Insights into the naturally acquired immune response to *Plasmodium vivax* malaria

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SUMMARY

Plasmodium vivax is the most geographically widespread of the malaria parasites causing human disease, yet it is comparatively understudied compared with *Plasmodium falciparum*. In this article we review what is known about naturally acquired immunity to *P. vivax*, and importantly, how this differs to that acquired against *P. falciparum*. Immunity to clinical *P. vivax* infection is acquired more quickly than to *P. falciparum*, and evidence suggests humans in endemic areas also have a greater capacity to mount a successful immunological memory response to this pathogen. Both of these factors give promise to the idea of a successful *P. vivax* vaccine. We review what is known about both the cellular and humoral immune response, including the role of cytokines, antibodies, immunoregulation, immune memory and immune dysfunction. Furthermore, we discuss where the future lies in terms of advancing our understanding of naturally acquired immunity to *P. vivax*, through the use of well-designed longitudinal epidemiological studies and modern tools available to immunologists.

Key words: *Plasmodium vivax*, naturally acquired immunity, antibodies, T cells.

INTRODUCTION

Plasmodium vivax is being increasingly recognized as a significant source of morbidity throughout an extremely wide geographic region, with more than 80 million people infected each year and more than 40% of the world's population at risk (Price *et al*. 2007) (estimated recently at 2·5 billion people at risk (Gething *et al*. 2012)). Epidemiological studies have indicated that individuals living in endemic areas can acquire natural immunity to clinical infections, and intriguingly, at a faster rate than acquisition of protective immunity to *Plasmodium falciparum*. In this article we will review what is known about the underlying immunology providing clinical protection (both cellular, humoral and memory) and what still remains to be discovered.

EPIDEMIOLOGICAL OBSERVATIONS OF NATURALLY ACQUIRED IMMUNITY TO *P. VIVAX*

Naturally acquired immunity to *P. vivax* was first proposed due to epidemiological observations in which children were more likely to be susceptible to clinical disease compared with adults living in the same area (Koch, 1900; Taliaferro, 1949). Three detailed epidemiological studies conducted in the late 1900s demonstrated this effect quite clearly: in Vanuatu, morbidity due to *P. vivax* peaked in children aged 0–2 years whilst little morbidity was detected beyond the age of 6 years (Maitland *et al*. 1996). In the Karen population in western Thailand, the peak incidence of *P. vivax* was in children aged 0–4 years, decreasing to a plateau after 25 years of age (Luxemburger *et al*. 1996). In Sri Lanka, rather than an association with age, the authors noted a decrease in the measures of malaria morbidity following successive *P. vivax* infections (Gunewardena *et al*. 1994). Since then, similar observations have again been documented in Sri Lanka (Karunaweera *et al*. 1998), Vanautu (Kaneko *et al*. 2014), Papau New Guinea (PNG) (Michon *et al*. 2007), the Solomon Islands (Harris *et al*. 2010), Vietnam (Nguyen *et al*. 2012), Peru (Branch *et al*. 2005) and Brazil (Camargo *et al*. 1999; Alves *et al*. 2002; da Silva-Nunes *et al*. 2008; Ladeia-Andrade *et al*. 2009). Hence, it is also possible to generate naturally acquired immunity in low-transmission regions.

Epidemiological studies have also demonstrated that this acquisition of natural immunity to clinical *P. vivax* infection actually occurs more rapidly than for *P. falciparum*. This was demonstrated in a number of the epidemiological studies mentioned above (Gunewardena *et al*. 1994; Luxemburger *et al*. 1996; Maitland *et al*. 1996; Michon *et al*. 2007), amongst others (Phimpraphi *et al*. 2008; Lin *et al*. 2010; Koepfli *et al*. 2013), as well as in

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historical trials wherein syphilis patients were infected with *Plasmodium* parasites as a curative measure (Boyd, 1947). The difference in speed of acquisition of naturally acquired immunity suggests that the functional immunology is likely quite different between the two species. It has been suggested that this may be due to different biological characteristics (Mueller *et al*. 2013), such as the presence of hypnozoites or the large reliance on the duffy binding protein (DBP) for invasion, or due to the increased force of genetically distinct blood-stage infection seen with *P. vivax* (Koepfli *et al*. 2013).

NATURALLY ACQUIRED CELLULAR IMMUNITY

The role of naturally acquired cellular immunity to *P. vivax* remains poorly understood. Clearly, cellular responses are instrumental in a functional and effective response to a pathogen, however these have been difficult to define for *P. vivax*. Whilst induction of $CD4^+$ T cells are critical in providing 'help' for B cells to produce antigen-specific antibodies, there is also evidence of cellular immunity to *P. vivax* being an important part of the immunoregulatory response. Herein, we first review evidence of naturally acquired *P. vivax*-specific cells and their relationship with protection from clinical infection, discuss the inflammatory response to *P. vivax* and the role of such cytokines in immunoregulation, and provide evidence of dendritic cell dysfunction during *P. vivax* infection. Finally, we compare the *P. vivax* response with the known cellular response induced by *P. falciparum*.

Induction of P. vivax*-specific T and B cells*

Whilst there is increasing recognition of the role T cells play during blood-stage infection (Riley *et al*. 1992; Carvalho *et al*. 1999; Pombo *et al*. 2002), we know that T cells are essential to eliminate liver-stages given the parasite hides within the cell (Hoffman *et al*. 1989; Weiss *et al*. 1990; Renia *et al*. 1991, 1993). It is therefore surprising that assessment of *P. vivax*–specific T and B cells has largely been conducted for blood-stage proteins. There have been multiple reports of the detection of specific cellular responses to the *P. vivax* (Pv) antigens tryptophan rich antigens (TRAgs) (Alam *et al*. 2008a, b; Garg *et al*. 2008; Siddiqui *et al*. 2008; Mittra *et al*. 2010; Zeeshan *et al*. 2013, 2015) and merozoite surface protein 1 (MSP1) (Soares *et al*. 1997; Seth *et al*. 2010; Riccio *et al*. 2013), and limited reports for the antigens apical membrane antigen 1 (AMA1) (Bueno *et al*. 2009; Seth *et al*. 2010), MSP9 (Lima-Junior *et al*. 2008) and DBP region II (DBPII) (Xainli *et al*. 2002). These antigens are all expressed during the blood-stage of infection: the large number of PvTRAgs can be expressed on both merozoites and schizonts (Zeeshan *et al*. 2015), PvMSP1 is located on the surface of the merozoite (Mueller *et al*. 2013), PvAMA1 is translocated to the micronemes of the merozoite near the end of asexual replication and eventually to the surface prior to invasion of erythrocytes (Bueno *et al*. 2009), and PvDBP is secreted by the micronemes as the merozoite invades erythrocytes (Adams *et al*. 1992). The frequency of positive responders varied in all studies, likely reflecting the protein or peptide used for stimulation, the different assays employed, the transmission intensity in the region and the level of past exposure. The most extensively studied non-blood-stage protein is the circumsporozoite protein (CSP) (Rodrigues *et al*. 1991; Herrera *et al*. 1992; Bilsborough *et al*. 1993; Migot *et al*. 1993; Carvalho *et al*. 1997; Suphavilai *et al*. 2004; Seth *et al*. 2010), however, the majority of these studies were conducted more than 10 years ago and hence cellular responses were assessed using immune assays that are now considered outdated. Nevertheless, the frequency of recognition of various *P. vivax* CSP epitopes varied from less than 20% in Thai individuals (Suphavilai *et al*. 2004), to almost 60% in a region of the Colombian Pacific Coast (Herrera *et al*. 1992) and in Caucasian volunteers who had previously lived for more than 7 years in a malaria endemic region (either PNG, the Solomon Islands or south-east Asia) (Bilsborough *et al*. 1993). These findings potentially reflect differences in transmission intensity.

In addition, a study in northern India by Seth *et al*. in 2010 assessed lymphocyte responses to B and T cell epitopes of four proteins, AMA1, MSP1, CSP and gametocyte surface antigen 1 (GAM1), covering each stage of the parasite's life-cycle (Seth *et al*. 2010). They were able to identify positive responses to these epitopes amongst their study population; however, given the study design was cross-sectional, and the cellular assay used (lymphocyte proliferation assay), no further conclusions can be drawn. In fact, all the above studies have been cross-sectional in design, and hence severely limit the ability to be able to determine the relationship of *P. vivax*-specific cellular responses with clinical infection, and as such the need for longitudinal studies is clearly evident. Furthermore, more attention needs to be paid to the pre-erythrocytic stages of infection, particularly given the potential for *P. vivax* hypnozoites to lay dormant in the liver, to determine whether any cellular responses are induced to this stage during natural infection (and whether this is similar or different to that observed following *P. falciparum* infection).

Induction of cytokines and role of immunoregulation

It has long been recognized that the host responds to *P. falciparum* infection through the release of pro-inflammatory cytokines into the blood stream, with

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most focus on the role of tumour necrosis factor (TNF) (Grau *et al*. 1989). Whilst this response acts to limit the infection (Kremsner *et al*. 1995; Dodoo *et al*. 2002), it can also induce immunopathology (Kwiatkowski *et al*. 1993), and has been associated with severe *P. falciparum* infections and fatal outcomes (Grau *et al*. 1989; Kwiatkowski *et al*. 1990; Day *et al*. 1999). The role of cytokine release in *P. vivax* was not assessed until the early 1990s (Karunaweera *et al*. 1992), when it was recognized that this infection also induced a TNF response, which was in fact comparatively stronger than that seen during *P. falciparum* infection. The induction of a strong pro-inflammatory response (cytokines such as TNF, interferon-gamma (IFN-γ), interleukin-12 (IL-12), IL-6, IL-1β and IL-8) during *P. vivax* infection compared with uninfected controls has since been shown in multiple studies (Torre *et al*. 1998; Praba-Egge *et al*. 2003; Hemmer *et al*. 2006; Zeyrek *et al*. 2006; Fernandes *et al*. 2008; Andrade *et al*. 2010; Jain *et al*. 2010; Medina *et al*. 2011; Goncalves *et al*. 2012; Leoratti *et al*. 2012; Mendonca *et al*. 2013; Raza *et al*. 2013; Silva *et al*. 2013; da Costa *et al*. 2014; Rodrigues-da-Silva *et al*. 2014), in some cases with comparatively higher levels than seen during *P. falciparum* infection (Praba-Egge *et al*. 2003; Hemmer *et al*. 2006), although other reports are in contradiction (Fernandes *et al*. 2008; Goncalves *et al*. 2012; Rodrigues-da-Silva *et al*. 2014). The key differences between the two studies that observed higher levels of pro-inflammatory cytokines (namely TNF) and the three studies that did not were the geographic region. The studies that identified similar levels of pro-inflammatory cytokines in plasma samples from acute *P. vivax* and *P. falciparum* patients were all from the Brazilian Amazon, a region of low or unstable transmission (Fernandes *et al*. 2008; Goncalves *et al*. 2012; Rodrigues-da-Silva *et al*. 2014). In comparison, the study by Hemmer *et al*. and that of Praba-Egge *et al*. were conducted in non-immune European patients and in Colombians with an average of 1–2 prior episodes of malaria, respectively.

Induction of prominent secretion of the anti-inflammatory cytokine IL-10 has also been reported (Praba-Egge *et al*. 2003; Zeyrek *et al*. 2006; Fernandes *et al*. 2008; Jangpatarapongsa *et al*. 2008; Andrade *et al*. 2010; Bueno *et al*. 2010; Goncalves *et al*. 2010, 2012; Jain *et al*. 2010; Yeo *et al*. 2010; Medina *et al*. 2011; Leoratti *et al*. 2012; Borges *et al*. 2013; Mendonca *et al*. 2013; Raza *et al*. 2013; Silva *et al*. 2013; da Costa *et al*. 2014; Rodrigues-da-Silva *et al*. 2014), again with multiple reports of higher levels during acute *P. vivax* than *P. falciparum* infection (Praba-Egge *et al*. 2003; Fernandes *et al*. 2008; Goncalves *et al*. 2010, 2012; Yeo *et al*. 2010) and one report of equal levels between the two species (Rodrigues-da-Silva *et al*. 2014). IL-10 can have an immunoregulatory, or immunosuppressive, effect and the outcome of IL-10 secretion during *P. falciparum* infection is still an area of contention (reviewed in (Hansen and Schofield, 2010)). Interestingly, Andrade *et al*. found that IL-10 levels were lower in severe cases of *P. vivax* malaria compared with asymptomatic cases (Andrade *et al*. 2010), potentially suggesting high IL-10 levels could mediate clinical immunity by reducing the effect of harmful pro-inflammatory cytokines. However, this was not confirmed in two more recent studies similarly conducted in Brazil (Goncalves *et al*. 2012; Mendonca *et al*. 2013). The contradicting hypothesis is that high levels of IL-10 allow the parasite to escape the host immune response, resulting in the manifestation of symptomatic or severe malaria (Hugosson *et al*. 2004). Alternatively, another possible explanation is that high levels of IL-10 are not induced until a high threshold level of pro-inflammatory cytokines has been achieved; this is supported by studies that have found strong correlations between the level of pro-inflammatory cytokines produced (such as IFN-γ, TNF or IL-16) and the level of IL-10 (Raza *et al*. 2013; Silva *et al*. 2013; da Costa *et al*. 2014). Unfortunately, as these studies are cross-sectional, causal relationships cannot be inferred, so the true mechanism remains unknown.

In most studies assessing the cytokine responses, levels have been measured in the plasma and hence the cellular source of these cytokines is unknown. Whilst pro-inflammatory cytokines are likely secreted by monocytes or macrophages and are part of the innate response to *P. vivax* (Leoratti *et al*. 2012; Antonelli *et al*. 2014), they can also be secreted from *P. vivax*-specific T cells (Salwati *et al*. 2011), including memory cells (Wipasa *et al*. 2011; Silva *et al*. 2013), and there has also been the recent suggestion that active atypical memory B cells could secrete pro-inflammatory cytokines (Requena *et al*. 2014). Regulatory cytokines such as IL-10 or transforming growth factor-beta (TGF-β) are likely secreted by T regulatory cells (Tregs), however little is known about the Treg response during *P. vivax* infection. Jangpatarapongsa *et al*. first identified significant upregulation of total $CD4^+$ $CD25^+$ (and also $FOX3P^+$) Tregs during acute *P. vivax* infection in the Tak province, Thailand, and demonstrated that these cells were likely the cellular source of IL-10 (Jangpatarapongsa *et al*. 2008). Augmented $CD4^+$ $CD25^+$ $FOX3P^+$ Tregs have since been similarly observed in two other acute *P. vivax* infected populations in Brazil (Bueno *et al*. 2010; Goncalves *et al*. 2010), although whether these cells are protective or harmful to the host has not been successfully elucidated. There is a clear need to solve this question in order to fully understand the development of naturally acquired immunity.

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In summary, whilst a multitude of studies have assessed the production of cytokines during acute *P. vivax* infection, little information has been gathered on what cells are producing these cytokines and the relationship of pro- and anti-inflammatory cytokines with pathology or protection to the host. Furthermore, the majority of these studies were conducted in the Brazilian Amazon and in subjects greater than 13 years of age, limiting the applicability of the results. Only two studies assessed differences in the cytokine responses between adults and children. Zeyrek *et al*. found that there was a positive correlation between age and IL-8 levels in Turkish *P. vivax* infected patients, whilst there was a negative correlation between age and IL-12 levels (Zeyrek *et al*. 2006). Jain *et al*. identified significantly higher IFN-γ levels in children in India than adults, and also found higher levels of IL-10 and the IFN-γ induced protein (IP-10) in children with chills and rigors compared with those without, but interestingly the same relationship was not found in adults (Jain *et al*. 2010). Hence, further work is required to clarify the relationship of cytokines with protective immunity and to extend these findings over greater geographical areas, transmission levels and age groups.

