09.059 [PubMed: 26428458]

Dent AE, Bergmann-Leitner ES, Wilson DW, Tisch DJ, Kimmel R, Vulule J, Sumba PO, Beeson JG, Angov E, Moormann AM, Kazura JW. Antibody-mediated growth inhibition of *Plasmodium falciparum*: relationship to age and protection from parasitemia in Kenyan children and adults. PLoS One. 2008; 3:e3557.doi: 10.1371/journal.pone.0003557 [PubMed: 18958285]

Dent AE, Nakajima R, Liang L, Baum E, Moormann AM, Sumba PO, Vulule J, Babineau D, Randall A, Davies DH, Felgner PL, et al. *Plasmodium falciparum* protein microarray antibody profiles correlate with protection from symptomatic malaria in Kenya. J Infect Dis. 2015; 212:1429–1438. DOI: 10.1093/infdis/jiv224 [PubMed: 25883384]

Diop F, Richard V, Diouf B, Sokhna C, Diagne N, Trape J-F, Faye MM, Tall A, Diop G, Balde AT. Dramatic declines in seropositivity as determined with crude extracts of *Plasmodium falciparum* schizonts between 2000 and 2010 in Dielmo and Ndiop, Senegal. Malar J. 2014; 13:83.doi: 10.1186/1475-2875-13-83 [PubMed: 24602390]

Doolan DL, Mu Y, Unal B, Sundaresh S, Hirst S, Valdez C, Randall A, Molina D, Liang X, Freilich DA, Oloo JA, et al. Profiling humoral immune responses to *P. falciparum* infection with protein microarrays. Proteomics. 2008; 8:4680–4694. DOI: 10.1002/pmic.200800194 [PubMed: 18937256]

Douglas AD, Williams AR, Knuepfer E, Illingworth JJ, Furze JM, Crosnier C, Choudhary P, Bustamante LY, Zakutansky SE, Awuah DK, Alanine DGW, et al. Neutralization of *Plasmodium falciparum* merozoites by antibodies against PfRH5. J Immunol. 2014; 192:245–258. DOI: 10.4049/jimmunol.1302045 [PubMed: 24293631]

Egan AF, Burghaus P, Druilhe P, Holder AA, Riley EM. Human antibodies to the 19kDa C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 inhibit parasite growth in vitro. Parasite Immunol. 1999; 21:133–139. [PubMed: 10205793]

Finney OC, Danziger SA, Molina DM, Vignali M, Takagi A, Ji M, Stanisic DI, Siba PM, Liang X, Aitchison JD, Mueller I, et al. Predicting antidisease immunity using proteome arrays and sera from children naturally exposed to malaria. Mol Cell Proteomics. 2014; 13:2646–2660. DOI: 10.1074/mcp.M113.036632 [PubMed: 25023128]

Flück C, Smith T, Beck H-P, Irion A, Betuela I, Alpers MP, Anders R, Saul A, Genton B, Felger I. Strain-specific humoral response to a polymorphic malaria vaccine. Infect Immun. 2004; 72:6300–6305. DOI: 10.1128/IAI.72.11.6300-6305.2004 [PubMed: 15501757]

Fowkes FJI, Richards JS, Simpson JA, Beeson JG. The relationship between anti-merozoite antibodies and incidence of *Plasmodium falciparum* malaria: a systematic review and meta-analysis. PLoS Med. 2010; 7:e1000218.doi: 10.1371/journal.pmed.1000218 [PubMed: 20098724]

França CT, Hostetler JB, Sharma S, White MT, Lin E, Kiniboro B, Waltmann A, Darcy AW, Li Wai Suen CSN, Siba P, King CL, et al. An antibody screen of a *Plasmodium vivax* antigen library identifies novel merozoite proteins associated with clinical protection. PLoS Negl Trop Dis. 2016; :10.doi: 10.1371/journal.pntd.0004639

Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, Rare L, Baisor M, Lorry K, Brown GV, Pye D, et al. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1–2b trial in Papua New Guinea. J Infect Dis. 2002; 185:820–827. DOI: 10.1086/339342 [PubMed: 11920300]

