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Invited Review

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

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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 Here we discuss recent published findings, and unpublished work presented at the 2016 Molecular Approaches to Malaria conference, that highlight advancements in understanding humoral immune responses in the context of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.

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

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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 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,

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