Dendritic cell dysfunction in P. vivax *infection*

Dendritic cells (DCs) act to link the innate and adaptive immune responses, and hence play an extremely important role in the response to infection. Studies conducted on *P. falciparum* infected patients have found evidence of decreased numbers of circulating DCs (Pichyangkul *et al*. 2004) or DC modulation (Urban *et al*. 1999, 2001, 2006; Skorokhod *et al*. 2004). Similarly, during *P. vivax* infection the majority of studies conducted have suggested that DC maturation is inhibited (Jangpatarapongsa *et al*. 2008; Bueno *et al*. 2009; Goncalves *et al*. 2010; Pinzon-Charry *et al*. 2013). Such a response was first identified within *P. vivax*-infected patients from Thailand, where a reduction was observed compared with uninfected controls in the total number of circulating DCs (Jangpatarapongsa *et al*. 2008). Further examination indicated this reduction also changed the ratio of myeloid ($CD11c^+$) and plasmacytoid ($CD123^+$) DCs, with a greater reduction in myeloid DCs overall. This finding was also supported by two studies in Brazil (Bueno *et al*. 2009; Goncalves *et al*. 2010), where more extensive phenotyping also revealed down-modulation of antigen presenting molecules such as CD1a and HLA-DR, as well as accessory molecules such as CD80 and CD86.

It has been suggested that the reduction of DCs during malarial infection could reflect sequestration of such cells to lymphoid tissues (Jangpatarapongsa *et al*. 2008; Pinzon-Charry *et al*. 2013), however one study conducted in Indonesian Papua provides an alternative theory. Pinzon-Charry *et al*. demonstrated clearly that the reduction in circulating DCs in patients infected with either *P. vivax* or *P. falciparum* was due to induced apoptosis of DCs (Pinzon-Charry *et al*. 2013). They also extended the phenotypic analysis of the remaining DCs by demonstrating the functional effect: impairment of these DCs to mature, as well as their ability to capture and present antigen to T cells. Furthermore, they also described a clear role for IL-10 in this process, whereby IL-10 levels in the plasma not only correlated with DC apoptosis but also blocking of IL-10 could prevent apoptosis *in vitro* (Pinzon-Charry *et al*. 2013). Pinzon-Charry *et al*. described a similar effect on DCs from both *P. vivax* and *P. falciparum*; in contrast, Goncalves *et al*. had only found impaired maturation within *P. vivax* and not *P. falciparum* infected patients (Goncalves *et al*. 2010).

There have also been two studies on monocyte/macrophage populations during *P. vivax* infection. DCs likely arise from monocytes (Guilliams *et al*. 2014) and they are differentiated based on the expression of different CD markers; however, both have a role in phagocytosis. Antonelli *et al*. recently found that the absolute numbers of monocytes ($CD14^+$ $CD16^-$, $CD14^{low}$ $CD16^+$ and $CD14^-$ $CD16^+$) increase during *P. vivax* infection and that they have a greater ability for phagocytosis, compared to cells of uninfected controls (Antonelli *et al*. 2014). However, Fernandez-Arias *et al*. previously described a reduction in the number of monocytes/macrophages ($CD16^+$ $CD10^-$) and a decrease in expression of surface complement receptor 1 (CR1) (which they related to decreased clearance of immune complexes) (Fernandez-Arias *et al*. 2013). As the two studies utilized different methods of classifying monocytes further analysis needs to be performed, in particular to determine whether an increase in monocytes and subsequent phagocytosis could be complementary to the decreased function of DCs.

Comparison with the cellular response induced by P. falciparum

As *P. falciparum* has long been considered the most clinically important species of *Plasmodium*, given its greater lethality than *P. vivax*, more extensive research has been conducted into the immunological response to this infection. This is also due to the greater ease of working with *P. falciparum* parasites (i.e. well established *in vitro* culture) and hence being able to determine *P. falciparum*-specific responses. A relatively limited number of studies have directly compared responses between *P. falciparum* and *P. vivax* infected patients, likely because in most areas of the world one species is dominant

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(Baird, 2013). Whilst it is difficult to directly compare antigen-specific B and T cell responses between species given the antigens do differ, it is possible to directly compare cytokine responses. It has largely been accepted that *P. vivax* is more pyrogenic than *P. falciparum,* in that it induces a greater cytokine response leading to fever at relatively lower parasite loads (Price *et al*. 2007). However the actual evidence is somewhat contradictory, as discussed above (whilst not all studies assessed cytokine concentration per parasite, in those that did not the parasitaemia was not significantly different between *P. falciparum* and *P. vivax* infected patients (Fernandes *et al*. 2008; Rodrigues-da-Silva *et al*. 2014)). Whilst IL-10 responses clearly seem to be greater in response to *P. vivax* infection (Praba-Egge *et al*. 2003; Fernandes *et al*. 2008; Goncalves *et al*. 2010, 2012; Yeo *et al*. 2010), the evidence is less certain for pro-inflammatory cytokines. A number of reasons could account for any observed differences, such as the age of the subjects, the transmission level and geographical location, the stage of infection at which the sample was taken and the method used for enumerating the relative cytokine concentration. Importantly, a greater effort needs to be made to use standardized assays for measurement of cytokine responses, as recently described (Moncunill *et al*. 2013).

NATURALLY ACQUIRED HUMORAL IMMUNITY

Antibodies are considered to provide what is known as 'naturally acquired immunity' to malaria. This is largely based upon a pivotal study conducted in 1961 that demonstrated that transferring sera from *P. falciparum* immune adults to children could subsequently protect those children from clinical disease (Cohen *et al*. 1961); this finding was independently confirmed 30 years later (Sabchareon *et al*. 1991). Antibody-mediated effector functions can include the inhibition of red blood cell (RBC) invasion, neutralization, opsonization and antibody-dependent cellular inhibition (Beeson *et al*. 2008). In this section we will focus on what is known about the production of *P. vivax*-specific antibodies, focusing on DBP as well as some other proteins of vaccine interest, in addition to the potential role of *P. vivax*-specific antibodies in blocking transmission.

Global production of antibodies

The *P. vivax* parasite contains over 5000 proteins (Aurrecoechea *et al*. 2009); whilst a number of these have been selected as vaccine candidates by various methods, we still do not know which are responsible for clinical immunity observed in endemic areas, nor do we have a suitable, highly efficacious candidate for a blood-stage malaria vaccine (for either *P. vivax* or *P. falciparum*). Therefore, whilst some protein-specific responses will be discussed in more detail below, we first wanted to review what has been learnt from studies where the focus was on the global production of antibodies. This generally involves using whole *P. vivax* lysate as a stimulant, or more recently, large-scale protein arrays.

An early study by Ray *et al*. demonstrated that a high proportion of Indian patients (94%), positive for *P. vivax*, responded with antibodies against *P. vivax* blood-stage lysate (Ray *et al*. 1994). They also demonstrated a high proportion of responsiveness in non-infected patients living in India (50%), compared with zero of nine controls from abroad. This demonstrated that sero-conversion following *P. vivax* infection is highly likely, and that potentially these responses can be maintained, which would account for the high rate in non-infected Indian controls. Whilst this study was useful in indicating that *P. vivax*-specific antibodies are highly prevalent, it did not shed any light on how many of the 5000-odd proteins are immunogenic, or whether this response was due to one immunodominant antigen.

Since this study was undertaken the *P. vivax* genome and transcriptome has been completed (Bozdech *et al*. 2008; Carlton *et al*. 2008; Westenberger *et al*. 2010). Together with the development of efficient systems for protein production (Davies *et al*. 2005; Tsuboi *et al*. 2008), this has enabled the development of *P. vivax*-specific protein arrays. Three such studies, screening between 89 and 152 proteins with sera from 20 to 60 patients, identified the rate of immunogenic proteins to be between 11 and 27·5% (Chen *et al*. 2010; Molina *et al*. 2012; Lu *et al*. 2014). However, a much larger study was recently conducted. Finney *et al*. screened 1936 *P. vivax* proteins (approximately 40% of the predicted 5000 proteins in *P. vivax*) with sera from 224 PNG children (Finney *et al*. 2014). They demonstrated that over 50% of these proteins were recognized in these children, which far outweighs the number of proteins that have traditionally been assessed for antibody responses (discussed below). Interestingly, the authors also had the opportunity to assess the contribution of these responses to clinical immunity, by comparing symptomatic and asymptomatic children, and did find that symptomatic children carried fewer antibodies (Finney *et al*. 2014). Such protein arrays are likely to provide useful information in the future, not only for identifying protective antibodies, but also for identifying those that act as markers of exposure and increasing our understanding of the humoral immune response to *P. vivax* in general.

The role of DBP antibodies and the association with protective immunity

DBP is the ligand the *P. vivax* parasite uses to bind to the Duffy antigen receptor for chemokines

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(DARC) on RBCs in order to invade. However, more recently it has been shown that this interaction is not essential for invasion, as the parasite can also infect Duffy negative individuals (although this is extremely rare) (Ryan *et al*. 2006; Cavasini *et al*. 2007; Menard *et al*. 2010; Mendes *et al*. 2011; Woldearegai *et al*. 2013). DBP is a type 1 membrane protein and it is the amino cysteine-rich domain, known as region II, which binds to DARC (Chitnis and Miller, 1994; Chitnis *et al*. 1996). Given the importance of this protein in invasion, there has been strong interest in using DBP as a vaccine target. In 1997 it was demonstrated that naturally infected individuals do make antibodies to DBP region II (DBPII) (Fraser *et al*. 1997), and subsequently it was also shown that such antibodies could block binding of DBPII to RBCs (Michon *et al*. 2000). The level of inhibition correlated with the antibody response, suggesting that with the development of enough antibodies against DBP this could result in protective immunity. This was further supported by the association of increased levels of antibodies to DBPII with age (Michon *et al*. 1998; Xainli *et al*. 2003) or with level of exposure (Ceravolo *et al*. 2005). Many subsequent studies have provided further support for the development of antibodies against DBP in various geographical regions (Cole-Tobian *et al*. 2002; Suh *et al*. 2003; Tran *et al*. 2005; Barbedo *et al*. 2007; Maestre *et al*. 2010; Souza-Silva *et al*. 2010; Zakeri *et al*. 2011; Kano *et al*. 2012; Valizadeh *et al*. 2014), with a subset also assessing and demonstrating inhibitory activity (Ceravolo *et al*. 2008, 2009; Grimberg *et al*. 2007; King *et al*. 2008; Chootong *et al*. 2010; Chootong *et al*. 2012; Souza-Silva *et al*. 2014).

However, whilst these responses have been identified in *P. vivax* infected individuals, in each study conducted, a significant proportion of infected or exposed individuals did not make antibodies against DBP. Similarly, of the studies listed above that described inhibition of binding or invasion of RBCs, the proportions of individuals with antibodies capable of this functionality in each study were low. Such variability could be due to the polymorphism within DBPII (Tsuboi *et al*. 1994; Cole-Tobian and King, 2003), which may favour immune evasion (and be the result of earlier selection), or due to difference in host genotype of the DARC molecule. DARC is a glycosylated membrane protein encoded by five common genotypes, resulting in three phenotypes that are associated with differential levels of expression of DARC (Maestre *et al*. 2010). It has been demonstrated that antibody responses against DBPII are greater within individuals with suspected lower levels of DARC (Maestre *et al*. 2010). This is potentially due to the suggested ability of DARC to down regulate the host immune response, although this has yet to be fully elucidated and more recent studies have disputed this finding (King *et al*. 2011; Souza-Silva *et al*. 2014).

The above findings make it clear that there is questionable evidence over whether DBP, or DBPII, would make a successful vaccine target. However, a study by King *et al*. did find an association between the presence of high-level inhibitory antibodies to DBPII and a delayed time to *P. vivax* re-infection, compared with subjects with low-level inhibitory antibodies (King *et al*. 2008). Perhaps most importantly, the authors also found that such high-level inhibitory antibodies associated with protection were able to overcome strain-specific responses, with similar levels of inhibition against *P. vivax* with six different DBPII haplotypes. These findings suggest that a vaccine may be successful, if predominantly protective, high-level inhibitory antibodies can be induced over non-, or low-level, inhibitory antibodies for DBPII.

Production of antigen-specific antibodies

Apart from the interest in DBP as a vaccine candidate, a number of other *P. vivax* blood-stage proteins have received a significant amount of interest. The majority of studies have been conducted on MSP1 (Soares *et al*. 1997, 1999b; Ak *et al*. 1998; Egan *et al*. 1999; Park *et al*. 2001; Soares and Rodrigues, 2002; Braga *et al*. 2002a; Rodrigues *et al*. 2003; Suh *et al*. 2003; Lim *et al*. 2004; Morais *et al*. 2005; Valderrama-Aguirre *et al*. 2005; Nogueira *et al*. 2006; Barbedo *et al*. 2007; Bastos *et al*. 2007; Ladeia-Andrade *et al*. 2007; Pitabut *et al*. 2007; Wickramarachchi *et al*. 2007; Zeyrek *et al*. 2008; Mehrizi *et al*. 2009; Seth *et al*. 2010; Fernandez-Becerra *et al*. 2010; Storti-Melo *et al*. 2011; Lima-Junior *et al*. 2012; Mourao *et al*. 2012; Riccio *et al*. 2013; Versiani *et al*. 2013). This is largely due to the extensive interest in a *P. falciparum* MSP1 vaccine given the presence of naturally induced antibodies and some associations with protection (reviewed in (Holder, 2009)), and the identification of blocks of conserved sequence between *P. falciparum* and *P. vivax* (del Portillo *et al*. 1991). Of the studies measuring antibody responses against *P. vivax* MSP1, only two were able to associate MSP1-specific antibodies with protection (Nogueira *et al*. 2006; Versiani *et al*. 2013). However, all studies detected a relatively high frequency of *P. vivax* infected or previously infected individuals who were sero-positive for MSP1 (30–98%, largely dependent on the region of PvMSP1 assessed), suggesting such antibodies are rather more reflective of exposure than clinical protection.

Other studies assessing naturally induced antibodies have been against blood-stage proteins including MSP3 (Lima-Junior *et al*. 2011, 2012; Mourao *et al*. 2012; Stanisic *et al*. 2013), MSP9

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(Soares and Rodrigues, 2002; Lima-Junior *et al*. 2008; Stanisic *et al*. 2013), AMA1 (Rodrigues *et al*. 2005; Wickramarachchi *et al*. 2006; Barbedo *et al*. 2007; Mufalo *et al*. 2008; Seth *et al*. 2010; Bueno *et al*. 2011; Dias *et al*. 2011) and various TRAgs (Garg *et al*. 2008; Siddiqui *et al*. 2008; Mittra *et al*. 2010; Zeeshan *et al*. 2013). Similar studies have focused on the *P. vivax* variant proteins (VIRs) (Oliveira *et al*. 2006), the reticulocyte binding proteins (RBPs) (Tran *et al*. 2005), the sporozoite antigen CSP (Migot *et al*. 1993; Carvalho *et al*. 1997; Arevalo-Herrera *et al*. 1998; Suh *et al*. 2003; Seth *et al*. 2010) and the gametocyte antigen GAM1 (Seth *et al*. 2010). However, it is difficult to determine the relative utility of such studies, when antibodies to only a small number of proteins are measured at only one time-point and a detailed history of malaria is unknown. A systematic review and meta-analysis of 18 studies (mostly cross-sectional) was recently conducted, in an attempt to combine all the relevant *P. vivax* antibody data. Overall, IgG responses to PvCSP, PvMSP1, PvMSP9 and PvAMA1 were associated with increased odds of *P. vivax* infection in a diverse range of populations (Cutts *et al*. 2014), hence acting as markers of exposure. Only PvMSP1 (described above), PvMSP3 and PvMSP9 had associations with protection from *P. vivax* malaria.

