

# Immunity to malaria in an era of declining malaria transmission

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## SUMMARY

With increasing malaria control and goals of malaria elimination, many endemic areas are transitioning from high-to-low-to-no malaria transmission. Reductions in transmission will impact on the development of naturally acquired immunity to malaria, which develops after repeated exposure to *Plasmodium* spp. However, it is currently unclear how declining transmission and malaria exposure will affect the development and maintenance of naturally acquired immunity. Here we review the key processes which underpin this knowledge; the amount of *Plasmodium* spp. exposure required to generate effective immune responses, the longevity of antibody responses and the ability to mount an effective response upon re-exposure through memory responses. Lastly we identify research priorities which will increase our understanding of how changing transmission will impact on malarial immunity.

Key words: Malaria, *falciparum*, vivax, immunity, antibodies, transmission.

## THE CHANGING EPIDEMIOLOGY OF MALARIA

Over the past decade there has been unprecedented investment into malaria control and elimination. Increased coverage and access to malaria control measures such as bed nets, indoor residual spraying, preventive treatment and the introduction of the highly efficacious artemisinin drugs has had considerable impact on the malaria burden. Between 2000 and 2013, estimated malaria mortality rates decreased by 47% worldwide (to 584 000 in 2013) predominantly in African children under the age of 5 years (WHO, 2014). The prevalence of *Plasmodium* spp. infection (symptomatic and asymptomatic) has also decreased with recent analysis showing a relative decline of 48% in average infection prevalence in children aged 2–10 years in sub-Saharan Africa and a 26% reduction overall (WHO, 2014). Since 2000, 55 countries have recorded >75% decrease in case incidence, and while the greatest gains have been observed in areas of high stable transmission in sub-Saharan Africa, impressive gains have also been observed in areas of relatively low transmission areas in Asia (>50% reduction in reported malaria incidence rates between 2000 and 2013); it is in low transmission areas where efforts have focussed on achieving the end goal of malaria elimination.

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Geographically, malaria transmission is highest in sub-Saharan Africa and parts of the Pacific (up to 1000 infectious bites/year) and *Plasmodium falciparum* is the dominant species (Gething *et al.* 2012, 2011). In higher transmission areas, malaria is holoendemic and symptomatic disease is confined to young children while older children and adults are typically protected from malaria illness and often asymptomatic parasitemias. Conversely, in Asia and South and Central America, transmission is typically low ( $\leq 1$  infectious bites/year), and seasonal and *P. falciparum* and *Plasmodium vivax* are often both prevalent (Gething *et al.* 2012, 2011). In these low transmission areas, transmission is typically unstable and symptomatic disease occurs in all age groups. The differences in the clinical consequences of *Plasmodium* spp. infection according to transmission is due to differences in naturally acquired immunity.

Naturally acquired immunity to malaria protects against the development of high density infections and clinical symptoms rather than infection *per se* (reviewed in Marsh and Kinyanjui, 2006). Naturally acquired immunity develops after repeated exposure, with faster rates of acquisition in high compared with low transmission areas. Antibodies are an important component of naturally acquired immunity to malaria as evidenced by experimental animal models and, most importantly, passive transfer studies in

which antibodies from malaria-immune adults were successfully used to treat patients with symptomatic malaria (Cohen *et al.* 1961; Sabchareon *et al.* 1991). Serum antibodies, which are made by plasma cells, mediate protection by acting predominantly against parasites of the asexual blood-stages that cause the clinical symptoms of malaria. Blood-stage targets include those expressed by the merozoite stage of *P. falciparum* and *P. vivax*, which invades the erythrocyte (specific targets reviewed in Richards and Beeson, 2009; Mueller *et al.* 2013), as well as variant surface antigens (predominantly *P. falciparum* erythrocyte membrane protein 1; PfEMP1) expressed on the surface of the *P. falciparum*-infected erythrocyte (IE) (specific targets reviewed in Chan *et al.* 2014) and potentially vir on the surface of *P. vivax*-IE (reviewed in Mueller *et al.* 2013). The combination of responses as well as breadth and magnitude of blood-stage responses are important in terms of the protective responses, with individuals who possess a greater repertoire are more protected against clinical malaria, although specific targets or patterns of responses are important (Gray *et al.* 2007; Osier *et al.* 2008; Crompton *et al.* 2010; Richards *et al.* 2013; Rono *et al.* 2013). Antibodies to blood-stages can act by directly blocking merozoite invasion and inhibiting growth (Cohen *et al.* 1969; Brown *et al.* 1982; Guevara Patino *et al.* 1997; O'Donnell *et al.* 2001; Singh *et al.* 2006; Dutta *et al.* 2009; Duncan *et al.* 2012), acting together with complement to inhibit invasion and lyse merozoites (Boyle *et al.* 2015), and by clearing merozoites and *P. falciparum*-IE by antibody dependent cellular mechanisms (Khusmith and Druilhe, 1983; Druilhe and Perignon, 1994) and opsonic phagocytosis (Celada *et al.* 1982; Hill *et al.* 2013; Chan *et al.* 2014; Osier *et al.* 2014a), thereby reducing parasite density and clinical symptoms. Additional *Plasmodium* spp. targets include the pre-erythrocytic sporozoite stage (specific targets reviewed in Dups *et al.* 2014) and sexual gametocyte stage (Riley *et al.* 1995; Milek *et al.* 1998; Chan *et al.* 2014). Antibodies to sporozoites would be expected to prevent infection; however, available evidence suggests that the acquisition of protective pre-erythrocytic immunity may be limited (Hoffman *et al.* 1987; Webster *et al.* 1988; Wongsrichanalai *et al.* 1991; Michon *et al.* 2007; Tran *et al.* 2013). Antibodies to gametocytes are thought to prevent the infectious spread between human and mosquito vectors (Healer *et al.* 1997; Bousema *et al.* 2006, 2010b).