Griffin JT, Ferguson NM, Ghani AC. Estimates of the changing age-burden of *Plasmodium falciparum* malaria disease in sub-Saharan Africa. Nat Commun. 2014; 5doi: 10.1038/ncomms4136

Helb DA, Tetteh KKA, Felgner PL, Skinner J, Hubbard A, Arinaitwe E, Mayanja-Kizza H, Ssewanyana I, Kamya MR, Beeson JG, Tappero J, et al. Novel serologic biomarkers provide

accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities. Proc Natl Acad Sci USA. 2015; 112:E4438–E4447. DOI: 10.1073/pnas.1501705112 [PubMed: 26216993]

Hill DL, Eriksson EM, Carmagnac AB, Wilson DW, Cowman AF, Hansen DS, Schofield L. Efficient measurement of opsonising antibodies to *Plasmodium falciparum* merozoites. PLoS One. 2012; 7:e51692.doi: 10.1371/journal.pone.0051692 [PubMed: 23300556]

Hill DL, Eriksson EM, Li Wai Suen CSN, Chiu CY, Ryg-Cornejo V, Robinson LJ, Siba PM, Mueller I, Hansen DS, Schofield L. Opsonising antibodies to *P. falciparum* merozoites associated with immunity to clinical malaria. PLoS One. 2013; 8:e74627.doi: 10.1371/journal.pone.0074627 [PubMed: 24040299]

Hostetler JB, Sharma S, Bartholdson SJ, Wright GJ, Fairhurst RM, Rayner JC. A library of *Plasmodium vivax* recombinant merozoite proteins reveals new vaccine candidates and protein–protein interactions. PLoS Negl Trop Dis. 2015; 9:e0004264.doi: 10.1371/journal.pntd.0004264 [PubMed: 26701602]

Irani V, Ramsland PA, Guy AJ, Siba PM, Mueller I, Richards JS, Beeson JG. Acquisition of functional antibodies that block the binding of erythrocyte-binding antigen 175 and protection against *Plasmodium falciparum* malaria in children. Clin Infect Dis. 2015; 61:1244–1252. DOI: 10.1093/cid/civ525 [PubMed: 26136391]

Jepsen MPG, Jogdand PS, Singh SK, Esen M, Christiansen M, Issifou S, Hounkpatin AB, Ateba-Ngoa U, Kremsner PG, Dziegiel MH, Olesen-Larsen S, et al. The malaria vaccine candidate GMZ2 elicits functional antibodies in individuals from malaria endemic and non-endemic areas. J Infect Dis. 2013; 208:479–488. DOI: 10.1093/infdis/jit185 [PubMed: 23624363]

Joos C, Marrama L, Polson HEJ, Corre S, Diatta A-M, Diouf B, Trape J-F, Tall A, Longacre S, Perraut R. Clinical protection from falciparum malaria correlates with neutrophil respiratory bursts induced by merozoites opsonized with human serum antibodies. PLoS One. 2010; 5:e9871.doi: 10.1371/journal.pone.0009871 [PubMed: 20360847]

Joos C, Varela M-L, Mbengue B, Mansourou A, Marrama L, Sokhna C, Tall A, Trape J-F, Touré A, Mercereau-Puijalon O, Perraut R. Antibodies to *Plasmodium falciparum* merozoite surface protein-1p19 malaria vaccine candidate induce antibody-dependent respiratory burst in human neutrophils. Malar J. 2015; 14:409.doi: 10.1186/s12936-015-0935-5 [PubMed: 26471813]

Khusmith S, Druilhe P, Gentilini M. Enhanced *Plasmodium falciparum* merozoite phagocytosis by monocytes from immune individuals. Infect Immun. 1982; 35:874–879. [PubMed: 7040248]

King CL, Michon P, Shakri AR, Marcotty A, Stanisic D, Zimmerman PA, Cole-Tobian JL, Mueller I, Chitnis CE. Naturally acquired Duffy-binding protein-specific binding inhibitory antibodies confer protection from blood-stage *Plasmodium vivax* infection. Proc Natl Acad Sci USA. 2008; 105:8363–8368. DOI: 10.1073/pnas.0800371105 [PubMed: 18523022]