The main determinant of whether antibodies associate with increased risk of infections or of protection is likely to be the previous lifetime exposure (Koepfli *et al*. 2013). In a recent longitudinal study in PNG children 1–4 years of age that acquired approximately 2·5-times as many distinct *P. vivax* than *P. falciparum* infections (Mueller *et al*. 2012), antibodies to PvMSP3 block II and PvMSP9 N-terminus were found to be independently associated with reduced risk of clinical malaria even after correction for individual variation in exposure (Stanisic *et al*. 2013). In the same children antibodies to a panel of *P. falciparum* merozoite antigens, however, predicted an increased risk of *P. falciparum* malaria, an association that disappeared when correcting for individual difference in exposure (Stanisic *et al*. 2015).

In order to investigate antibodies as potential markers of exposure or as correlates of (clinical) protection, it is therefore essential to account both for difference in exposure as well as for the boosting of antibody responses by concurrent infections. By combining such well designed cohort studies with cutting-edge approaches (e.g. protein arrays) it will be become possible to screen a much larger part of the *P. vivax* proteome and rationally prioritize antigens for further evaluation as potential vaccine candidates. However, the lack of a continuous *P. vivax in vitro* culture system makes it significantly more difficult to develop an assay that can evaluate not only the presence but also investigate the functionality of antibodies against *P. vivax*.

Role of P. vivax*-specific antibodies in blocking transmission*

Interestingly, as the presence of malarial infections diminishes with age, so does the presence of gametocytes (Nguyen *et al*. 2012). Whilst this could be simply due to a decrease in the number of total parasites (and hence gametocytes included), there also could be an induction of antibodies specific to sexual-stage antigens and hence naturally acquired transmission-blocking immunity. Early studies conducted in Sri Lanka indicated that this could well be the case (Mendis *et al*. 1987; de Zoysa *et al*. 1988; Peiris *et al*. 1988; Ranawaka *et al*. 1988; Gamage-Mendis *et al*. 1992). These studies demonstrated that sera from *P. vivax* infected patients were very effective at suppressing infection of the parasites in mosquitoes. They identified that this was likely due to the presence of antibodies against surface antigens of extracellular gametes or zygotes, as the level of these antibodies correlated closely with the level of suppression of infectivity. They also demonstrated that both the reduction in infectivity and the level of antibodies increased when the patient had frequent, recent *P. vivax* infections. However, beyond 4 months since the last infection this pattern was not present, suggesting that there was no true maintenance (or perhaps, generation) of memory to this form of transmission-blocking immunity.

However, following these studies in Sri Lanka a similar experiment was conducted along the southern coast of Mexico (Ramsey *et al*. 1996), and the authors found slightly different results. They also identified transmission-blocking activity using sera from patients with acute *P. vivax* malaria, however only in those who had experienced at least one previous malaria episode, and the duration since this past episode was not important (i.e. even if the previous episode was more than 7 months prior, they still exhibited good transmission-blocking activity). Transmission-blocking activity has since also been identified using *P. vivax* infected patients from western Thailand (Sattabongkot *et al*. 2003; Coleman *et al*. 2004) and Colombia (Arevalo-Herrera *et al*. 2005). Nearly all such studies have been conducted on symptomatic patients, clearly demonstrating that such transmission-blocking activity may be of benefit to the community, but not to the individual patient.

LONGEVITY OF IMMUNITY: EVIDENCE OF PREMUNITION, MEMORY OR EXHAUSTION

The longevity of immunity to malaria has long been a hot topic, with observations for *P. falciparum* pointing to a situation of 'premunition', whereby constant antigenic stimulation is required to maintain the immune response (Smith *et al*. 1999).

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There has also been more recent evidence for *P. falciparum* that the immune response may be dysfunctional, with studies identifying atypical B cells and exhausted T cells (Weiss *et al*. 2009, 2011; Illingworth *et al*. 2013; Scholzen *et al*. 2014), and we know that memory responses to all pathogens or vaccines are not equal (Bottiger *et al*. 1998; Amanna and Slifka, 2010). How this contributes to the overall immune response and whether this is related to the perceived lack of memory for *P. falciparum* has not yet been fully quantified, however some evidence suggests that these cells are not, in fact, 'dysfunctional' (Muellenbeck *et al*. 2013; Speiser *et al*. 2014). Conversely, the response to *P. vivax* appears to be different, with multiple lines of evidence pointing to sustained immunological memory in the absence of concurrent infection, an area of wide interest within the field of immunology.

There is still debate over what constitutes the basis of immunological memory in humans (Crotty and Ahmed, 2004). In regards to malarial infections, it has been suggested that constant antigenic stimulation is required to maintain memory T and B cell populations (Bilsborough *et al*. 1993; Berenzon *et al*. 2003), and that dormant hypnozoites may provide this stimulation for *P. vivax*. In fact, whether antigenic stimulation is required for maintenance of memory cells has been historically controversial (Gray and Skarvall, 1988; Schittek and Rajewsky, 1990; Maruyama *et al*. 2000; Zinkernagel, 2002). However, a study of the successful smallpox vaccine found this was not the case, and that memory B cells could be maintained for many years without specific antigenic stimulation (Crotty *et al*. 2003). Interestingly, it has also been demonstrated that memory B cells were still identifiable when plasma antibodies no longer existed (Bauer and Jilg, 2006; Ndungu *et al*. 2012), providing further support for the maintenance of memory cells without antigenic stimulation. Clearly, if there was stimulation, memory B cells would differentiate into antibody secreting cells and hence antibodies should be detectable.

Whilst memory B cells may survive without antigenic stimulation, to maintain circulating antibodies either of three scenarios must exist: memory B cells must be stimulated to differentiate into antibody secreting cells by specific antigen, either through re-infection or from that captured by follicular DCs (Tew *et al*. 1980; Ochsenbein *et al*. 2000); alternatively, memory B cells may be stimulated by some form of polyclonal activation (Bernasconi *et al*. 2002); or, finally, long-lived plasma cells must be generated. Long-lived plasma cells can survive for many years without antigenic stimulation (Manz *et al*. 1997, 1998; Slifka *et al*. 1998), however their location (in the bone marrow) makes them unsuitable for measurement in human studies. The presence of antibodies in the absence of memory B cells is generally considered evidence of long-lived plasma cells, and other indirect evidence exists (Sfikakis *et al*. 2005). Apart from the maintenance of antibodies, the generation of memory T cells is also extremely important (Hammarlund *et al*. 2003; Tsai and Yu, 2014).

In summary, it seems appropriate that where possible, all such measures should be considered as potential surrogates of immunological memory, including the generation of memory T and B cells and the maintenance of antigen-specific antibodies. All such factors will be discussed below in relation to the generation of memory to *P. vivax*. Secondly, we will also consider whether there is any evidence of dysfunctional memory to *P. vivax*, as has been suggested for *P. falciparum*.

Memory responses to P. vivax

Memory (or in some cases, persisting) T cells have been identified in a number of malaria endemic populations. In 1993, Bilsborough *et al*. identified *vivax*-specific T cell responses (to epitopes of CSP) in individuals who had not experienced a malaria episode for up to 49 years (Bilsborough *et al*. 1993). This study was conducted in volunteers who no longer lived in a malaria endemic area, but had done so previously for an average of 7 years. A similar result was found the following year in a study conducted in Thailand (Zevering *et al*. 1994). The authors hypothesized that the maintenance of these *P. vivax* CSP-specific T cell responses in the absence of infection was due to the presence of hypnozoites, given the same pattern was not found for *P. falciparum* CSP. Further studies have also identified a greater percentage of memory T cells (largely $CD4^+$) in acute patients, as well as uninfected patients who lived in endemic areas, compared with un-exposed controls. However, it is difficult to determine whether these were (or would be) long-lived responses or not (Jangpatarapongsa *et al*. 2006, 2012; Silva *et al*. 2013; Zeeshan *et al*. 2013).

Despite the obvious interest from the vaccine community in inducing memory B cells or long-lived plasma cells, this remains an area of limited research in studies of naturally acquired immunity. Wipasa *et al*. investigated the response to some well-known *P. vivax* antigens, AMA1, MSP1-19 and DBP, and found that within a group of 26 individuals from Northern Thailand with previous malaria exposure, 35% had memory B cells specific to one or more of these *P. vivax* antigens (Wipasa *et al*. 2010). Requena *et al*. recently identified an increase in the percentage of atypical memory B cells (active and resting, defined as IgD^- $CD27^-$ $CD21^-$ and IgD^- $CD27^-$ $CD21^+$, respectively) and active classical memory B cells (IgD $CD27^+$ $CD21^-$) in a

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malaria exposed population (both *P. vivax* and *P. falciparum*) compared with a non-exposed population (Requena *et al*. 2014). It will be of great interest to the field to follow-up these interesting studies with further longitudinal experiments, including the addition of peripheral blood mononuclear cells stimulated with whole *P. vivax* parasites or lysate, to determine whether the response is truly specific.

Wipasa *et al*. also measured *P. vivax*-specific antibodies, and whilst the overall level of seropositivity was low (25% of volunteers previously exposed to *P. vivax*, with no significant difference to those with no previous exposure), they found that of those who were positive there was no significant decline in titre over the 12 months of the study (Wipasa *et al*. 2010). Other studies have also indicated that there is generally a good maintenance of positive antibody responses to *P. vivax* antigens (Braga *et al*. 1998; Park *et al*. 2001; Lim *et al*. 2004; Morais *et al*. 2006; Barbedo *et al*. 2007), although in general the titres did decline over time. However, there was significant variation in the amount of time these studies classified as 'maintenance', varying from 1 year post-exposure (Park *et al*. 2001), to up to 30 years (Lim *et al*. 2004). This could depend on numerous factors including the antigen studied or the age of the subjects, as well as the underlying level of transmission subjects were exposed to or the number of malarial infections they experienced prior to the study. Conversely, another study found that *P. vivax* MSP1-specific antibodies declined rapidly over a period of 2–4 months (Soares *et al*. 1999a), and inhibitory antibodies against DBPII are also known to be short-lived (Ceravolo *et al*. 2009). This could again be attributed to some of the factors outlined above.

Even though the underlying immunological evidence demonstrating a successful memory response against *P. vivax* may not yet be clear, a strong epidemiological study supports this finding. In 1991, a malaria elimination program was initiated on Aneityum Island in Vanuatu, and by 1996 *P. vivax* was considered to be eliminated. However, in 2002 an outbreak occurred, with 77 infections identified from 759 individuals (Kaneko *et al*. 2014). The infections showed a clear age-structure, with adults remaining largely *P. vivax* free. This suggested that those who were born before the elimination strategy begun still retained immunity from exposure prior to 1996. Combining all evidence, it does seem that immunological memory to *P. vivax* is a strong possibility, however the exact mechanism by which this occurs (and to which antigens this is directed) still needs to be uncovered.

Dysfunctional immunity

Interestingly, there has clearly been an inability of some populations to maintain antibody responses to specific antigens, such as MSP1 or DBP, as mentioned earlier (Soares *et al*. 1999a, b; Ceravolo *et al*. 2009). One hypothesis is that memory generation to immunodominant antigens is dysfunctional in *P. vivax* infection, conceivably as an immune defence mechanism or evasion strategy of the parasite. Perhaps surprisingly, this has also been evident for cellular responses. A small study of 33 people in Sri Lanka found that residents who had lived their entire life in a malaria endemic area were less able to respond to stimulation with a soluble extract of *P. vivax* infected erythrocytes than individuals who did not have life-long exposure but had acquired malaria on a visit to an endemic area (Goonewardene *et al*. 1990). This suggests that life-long exposure had led to immunosuppression in response to *P. vivax* antigens. Again, these results could be interpreted in another manner: perhaps such 'immunosuppression' is required in individuals with life-long exposure in order to not overwhelm the immune response, and would hence then be referred to as immune adaptation. However, in this particular study, the volunteers were all symptomatic, even though they all reported having experienced 'repeated malaria infections'. This finding contradicts the evidence demonstrating effective immunity against clinical *P. vivax* symptoms in endemic areas (including those of low-transmission intensity). A similar study more than 10 years later in Brazil identified the same phenomenon: individuals who had been resident for more than 10 years in an endemic area had a lower proliferative response to *P. vivax* CSP than those with less than 1 year total in an endemic area (Braga *et al*. 2002b). In this case given only CSP was studied, immunosuppression cannot be as easily assigned; particularly given the antibody response to CSP was comparable between the two groups. Furthermore, as both studies focused on patients with acute malaria, they could be considered biased towards individuals with a poorer immune response to malaria. Clearly, further studies are required, but there does seem to be some evidence of immune dysfunction in particular groups of patients.

CROSS-SPECIES IMMUNITY

Another important aspect of naturally acquired immunity to *P. vivax* is whether this immunity is species specific, or whether exposure to *P. vivax* could also protect against *P. falciparum* (or vice versa). A number of the highly immunogenic *P. vivax* antigens mentioned so far, such as AMA1, CSP, MSP1 and MSP3, have homologs in *P. falciparum*. Whilst it is still unknown as to whether cross-species immunity could actually provide functional protection, there has long been evidence of cross-species recognition of immune determinants (Diggs and Sadun, 1965).

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It has been shown through the use of a competition enzyme-linked immunosorbent assay that cross-reactive antibody responses can be induced by natural infection between Pv and PfMSP5 (Woodberry *et al*. 2008). The same group subsequently demonstrated that cellular responses specific to PfMSP5 can also be boosted by *P. vivax* infection (Salwati *et al*. 2011). This is supported by other studies where infection with either species seemed to be capable of boosting existing responses to either *P. vivax* or *P. falciparum* antigens or crude lysate (del Portillo *et al*. 1992; Carvalho *et al*. 1997; Chuangchaiya *et al*. 2010; Wipasa *et al*. 2011). Furthermore, in an area of only *P. vivax* transmission, *P. falciparum* antigens were also able to stimulate the immune response in *P. vivax* infected patients (Jangpatarapongsa *et al*. 2012). This would suggest that there are conserved regions between the two species that the human immune system can respond to; however, we do not know whether these are protective determinants. However, two papers have proposed the theory that prior exposure of *P. vivax* may ameliorate a subsequent course of *P. falciparum* infection, based largely on the unusual finding that malaria specific mortality was very low in the two populations studied (Gunewardena *et al*. 1994; Maitland *et al*. 1996). This hypothesis remains to be experimentally proven.

