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The Immunology of Malaria

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1. Introduction

The main impetus for trying to understand immunity to malaria is the need to develop effective malaria vaccines. Despite years of knowing that humans can be immune to malaria the mechanisms underlying this immunity are yet to be properly understood. This is, in part, attributable to the complexity of the malaria parasite and its life cycle resulting in a complex parasite-host relation. The outcome of a malaria infection is a consequence of interactions between host, parasite and environmental factors. As such attempts to correlate outcome with a single immunological parameter often results in spurious associations that do not hold in different circumstances. This situation is exacerbated by the lack of natural animal models for human malaria from which observations could reliably be extrapolated. Consequently, much of our understanding of malaria immunity is based on extrapolation of in vitro observations or deduced from phenomenological observations.

As is the case with immunity to other infections, immunity to malaria is the result of a combination of genetic resistance, non-adaptive immunity, and acquired or adaptive immunity. This chapter will mainly focus on immunity to *Plasmodium falciparum* malaria because it accounts for largest proportion of disease and practically all malaria mortality.

2. Genetic resistance to malaria

Population genetics studies suggest that a large proportion of the variability in malaria incidence among people residing in a malaria endemic area may be attributable to genetic factors (Mackinnon *et al* 2005). This indicates that in addition to the well known genetic variations in red cell components there are a number of other genetic variations, albeit with less obvious phenotype, that also affect susceptibility to malaria. The discovery of these variations has accelerated in the recent while thanks to advances in molecular biology techniques especially the capacity to do high throughput DNA sequencing (Williams 2009).

2.1 Protection against malaria by haemoglobinopathies and other red cell mutations

Haldane in 1949 was the first to hypothesize that certain red cell mutations reached unexpectedly high prevalence in malaria endemic areas because these mutations protect against malaria and hence confer survival advantage over non-carriers (Haldane 1949, Piel *et al* 2010, Weatherall 1997). This hypothesis has since been confirmed through a number of studies that have reported over 80% protection against severe malaria among sickle cell

heterozygotes (Hill *et al* 1991, Williams *et al* 2005b) and haemoglobin C homozygotes (Modiano *et al* 2001) and between 40-60% protection among α+ thalassaemia heterozygotes (Allen *et al* 1997, Wambua *et al* 2006, Williams *et al* 2005d). Interestingly, when thalassaemia and sickle cell are co-inherited the protection provided by each trait separately was lost (Penman *et al* 2009, Williams *et al* 2005c). Mutations that affect other components of the red cell have also been shown to provide protection against malaria. Although some studies suggest that only hemizygote Glucose-6-Phosphate Dehydrogenase (G6PD) deficient male are protected against malaria (Guindo *et al* 2007) other studies found that female homozygotes also enjoy significant protection against malaria (Clark *et al* 2009, Ruwende *et al* 1995).

The mechanisms by which haemoglobinopathies protect against malaria are poorly understood. Decreased parasite invasion and growth, possibly due to altered membrane characteristics and physiology in abnormal cells has been reported (Senok *et al* 1997). The susceptibility of G6PD deficient and thalassaemic cells to oxidative damage which in turn kills the parasite inside has been cited as a possible explanation for their protection against malaria (Friedman 1978, Golenser and Chevion 1989, Mendez *et al* 2011) At the same time, infected abnormal red cells exhibit reduced cytoadherence and rosetting, two phenomena that have been implicated in pathogenesis of cerebral malaria (Carlson *et al* 1994, Cholera *et al* 2008, Fairhurst *et al* 2005).

However, protection by haemoglobinopathies might not be entirely passive; a study by Williams et al (2005) found that protection by sickle cell trait against all forms of clinical malaria increased with age over the first ten years of life suggesting that the mechanisms cited above may interact with age-acquired immunity to enhance protection against malaria (Williams *et al* 2005a). Indeed, increased phagocytosis of infected mutant cells has been observed in the presence of otherwise normal parasite growth (Ayi *et al* 2004, Gallo *et al* 2009, Yuthavong *et al* 1990) further supporting the idea of synergy between natural and active immunity.