In this era of increased malaria control and goals of malaria elimination, many endemic areas are transitioning from high-to-low-to-no malaria transmission (e.g. O'Meara *et al.* 2008a, b; O'Meara *et al.* 2010; Kalayjian *et al.* 2013; Snow *et al.* 2015) and the impact on immunity is evident epidemiologically; rebounds of malaria in previously eliminated

areas and shifts in case distribution to older age groups in areas where malaria incidence is decreasing (Ceesay *et al.* 2008; Brasseur *et al.* 2011). To fully understand the impact of changing transmission on immunity, a greater knowledge of the development and maintenance of naturally acquired immunity to malaria immunity is required. Here we review the key processes that underpin this knowledge; the amount of *Plasmodium* spp. exposure required to generate effective immune responses, the longevity of antibody responses and the ability to mount an effective response upon re-exposure through memory responses. This review will focus largely on humoral immunity, since knowledge on longevity of T-cell responses and the potential impact of declining transmission on these responses has been greatly under-studied.

#### HOW MUCH *PLASMODIUM* SPP. EXPOSURE IS REQUIRED TO BECOME IMMUNE?

The acquisition of immunity to malaria in populations is evident by the declining incidence of uncomplicated and severe malaria with increasing age, and a reduction in average malaria parasite density with increasing age (reviewed in Marsh and Kinyanjui, 2006). It is believed that immunity that reduces the risk of severe and life-threatening malaria is acquired more quickly than robust immunity that protects against all forms of malaria, including uncomplicated illness (reviewed in Marsh and Kinyanjui, 2006). This paradigm of the acquisition of immunity is supported by epidemiologic evidence and modelling studies (e.g. Snow *et al.* 1997; Gupta *et al.* 1999; Reyburn *et al.* 2005; Michon *et al.* 2007; Carneiro *et al.* 2010; Griffin *et al.* 2015). However, the rate at which immunity to severe malaria is acquired relative to broader immunity, and the extent to which this is influenced by the intensity and nature of malaria transmission and host factors is not entirely clear. More rapid acquisition of immunity to severe malaria may be because some level of immunity is sufficient to prevent severe disease, or there may be specific immune mechanisms mediating protection against severe disease; studies also suggest that host-age is an important factor in susceptibility to severe malaria (e.g. Reyburn *et al.* 2005; Griffin *et al.* 2015). In populations exposed to stable malaria transmission of medium-high intensity, malaria is typically uncommon in older children and adults, and severe malaria is rare (reviewed in Marsh and Kinyanjui, 2006; Carneiro *et al.* 2010). In settings of high transmission, severe malaria is largely restricted to young children (under 5 years), and severe malaria continues to occur later in childhood in settings where malaria transmission is lower (Snow *et al.* 1997; Carneiro *et al.* 2010). Reductions in malaria transmission would be expected to shift the peak

incidence of severe malaria to later in childhood or adulthood (Griffin *et al.* 2014), and in populations where malaria transmission is low and unstable people may remain at risk of severe malaria throughout their life.

Interestingly, epidemiological studies of immunity where *P. falciparum* and *P. vivax* are co-endemic have suggested that the rate of acquisition of immunity to *P. vivax* is faster than for *P. falciparum* (Maitland *et al.* 1996; Bruce *et al.* 2000b; Michon *et al.* 2007; Lin *et al.* 2010). Immunity to *P. vivax* malaria is evident at a younger age compared with *P. falciparum* in populations exposed to similar levels of transmission of the two species. Recent studies have suggested that this may be explained by a higher force-of-infection for *P. vivax*, compared with *P. falciparum*, for a given entomologic inoculation rate, due to the ability of *P. vivax* to cause relapses from dormant hypnozoites (Koepfli *et al.* 2013). It is possible that there are also underlying differences in the acquisition and nature of protective responses to the two species, but these are yet to be defined and immunity to *P. vivax* malaria has been greatly under-studied.