CONCLUSIONS/FUTURE DIRECTIONS

Gaps in our knowledge of naturally acquired immunity to P. vivax

Whilst many immuno-epidemiological studies have now been undertaken in the hope of understanding naturally acquired immunity or identifying promising vaccine candidates, much remains unknown. We have identified several key questions that remain unanswered:

(i) Are cellular responses induced to pre-erythrocytic antigens, and is this to a greater extent than seen for *P. falciparum* (given the potential for an extended liver-stage)?
(ii) Do the newly identified innate lymphoid cells respond to *P. vivax*? If so, could this partly be responsible for the high cytokine response?
(iii) How does immunoregulation influence naturally acquired immunity? What are the roles of regulatory T cells and IL-10, and how do they change with acute compared with asymptomatic infections?
(iv) What role do DCs and phagocytes play in naturally acquired immunity, and are their roles interchangeable?
(v) What other proteins does the parasite use for invasion of RBCs in addition to DBP, and are they targets of naturally induced antibodies?
(vi) What are the targets of antibodies that provide naturally acquired transmission-blocking immunity?
(vii) What constitutes immunological memory to *P. vivax* and what cell types are involved in generating such a long-lived response? Alternatively, does *P. vivax* infection lead to atypical B cells and exhausted T cells?
(viii) Are follicular helper T cells essential for naturally acquired immunity, given their importance in generating high-affinity antibodies? Do they contribute to the longevity of immunity?
(ix) Is cross-species protection a true phenomenon, and can this be answered through studies of natural immunity?

Answering such questions might give further input to the overall aim of many immuo-epidemiological studies: defining key targets of long-lasting natural immunity and ultimately identifying correlates of protection. To answer such questions, researchers will need to use the right tools, such as protein arrays, multi-parameter flow cytometry, the generation of *P. vivax* lysate (from multiple strains) to determine specific responses and the use of samples from well defined, preferentially longitudinal, studies. Furthermore, collaborative efforts will be required to draw all such information together to build a working model of naturally acquired immunity to *P. vivax*.

Lessons learned: applications in vaccine development and sero-diagnostics

Although many questions surrounding the development and maintenance of naturally acquired immunity remain to be answered, what we know so far gives great promise for the development of a *P. vivax* vaccine. We know that immunity to clinical infection is acquired faster for *P. vivax* than for *P. falciparum*, and that long-lasting immune responses to defined antigens can be induced following *P. vivax* infection (Bilsborough *et al*. 1993; Zevering *et al*. 1994; Wipasa *et al*. 2010). The difficulty will be choosing what targets to incorporate into a successful vaccine, and until we uncover the mechanisms of naturally acquired immunity, defining what type of immune response we want such a vaccine to induce will be difficult. Furthermore, more effort needs to be placed into understanding naturally acquired immunity to *P. vivax* in children, rather than adults, given children encompass the target demographic for a malaria vaccine in endemic regions.

Apart from contributing to rational vaccine design, a greater understanding of naturally acquired immunity can also be used to design new sero-diagnostic tools (Cutts *et al*. 2014). Whilst *P. vivax* antigens used for targets in vaccines will need to induce

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long-lived immune responses, those that only induce short-lived antibody responses may prove useful for population-level surveillance. Sero-surveillance has the potential to identify areas of remaining *P. vivax* transmission, asymptomatic individuals and potentially individuals harbouring hypnozoites, and would be a unique and useful tool for monitoring the success of malaria elimination programs. In order to develop such a tool, detailed information must be learned about antigen-specific antibody responses and their longevity in various geographical regions, transmission settings and age groups.

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REFERENCES

Adams, J. H., Sim, B. K., Dolan, S. A., Fang, X., Kaslow, D. C. and Miller, L. H. (1992). A family of erythrocyte binding proteins of malaria parasites. *Proceedings of the National Academy of Sciences USA* **89**, 7085–7089.

Ak, M., Jones, T. R., Charoenvit, Y., Kumar, S., Kaslow, D. C., Maris, D., Marwoto, H., Masbar, S. and Hoffman, S. L. (1998). Humoral immune responses against *Plasmodium vivax* MSP1 in humans living in a malaria endemic area in Flores, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **29**, 685–691.

Alam, M. T., Bora, H., Mittra, P., Singh, N. and Sharma, Y. D. (2008a). Cellular immune responses to recombinant *Plasmodium vivax* tryptophan-rich antigen (PvTRAg) among individuals exposed to *vivax* malaria. *Parasite Immunology* **30**, 379–383.

Alam, M. T., Bora, H., Singh, N. and Sharma, Y. D. (2008b). High immunogenecity and erythrocyte-binding activity in the tryptophan-rich domain (TRD) of the 74-kDa *Plasmodium vivax* alanine-tryptophan-rich antigen (PvATRAg74). *Vaccine* **26**, 3787–3794.

Alves, F. P., Durlacher, R. R., Menezes, M. J., Krieger, H., Silva, L. H. and Camargo, E. P. (2002). High prevalence of asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections in native Amazonian populations. *American Journal of Tropical Medicine and Hygiene* **66**, 641–648.

Amanna, I. J. and Slifka, M. K. (2010). Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. *Immunological Reviews* **236**, 125–138.

Andrade, B. B., Reis-Filho, A., Souza-Neto, S. M., Clarencio, J., Camargo, L. M., Barral, A. and Barral-Netto, M. (2010). Severe *Plasmodium vivax* malaria exhibits marked inflammatory imbalance. *Malaria Journal* **9**, 13.

Antonelli, L. R., Leoratti, F. M., Costa, P. A., Rocha, B. C., Diniz, S. Q., Tada, M. S., Pereira, D. B., Teixeira-Carvalho, A., Golenbock, D. T., Goncalves, R. and Gazzinelli, R. T. (2014). The CD14+CD16+ inflammatory monocyte subset displays increased mitochondrial activity and effector function during acute *Plasmodium vivax* malaria. *PLoS Pathogens* **10**, e1004393.

Arevalo-Herrera, M., Roggero, M. A., Gonzalez, J. M., Vergara, J., Corradin, G., Lopez, J. A. and Herrera, S. (1998). Mapping and comparison of the B-cell epitopes recognized on the *Plasmodium vivax* circumsporozoite protein by immune Colombians and immunized Aotus monkeys. *Annals of Tropical Medecine and Parasitology* **92**, 539–551.

Arevalo-Herrera, M., Solarte, Y., Zamora, F., Mendez, F., Yasnot, M. F., Rocha, L., Long, C., Miller, L. H. and Herrera, S. (2005). *Plasmodium vivax*: transmission-blocking immunity in a malaria-endemic area of Colombia. *American Journal of Tropical Medicine and Hygiene* **73**, 38–43.

Aurrecoechea, C., Brestelli, J., Brunk, B. P., Dommer, J., Fischer, S., Gajria, B., Gao, X., Gingle, A., Grant, G., Harb, O. S., Heiges, M., Innamorato, F., Iodice, J., Kissinger, J. C., Kraemer, E., Li, W., Miller, J. A., Nayak, V., Pennington, C., Pinney, D. F., Roos, D. S., Ross, C., Stoeckert, C. J., Jr., Treatman, C. and Wang, H. (2009). PlasmoDB: a functional genomic database for malaria parasites. *Nucleic Acids Research* **37**, D539–D543.

Baird, J. K. (2013). Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. *Clinical Microbiology Reviews* **26**, 36–57.

Barbedo, M. B., Ricci, R., Jimenez, M. C., Cunha, M. G., Yazdani, S. S., Chitnis, C. E., Rodrigues, M. M. and Soares, I. S. (2007). Comparative recognition by human IgG antibodies of recombinant proteins representing three asexual erythrocytic stage vaccine candidates of *Plasmodium vivax*. *Memorias do Instituto Oswaldo Cruz* **102**, 335–339.

Bastos, M. S., da Silva-Nunes, M., Malafronte, R. S., Hoffmann, E. H., Wunderlich, G., Moraes, S. L. and Ferreira, M. U. (2007). Antigenic polymorphism and naturally acquired antibodies to *Plasmodium vivax* merozoite surface protein 1 in rural Amazonians. *Clinical Vaccine Immunology* **14**, 1249–1259.

Bauer, T. and Jilg, W. (2006). Hepatitis B surface antigen-specific T and B cell memory in individuals who had lost protective antibodies after hepatitis B vaccination. *Vaccine* **24**, 572–577.

Beeson, J. G., Osier, F. H. and Engwerda, C. R. (2008). Recent insights into humoral and cellular immune responses against malaria. *Trends in Parasitology* **24**, 578–584.

Berenzon, D., Schwenk, R. J., Letellier, L., Guebre-Xabier, M., Williams, J. and Krzych, U. (2003). Protracted protection to *Plasmodium berghei* malaria is linked to functionally and phenotypically heterogeneous liver memory $CD8^+$ T cells. *Journal of Immunology* **171**, 2024–2034.

Bernasconi, N. L., Traggiai, E. and Lanzavecchia, A. (2002). Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* **298**, 2199–2202.

Bilsborough, J., Carlisle, M. and Good, M. F. (1993). Identification of Caucasian CD4 T cell epitopes on the circumsporozoite protein of *Plasmodium vivax*. T cell memory. *Journal of Immunology* **151**, 890–899.

Borges, Q. I., Fontes, C. J. and Damazo, A. S. (2013). Analysis of lymphocytes in patients with *Plasmodium vivax* malaria and its relation to the annexin-A1 and IL-10. *Malaria Journal* **12**, 455.

Bottiger, M., Gustavsson, O. and Svensson, A. (1998). Immunity to tetanus, diphtheria and poliomyelitis in the adult population of Sweden in 1991. *International Journal of Epidemiology* **27**, 916–925.

Boyd, M. F. (1947). A review of studies on immunity to vivax malaria. *Journal of National Malaria Society* **6**, 12–31.

Bozdech, Z., Mok, S., Hu, G., Imwong, M., Jaidee, A., Russell, B., Ginsburg, H., Nosten, F., Day, N. P., White, N. J., Carlton, J. M. and Preiser, P. R. (2008). The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites. *Proceedings of the National Academy of Sciences USA* **105**, 16290–16295.

Braga, E. M., Fontes, C. J. and Krettli, A. U. (1998). Persistence of humoral response against sporozoite and blood-stage malaria antigens 7 years after a brief exposure to *Plasmodium vivax*. *Journal of Infectious Diseases* **177**, 1132–1135.

Braga, E. M., Barros, R. M., Reis, T. A., Fontes, C. J., Morais, C. G., Martins, M. S. and Krettli, A. U. (2002a). Association of the IgG response to *Plasmodium falciparum* merozoite protein (C-terminal 19 kD) with clinical immunity to malaria in the Brazilian Amazon region. *American Journal of Tropical Medicine and Hygiene* **66**, 461–466.

Braga, E. M., Carvalho, L. H., Fontes, C. J. and Krettli, A. U. (2002b). Low cellular response *in vitro* among subjects with long-term exposure to malaria transmission in Brazilian endemic areas. *American Journal of Tropical Medicine and Hygiene* **66**, 299–303.

Branch, O., Casapia, W. M., Gamboa, D. V., Hernandez, J. N., Alava, F. F., Roncal, N., Alvarez, E., Perez, E. J. and Gotuzzo, E. (2005). Clustered local transmission and asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* malaria infections in a recently emerged, hypoendemic Peruvian Amazon community. *Malaria Journal* **4**, 27.

Bueno, L. L., Morais, C. G., Soares, I. S., Bouillet, L. E., Bruna-Romero, O., Fontes, C. J., Fujiwara, R. T. and Braga, E. M. (2009).

Plasmodium vivax recombinant vaccine candidate AMA-1 plays an important role in adaptive immune response eliciting differentiation of dendritic cells. *Vaccine* **27**, 5581–5588.

Bueno, L. L., Morais, C. G., Araujo, F. F., Gomes, J. A., Correa-Oliveira, R., Soares, I. S., Lacerda, M. V., Fujiwara, R. T. and Braga, E. M. (2010). *Plasmodium vivax*: induction of $CD4^+CD25^+FoxP3^+$ regulatory T cells during infection are directly associated with level of circulating parasites. *PLoS ONE* **5**, e9623.

Bueno, L. L., Lobo, F. P., Morais, C. G., Mourao, L. C., de Avila, R. A., Soares, I. S., Fontes, C. J., Lacerda, M. V., Chavez Olortegui, C., Bartholomeu, D. C., Fujiwara, R. T. and Braga, E. M. (2011). Identification of a highly antigenic linear B cell epitope within *Plasmodium vivax* apical membrane antigen 1 (AMA-1). *PLoS ONE* **6**, e21289.

Camargo, E. P., Alves, F. and Pereira da Silva, L. H. (1999). Symptomless *Plasmodium vivax* infections in native Amazonians. *Lancet* **353**, 1415–1416.

Carlton, J. M., Adams, J. H., Silva, J. C., Bidwell, S. L., Lorenzi, H., Caler, E., Crabtree, J., Angiuoli, S. V., Merino, E. F., Amedeo, P., Cheng, Q., Coulson, R. M., Crabb, B. S., Del Portillo, H. A., Essien, K., Feldblyum, T. V., Fernandez-Becerra, C., Gilson, P. R., Gueye, A. H., Guo, X., Kang'a, S., Kooij, T. W., Korsinczky, M., Meyer, E. V., Nene, V., Paulsen, I., White, O., Ralph, S. A., Ren, Q., Sargeant, T. J. *et al.* (2008). Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature* **455**, 757–763.

Carvalho, L. H., Fontes, C. J., Fernandes, A. A., Marinuzzi, H. C. and Krettli, A. U. (1997). Cross-reactive cellular immune response to circumsporozoite proteins of *Plasmodium vivax* and *P. falciparum* in malaria-exposed individuals. *Parasite Immunology* **19**, 47–59.

Carvalho, L. H., Fontes, C. J. and Krettli, A. U. (1999). Cellular responses to *Plasmodium falciparum* major surface antigens and their relationship to human activities associated with malaria transmission. *American Journal of Tropical Medicine and Hygiene* **60**, 674–679.

Cavasini, C. E., Mattos, L. C., Couto, A. A., Bonini-Domingos, C. R., Valencia, S. H., Neiras, W. C., Alves, R. T., Rossit, A. R., Castilho, L. and Machado, R. L. (2007). *Plasmodium vivax* infection among Duffy antigen-negative individuals from the Brazilian Amazon region: an exception? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**, 1042–1044.

Ceravolo, I. P., Bruna-Romero, O., Braga, E. M., Fontes, C. J., Brito, C. F., Souza, J. M., Krettli, A. U., Adams, J. H. and Carvalho, L. H. (2005). Anti-*Plasmodium vivax* duffy binding protein antibodies measure exposure to malaria in the Brazilian Amazon. *American Journal of Tropical Medicine and Hygiene* **72**, 675–681.

Ceravolo, I. P., Souza-Silva, F. A., Fontes, C. J., Braga, E. M., Madureira, A. P., Krettli, A. U., Souza, J. M., Brito, C. F., Adams, J. H. and Carvalho, L. H. (2008). Inhibitory properties of the antibody response to *Plasmodium vivax* Duffy binding protein in an area with unstable malaria transmission. *Scandinavian Journal of Immunology* **67**, 270–278.

Ceravolo, I. P., Sanchez, B. A., Sousa, T. N., Guerra, B. M., Soares, I. S., Braga, E. M., McHenry, A. M., Adams, J. H., Brito, C. F. and Carvalho, L. H. (2009). Naturally acquired inhibitory antibodies to *Plasmodium vivax* Duffy binding protein are short-lived and allele-specific following a single malaria infection. *Clinical & Experimental Immunology* **156**, 502–510.