King CL, Davies DH, Felgner P, Baum E, Jain A, Randall A, Tetteh K, Drakeley CJ, Greenhouse B. Biosignatures of exposure/transmission and immunity. Am J Trop Med Hyg. 2015; 93:16–27. DOI: 10.4269/ajtmh.15-0037 [PubMed: 26259938]

Kumaratilake LM, Ferrante A, Jaeger T, Rzepczyk CM. Effects of cytokines, complement, and antibody on the neutrophil respiratory burst and phagocytic response to *Plasmodium falciparum* merozoites. Infect Immun. 1992; 60:3731–3738. [PubMed: 1500183]

Longley RJ, Reyes-Sandoval A, Montoya-Diaz E, Dunachie S, Kumpitak C, Nguitragool W, Mueller I, Sattabongkot J. Acquisition and longevity of antibodies to *Plasmodium vivax* preerythrocytic antigens in Western Thailand. Clin Vaccine Immunol. 2015; 23:117–124. DOI: 10.1128/CVI.00501-15 [PubMed: 26656115]

Marsh K, Howard RJ. Antigens induced on erythrocytes by *P. falciparum*: expression of diverse and conserved determinants. Science. 1986; 231:150–153. [PubMed: 2417315]

Marsh K, Kinyanjui S. Immune effector mechanisms in malaria. Parasite Immunol. 2006; 28:51–60. DOI: 10.1111/j.1365-3024.2006.00808.x [PubMed: 16438676]

Marsh K, Otoo L, Hayes RJ, Carson DC, Greenwood BM. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. Trans R Soc Trop Med Hyg. 1989; 83:293–303. [PubMed: 2694458]

McCallum FJ, Persson KEM, Mugyenyi CK, Fowkes FJI, Simpson JA, Richards JS, Williams TN, Marsh K, Beeson JG. Acquisition of growth-inhibitory antibodies against blood-stage *Plasmodium falciparum*. PLoS One. 2008; 3:e3571.doi: 10.1371/journal.pone.0003571 [PubMed: 18958278]

McCarthy JS, Marjason J, Elliott S, Fahey P, Bang G, Malkin E, Tierney E, Aked-Hurditch H, Adda C, Cross N, Richards JS, et al. A phase 1 trial of MSP2-C1, a blood-stage malaria vaccine containing 2 isoforms of MSP2 formulated with Montanide® ISA 720. PLoS One. 2011; 6:e24413.doi: 10.1371/journal.pone.0024413 [PubMed: 21949716]

Migot F, Chougnet C, Raharimalala L, Astagneau P, Lepers JP, Deloron P. Human immune responses to the *Plasmodium falciparum* ring-infected erythrocyte surface antigen (Pf155/RESA) after a decrease in malaria transmission in Madagascar. Am J Trop Med Hyg. 1993; 48:432–439. [PubMed: 8470778]

Mueller I, Galinski MR, Tsuboi T, Arevalo-Herrera M, Collins WE, King CL. Natural acquisition of immunity to *Plasmodium vivax*: epidemiological observations and potential targets. Adv Parasitol. 2013; 81:77–131. DOI: 10.1016/B978-0-12-407826-0.00003-5 [PubMed: 23384622]

Nkuo TK, Deas JE. Sera from Cameroon induce crisis forms during *Plasmodium falciparum* growth inhibition studies in vitro. Trans R Soc Trop Med Hyg. 1988; 82:380–383. DOI: 10.1016/0035-9203(88)90125-3 [PubMed: 3068849]

Nnedu ON, O'Leary MP, Mutua D, Mutai B, Kalantari-Dehaghi M, Jasinskas A, Nakajima-Sasaki R, John-Stewart G, Otieno P, Liang X, Waitumbi J, et al. Humoral immune responses to *Plasmodium falciparum* among HIV-1-infected Kenyan adults. Proteomics Clin Appl. 2011; 5:613–623. DOI: 10.1002/prca.201100021 [PubMed: 21956928]