Currently there is not a clear understanding of how much exposure is required for the development of immunity. In broad terms, the age at which effective immunity is evident is related to the level of transmission, such that substantial immunity may be acquired by age 10 in areas of medium-high transmission, whereas immunity may not be acquired until teenage years or early adulthood in areas with lower transmission (e.g. Mwangi *et al.* 2005; Marsh and Kinyanjui, 2006; Carneiro *et al.* 2010). While this pattern of acquisition has been reported from many settings, there are some exceptions to this general picture and the development of protective immunity after relatively few infections. For example, studies of transmigrants moving from malaria-free areas to malaria-endemic regions of Indonesia suggested that effective immunity against symptomatic *P. falciparum* malaria could be acquired within 2 years and was dependent on a threshold number of episodes over that time, and was acquired more rapidly among adults than children (Baird *et al.* 1991, 1993). Repeated exposure does result in more clinical immunity as studies have shown that within the same region, individuals repeatedly exposed have lower parasite densities and less frequent clinical episodes than less exposed individuals (Thomas and Lindsay, 2000; Bejon *et al.* 2009, 2014; Mosha *et al.* 2013; Ndungu *et al.* 2015). In many regions of low transmission, the extent and frequency of exposure may be low to result in effective immunity, such that adults may remain at risk of symptomatic and severe malaria (e.g. Creasey *et al.* 2004; Reyburn *et al.* 2005), which is particularly relevant in the context of intensified malaria control efforts and

declining malaria globally. The implication from these observations is that extensive exposure to blood-stage infection (e.g. dozens of episodes, or persistent low-grade blood-stage infection) is required for the development of protective immunity. It has also been proposed that chronic parasitemia, or repeated and frequent parasitemia is required to maintain robust immunity (a phenomenon referred to as premunition). Infections can persist for many months in the absence of symptoms (Bruce *et al.* 2000a; Franks *et al.* 2001; Njama-Meya *et al.* 2004; Nsobyia *et al.* 2004) and asymptomatic infections have been reported to provide protection against symptomatic disease (Farnert *et al.* 1999). However, several studies have shown that asymptomatic infections predict symptomatic disease and teasing out the effects of exposure and protective immunity have been challenging (Njama-Meya *et al.* 2004; Bejon *et al.* 2010; Greenhouse *et al.* 2011; Liljander *et al.* 2011; Loucoubar *et al.* 2013). Some longitudinal data supports the notion that asymptomatic infections may be important in maintaining antibody responses (Shekalaghe *et al.* 2009; Fowkes *et al.* 2012; Ibison *et al.* 2012; Proietti *et al.* 2013; Daou *et al.* 2015; Rono *et al.* 2015), but this has not been extensively studied or clearly established. Removal of these antigenic stimuli may have significant effects on the maintenance of immunity in malaria endemic populations (detailed below).

Acquisition of immunity is influenced by multiple factors, which are reflected in different rates of immune acquisition reported across various populations. Clearly the extent and frequency of exposure is important (reviewed in Doolan *et al.* 2009), but immunity is not simply determined by the total number of episodes of infections, or the cumulative exposure to blood-stage infection. Host and parasite factors may influence acquisition of immunity. Specific genetic traits influence susceptibility to malaria and potentially immune responses (Edozien *et al.* 1960; Marsh *et al.* 1989; Cabrera *et al.* 2005; Verra *et al.* 2007), and the prevalence of these traits vary substantially between populations (Howes *et al.* 2011, 2012; 2013; Piel *et al.* 2013a, b, 2010). Parasite diversity is also important and immunity may be acquired more quickly where genetic diversity is limited, since many of the key targets of protective immunity are polymorphic. Reduced parasite population genetic diversity may be a consequence of intensified malaria control activities with implications for development of immunity (Gray *et al.* 2013; Kaneko *et al.* 2014). Significantly, most studies of immune acquisition have studied people living in malaria-endemic regions since birth, but the impact of a shift upwards in the age of first exposure with declining transmission on the acquisition of immunity is unclear. Firstly, infection of mothers during pregnancy can influence immune responses in infants

(Desowitz, 1988; Desowitz *et al.* 1992; King *et al.* 2002; Malhotra *et al.* 2009) and the development of immunity in the absence of *in utero* exposure is unknown. Secondly, the acquisition of immunity may vary according to age of first exposure. Early studies in transmigrants demonstrated that antibody responses are acquired more quickly in adults than children (Baird *et al.* 1991, 1993), and analyses have suggested that age is an important factor influencing susceptibility to severe malaria (Carneiro *et al.* 2010; Griffin *et al.* 2015). However, whether this relates to the nature of the immune response is not presently known. Conversely, studies in malaria endemic areas of Africa have suggested that infants acquire immunity faster than older children (Aponte *et al.* 2007), however other studies in the region have demonstrated no association between the rate of antibody acquisition and age of first exposure (Guinovart *et al.* 2012; Moncunill *et al.* 2013b). The effect of the age shift with declining transmission on the development of immunity across the age spectra warrants further investigation.