Chen, J. H., Jung, J. W., Wang, Y., Ha, K. S., Lu, F., Lim, C. S., Takeo, S., Tsuboi, T. and Han, E. T. (2010). Immunoproteomics profiling of blood stage *Plasmodium vivax* infection by high-throughput screening assays. *Journal of Proteome Research* **9**, 6479–6489.

Chitnis, C. E. and Miller, L. H. (1994). Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion. *Journal of Experimental Medicine* **180**, 497–506.

Chitnis, C. E., Chaudhuri, A., Horuk, R., Pogo, A. O. and Miller, L. H. (1996). The domain on the Duffy blood group antigen for binding *Plasmodium vivax* and *P. knowlesi* malarial parasites to erythrocytes. *Journal of Experimental Medicine* **184**, 1531–1536.

Chootong, P., Ntumngia, F. B., VanBuskirk, K. M., Xainli, J., Cole-Tobian, J. L., Campbell, C. O., Fraser, T. S., King, C. L. and Adams, J. H. (2010). Mapping epitopes of the *Plasmodium vivax* Duffy binding protein with naturally acquired inhibitory antibodies. *Infection and Immunity* **78**, 1089–1095.

Chootong, P., Panichakul, T., Permmongkol, C., Barnes, S. J., Udomsangpetch, R. and Adams, J. H. (2012). Characterization of inhibitory anti-Duffy binding protein II immunity: approach to *Plasmodium vivax* vaccine development in Thailand. *PLoS ONE* **7**, e35769.

Chuangchaiya, S., Jangpatarapongsa, K., Chootong, P., Sirichaisinthop, J., Sattabongkot, J., Pattanapanyasat, K., Chotivanich, K., Troye-Blomberg, M., Cui, L. and Udomsangpetch, R. (2010). Immune response to *Plasmodium vivax* has a potential to reduce malaria severity. *Clinical & Experimental Immunology* **160**, 233–239.

Cohen, S., Mc, G. I. and Carrington, S. (1961). Gamma-globulin and acquired immunity to human malaria. *Nature* **192**, 733–737.

Cole-Tobian, J. and King, C. L. (2003). Diversity and natural selection in *Plasmodium vivax* Duffy binding protein gene. *Molecular and Biochemical Parasitology* **127**, 121–132.

Cole-Tobian, J. L., Cortes, A., Baisor, M., Kastens, W., Xainli, J., Bockarie, M., Adams, J. H. and King, C. L. (2002). Age-acquired immunity to a *Plasmodium vivax* invasion ligand, the duffy binding protein. *Journal of Infectious Diseases* **186**, 531–539.

Coleman, R. E., Kumpitak, C., Ponlawat, A., Maneechai, N., Phunkitchar, V., Rachapaew, N., Zollner, G. and Sattabongkot, J. (2004). Infectivity of asymptomatic *Plasmodium*-infected human populations to Anopheles dirus mosquitoes in western Thailand. *Journal of Medical Entomology* **41**, 201–208.

Crotty, S. and Ahmed, R. (2004). Immunological memory in humans. *Seminars in Immunology* **16**, 197–203.

Crotty, S., Felgner, P., Davies, H., Glidewell, J., Villarreal, L. and Ahmed, R. (2003). Cutting edge: long-term B cell memory in humans after smallpox vaccination. *Journal of Immunology* **171**, 4969–4973.

Cutts, J. C., Powell, R., Agius, P. A., Beeson, J. G., Simpson, J. A. and Fowkes, F. J. (2014). Immunological markers of *Plasmodium vivax* exposure and immunity: a systematic review and meta-analysis. *BMC Medicine* **12**, 150.

da Costa, A. G., Antonelli, L. R., Costa, P. A., Pimentel, J. P., Garcia, N. P., Tarrago, A. M., dos Santos Mdo, P., Nogueira, P. A., Hekcmann, M. I., Sadahiro, A., Teixeira-Carvalho, A., Martins-Filho, O. A. and Malheiro, A. (2014). The robust and modulated biomarker network elicited by the *Plasmodium vivax* infection is mainly mediated by the IL-6/IL-10 axis and is associated with the parasite load. *Journal of Immunology Research* **2014**, 318250.

da Silva-Nunes, M., Codeco, C. T., Malafronte, R. S., da Silva, N. S., Juncansen, C., Muniz, P. T. and Ferreira, M. U. (2008). Malaria on the Amazonian frontier: transmission dynamics, risk factors, spatial distribution, and prospects for control. *American Journal of Tropical Medicine and Hygiene* **79**, 624–635.

Davies, D. H., Liang, X., Hernandez, J. E., Randall, A., Hirst, S., Mu, Y., Romero, K. M., Nguyen, T. T., Kalantari-Dehaghi, M., Crotty, S., Baldi, P., Villarreal, L. P. and Felgner, P. L. (2005). Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. *Proceedings of National Academy of Sciences U S A* **102**, 547–552.

Day, N. P., Hien, T. T., Schollaardt, T., Loc, P. P., Chuong, L. V., Chau, T. T., Mai, N. T., Phu, N. H., Sinh, D. X., White, N. J. and Ho, M. (1999). The prognostic and pathophysiologic role of pro- and anti-inflammatory cytokines in severe malaria. *Journal of Infectious Diseases* **180**, 1288–1297.

de Zoysa, A. P., Herath, P. R., Abhayawardana, T. A., Padmalal, U. K. and Mendis, K. N. (1988). Modulation of human malaria transmission by anti-gamete transmission blocking immunity. *Transactions of the Royal Society of Tropical Medicines and Hygiene* **82**, 548–553.

del Portillo, H. A., Longacre, S., Khouri, E. and David, P. H. (1991). Primary structure of the merozoite surface antigen 1 of *Plasmodium vivax* reveals sequences conserved between different *Plasmodium* species. *Proceedings of the National Academy of Sciences U S A* **88**, 4030–4034.

del Portillo, H. A., Levitus, G., Camargo, L. M., Ferreira, M. U. and Mertens, F. (1992). Human IgG responses against the N-terminal region of the Merozoite Surface Protein 1 of *Plasmodium vivax*. *Memorias do Instituto Oswaldo Cruz* **87** (Suppl 3), 77–84.

Dias, S., Somarathna, M., Manamperi, A., Escalante, A. A., Gunasekera, A. M. and Udagama, P. V. (2011). Evaluation of the genetic diversity of domain II of *Plasmodium vivax* Apical Membrane Antigen 1 (PvAMA-1) and the ensuing strain-specific immune responses in patients from Sri Lanka. *Vaccine* **29**, 7491–7504.

Diggs, C. L. and Sadun, E. H. (1965). Serological cross-reactivity between *Plasmodium vivax* and *plasmodium falciparum* as determined by a modified fluorescent antibody test. *Experimental Parasitology* **16**, 217–223.

Dodoo, D., Omer, F. M., Todd, J., Akanmori, B. D., Koram, K. A. and Riley, E. M. (2002). Absolute levels and ratios of pro-inflammatory and anti-inflammatory cytokine production *in vitro* predict clinical immunity to *Plasmodium falciparum* malaria. *Journal of Infectious Diseases* **185**, 971–979.

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

Fernandes, A. A., Carvalho, L. J., Zanini, G. M., Ventura, A. M., Souza, J. M., Cotias, P. M., Silva-Filho, I. L. and Daniel-Ribeiro, C. T. (2008). Similar cytokine responses and degrees of anemia in patients with *Plasmodium falciparum* and *Plasmodium vivax* infections in the Brazilian Amazon region. *Clinical and Vaccine Immunology* **15**, 650–658.

Fernandez-Arias, C., Lopez, J. P., Hernandez-Perez, J. N., Bautista-Ojeda, M. D., Branch, O. and Rodriguez, A. (2013). Malaria inhibits surface expression of complement receptor 1 in monocytes/macrophages, causing decreased immune complex internalization. *Journal of Immunology* **190**, 3363–3372.

Fernandez-Becerra, C., Sanz, S., Brucet, M., Stanisic, D. I., Alves, F. P., Camargo, E. P., Alonso, P. L., Mueller, I. and del Portillo, H. A. (2010). Naturally-acquired humoral immune responses against the N- and C-termini of the *Plasmodium vivax* MSP1 protein in endemic regions of Brazil and Papua New Guinea using a multiplex assay. *Malaria Journal* **9**, 29.

Finney, O. C., Danziger, S. A., Molina, D. M., Vignali, M., Takagi, A., Ji, M., Stanisic, D. I., Siba, P. M., Liang, X., Aitchison, J. D., Mueller, I., Gardner, M. J. and Wang, R. (2014). Predicting anti-disease immunity using proteome arrays and sera from children naturally exposed to malaria. *Molecular & Cellular Proteomics* **13**, 2646–2660.

Fraser, T., Michon, P., Barnwell, J. W., Noe, A. R., Al-Yaman, F., Kaslow, D. C. and Adams, J. H. (1997). Expression and serologic activity of a soluble recombinant *Plasmodium vivax* Duffy binding protein. *Infection and Immunity* **65**, 2772–2777.

Gamage-Mendis, A. C., Rajakaruna, J., Carter, R. and Mendis, K. N. (1992). Transmission blocking immunity to human *Plasmodium vivax* malaria in an endemic population in Kataragama, Sri Lanka. *Parasite Immunology* **14**, 385–396.

Garg, S., Chauhan, S. S., Singh, N. and Sharma, Y. D. (2008). Immunological responses to a 39·8kDa *Plasmodium vivax* tryptophan-rich antigen (PvTRAg39·8) among humans. *Microbes and Infection* **10**, 1097–1105.

Gething, P. W., Elyazar, I. R., Moyes, C. L., Smith, D. L., Battle, K. E., Guerra, C. A., Patil, A. P., Tatem, A. J., Howes, R. E., Myers, M. F., George, D. B., Horby, P., Wertheim, H. F., Price, R. N., Mueller, I., Baird, J. K. and Hay, S. I. (2012). A long neglected world malaria map: *Plasmodium vivax* endemicity in 2010. *PLoS Neglected Tropical Diseases* **6**, e1814.

Goncalves, R. M., Salmazi, K. C., Santos, B. A., Bastos, M. S., Rocha, S. C., Boscardin, S. B., Silber, A. M., Kallas, E. G., Ferreira, M. U. and Scopel, K. K. (2010). $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells, dendritic cells, and circulating cytokines in uncomplicated malaria: do different parasite species elicit similar host responses? *Infection and Immunity* **78**, 4763–4772.

Goncalves, R. M., Scopel, K. K., Bastos, M. S. and Ferreira, M. U. (2012). Cytokine balance in human malaria: does *Plasmodium vivax* elicit more inflammatory responses than *Plasmodium falciparum*? *PLoS ONE* **7**, e44394.

Goonewardene, R., Carter, R., Gamage, C. P., Del Giudice, G., David, P. H., Howie, S. and Mendis, K. N. (1990). Human T cell proliferative responses to *Plasmodium vivax* antigens: evidence of immunosuppression following prolonged exposure to endemic malaria. *European Journal of Immunology* **20**, 1387–1391.

Grau, G. E., Taylor, T. E., Molyneux, M. E., Wirima, J. J., Vassalli, P., Hommel, M. and Lambert, P. H. (1989). Tumor necrosis factor and disease severity in children with *falciparum* malaria. *New England Journal of Medicine* **320**, 1586–1591.

Gray, D. and Skarvall, H. (1988). B-cell memory is short-lived in the absence of antigen. *Nature* **336**, 70–73.

Grimberg, B. T., Udomsangpetch, R., Xainli, J., McHenry, A., Panichakul, T., Sattabongkot, J., Cui, L., Bockarie, M., Chitnis, C., Adams, J., Zimmerman, P. A. and King, C. L. (2007). *Plasmodium vivax* invasion of human erythrocytes inhibited by antibodies directed against the Duffy binding protein. *PLoS Medicine* **4**, e337.

Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S. H., Onai, N., Schraml, B. U., Segura, E., Tussiwand, R. and Yona, S. (2014). Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nature Reviews Immunology* **14**, 571–578.

Gunewardena, D. M., Carter, R. and Mendis, K. N. (1994). Patterns of acquired anti-malarial immunity in Sri Lanka. *Memorias do Instituto Oswaldo Cruz* **89** (Suppl 2), 63–65.

Hammarlund, E., Lewis, M. W., Hansen, S. G., Strelow, L. I., Nelson, J. A., Sexton, G. J., Hanifin, J. M. and Slifka, M. K. (2003). Duration of antiviral immunity after smallpox vaccination. *Nature Medicine* **9**, 1131–1137.

Hansen, D. S. and Schofield, L. (2010). Natural regulatory T cells in malaria: host or parasite allies? *PLoS Pathogens* **6**, e1000771.

Harris, I., Sharrock, W. W., Bain, L. M., Gray, K. A., Bobogare, A., Boaz, L., Lilley, K., Krause, D., Vallely, A., Johnson, M. L., Gatton, M. L., Shanks, G. D. and Cheng, Q. (2010). A large proportion of asymptomatic *Plasmodium* infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. *Malaria Journal* **9**, 254.

Hemmer, C. J., Holst, F. G., Kern, P., Chiwakata, C. B., Dietrich, M. and Reisinger, E. C. (2006). Stronger host response per parasitized erythrocyte in *Plasmodium vivax* or ovale than in *Plasmodium falciparum* malaria. *Tropical Medicine & International Health* **11**, 817–823.

Herrera, S., Escobar, P., de Plata, C., Avila, G. I., Corradin, G. and Herrera, M. A. (1992). Human recognition of T cell epitopes on the *Plasmodium vivax* circumsporozoite protein. *Journal of Immunology* **148**, 3986–3990.

Hoffman, S. L., Isenbarger, D., Long, G. W., Sedegah, M., Szarfman, A., Waters, L., Hollingdale, M. R., van der Meide, P. H., Finbloom, D. S. and Ballou, W. R. (1989). Sporozoite vaccine induces genetically restricted T cell elimination of malaria from hepatocytes. *Science* **244**, 1078–1081.

Holder, A. A. (2009). The carboxy-terminus of merozoite surface protein 1: structure, specific antibodies and immunity to malaria. *Parasitology* **136**, 1445–1456.

Hugosson, E., Montgomery, S. M., Premji, Z., Troye-Blomberg, M. and Bjorkman, A. (2004). Higher IL-10 levels are associated with less effective clearance of *Plasmodium falciparum* parasites. *Parasite Immunology* **26**, 111–117.

Illingworth, J., Butler, N. S., Roetynck, S., Mwacharo, J., Pierce, S. K., Bejon, P., Crompton, P. D., Marsh, K. and Ndungu, F. M. (2013). Chronic exposure to *Plasmodium falciparum* is associated with phenotypic evidence of B and T cell exhaustion. *Journal of Immunology* **190**, 1038–1047.

Jain, V., Singh, P. P., Silawat, N., Patel, R., Saxena, A., Bharti, P. K., Shukla, M., Biswas, S. and Singh, N. (2010). A preliminary study on pro- and anti-inflammatory cytokine profiles in *Plasmodium vivax* malaria patients from central zone of India. *Acta Tropica* **113**, 263–268.

Jangpatarapongsa, K., Sirichaisinthop, J., Sattabongkot, J., Cui, L., Montgomery, S. M., Looareesuwan, S., Troye-Blomberg, M. and Udomsangpetch, R. (2006). Memory T cells protect against *Plasmodium vivax* infection. *Microbes and Infection* **8**, 680–686.