Osier FH, Feng G, Boyle MJ, Langer C, Zhou J, Richards JS, McCallum FJ, Reiling L, Jaworowski A, Anders RF, Marsh K, et al. Opsonic phagocytosis of *Plasmodium falciparum* merozoites: mechanism in human immunity and a correlate of protection against malaria. BMC Med. 2014a; 12:108.doi: 10.1186/1741-7015-12-108 [PubMed: 24980799]

Osier FH, Mackinnon MJ, Crosnier C, Fegan G, Kamuyu G, Wanaguru M, Ogada E, McDade B, Rayner JC, Wright GJ, Marsh K. New antigens for a multicomponent blood-stage malaria vaccine. Sci Transl Med. 2014b; :6.doi: 10.1126/scitranslmed.3008705

Persson KEM, Lee CT, Marsh K, Beeson JG. Development and optimization of high-throughput methods to measure *Plasmodium falciparum*-specific growth inhibitory antibodies. J Clin Microbiol. 2006; 44:1665–1673. DOI: 10.1128/JCM.44.5.1665-1673.2006 [PubMed: 16672391]

Persson KEM, McCallum FJ, Reiling L, Lister NA, Stubbs J, Cowman AF, Marsh K, Beeson JG. Variation in use of erythrocyte invasion pathways by *Plasmodium falciparum* mediates evasion of human inhibitory antibodies. J Clin Invest. 2008; 118:342–351. DOI: 10.1172/JCI32138 [PubMed: 18064303]

Persson KEM, Fowkes FJI, McCallum FJ, Gicheru N, Reiling L, Richards JS, Wilson DW, Lopaticki S, Cowman AF, Marsh K, Beeson JG. Erythrocyte-binding antigens of *Plasmodium falciparum* are targets of human inhibitory antibodies and function to evade naturally acquired immunity. J Immunol. 2013; 191:785–794. DOI: 10.4049/jimmunol.1300444 [PubMed: 23776178]

Polley SD, Conway DJ, Cavanagh DR, McBride JS, Lowe BS, Williams TN, Mwangi TW, Marsh K. High levels of serum antibodies to merozoite surface protein 2 of *Plasmodium falciparum* are associated with reduced risk of clinical malaria in coastal Kenya. Vaccine. 2006; 24:4233–4246. DOI: 10.1016/j.vaccine.2005.06.030 [PubMed: 16111789]

Reiling L, Richards JS, Fowkes FJI, Barry AE, Triglia T, Chokejindachai W, Michon P, Tavul L, Siba PM, Cowman AF, Mueller I, et al. Evidence that the erythrocyte invasion ligand PfRh2 is a target of protective immunity against *Plasmodium falciparum* malaria. J Immunol. 2010; 185:6157–6167. DOI: 10.4049/jimmunol.1001555 [PubMed: 20962255]

Reiling L, Richards JS, Fowkes FJI, Wilson DW, Chokejindachai W, Barry AE, Tham W-H, Stubbs J, Langer C, Donelson J, Michon P, et al. The *Plasmodium falciparum* erythrocyte invasion ligand Pfrh4 as a target of functional and protective human antibodies against malaria. PLoS One. 2012; 7:e45253.doi: 10.1371/journal.pone.0045253 [PubMed: 23028883]

Richards JS, Stanisic DI, Fowkes FJI, Tavul L, Dabod E, Thompson JK, Kumar S, Chitnis CE, Narum DL, Michon P, Siba PM, et al. Association between naturally acquired antibodies to erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and high-density parasitemia. Clin Infect Dis. 2010; 51:e50–e60. DOI: 10.1086/656413 [PubMed: 20843207]

Richards JS, Arumugam TU, Reiling L, Healer J, Hodder AN, Fowkes FJI, Cross N, Langer C, Takeo S, Uboldi AD, Thompson JK, et al. Identification and prioritization of merozoite antigens as targets of protective human immunity to *Plasmodium falciparum* malaria for vaccine and biomarker development. J Immunol. 2013; 191:795–809. DOI: 10.4049/jimmunol.1300778 [PubMed: 23776179]

Roussilhon C, Oeuvray C, Müller-Graf C, Tall A, Rogier C, Trape J-F, Theisen M, Balde A, Pérignon J-L, Druilhe P. Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. PLoS Med. 2007; 4:e320.doi: 10.1371/journal.pmed.0040320 [PubMed: 18001147]