The challenges in predicting and measuring immunity in populations highlights the need for immune correlates or biomarkers of immunity that would enable the immune status of populations to be monitored to evaluate the impact of interventions and identify populations or sub-groups at risk. With intensified control activities and reducing malaria in many regions, research to address this needs to be a high priority. The utility of serosurveillance has been assessed in several studies using enzyme-linked immunosorbent assay (ELISA) and microarray assays (Drakeley *et al.* 2005; Satoguina *et al.* 2009; Stewart *et al.* 2009; Bousema *et al.* 2010a; Cook *et al.* 2011, 2010; Crompton *et al.* 2010; Badu *et al.* 2012; Elliott *et al.* 2014). Simple immunoassays may be broadly informative, but the use of simplified and standardized functional assays may be more indicative of immunity. A growing body of data supports the utility of antibodies to merozoite antigens as biomarkers of immunity (Osier *et al.* 2008, 2014b; Fowkes *et al.* 2010; Richards *et al.* 2013; Cutts *et al.* 2014), and recent studies have reported that opsonic phagocytosis and complement fixation with anti-merozoite responses may be valuable functional assays (Hill *et al.* 2013; Osier *et al.* 2014a; Boyle *et al.* 2015). Recent longitudinal studies in Kenya and Papua New Guinea, comparing children with different levels of malaria exposure, support the hypothesis that there is a threshold level of immunity required to mediate protection from malaria (Murungi *et al.* 2013; Stanisic *et al.* 2015). In these studies older children, or children from higher transmission settings, had substantially higher antibodies and significant clinical immunity compared with young children who had low levels of antibodies which were not associated with protection against from malaria (Murungi

*et al.* 2013; Stanisic *et al.* 2015). These approaches may be valuable in defining threshold antibody levels that act as markers of protective immunity. Determining what constitutes a protective immune response, together with factors required to generate it (number of exposures, antigenic diversity), is critical in understanding how changes in malaria transmission will impact on the acquisition of naturally acquired immunity to malaria.

#### HOW LONG DO *PLASMODIUM* SPP. ANTIBODY RESPONSES LAST?

Once immunity is acquired the next question is how long does it last? Investigations into the longevity of antibody responses have spanned almost 50 years and have included studies in malaria endemic areas and studies in previously immune immigrants and neurosyphilis patients, which offer a classic experimental approach to analyse antibody longevity in the absence of intermittent exposure to infection. The question of the longevity of antibody responses is hotly debated, with evidence for and against short- and long-lived antibody responses. It became dogma that antibody responses were relatively short-lived from a number of studies in malaria-endemic areas; *P. falciparum* and *P. vivax* antibody responses (sporozoites, merozoites, *P. falciparum*-IE and gametocytes) have been shown to rapidly decline after a few months following drug treatment and parasite clearance in subjects with symptomatic infections in both children and adults (Cavanagh *et al.* 1998; Fonjongo *et al.* 1999; Giha *et al.* 1999; Soares *et al.* 1999; Kinyanjui *et al.* 2007; Weiss *et al.* 2010; Bousema *et al.* 2010b) and rapidly declined over the dry season in areas of seasonal *P. falciparum* transmission (Fruh *et al.* 1991; Ramasamy *et al.* 1994; Cavanagh *et al.* 1998; Perraut *et al.* 2000; Akpogheneta *et al.* 2008; Weiss *et al.* 2010). These studies suggest that regular exposure is needed for maintenance of antibodies (premunition) and, in the absence of exposure, antibody responses are relatively short-lived. Indeed in the context of declining transmission, *P. falciparum* and *P. vivax* antibodies have also been shown to decline with the implementation of malaria control interventions such as chemoprophylaxis, indoor residual spraying and bednets (Warren *et al.* 1983; Staalsoe *et al.* 2004; Aitken *et al.* 2010, 2012; Cook *et al.* 2011; Diop *et al.* 2014) and antibody levels have reflected declines in malaria transmission in studies spanning several years (Migot *et al.* 1993; Ceesay *et al.* 2010; Diop *et al.* 2014).

However, it is important to note that while the afore-mentioned studies show antibody decline post *Plasmodium* spp. exposure, antibodies still largely persist, and other studies provide evidence for long-lived antibody responses. Stable antibody responses have been shown over a 4 year period in



an area of seasonal *P. falciparum* transmission (Taylor *et al.* 1996), and in areas of low *P. falciparum* and *P. vivax* transmission with little/no detectable infections over several months and years (up to 6 years) (Warren *et al.* 1975; Chougnnet *et al.* 1990; Migot *et al.* 1993; Torres *et al.* 2008; Bousema *et al.* 2010c; Wipasa *et al.* 2010; Clark *et al.* 2012; Fowkes *et al.* 2012; Ayieko *et al.* 2013). Persistent life-long *P. falciparum* merozoite responses have been reported in population seroconversion studies (antibody response half-life 50 years, 95% CI 36, 73) (Drakeley *et al.* 2005). In another instance, long-lived *P. vivax* merozoite antibodies have been reported more than 30 years after malaria elimination (Lim *et al.* 2004), although with *P. vivax* it is hard to separate out the effect of relapses. Studies of VAR2CSA have suggested antibodies may persist for decades (Fowkes *et al.* 2012), and VAR2CSA antibodies have been detected 20 years after the last pregnancy (Ampomah *et al.* 2014a). Long-lived responses may explain epidemiological observations of long-lived protection against clinical disease in Madagascan adults during malaria epidemics, despite having been exposed to malaria 30 years previously (Deloron and Chougnnet, 1992; Kleinschmidt and Sharp, 2001).