2.2 Other genetic polymorphisms that influence susceptibility to malaria

In addition to red cell polymorphism, several other genetic polymorphisms have also been implicated in natural resistance to malaria. The majority of these are in DNA regions that encode or control the encoding of components of the immune system and cellular adhesion proteins (Lopez *et al* 2010). The latter are important with regard to malaria as they have been implicated in the adherence of malaria infected red cells in the microvasculature of organs such as the brain; a process that contributes to the pathology of malaria. While some of these polymorphisms such as class 1 HLA-Bw53 allele have large effects; about 40% protection against severe malarial anaemia and cerebral malaria (Hill *et al* 1991), the others have subtle effect and are difficult to detect except in very large studies. The relationship between genetic polymorphisms and susceptibility to malaria is complex. Different polymorphism have varying influence on different syndromes of malaria some affect susceptibility to severe but not mild malaria or asymptomatic infection. Furthermore, the association with susceptibility to malaria for some polymorphism is only evident in one geographic region (West Africa only in the case of HLABw53 allele) but absent in other regions (Hill *et al* 1991). Although this could reflect some methodological difference in studies done in different regions, it also suggests that other unidentified genetic and environmental factors may modify the association between a known polymorphism and malaria outcome. Table 1 lists

out some of the polymorphism identified so far, and illustrates that a large number of genes are involved in determining susceptibility to malaria.

Functions	Gene (Protein)
Major Histocompatibility complex antigens	HLA-B53, HLA-DRB1
Pro-inflammatory cytokine and cytokine receptors	Interferon alpha receptor 1 (IFNαR1); interferon gamma and interferon gamma receptor (IFN-γR & IFNγR); Tumor Necrotic Factor alpha (TNF-α); Interleukin 1α, 1β ,4 and 12b (IL-1α/β, 4, & 12b)
Anti-inflammatory cytokines	Interleukin 10 (IL-10)
B-cell function regulation	Interleukin 4 (IL-4), TNFSF5 (CD40L)
Complement pathway components	Complement receptor 1 (CR1); Mannose Binding Lectin 2 (MBL2)
Nitric oxide (NO) pathway (immunoregulatory and microbicidal)	Nitric oxide synthase 2A (NOS2A)
Components of innate immunity	Toll-like receptors 1, 4, 9 (TLR1, TLR4, TLR9)
Macrophage receptor for antibodies	Fc gama receptor 2A and 3B (FcγRIIA & FcγRIIIB)
Blood cells development	Stem Cell Growth Factor (SCGF)
Blood Group antigens	Groups A, O, B
Acute Phase proteins	Haptoglobin
Cellular Adhesion Molecules	Thrombospondin receptor (CD36), Intercellular adhesion molecule1 (ICAM1); Platelet-endothelial cell adhesion molecule (PECAM)
Chromosomal region with immune genes cluster	Chromosome 5 region 5q31-q33

Table 1. Genes Reported to Be Associated with Susceptibility to Malaria

3. Innate and acquired immunity

Decreasing frequency and severity of malaria episodes with age among endemic populations is the best indicator that people do acquire immunity to malaria following repeated exposure. However, because both the truly protective immune response to malaria and those that simply reflect exposure to malaria increase concurrently with age, many putative in vitro measure of "immunity" to malaria show no correlation with protection against malaria. As such disentangling protective responses from non-protective ones in the complex milieu of responses provoked by malaria parasites is a major objective of malaria immunity studies. Unfortunately, differences in study methodology, polymorphism of target antigens or epitopes and other factors, such as variation in transmission in different study settings and even microvariations in transmission within a given study setting makes it difficult to develop a consistent picture of the efficacy of a given natural or vaccine-induced immune response in protecting against malaria (Bejon *et al* 2009, Kinyanjui *et al* 2009, Marsh and Kinyanjui 2006).

3.1 Natural history of acquired immunity to malaria

As shown in figure 1, in areas of stable malaria transmission, the majority of severe disease and death due to malaria occur mainly in children but parasite prevalence continue to rise well beyond childhood (Roca-Feltrer *et al* 2010, Snow *et al* 1997, Snow *et al* 1994). This has led to the suggestion that acquisition of immunity to malaria may be biphasic with immunity to disease being acquired before immunity to infection. Observations from experimental infection studies lend support to this suggestion. Records from malariotherapy in the 1940s which involved deliberate infection of syphilis patients with malaria so that the fever induced can kill syphilis spirochetes, show that in most patients, fever and high parasitaemia occurred in the first 25 days after which a low-density asymptomatic infection persisted for many months (Collins and Jeffery 1999b). More recently some modelling have suggested that immunity to severe disease may develop after only one or two episodes of disease (Gupta *et al* 1999a, Gupta *et al* 1999b). In reality, there is an overlap between the two phases of immunity otherwise anti-disease immunity acting in the absence of anti-parasite immunity would not prevent the parasites from multiplying and eventually overwhelming the patient. Furthermore, the risk of disease is proportional to parasite density (Rougemont *et al* 1991, Smith *et al* 1994) therefore immune mechanisms that clear parasites will also reduce the risk of disease.