Jangpatarapongsa, K., Chootong, P., Sattabongkot, J., Chotivanich, K., Sirichaisinthop, J., Tungpradabkul, S., Hisaeda, H., Troye-Blomberg, M., Cui, L. and Udomsangpetch, R. (2008). *Plasmodium vivax* parasites alter the balance of myeloid and plasmacytoid dendritic cells and the induction of regulatory T cells. *European Journal of Immunology* **38**, 2697–2705.

Jangpatarapongsa, K., Xia, H., Fang, Q., Hu, K., Yuan, Y., Peng, M., Gao, Q., Sattabongkot, J., Cui, L., Li, B. and Udomsangpetch, R. (2012). Immunity to malaria in *Plasmodium vivax* infection: a study in central China. *PLoS ONE* **7**, e45971.

Kaneko, A., Chaves, L. F., Taleo, G., Kalkoa, M., Isozumi, R., Wickremasinghe, R., Perlmann, H., Takeo, S., Tsuboi, T., Tachibana, S., Kimura, M., Bjorkman, A., Troye-Blomberg, M., Tanabe, K. and Drakeley, C. (2014). Characteristic age distribution of *Plasmodium vivax* infections after malaria elimination on Aneityum Island, Vanuatu. *Infection and Immunity* **82**, 243–252.

Kano, F. S., Sanchez, B. A., Sousa, T. N., Tang, M. L., Saliba, J., Oliveira, F. M., Nogueira, P. A., Goncalves, A. Q., Fontes, C. J., Soares, I. S., Brito, C. F., Rocha, R. S. and Carvalho, L. H. (2012). *Plasmodium vivax* Duffy binding protein: baseline antibody responses and parasite polymorphisms in a well-consolidated settlement of the Amazon Region. *Tropical Medicine & International Health* **17**, 989–1000.

Karunaweera, N. D., Grau, G. E., Gamage, P., Carter, R. and Mendis, K. N. (1992). Dynamics of fever and serum levels of tumor necrosis factor are closely associated during clinical paroxysms in *Plasmodium vivax* malaria. *Proceedings of the National Academy of Sciences USA* **89**, 3200–3203.

Karunaweera, N. D., Carter, R., Grau, G. E. and Mendis, K. N. (1998). Demonstration of anti-disease immunity to *Plasmodium vivax* malaria in Sri Lanka using a quantitative method to assess clinical disease. *American Journal of Tropical Medicine and Hygiene* **58**, 204–210.

King, C. L., Michon, P., Shakri, A. R., Marcotty, A., Stanisic, D., Zimmerman, P. A., Cole-Tobian, J. L., Mueller, I. and Chitnis, C. E. (2008). Naturally acquired Duffy-binding protein-specific

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binding inhibitory antibodies confer protection from blood-stage *Plasmodium vivax* infection. *Proceedings of the National Academy of Sciences U S A* **105**, 8363–8368.

King, C. L., Adams, J. H., Xianli, J., Grimberg, B. T., McHenry, A. M., Greenberg, L. J., Siddiqui, A., Howes, R. E., da Silva-Nunes, M., Ferreira, M. U. and Zimmerman, P. A. (2011). Fy(a)/Fy(b) antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to *Plasmodium vivax* malaria. *Proceedings of the National Academy of Sciences U S A* **108**, 20113–20118.

Koch, R. (1900). Professor Koch's investigations on Malaria: fourth report to the Colonial Department of the German Colonial Office. *British Medical Journal* **1**, 1597–1598.

Koepfli, C., Colborn, K. L., Kiniboro, B., Lin, E., Speed, T. P., Siba, P. M., Felger, I. and Mueller, I. (2013). A high force of *Plasmodium vivax* blood-stage infection drives the rapid acquisition of immunity in papua new guinean children. *PLoS Neglected Tropical Diseases* **7**, e2403.

Kremsner, P. G., Winkler, S., Brandts, C., Wildling, E., Jenne, L., Graninger, W., Prada, J., Bienzle, U., Juillard, P. and Grau, G. E. (1995). Prediction of accelerated cure in *Plasmodium falciparum* malaria by the elevated capacity of tumor necrosis factor production. *American Journal of Tropical Medicines and Hygiene* **53**, 532–538.

Kwiatkowski, D., Hill, A. V., Sambou, I., Twumasi, P., Castracane, J., Manogue, K. R., Cerami, A., Brewster, D. R. and Greenwood, B. M. (1990). TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet* **336**, 1201–1204.

Kwiatkowski, D., Molyneux, M. E., Stephens, S., Curtis, N., Klein, N., Pointaire, P., Smit, M., Allan, R., Brewster, D. R., Grau, G. E. and Greenwood, B. M. (1993). Anti-TNF therapy inhibits fever in cerebral malaria. *Quarterly Journal of Medicine* **86**, 91–98.

Ladeia-Andrade, S., Ferreira, M. U., Scopel, K. K., Braga, E. M., Bastos Mda, S., Wunderlich, G. and Coura, J. R. (2007). Naturally acquired antibodies to merozoite surface protein (MSP)-1(19) and cumulative exposure to *Plasmodium falciparum* and *Plasmodium vivax* in remote populations of the Amazon Basin of Brazil. *Memorias do Instituto Oswaldo Cruz* **102**, 943–951.

Ladeia-Andrade, S., Ferreira, M. U., de Carvalho, M. E., Curado, I. and Coura, J. R. (2009). Age-dependent acquisition of protective immunity to malaria in riverine populations of the Amazon Basin of Brazil. *American Journal of Tropical Medicine and Hygiene* **80**, 452–459.

Leoratti, F. M., Trevelin, S. C., Cunha, F. Q., Rocha, B. C., Costa, P. A., Gravina, H. D., Tada, M. S., Pereira, D. B., Golenbock, D. T., Antonelli, L. R. and Gazzinelli, R. T. (2012). Neutrophil paralysis in *Plasmodium vivax* malaria. *PLoS Neglected Tropical Diseases* **6**, e1710.

Lim, K. J., Park, J. W., Yeom, J. S., Lee, Y. H., Yoo, S. B., Oh, J. H., Sohn, M. J., Bahk, Y. Y. and Kim, Y. S. (2004). Humoral responses against the C-terminal region of merozoite surface protein 1 can be remembered for more than 30 years in persons exposed to *Plasmodium vivax*. *Parasitology Research* **92**, 384–389.

Lima-Junior, J. C., Tran, T. M., Meyer, E. V., Singh, B., De-Simone, S. G., Santos, F., Daniel-Ribeiro, C. T., Moreno, A., Barnwell, J. W., Galinski, M. R. and Oliveira-Ferreira, J. (2008). Naturally acquired humoral and cellular immune responses to *Plasmodium vivax* merozoite surface protein 9 in Northwestern Amazon individuals. *Vaccine* **26**, 6645–6654.

Lima-Junior, J. C., Jiang, J., Rodrigues-da-Silva, R. N., Banic, D. M., Tran, T. M., Ribeiro, R. Y., Meyer, V. S., De-Simone, S. G., Santos, F., Moreno, A., Barnwell, J. W., Galinski, M. R. and Oliveira-Ferreira, J. (2011). B cell epitope mapping and characterization of naturally acquired antibodies to the *Plasmodium vivax* merozoite surface protein-3alpha (PvMSP-3alpha) in malaria exposed individuals from Brazilian Amazon. *Vaccine* **29**, 1801–1811.

Lima-Junior, J. C., Rodrigues-da-Silva, R. N., Banic, D. M., Jiang, J., Singh, B., Fabricio-Silva, G. M., Porto, L. C., Meyer, E. V., Moreno, A., Rodrigues, M. M., Barnwell, J. W., Galinski, M. R. and de Oliveira-Ferreira, J. (2012). Influence of HLA-DRB1 and HLA-DQB1 alleles on IgG antibody response to the *P. vivax* MSP-1, MSP-3alpha and MSP-9 in individuals from Brazilian endemic area. *PLoS ONE* **7**, e36419.

Lin, E., Kiniboro, B., Gray, L., Dobbie, S., Robinson, L., Laumaea, A., Schopflin, S., Stanisic, D., Betuela, I., Blood-Zikursh, M., Siba, P., Felger, I., Schofield, L., Zimmerman, P. and Mueller, I. (2010). Differential patterns of infection and disease with *P. falciparum* and *P. vivax* in young Papua New Guinean children. *PLoS ONE* **5**, e9047.

Lu, F., Li, J., Wang, B., Cheng, Y., Kong, D. H., Cui, L., Ha, K. S., Sattabongkot, J., Tsuboi, T. and Han, E. T. (2014). Profiling the humoral immune responses to *Plasmodium vivax* infection and identification of candidate immunogenic rhoptry-associated membrane antigen (RAMA). *Journal of Proteomics* **102**, 66–82.

Luxemburger, C., Thwai, K. L., White, N. J., Webster, H. K., Kyle, D. E., Maelankirri, L., Chongsuphajaisiddhi, T. and Nosten, F. (1996). The epidemiology of malaria in a Karen population on the western border of Thailand. *Transactions of the Royal Society of Tropical Medicines and Hygiene* **90**, 105–111.

Maestre, A., Muskus, C., Duque, V., Agudelo, O., Liu, P., Takagi, A., Ntumngia, F. B., Adams, J. H., Sim, K. L., Hoffman, S. L., Corradin, G., Velez, I. D. and Wang, R. (2010). Acquired antibody responses against *Plasmodium vivax* infection vary with host genotype for duffy antigen receptor for chemokines (DARC). *PLoS ONE* **5**, e11437.

Maitland, K., Williams, T. N., Bennett, S., Newbold, C. I., Peto, T. E., Viji, J., Timothy, R., Clegg, J. B., Weatherall, D. J. and Bowden, D. K. (1996). The interaction between *Plasmodium falciparum* and *P. vivax* in children on Espiritu Santo island, Vanuatu. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 614–620.

Manz, R. A., Thiel, A. and Radbruch, A. (1997). Lifetime of plasma cells in the bone marrow. *Nature* **388**, 133–134.

Manz, R. A., Lohning, M., Cassese, G., Thiel, A. and Radbruch, A. (1998). Survival of long-lived plasma cells is independent of antigen. *International Immunology* **10**, 1703–1711.

Maruyama, M., Lam, K. P. and Rajewsky, K. (2000). Memory B-cell persistence is independent of persisting immunizing antigen. *Nature* **407**, 636–642.

Medina, T. S., Costa, S. P., Oliveira, M. D., Ventura, A. M., Souza, J. M., Gomes, T. F., Vallinoto, A. C., Povoa, M. M., Silva, J. S. and Cunha, M. G. (2011). Increased interleukin-10 and interferon-gamma levels in *Plasmodium vivax* malaria suggest a reciprocal regulation which is not altered by IL-10 gene promoter polymorphism. *Malaria Journal* **10**, 264.

Mehrizi, A. A., Zakeri, S., Salmanian, A. H., Sanati, M. H. and Djadid, N. D. (2009). IgG subclasses pattern and high-avidity antibody to the C-terminal region of merozoite surface protein 1 of *Plasmodium vivax* in an unstable hypoendemic region in Iran. *Acta Tropica* **112**, 1–7.

Menard, D., Barnadas, C., Bouchier, C., Henry-Halldin, C., Gray, L. R., Ratsimbasoa, A., Thonier, V., Carod, J. F., Domarle, O., Colin, Y., Bertrand, O., Picot, J., King, C. L., Grimberg, B. T., Mercereau-Puijalon, O. and Zimmerman, P. A. (2010). *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people. *Proceedings of the National Academy of Sciences U S A* **107**, 5967–5971.

Mendes, C., Dias, F., Figueiredo, J., Mora, V. G., Cano, J., de Sousa, B., do Rosario, V. E., Benito, A., Berzosa, P. and Arez, A. P. (2011). Duffy negative antigen is no longer a barrier to *Plasmodium vivax* –molecular evidences from the African West Coast (Angola and Equatorial Guinea). *PLoS Neglected Tropical Diseases* **5**, e1192.

Mendis, K. N., Munesinghe, Y. D., de Silva, Y. N., Keragalla, I. and Carter, R. (1987). Malaria transmission-blocking immunity induced by natural infections of *Plasmodium vivax* in humans. *Infection and Immunity* **55**, 369–372.

Mendonca, V. R., Queiroz, A. T., Lopes, F. M., Andrade, B. B. and Barral-Netto, M. (2013). Networking the host immune response in *Plasmodium vivax* malaria. *Malaria Journal* **12**, 69.

Michon, P., Fraser, T. and Adams, J. H. (2000). Naturally acquired and vaccine-elicited antibodies block erythrocyte cytoadherence of the *Plasmodium vivax* Duffy binding protein. *Infection and Immunity* **68**, 3164–3171.

Michon, P., Cole-Tobian, J. L., Dabod, E., Schoepflin, S., Igu, J., Susapu, M., Tarongka, N., Zimmerman, P. A., Reeder, J. C., Beeson, J. G., Schofield, L., King, C. L. and Mueller, I. (2007). The risk of malarial infections and disease in Papua New Guinean children. *American Journal of Tropical Medicines and Hygiene* **76**, 997–1008.

Michon, P. A., Arevalo-Herrera, M., Fraser, T., Herrera, S. and Adams, J. H. (1998). Serologic responses to recombinant *Plasmodium vivax* Duffy binding protein in a Colombian village. *American Journal of Tropical Medicine and Hygiene* **59**, 597–599.

Migot, F., Millet, P., Chougnet, C., Lepers, J. P. and Deloron, P. (1993). Humoral and cellular immune responses to the circumsporozoite protein of *Plasmodium vivax* in Madagascar. *American Journal of Tropical Medicine and Hygiene* **48**, 524–529.

Mittra, P., Singh, N. and Sharma, Y. D. (2010). *Plasmodium vivax*: immunological properties of tryptophan-rich antigens PvTRAg 35·2 and PvTRAg 80·6. *Microbes and Infection* **12**, 1019–1026.

Molina, D. M., Finney, O. C., Arevalo-Herrera, M., Herrera, S., Felgner, P. L., Gardner, M. J., Liang, X. and Wang, R. (2012). *Plasmodium vivax* pre-erythrocytic-stage antigen discovery: exploiting naturally acquired humoral responses. *American Journal of Tropical Medicine and Hygiene* **87**, 460–469.

Moncunill, G., Aponte, J. J., Nhabomba, A. J. and Dobano, C. (2013). Performance of multiplex commercial kits to quantify cytokine and chemokine responses in culture supernatants from *Plasmodium falciparum* stimulations. *PLoS ONE* **8**, e52587.

Morais, C. G., Soares, I. S., Carvalho, L. H., Fontes, C. J., Krettli, A. U. and Braga, E. M. (2005). IgG isotype to C-terminal 19 kDa of *Plasmodium vivax* merozoite surface protein 1 among subjects with different levels of exposure to malaria in Brazil. *Parasitology Research* **95**, 420–426.

Morais, C. G., Soares, I. S., Carvalho, L. H., Fontes, C. J., Krettli, A. U. and Braga, E. M. (2006). Antibodies to *Plasmodium vivax* apical membrane antigen 1: persistence and correlation with malaria transmission intensity. *American Journal of Tropical Medicine and Hygiene* **75**, 582–587.