Long-lived responses are also supported in studies of previously immune migrants and neurosyphilis patients (who are infected with malaria as a form of treatment), which offer a classic experimental approach to analyse antibody longevity in the absence of intermittent exposure to infection. Early serological investigations showed detectable *P. falciparum* and *P. vivax* antibodies in African and Central Asian immigrants and neurosyphilis patients 3–15 years post exposure (Collins *et al.* 1968; Bruce-Chwatt *et al.* 1972; Druilhe *et al.* 1986). Responses in immigrants appear not to wane completely, with several studies showing that antibody levels are independent of length of residence, which has ranged from 1 to 4 years to almost 4 decades (Bouchaud *et al.* 2005; Moncunill *et al.* 2013a). These studies show that antibody responses can be long-lived in the absence of exposure, and may still provide a degree of protective immunity; immigrants have milder *P. falciparum* and *P. vivax* disease and lower parasite densities compared with naïve non-immigrant travellers (Matteelli *et al.* 1999; Jelinek *et al.* 2002; Bouchaud *et al.* 2005; Mascarello *et al.* 2008; Salvado *et al.* 2008; Gonzalez *et al.* 2009; Monge-Maillo *et al.* 2012; Farnert *et al.* 2014; Pistone *et al.* 2014). However, despite having some immunity, a degree of immunity may have been lost, with data from recent studies showing that immigrants contracting malaria from visits to malaria endemic areas have lower levels of antibodies than semi-immune adults (Moncunill *et al.* 2013a). Furthermore the fact that they have developed malaria in the first place provides evidence for the shorter-lived responses however, this is hard to

separate this from possible temporal changes in circulating *Plasmodium* spp. strains in the source population.

A major factor contributing to diverging results and conclusions on antibody longevity is the difference in sampling on the antibody response curve between studies. After antigenic stimulus antibody titres rapidly increase peak, and then enter a biphasic decay consisting of a rapid decay from the initial peak followed by a slower decay lasting for several years, or providing life-long immunity depending on antigen (reviewed in Amanna and Slifka, 2010). Recently, there have been several longitudinal studies estimating *Plasmodium* spp. antibody response half-life, which is the product of both antibody production and antibody decay (IgG molecules have a half-life of ~21 days Morell *et al.* 1970). Estimates on antibody response half-life in African children following the peak antibody response after clinical malaria (the first phase of decay) have estimated short antibody response half-lives for *P. falciparum* merozoite and gametocyte antigens ranging from <2 weeks (Kinyanjui *et al.* 2007) to ~12 weeks (Bousema *et al.* 2010b). Similar *P. falciparum* merozoite antibody response half-lives ranges (2–8 weeks) were found in asymptomatic Gambian children over the dry season (Akpogheneta *et al.* 2008). Studies sampling in the second phase of decay have reported much longer half-lives. Studies in low transmission areas of Thailand, in the absence of *Plasmodium* spp. exposure, have shown antibody response half-lives for *P. falciparum* and *P. vivax* merozoite antigens to be 1–10 years (Wipasa *et al.* 2010; Fowkes *et al.* 2012). Given that there are few exposures in low transmission areas the development of long-lived responses may be acquired after relatively few infections. Studies estimating half-life from seroconversion models (which will be at the tail end of the antibody response) have estimated of the antibody response of 49.8 year (95% CI 36.4, 72.7 years for *P. falciparum*-merozoite surface protein 1 (MSP1<sub>19</sub>)) (Drakeley *et al.* 2005), that is life-long immunity. Recent mathematical modelling of antibody responses in African children have shown that the biphasic decay may be the result of short-lived antibody secreting cells (half-life 2–10 days) which boost antibody levels post infection, and long-lived antibody secreting cells (half-life 3–9 years) which maintain persistent antibody responses (White *et al.* 2014b).

Other factors will also contribute to varying estimates of antibody longevity such as methodology (study design, analysis, antigen/antibody investigated), malaria transmission and study populations. There may also be differences according to the type of antigen. Within individual studies, similar antibody longevity is observed for merozoite antigens, despite antigenic and species diversity (Kinyanjui *et al.* 2007; Wipasa *et al.* 2010; Fowkes *et al.* 2012), and merozoites and gametocytes (Bousema *et al.* 2010b). However there is some

evidence that responses to *P. falciparum*-IE may be longer than *P. falciparum* merozoite antibody responses (Fowkes *et al.* 2012) although this is not consistent in all studies (Perraut *et al.* 2000). Further studies are required to validate these findings and to dissect out whether specific antigens elicit longer-lived responses. Different antigens also have different IgG1 to IgG3 ratios and the extent of this can vary with age and exposure (Tongren *et al.* 2006; Stanisic *et al.* 2009). Differences in this ratio and the kinetics may contribute to some of the variation seen. The longevity of antibody responses are dependent on exposure to *Plasmodium* spp. Studies have shown that antibody longevity is positively correlated with transmission (Drakeley *et al.* 2005) and persist for longer in those with recent documented infection compared with those unexposed to *Plasmodium* spp (Akpogheneta *et al.* 2008; Fowkes *et al.* 2012). This may reflect a cumulative exposure effect as antibody response longevity increases with age in both high and low transmission areas (Perraut *et al.* 2000; Akpogheneta *et al.* 2008; Torres *et al.* 2008; Bousema *et al.* 2010b; Clark *et al.* 2012; Diop *et al.* 2014).