RTS,S Clinical Trials Partnership. Efficacy and safety of RTS, S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet. 2015; 386:31–45. DOI: 10.1016/S0140-6736(15)60721-8 [PubMed: 25913272]

Shi YP, Udhayakumar V, Oloo AJ, Nahlen BL, Lal AA. Differential effect and interaction of monocytes, hyperimmune sera, and immunoglobulin G on the growth of asexual stage *Plasmodium falciparum* parasites. Am J Trop Med Hyg. 1999; 60:135–141. [PubMed: 9988337]

Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, Weber MW, Pinder M, Nahlen B, Obonyo C, Newbold C, et al. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. Lancet. 1997; 349:1650–1654. DOI: 10.1016/S0140-6736(97)02038-2 [PubMed: 9186382]

Stanisic DI, Richards JS, McCallum FJ, Michon P, King CL, Schoepflin S, Gilson PR, Murphy VJ, Anders RF, Mueller I, Beeson JG. Immunoglobulin G subclass-specific responses against *Plasmodium falciparum* merozoite antigens are associated with control of parasitemia and protection from symptomatic illness. Infect Immun. 2009; 77:1165–1174. DOI: 10.1128/IAI.01129-08 [PubMed: 19139189]

Tan J, Pieper K, Piccoli L, Abdi A, Foglierini M, Geiger R, Tully CM, Jarrossay D, Ndungu FM, Wambua J, Bejon P, et al. A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens. Nature. 2016; 529:105–109. DOI: 10.1038/nature16450 [PubMed: 26700814]

Taylor RR, Allen SJ, Greenwood BM, Riley EM. IgG3 antibodies to *Plasmodium falciparum* merozoite surface protein 2 (MSP2): increasing prevalence with age and association with clinical immunity to malaria. Am J Trop Med Hyg. 1998; 58:406–413. [PubMed: 9574783]

Tran TM, Ongoiba A, Coursen J, Crosnier C, Diouf A, Huang C-Y, Li S, Doumbo S, Doumtabe D, Kone Y, Bathily A, et al. Naturally acquired antibodies specific for *Plasmodium falciparum* reticulocyte-binding protein homologue 5 inhibit parasite growth and predict protection from malaria. J Infect Dis. 2014; 209:789–798. DOI: 10.1093/infdis/jit553 [PubMed: 24133188]

Trape J-F, Tall A, Diagne N, Ndiath O, Ly AB, Faye J, Dieye-Ba F, Roucher C, Bouganali C, Badiane A, Sarr FD, et al. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. Lancet Infect Dis. 2011; 11:925–932. DOI: 10.1016/S1473-3099(11)70194-3 [PubMed: 21856232]

Wilson DW, Crabb BS, Beeson JG. Development of fluorescent *Plasmodium falciparum* for in vitro growth inhibition assays. Malar J. 2010; 9:152.doi: 10.1186/1475-2875-9-152 [PubMed: 20525251]

Wilson DW, Fowkes FJI, Gilson PR, Elliott SR, Tavul L, Michon P, Dabod E, Siba PM, Mueller I, Crabb BS, Beeson JG. Quantifying the importance of MSP1-19 as a target of growth-inhibitory and protective antibodies against *Plasmodium falciparum* in humans. PLoS One. 2011; 6:e27705.doi: 10.1371/journal.pone.0027705 [PubMed: 22110733]

World Health Organization. World malaria report. WHO Press; Geneva, Switzerland: 2015.

The Joint Technical Expert Group on Malaria and World Health Organization Secretariat. , editor. World Health Organization Secretariat. Background Paper on the RTS, S/ASO1 Malaria Vaccine. WHO Press; Geneva, Switzerland: 2015.

Wright KE, Hjerrild KA, Bartlett J, Douglas AD, Jin J, Brown RE, Illingworth JJ, Ashfield R, Clemmensen SB, de Jongh WA, Draper SJ, et al. Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies. Nature. 2014; 515:427–430. DOI: 10.1038/nature13715 [PubMed: 25132548]