Mourao, L. C., Morais, C. G., Bueno, L. L., Jimenez, M. C., Soares, I. S., Fontes, C. J., Guimaraes Lacerda, M. V., Xavier, M. S., Barnwell, J. W., Galinski, M. R. and Braga, E. M. (2012). Naturally acquired antibodies to *Plasmodium vivax* blood-stage vaccine candidates (PvMSP-1(1)(9) and PvMSP-3alpha(3)(5)(9)(-)(7)(9)(8) and their relationship with hematological features in malaria patients from the Brazilian Amazon. *Microbes and Infection* **14**, 730–739.

Muellenbeck, M. F., Ueberheide, B., Amulic, B., Epp, A., Fenyo, D., Busse, C. E., Esen, M., Theisen, M., Mordmuller, B. and Wardemann, H. (2013). Atypical and classical memory B cells produce *Plasmodium falciparum* neutralizing antibodies. *Journal of Experimental Medicine* **210**, 389–399.

Mueller, I., Schoepflin, S., Smith, T. A., Benton, K. L., Bretscher, M. T., Lin, E., Kiniboro, B., Zimmerman, P. A., Speed, T. P., Siba, P. and Felger, I. (2012). Force of infection is key to understanding the epidemiology of *Plasmodium falciparum* malaria in Papua New Guinean children. *Proceedings of the National Academy of Sciences USA* **109**, 10030–10035.

Mueller, I., Galinski, M. R., Tsuboi, T., Arevalo-Herrera, M., Collins, W. E. and King, C. L. (2013). Natural acquisition of immunity to *Plasmodium vivax*: epidemiological observations and potential targets. *Advances in Parasitology* **81**, 77–131.

Mufalo, B. C., Gentil, F., Bargieri, D. Y., Costa, F. T., Rodrigues, M. M. and Soares, I. S. (2008). *Plasmodium vivax* apical membrane antigen-1: comparative recognition of different domains by antibodies induced during natural human infection. *Microbes and Infection* **10**, 1266–1273.

Ndungu, F. M., Olotu, A., Mwacharo, J., Nyonda, M., Apfeld, J., Mramba, L. K., Fegan, G. W., Bejon, P. and Marsh, K. (2012). Memory B cells are a more reliable archive for historical antimalarial responses than plasma antibodies in no-longer exposed children. *Proceedings of the National Academy of Sciences USA* **109**, 8247–8252.

Nguyen, H. V., van den Eede, P., van Overmeir, C., Thang, N. D., Hung le, X., D'Alessandro, U. and Erhart, A. (2012). Marked age-dependent prevalence of symptomatic and patent infections and complexity of distribution of human *Plasmodium* species in central Vietnam. *American Journal of Tropical Medicines and Hygiene* **87**, 989–995.

Nogueira, P. A., Alves, F. P., Fernandez-Becerra, C., Pein, O., Santos, N. R., Pereira da Silva, L. H., Camargo, E. P. and del Portillo, H. A. (2006). A reduced risk of infection with *Plasmodium vivax* and clinical protection against malaria are associated with antibodies against the N terminus but not the C terminus of merozoite surface protein 1. *Infection and Immunity* **74**, 2726–2733.

Ochsenbein, A. F., Pinschewer, D. D., Sierro, S., Horvath, E., Hengartner, H. and Zinkernagel, R. M. (2000). Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proceedings of the National Academy of Sciences USA* **97**, 13263–13268.

Oliveira, T. R., Fernandez-Becerra, C., Jimenez, M. C., Del Portillo, H. A. and Soares, I. S. (2006). Evaluation of the acquired immune responses to *Plasmodium vivax* VIR variant antigens in individuals living in malaria-endemic areas of Brazil. *Malaria Journal* **5**, 83.

Park, J. W., Moon, S. H., Yeom, J. S., Lim, K. J., Sohn, M. J., Jung, W. C., Cho, Y. J., Jeon, K. W., Ju, W., Ki, C. S., Oh, M. D. and Choe, K. (2001). Naturally acquired antibody responses to the C-terminal region of merozoite surface protein 1 of *Plasmodium vivax* in Korea. *Clinical and Diagnostic Laboratory Immunology* **8**, 14–20.

Peiris, J. S., Premawansa, S., Ranawaka, M. B., Udagama, P. V., Munasinghe, Y. D., Nanayakkara, M. V., Gamage, C. P., Carter, R., David, P. H. and Mendis, K. N. (1988). Monoclonal and polyclonal antibodies both block and enhance transmission of human *Plasmodium vivax* malaria. *American Journal of Tropical Medicines and Hygiene* **39**, 26–32.

Phimpraphi, W., Paul, R. E., Yimsamran, S., Puangsa-art, S., Thanyavanich, N., Maneeboonyang, W., Prommongkol, S., Sornklom, S., Chaimungkun, W., Chavez, I. F., Blanc, H., Looareesuwan, S., Sakuntabhai, A. and Singhasivanon, P. (2008). Longitudinal study of *Plasmodium falciparum* and *Plasmodium vivax* in a Karen population in Thailand. *Malaria Journal* **7**, 99.

Pichyangkul, S., Yongvanitchit, K., Kum-arb, U., Hemmi, H., Akira, S., Krieg, A. M., Heppner, D. G., Stewart, V. A., Hasegawa, H., Looareesuwan, S., Shanks, G. D. and Miller, R. S. (2004). Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a Toll-like receptor 9-dependent pathway. *Journal of Immunology* **172**, 4926–4933.

Pinzon-Charry, A., Woodberry, T., Kienzle, V., McPhun, V., Minigo, G., Lampah, D. A., Kenangalem, E., Engwerda, C., Lopez, J. A., Anstey, N. M. and Good, M. F. (2013). Apoptosis and dysfunction of blood dendritic cells in patients with *falciparum* and *vivax* malaria. *Journal of Experimental Medicine* **210**, 1635–1646.

Pitabut, N., Panichakorn, J., Mahakunkijcharoen, Y., Hirunpetcharat, C., Looareesuwan, S. and Khusmith, S. (2007). IgG antibody profile to c-terminal region of *Plasmodium vivax* merozoite surface protein-1 in Thai individuals exposed to malaria. *Southeast Asian Journal of Tropical Medicine and Public Health* **38**, 1–7.

Pombo, D. J., Lawrence, G., Hirunpetcharat, C., Rzepczyk, C., Bryden, M., Cloonan, N., Anderson, K., Mahakunkijcharoen, Y., Martin, L. B., Wilson, D., Elliott, S., Elliott, S., Eisen, D. P., Weinberg, J. B., Saul, A. and Good, M. F. (2002). Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. *Lancet* **360**, 610–617.

Praba-Egge, A. D., Montenegro, S., Arevalo-Herrera, M., Hopper, T., Herrera, S. and James, M. A. (2003). Human cytokine responses to meso-endemic malaria on the Pacific Coast of Colombia. *Annals of Tropical Medicine and Parasitology* **97**, 327–337.

Price, R. N., Tjitra, E., Guerra, C. A., Yeung, S., White, N. J. and Anstey, N. M. (2007). *Vivax* malaria: neglected and not benign. *American Journal of Tropical Medicine and Hygiene* **77**, 79–87.

Ramsey, J. M., Salinas, E. and Rodriguez, M. H. (1996). Acquired transmission-blocking immunity to *Plasmodium vivax* in a population of southern coastal Mexico. *American Journal of Tropical Medicines and Hygiene* **54**, 458–463.

Ranawaka, M. B., Munesinghe, Y. D., de Silva, D. M., Carter, R. and Mendis, K. N. (1988). Boosting of transmission-blocking immunity during natural *Plasmodium vivax* infections in humans depends upon frequent reinfection. *Infection and Immunity* **56**, 1820–1824.

Ray, P., Ansari, M. A. and Sharma, Y. D. (1994). *Plasmodium vivax*: immune responses in a cross-section of the population in the Delhi area of India. *American Journal of Tropical Medicines and Hygiene* **51**, 436–443.

Raza, A., Ghanchi, N. K., Sarwar Zubairi, A., Raheem, A., Nizami, S. and Beg, M. A. (2013). Tumor necrosis factor-alpha, interleukin-10, intercellular and vascular adhesion molecules are possible biomarkers of disease severity in complicated *Plasmodium vivax* isolates from Pakistan. *PLoS ONE* **8**, e81363.

Renia, L., Marussig, M. S., Grillot, D., Pied, S., Corradin, G., Miltgen, F., Del Giudice, G. and Mazier, D. (1991). *In vitro* activity of $CD4^+$ and $CD8^+$ T lymphocytes from mice immunized with a synthetic malaria peptide. *Proceedings of the National Academy of Sciences USA* **88**, 7963–7967.

Renia, L., Grillot, D., Marussig, M., Corradin, G., Miltgen, F., Lambert, P. H., Mazier, D. and Del Giudice, G. (1993). Effector functions of circumsporozoite peptide-primed CD4+ T cell clones against *Plasmodium yoelii* liver stages. *Journal of Immunology* **150**, 1471–1478.

Requena, P., Campo, J. J., Umbers, A. J., Ome, M., Wangnapi, R., Barrios, D., Robinson, L. J., Samol, P., Rosanas-Urgell, A., Ubillos, I., Mayor, A., Lopez, M., de Lazzari, E., Arevalo-Herrera, M., Fernandez-Becerra, C., del Portillo, H., Chitnis, C. E., Siba, P. M., Bardaji, A., Mueller, I., Rogerson, S., Menendez, C. and Dobano, C. (2014). Pregnancy and malaria exposure are associated with changes in the B cell pool and in plasma eotaxin levels. *Journal of Immunology* **193**, 2971–2983.

Riccio, E. K., Totino, P. R., Pratt-Riccio, L. R., Ennes-Vidal, V., Soares, I. S., Rodrigues, M. M., de Souza, J. M., Daniel-Ribeiro, C. T. and Ferreira-da-Cruz Mde, F. (2013). Cellular and humoral immune responses against the *Plasmodium vivax* MSP-1(1)(9) malaria vaccine candidate in individuals living in an endemic area in north-eastern Amazon region of Brazil. *Malaria Journal* **12**, 326.

Riley, E. M., Allen, S. J., Wheeler, J. G., Blackman, M. J., Bennett, S., Takacs, B., Schonfeld, H. J., Holder, A. A. and Greenwood, B. M. (1992). Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced malaria morbidity. *Parasite Immunology* **14**, 321–337.

Rodrigues, M. M., Dutra, A. P. and Yoshida, N. (1991). Cellular immune response of humans to the circumsporozoite protein of *Plasmodium vivax*. *Memorias do Instituto Oswaldo Cruz* **86**, 153–158.

Rodrigues, M. H., Cunha, M. G., Machado, R. L., Ferreira, O. C., Jr., Rodrigues, M. M. and Soares, I. S. (2003). Serological detection of *Plasmodium vivax* malaria using recombinant proteins corresponding to the 19-kDa C-terminal region of the merozoite surface protein-1. *Malaria Journal* **2**, 39.

Rodrigues, M. H., Rodrigues, K. M., Oliveira, T. R., Comodo, A. N., Rodrigues, M. M., Kocken, C. H., Thomas, A. W. and Soares, I. S. (2005). Antibody response of naturally infected individuals to recombinant *Plasmodium vivax* apical membrane antigen-1. *International Journal of Parasitology* **35**, 185–192.

Rodrigues-da-Silva, R. N., Lima-Junior Jda, C., e Fonseca Bde, P., Antas, P. R., Baldez, A., Storer, F. L., Santos, F., Banic, D. M. and de Oliveira-Ferreira, J. (2014). Alterations in cytokines and haematological parameters during the acute and convalescent phases of *Plasmodium falciparum* and *Plasmodium vivax* infections. *Memorias do Instituto Oswaldo Cruz* **109**, 154–162.

Ryan, J. R., Stoute, J. A., Amon, J., Dunton, R. F., Mtalib, R., Koros, J., Owour, B., Luckhart, S., Wirtz, R. A., Barnwell, J. W. and Rosenberg, R. (2006). Evidence for transmission of *Plasmodium vivax* among a duffy antigen negative population in Western Kenya. *American Journal of Tropical Medicines and Hygiene* **75**, 575–581.

Sabchareon, A., Burnouf, T., Ouattara, D., Attanath, P., Bouharoun-Tayoun, H., Chantavanich, P., Foucault, C., Chongsuphajaisiddhi, T. and Druilhe, P. (1991). Parasitologic and clinical human response to immunoglobulin administration in *falciparum* malaria. *American Journal of Tropical Medicines and Hygiene* **45**, 297–308.

Salwati, E., Minigo, G., Woodberry, T., Piera, K. A., de Silva, H. D., Kenangalem, E., Tjitra, E., Coppel, R. L., Price, R. N., Anstey, N. M. and Plebanski, M. (2011). Differential cellular recognition of antigens during acute *Plasmodium falciparum* and *Plasmodium vivax* malaria. *Journal of Infectious Diseases* **203**, 1192–1199.

Sattabongkot, J., Maneechai, N., Phunkitchar, V., Eikarat, N., Khuntirat, B., Sirichaisinthop, J., Burge, R. and Coleman, R. E. (2003). Comparison of artificial membrane feeding with direct skin feeding to estimate the infectiousness of *Plasmodium vivax* gametocyte carriers to mosquitoes. *American Journal of Tropical Medicines and Hygiene* **69**, 529–535.

Schittek, B. and Rajewsky, K. (1990). Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. *Nature* **346**, 749–751.

Scholzen, A., Teirlinck, A. C., Bijker, E. M., Roestenberg, M., Hermsen, C. C., Hoffman, S. L. and Sauerwein, R. W. (2014). BAFF and BAFF receptor levels correlate with B cell subset activation and redistribution in controlled human malaria infection. *Journal of Immunology* **192**, 3719–3729.

Seth, R. K., Bhat, A. A., Rao, D. N. and Biswas, S. (2010). Acquired immune response to defined *Plasmodium vivax* antigens in individuals residing in northern India. *Microbes and Infection* **12**, 199–206.

Sfikakis, P. P., Boletis, J. N. and Tsokos, G. C. (2005). Rituximab anti-B-cell therapy in systemic lupus erythematosus: pointing to the future. *Current Opinion in Rheumatology* **17**, 550–557.

Siddiqui, A. A., Bora, H., Singh, N., Dash, A. P. and Sharma, Y. D. (2008). Expression, purification, and characterization of the immunological response to a 40-kilodalton *Plasmodium vivax* tryptophan-rich antigen. *Infection and Immunity* **76**, 2576–2586.

Silva, A. L., Lacerda, M. V., Fujiwara, R. T., Bueno, L. L. and Braga, E. M. (2013). *Plasmodium vivax* infection induces expansion of activated naive/memory T cells and differentiation into a central memory profile. *Microbes and Infection* **15**, 837–843.

Skorokhod, O. A., Alessio, M., Mordmuller, B., Arese, P. and Schwarzer, E. (2004). Hemozoin (malarial pigment) inhibits differentiation and maturation of human monocyte-derived dendritic cells: a peroxisome proliferator-activated receptor-gamma-mediated effect. *Journal of Immunology* **173**, 4066–4074.

Slifka, M. K., Antia, R., Whitmire, J. K. and Ahmed, R. (1998). Humoral immunity due to long-lived plasma cells. *Immunity* **8**, 363–372.

Smith, T., Felger, I., Tanner, M. and Beck, H. P. (1999). Premunition in *Plasmodium falciparum* infection: insights from the epidemiology of multiple infections. *Transactions of the Royal Society of Tropical Medicines and Hygiene* **93** (Suppl 1), 59–64.