Determining the longevity of antibody responses is challenging due to the dynamic kinetics and biphasic nature of antibody responses, particularly in high transmission areas with multiple reinfections. Further longitudinal studies, with accurate data on duration of infections, encompassing both parts of biphasic antibody decay are warranted. These studies should be performed in areas of varying transmission intensities to ensure generalizability and enable cross-transmission comparisons. Data from these studies will provide a better understanding for how changing transmission will impact on circulating antibodies and their ability to protect against symptomatic disease. Importantly, a greater understanding of the types of cells underpinning antibody dynamics remain to be elucidated as well as the contribution of immunological memory to antibody responses which remains hotly debated (Struik and Riley, 2004; Hviid *et al.* 2015; Portugal *et al.* 2015). Presently, it is unclear whether immunity depends on the maintenance of antibodies above a threshold level, or by B-cell memory and the ability to recall responses when re-infected. To date, longitudinal dynamics of immunity have been performed almost entirely with standard immunoassays (e.g. ELISA). Studies that measure functional activity of antibodies may reveal different patterns if there is a threshold antibody level for functional activity. A recent study of pregnant women suggested that functional antibodies may be more resilient to changes in malaria transmission (Teo *et al.* 2014).

#### IMMUNOLOGICAL MEMORY TO MALARIA

The antibodies first produced in response to a new antigen are of relatively low affinity and are secreted

by short-lived plasma cells generated following interaction between naïve B cells and antigen-specific helper T cells. Once the infection is controlled, the population of antigen-specific effector T cells and memory B cells (MBC) contracts leaving behind a small number of long-lived memory T and B cells, but the pool of these cells is thought to increase with each subsequent infection to increase capacity for subsequent responses. Some of the plasma cells differentiate into long-lived plasma cells that migrate to the bone marrow and continue to produce antibodies even in the absence of antigen. Evidence for malaria memory responses comes from the observation of rapid boosting of *P. falciparum*-specific antibody responses upon re-exposure to malaria following the dry season or prolonged periods of low transmission (Vande Waa *et al.* 1984; Migot *et al.* 1993) and data from controlled human malaria infection (CHMI) studies showing that previously exposed (>5 years prior) a parasitemic individuals showed a stronger increase in antibody titres than naïve volunteers, (Obiero *et al.* 2015) suggesting that individuals can generate and retain *P. falciparum*-specific MBC.

There is a paucity of studies on MBC, and the ability of *Plasmodium* spp. to produce a long-lived memory response is debated (Struik and Riley, 2004; Hviid *et al.* 2015; Portugal *et al.* 2015). Much of the debate has originated from the aforementioned reports of both short- and long-lived antibody responses in the absence of exposure. Epidemiological studies have shown that MBC, like malarial antibodies, are acquired slowly after repeated infections in malaria endemic areas suggesting that they play an important role in the acquired immune response (Dorfman *et al.* 2005; Wipasa *et al.* 2010; Nogaro *et al.* 2011; Weiss *et al.* 2011, 2009, 2010, 2012; Ndungu *et al.* 2012; Illingworth *et al.* 2013; Muellenbeck *et al.* 2013). However, the afore-mentioned epidemiological studies have shown that antigen-specific antibodies and MBC are not always correlated. Notably, studies of the immune response to VAR2CSA have shown that multigravid pregnant women may lack VAR2CSA-specific antibodies early on in pregnancy despite having detectable MBC spanning decades (Ampomah *et al.* 2014a). Similarly, antibodies to pre-erythrocytic and merozoite antigens have been shown to decline after *P. falciparum* transmission/exposure ceases, whereas MBC were maintained for decades (Wipasa *et al.* 2010; Ndungu *et al.* 2012). Furthermore, studies of apical membrane antigen 1 (AMA1) in Swedish travellers have also shown the presence of MBC up to 16 years post infection in the absence of antibody (Ndungu *et al.* 2013). These studies provide evidence for long-lived, potentially life-long, MBC responses and the observation that MBCs are detectable decades later in previously naïve individuals would suggest that

MBC responses are generated and maintained despite any re-exposure or persistent antigen stimulation. However, the role of MBCs in contributing to protective immunity has not yet been quantified.

The number of infections required to generate MBCs is unclear but evidence from MBC studies in CHMI and returned travellers would suggest that MBC can develop after a brief single exposure to malaria (Ndungu *et al.* 2013; Nahrendorf *et al.* 2014). These findings are supported by data on MBC in low transmission areas. A study in Peru showed that even one reported prior infection was sufficient to generate antigen-specific MBC and to maintain a positive antibody response for at least 5 months, in the absence of reinfection (Clark *et al.* 2012). Another study conducted in an area of very low malaria transmission in Northern Thailand showed that malaria-naïve individuals or those who had not had clinical episodes of malaria over the past 6 years had MBC response to *P. falciparum* (and *P. vivax*) (Wipasa *et al.* 2010). This suggests that the number of infections required to develop long-lived antibody responses is likely to be quite small.

However, protective antibody and MBC responses appear to be slow to develop and ineffectively maintained. This could be due to genetic and antigenic variation of the parasite (Scherf *et al.* 2008; Takala and Plowe, 2009) so that repeated exposure leads to a gradual expansion of the repertoire of *P. falciparum*-specific MBC (Weiss *et al.* 2010). However, recent evidence also shows that MBC responses may be dysregulated in malaria infections. Malarial MBC responses appear to be sub-optimally produced compared with other MBC responses. While the magnitude of MBC responses in naturally exposed individuals are in the same range as childhood vaccine-induced MBCs in the same populations, MBC prevalence is much lower (30–60% compared with 60–100% for vaccine antigens) even after many decades of exposure (Dorfman *et al.* 2005; Wipasa *et al.* 2010; Nogaro *et al.* 2011; Ndungu *et al.* 2012; Weiss *et al.* 2012, 2010). It has been shown that repeated exposure to infection, can lead to the large expansion of phenotypically similar ‘atypical’ MBC in children, adults and pregnant women in geographically diverse regions (West and East Africa, Peru, Papua New Guinea) of varying transmission (Weiss *et al.* 2009, 2011; Illingworth *et al.* 2013; Muellenbeck *et al.* 2013; Nogaro *et al.* 2011; Ampomah *et al.* 2014b; Requena *et al.* 2014; Subramaniam *et al.* 2015). CHMI studies have shown that out of all the MBC types, atypical MBCs have the strongest proliferative response and peak either immediately after blood-stage infection or convalescence (Scholzen *et al.* 2014). Evidence suggests that *P. falciparum* exposure drives the expansion of atypical MBC; atypical MBC are correlated with *P. falciparum*

transmission intensity (Weiss *et al.* 2009) and a study in Kenya showed that *P. falciparum* exposure drove the differential expansion of atypical MBCs in age-matched children (Illingworth *et al.* 2013). Atypical MBC are also found more frequently in children who asymptotically carry *P. falciparum* as compared with children who are *P. falciparum* free (Weiss *et al.* 2009). Furthermore, in the absence of *P. falciparum* infection (for 1 year) atypical and activated MBC subsets decrease (Ayieko *et al.* 2013) and studies in pregnant women also show a retraction of VAR2CSA-specific atypical MBCs in the postpartum period (Ampomah *et al.* 2014b).

The phenotype of atypical MBCs is unclear but recent studies have shown that atypical MBCs contribute to *P. falciparum*-specific IgG (Muellenbeck *et al.* 2013; Portugal *et al.* 2015) but they may have a significantly reduced signalling and effector function through a range of potential molecular modulators (Scholzen and Sauerwein, 2013; Scholzen *et al.* 2014; Portugal *et al.* 2015; Zinocker *et al.* 2015). The precise function and specificity of atypical MBCs, their potential regulatory role and the factors that drive their expansion are yet to be elucidated and requires further research. Furthermore the dynamics of MBCs and their contribution to antibody dynamics are unknown, and the basal levels and breadth of circulating antibodies needed for protection against disease need to be defined. The majority of research into MBC responses has centred on *P. falciparum*, and the role of MBC in *P. vivax* is also warranted.

The role of T cells in immunological memory is even less clear. T cells are thought to be involved in immune responses that clear pre-erythrocytic stages, and in providing T-cell help and regulatory responses in immunity against blood-stages (Beeson *et al.* 2008). The majority of T-cell literature pertains to murine models which have been the feature of several recent reviews (Corradin and Levitskaya, 2014; Doll and Harty, 2014; Krzych *et al.* 2014; Van Braeckel-Budimir and Harty, 2014). There is a paucity of data on T cell responses in humans, and data from CHMI and epidemiological studies are not complementary. In a CHMI study, DNA prime/adenovirus boost immunization of circumsporozoite protein (CSP) and AMA1 could induce sterile immunity which was associated with higher effector to central memory CSP- and AMA1-specific cytotoxic CD8+ T cells ratios, than non-protected volunteers (Sedegah *et al.* 2014). T cell responses to both *P. falciparum* sporozoites and IE are induced rapidly and remain almost undiminished up to 14 months even after a single malaria episode (Teirlinck *et al.* 2011). However, in naturally exposed individuals, CD8+ T cell responses to sporozoite epitopes are of a much lower magnitude than in experimentally infected individuals and CD8+ T cell memory appears to