Soares, I. S., Levitus, G., Souza, J. M., Del Portillo, H. A. and Rodrigues, M. M. (1997). Acquired immune responses to the N- and C-terminal regions of *Plasmodium vivax* merozoite surface protein 1 in individuals exposed to malaria. *Infection and Immunity* **65**, 1606–1614.

Soares, I. S., da Cunha, M. G., Silva, M. N., Souza, J. M., Del Portillo, H. A. and Rodrigues, M. M. (1999a). Longevity of naturally acquired antibody responses to the N- and C-terminal regions of *Plasmodium vivax* merozoite surface protein 1. *American Journal of Tropical Medicine and Hygiene* **60**, 357–363.

Soares, I. S., Oliveira, S. G., Souza, J. M. and Rodrigues, M. M. (1999b). Antibody response to the N and C-terminal regions of the *Plasmodium vivax* Merozoite Surface Protein 1 in individuals living in an area of exclusive transmission of *P. vivax* malaria in the north of Brazil. *Acta Tropica* **72**, 13–24.

Soares, I. S. and Rodrigues, M. M. (2002). Immunogenic properties of the *Plasmodium vivax* vaccine candidate MSP1(19) expressed as a secreted non-glycosylated polypeptide from Pichia pastoris. *Parasitology* **124**, 237–246.

Souza-Silva, F. A., da Silva-Nunes, M., Sanchez, B. A., Ceravolo, I. P., Malafronte, R. S., Brito, C. F., Ferreira, M. U. and Carvalho, L. H. (2010). Naturally acquired antibodies to *Plasmodium vivax* Duffy binding protein (DBP) in Brazilian Amazon. *American Journal of Tropical Medicines and Hygiene* **82**, 185–193.

Souza-Silva, F. A., Torres, L. M., Santos-Alves, J. R., Tang, M. L., Sanchez, B. A., Sousa, T. N., Fontes, C. J., Nogueira, P. A., Rocha, R. S., Brito, C. F., Adams, J. H., Kano, F. S. and Carvalho, L. H. (2014). Duffy antigen receptor for chemokine (DARC) polymorphisms and its involvement in acquisition of inhibitory anti-duffy binding protein II (DBPII) immunity. *PLoS ONE* **9**, e93782.

Speiser, D. E., Utzschneider, D. T., Oberle, S. G., Munz, C., Romero, P. and Zehn, D. (2014). T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? *Nature Reviews Immunology* **14**, 768–774.

Stanisic, D. I., Javati, S., Kiniboro, B., Lin, E., Jiang, J., Singh, B., Meyer, E. V., Siba, P., Koepfli, C., Felger, I., Galinski, M. R. and Mueller, I. (2013). Naturally acquired immune responses to *P. vivax* merozoite surface protein 3alpha and merozoite surface protein 9 are associated with reduced risk of *P. vivax* malaria in young Papua New Guinean children. *PLoS Neglected Tropical Diseases* **7**, e2498.

Stanisic, D. I., Fowkes, F. J., Koinari, M., Javati, S., Lin, E., Kiniboro, B., Richards, J. S., Robinson, L. J., Schofield, L., Kazura, J. W., King, C. L., Zimmerman, P., Felger, I., Siba, P. M., Mueller, I. and Beeson, J. G. (2015). Acquisition of antibodies against *Plasmodium falciparum* merozoites and malaria immunity in young children and the influence of age, force of infection, and magnitude of response. *Infection and Immunity* **83**, 646–660.

Storti-Melo, L. M., Souza-Neiras, W. C., Cassiano, G. C., Taveira, L. C., Cordeiro, A. J., Couto, V. S., Povoa, M. M., Cunha, M. G., Echeverry, D. M., Rossit, A. R., Arevalo-Herrera, M., Herrera, S. and Machado, R. L. (2011). Evaluation of the naturally acquired antibody immune response to the Pv200L N-terminal fragment of *Plasmodium vivax* merozoite surface protein-1 in four areas of the Amazon Region of Brazil. *American Journal of Tropical Medicines and Hygiene* **84**, 58–63.

Suh, I. B., Choi, H. K., Lee, S. W., Woo, S. K., Kang, H. Y., Won, Y. D., Cho, M. and Lim, C. S. (2003). Reactivity of sera from cases of *Plasmodium vivax* malaria towards three recombinant antigens based on the surface proteins of the parasite. *Annals of Tropical Medicine and Parasitology* **97**, 481–487.

Suphavilai, C., Looareesuwan, S. and Good, M. F. (2004). Analysis of circumsporozoite protein-specific immune responses following recent infection with *Plasmodium vivax*. *American Journal of Tropical Medicines and Hygiene* **71**, 29–39.

Taliaferro, W. H. (1949). Immunity to the Malaria infections. In *Malariology* (ed. Boyd, M. F.), pp. 935–965. W.B. Saunders, Philadelphia.

Tew, J. G., Phipps, R. P. and Mandel, T. E. (1980). The maintenance and regulation of the humoral immune response: persisting antigen and the role of follicular antigen-binding dendritic cells as accessory cells. *Immunology Reviews* **53**, 175–201.

Torre, D., Ferrario, G., Matteelli, A., Speranza, F., Giola, M., Pugliese, A., Cantamessa, C., Carosi, G. and Fiori, G. P. (1998). Levels of circulating nitrate/nitrite and gamma interferon not increased in uncomplicated malaria. *Infection* **26**, 301–303.

Tran, T. M., Oliveira-Ferreira, J., Moreno, A., Santos, F., Yazdani, S. S., Chitnis, C. E., Altman, J. D., Meyer, E. V., Barnwell, J. W. and Galinski, M. R. (2005). Comparison of IgG reactivities to *Plasmodium vivax* merozoite invasion antigens in a Brazilian Amazon population. *American Journal of Tropical Medicines and Hygiene* **73**, 244–255.

Tsai, L. M. and Yu, D. (2014). Follicular helper T-cell memory: establishing new frontiers during antibody response. *Immunology and Cell Biology* **92**, 57–63.

Tsuboi, T., Kappe, S. H., al-Yaman, F., Prickett, M. D., Alpers, M. and Adams, J. H. (1994). Natural variation within the principal adhesion

domain of the *Plasmodium vivax* duffy binding protein. *Infection and Immunity* **62**, 5581–5586.

Tsuboi, T., Takeo, S., Iriko, H., Jin, L., Tsuchimochi, M., Matsuda, S., Han, E. T., Otsuki, H., Kaneko, O., Sattabongkot, J., Udomsangpetch, R., Sawasaki, T., Torii, M. and Endo, Y. (2008). Wheat germ cell-free system-based production of malaria proteins for discovery of novel vaccine candidates. *Infection and Immunity* **76**, 1702–1708.

Urban, B. C., Ferguson, D. J., Pain, A., Willcox, N., Plebanski, M., Austyn, J. M. and Roberts, D. J. (1999). *Plasmodium falciparum*-infected erythrocytes modulate the maturation of dendritic cells. *Nature* **400**, 73–77.

Urban, B. C., Mwangi, T., Ross, A., Kinyanjui, S., Mosobo, M., Kai, O., Lowe, B., Marsh, K. and Roberts, D. J. (2001). Peripheral blood dendritic cells in children with acute *Plasmodium falciparum* malaria. *Blood* **98**, 2859–2861.

Urban, B. C., Cordery, D., Shafi, M. J., Bull, P. C., Newbold, C. I., Williams, T. N. and Marsh, K. (2006). The frequency of BDCA3-positive dendritic cells is increased in the peripheral circulation of Kenyan children with severe malaria. *Infection and Immunity* **74**, 6700–6706.

Valderrama-Aguirre, A., Quintero, G., Gomez, A., Castellanos, A., Perez, Y., Mendez, F., Arevalo-Herrera, M. and Herrera, S. (2005). Antigenicity, immunogenicity, and protective efficacy of *Plasmodium vivax* MSP1 PV200l: a potential malaria vaccine subunit. *American Journal of Tropical Medicines and Hygiene* **73**, 16–24.

Valizadeh, V., Zakeri, S., Mehrizi, A. A. and Djadid, N. D. (2014). Non-allele specific antibody responses to genetically distinct variant forms of *Plasmodium vivax* Duffy binding protein (PvDBP-II) in Iranians exposed to seasonal malaria transmission. *Acta Tropica* **136**, 89–100.

Versiani, F. G., Almeida, M. E., Melo, G. C., Versiani, F. O., Orlandi, P. P., Mariuba, L. A., Soares, L. A., Souza, L. P., da Silva Balieiro, A. A., Monteiro, W. M., Costa, F. T., del Portillo, H. A., Lacerda, M. V. and Nogueira, P. A. (2013). High levels of IgG3 anti ICB2-5 in *Plasmodium vivax* -infected individuals who did not develop symptoms. *Malaria Journal* **12**, 294.

Weiss, G. E., Crompton, P. D., Li, S., Walsh, L. A., Moir, S., Traore, B., Kayentao, K., Ongoiba, A., Doumbo, O. K. and Pierce, S. K. (2009). Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *Journal of Immunology* **183**, 2176–2182.

Weiss, G. E., Clark, E. H., Li, S., Traore, B., Kayentao, K., Ongoiba, A., Hernandez, J. N., Doumbo, O. K., Pierce, S. K., Branch, O. H. and Crompton, P. D. (2011). A positive correlation between atypical memory B cells and *Plasmodium falciparum* transmission intensity in cross-sectional studies in Peru and Mali. *PLoS ONE* **6**, e15983.

Weiss, W. R., Mellouk, S., Houghten, R. A., Sedegah, M., Kumar, S., Good, M. F., Berzofsky, J. A., Miller, L. H. and Hoffman, S. L. (1990). Cytotoxic T cells recognize a peptide from the circumsporozoite protein on malaria-infected hepatocytes. *Journal of Experimental Medicine* **171**, 763–773.

Westenberger, S. J., McClean, C. M., Chattopadhyay, R., Dharia, N. V., Carlton, J. M., Barnwell, J. W., Collins, W. E., Hoffman, S. L., Zhou, Y., Vinetz, J. M. and Winzeler, E. A. (2010). A systems-based analysis of *Plasmodium vivax* lifecycle transcription from human to mosquito. *PLoS Neglected Tropical Diseases* **4**, e653.

Wickramarachchi, T., Premaratne, P. H., Perera, K. L., Bandara, S., Kocken, C. H., Thomas, A. W., Handunnetti, S. M. and Udagama-Randeniya, P. V. (2006). Natural human antibody responses to *Plasmodium vivax* apical membrane antigen 1 under low transmission and unstable malaria conditions in Sri Lanka. *Infection and Immunity* **74**, 798–801.

Wickramarachchi, T., Illeperuma, R. J., Perera, L., Bandara, S., Holm, I., Longacre, S., Handunnetti, S. M. and Udagama-Randeniya, P. V. (2007). Comparison of naturally acquired antibody responses against the C-terminal processing products of *Plasmodium vivax* Merozoite Surface Protein-1 under low transmission and unstable malaria conditions in Sri Lanka. *International Journal of Parasitology* **37**, 199–208.

Wipasa, J., Suphavilai, C., Okell, L. C., Cook, J., Corran, P. H., Thaikla, K., Liewsaree, W., Riley, E. M. and Hafalla, J. C. (2010). Long-lived antibody and B Cell memory responses to the human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*. *PLoS Pathogens* **6**, e1000770.

Wipasa, J., Okell, L., Sakkhachornphop, S., Suphavilai, C., Chawansuntati, K., Liewsaree, W., Hafalla, J. C. and Riley, E. M. (2011). Short-lived IFN-gamma effector responses, but long-lived IL-10 memory responses, to malaria in an area of low malaria endemicity. *PLoS Pathogens* **7**, e1001281.

Woldearegai, T. G., Kremsner, P. G., Kun, J. F. and Mordmuller, B. (2013). *Plasmodium vivax* malaria in Duffy-negative individuals from Ethiopia. *Transactions of the Royal Society of Tropical Medicines and Hygiene* **107**, 328–331.

Woodberry, T., Minigo, G., Piera, K. A., Hanley, J. C., de Silva, H. D., Salwati, E., Kenangalem, E., Tjitra, E., Coppel, R. L., Price, R. N., Anstey, N. M. and Plebanski, M. (2008). Antibodies to *Plasmodium falciparum* and *Plasmodium vivax* merozoite surface protein 5 in Indonesia: species-specific and cross-reactive responses. *Journal of Infectious Diseases* **198**, 134–142.

Xainli, J., Baisor, M., Kastens, W., Bockarie, M., Adams, J. H. and King, C. L. (2002). Age-dependent cellular immune responses to *Plasmodium vivax* Duffy binding protein in humans. *Journal of Immunology* **169**, 3200–3207.

Xainli, J., Cole-Tobian, J. L., Baisor, M., Kastens, W., Bockarie, M., Yazdani, S. S., Chitnis, C. E., Adams, J. H. and King, C. L. (2003). Epitope-specific humoral immunity to *Plasmodium vivax* Duffy binding protein. *Infection and Immunity* **71**, 2508–2515.

Yeo, T. W., Lampah, D. A., Tjitra, E., Piera, K., Gitawati, R., Kenangalem, E., Price, R. N. and Anstey, N. M. (2010). Greater endothelial activation, Weibel-Palade body release and host inflammatory response to *Plasmodium vivax*, compared with *Plasmodium falciparum*: a prospective study in Papua, Indonesia. *Journal of Infectious Diseases* **202**, 109–112.

Zakeri, S., Babaeekhou, L., Mehrizi, A. A., Abbasi, M. and Djadid, N. D. (2011). Antibody responses and avidity of naturally acquired anti- *Plasmodium vivax* Duffy binding protein (PvDBP) antibodies in individuals from an area with unstable malaria transmission. *American Journal of Tropical Medicines and Hygiene* **84**, 944–950.

Zeeshan, M., Bora, H. and Sharma, Y. D. (2013). Presence of memory T cells and naturally acquired antibodies in *Plasmodium vivax* malaria-exposed individuals against a group of tryptophan-rich antigens with conserved sequences. *Journal of Infectious Diseases* **207**, 175–185.

Zeeshan, M., Tyagi, K. and Sharma, Y. D. (2015). CD4+ T cell response correlates with naturally acquired antibodies against *Plasmodium vivax* tryptophan-rich antigens. *Infection and Immunity* **83**, 2018–2029.

Zevering, Y., Khamboonruang, C., Rungruengthanakit, K., Tungviboonchai, L., Ruengpipattanapan, J., Bathurst, I., Barr, P. and Good, M. F. (1994). Life-spans of human T-cell responses to determinants from the circumsporozoite proteins of *Plasmodium falciparum* and *Plasmodium vivax*. *Proceedings of the National Academy of Sciences U S A* **91**, 6118–6122.

Zeyrek, F. Y., Kurcer, M. A., Zeyrek, D. and Simsek, Z. (2006). Parasite density and serum cytokine levels in *Plasmodium vivax* malaria in Turkey. *Parasite Immunology* **28**, 201–207.

Zeyrek, F. Y., Babaoglu, A., Demirel, S., Erdogan, D. D., Ak, M., Korkmaz, M. and Coban, C. (2008). Analysis of naturally acquired antibody responses to the 19-kd C-terminal region of merozoite surface protein-1 of *Plasmodium vivax* from individuals in Sanliurfa, Turkey. *American Journal of Tropical Medicines and Hygiene* **78**, 729–732.

Zinkernagel, R. M. (2002). On differences between immunity and immunological memory. *Current Opinion in Immunology* **14**, 523–536.