wane with time. The poor natural immunity to sporozoites could be due to the interference of erythrocytic stages on existing T cell responses described in rodent models of malaria (Ocana-Morgner *et al.* 2003). Alternatively, irradiated sporozoites (used in experimental infections) could also be more immunogenic than live sporozoites and their antigens could persist for longer, helping to maintain effector T cells in the liver (Scheller and Azad, 1995). CD4+ helper T cells primary function is to help the development of CD8+ T and B cell responses and MBCs. Murine studies have shown that CD4+ T cells retract and form a resting memory T cell population able to respond rapidly on re-infection, especially in the presence of antigen in the form of ongoing, sub-patent infection (reviewed in Stephens and Langhorne, 2006). Phase 2a RTS,S/AS trials in malaria-naïve individuals have shown that protected individuals can induce higher frequencies of effector and central memory T cells compared with unprotected individuals (Lumsden *et al.* 2011) and in malaria-exposed individuals vaccinated with the RTS,S vaccine, protection from re-infection (within 4 months of immunization) was associated with central memory CD4+ T cells reactive against an epitope of CSP (Reece *et al.* 2004).

There is a clear deficit of studies of T cell immunity in individuals living in malaria endemic areas. Given the importance of T cell responses in immunity against the transmissible sporozoite stage, further investigation in individuals living in malaria endemic areas of varying transmission is warranted. The available evidence suggests that continuous antigenic stimulation may help to maintain a protective effector T cell population, and therefore ongoing exposure to infectious bites may help maintain protection. Further studies in areas of declining transmission are warranted so that the full impact of malaria control on sporozoite immunity can be realized. This is particularly pertinent in the context of vaccination with RTS,S, the most advanced malaria vaccine, which is based on a construct containing B-cell and T-cell epitopes of the CSP protein (RTS, 2015). The efficacy of RTS,S is dependent on the level of pre-vaccination anti-CSP titres, with improved efficacy in individuals with higher anti-CSP titres (Bejon *et al.* 2013; White *et al.* 2014a), and exposed to higher transmission intensity (Bejon *et al.* 2013; Campo *et al.* 2015).

#### CONCLUSIONS AND RESEARCH PRIORITIES

It is clear that further research is required to fully understand the acquisition and maintenance of the naturally acquired immune response to malaria to fully comprehend the impact of changing transmission on malarial immunity (research priorities summarized in Box 1). We know that multiple

#### Box 1. Research priorities

- **Identifying correlates or biomarkers of immunity that can be measured in a standardized manner for population surveillance.**
- **Determining the number and frequency of exposures (including the role of submicroscopic infections and age at first exposure) in the development of effective clinical immunity and memory responses in high and low transmission areas.**
- **Longitudinal studies to understand the kinetics and longevity of antibody responses (both levels and clinical relevance) and the impact of declining malaria transmission on these responses.**
- **Understanding the impact of reducing parasite genetic diversity arising from malaria control activities on acquisition of immunity.**
- **Determining the impact of transmission intensity on the characteristics and subtypes of *Plasmodium*-specific memory B cells and plasma cells.**
- **Defining the role of T-cells in anti-sporozoite responses and understanding how changing transmission will impact on their role in naturally acquired immunity.**

*Plasmodium* spp. exposures are required but the number and frequency of exposures required is unknown, as is how this process varies across genetically diverse populations experiencing varying transmission and parasite species and genotypes. What constitutes an effective immune response is also not clear together with which assays can accurately assess and define protective clinical immunity. Identifying immune correlates of exposure and protective immunity are keys to further developing serological tools (including functional assays) that can be used to track the immunological consequences of declining malaria transmission. Furthermore these approaches can also be used to determine whether detectable long-lived antibodies still function to provide effective protection against clinical disease. Longitudinal studies encompassing the biphasic decay of antibodies to identify the cell types which produce long-lived antibody responses are also warranted as are investigations into the sub-optimal effectiveness of MBC and T cell responses. How all of the above differs according to species, life-cycle stage and antigenic diversity also



remains to be teased out. This is particularly important in the context of developing vaccine candidate antigens and understanding the efficacy, longevity and impact of vaccines in populations with declining immunity.

Importantly, when we think of declining transmission and less ‘exposure’ we simply think of a reduced number of infections, which we typically measure epidemiologically as an episode of clinical malaria or by detection of parasites by light microscopy (which was the case for many studies featured in this review). However, submicroscopic infections (diagnosed by polymerase chain reaction) often exceeds those detectable by light microscopy by several fold (Okell *et al.* 2009; Satoguina *et al.* 2009; Mosha *et al.* 2013; Baum *et al.* 2015; Tadesse *et al.* 2015; Thanh *et al.* 2015). Sub-microscopic infections will provide an antigenic stimulus to maintain immune responses but the role of sub-microscopic infections in maintaining immunity is yet to be quantified. It is critical that a greater understanding of submicroscopic infection in the maintenance of immunity is performed in the context of declining transmission given that the highest prevalences of submicroscopic carriage are found in areas of lowest transmission (Okell *et al.* 2009) including areas which have recently transitioned from high to low malaria transmission intensity (Satoguina *et al.* 2009; Kalayjian *et al.* 2013). However, whether the prevalence of submicroscopic infections increases when transmission declines in a geographical area is yet to be determined and will be pivotal to our understanding of how immunity changes in this era of increased malaria control and declining malaria transmission.

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