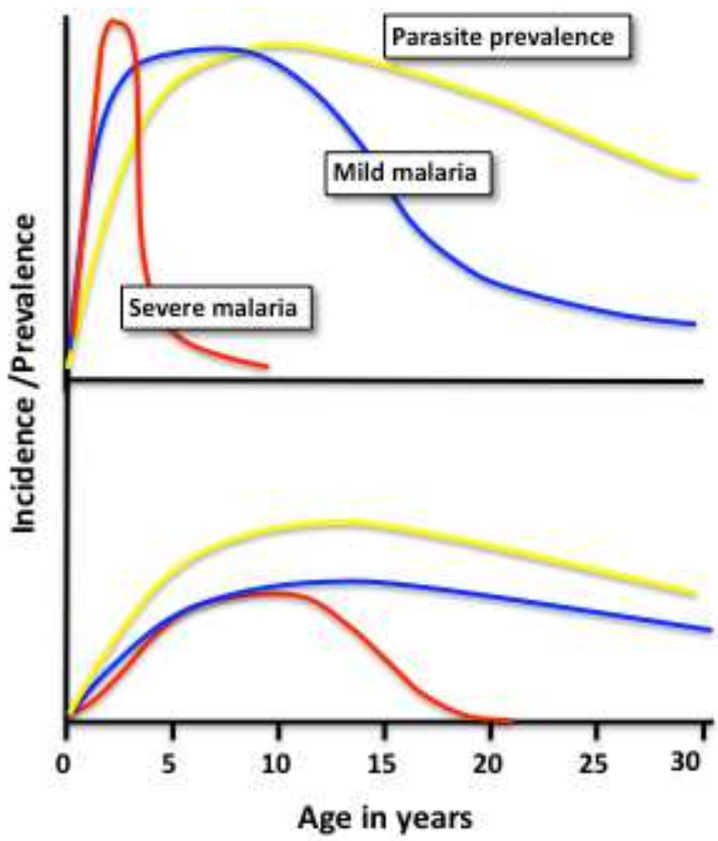

Fig. 1. A typical age pattern for incidence of severe and mild malaria and prevalence of asymptomatic malaria infection in an area of high (upper graph) and low (lower graph) malaria transmission

3.2 Strain specificity of malaria immunity

It has been suggested that the reason why immunity to malaria takes so long to acquire is because malaria is transmitted as a construct of many independent "strains" and one needs to accumulate immunity to all the circulating strains (Gupta and Day 1994, Gupta and Hill 1995). The observations in malariotherapy that infection with a given strain gave considerably more protection against re-infection by the same strain than by a different strain (Collins and Jeffery 1999a, Jeffery 1966) points to strain-specific immunity. However, unlike in the laboratory where cloned lines can be physically separated, maintaining such a population structure in the field despite sexual mixing is difficult (Babiker *et al* 1994, Hill and Babiker 1995, Ranford-Cartwright *et al* 1993). Nonetheless a number of models suggest that efficient immunity responses directed against a polymorphic antigenic determinant could constrain parasite populations into discrete non-overlapping strains with respect to that antigen (Gupta and Hill 1995, Recker *et al* 2008, Recker *et al* 2004).

Immune responses against the variant parasite antigens (VSA) exported to the surface of infected red cells, of which PfEMP1 is the best characterised, are an example of immunity that might be sufficiently efficient to structure malaria parasite population into "strains". These antigens are highly polymorphic and undergo clonal antigenic variation (Brannan *et al* 1994, Recker *et al* 2011, Roberts *et al* 1992). Antibodies to VSA provide variant-specific protection against malaria (Bull *et al* 1999, Bull *et al* 1998, Marsh *et al* 1989, Newbold *et al* 1992). The number of VSA variants against which an individual has antibodies increases with age (Bull *et al* 1998, Iqbal *et al* 1993, Reeder *et al* 1994). Thus, acquisition of immunity to malaria might, in part, involve the accumulation of antibodies against the circulating repertoire of VSA variants. It is thought that the variation of these antigens serve as an parasite immune evasion mechanism and therefore the need to avoid the generation of cross-reactive responses might provide the selection pressure necessary to maintain the circulation of distinct variants within a parasite population (Gupta and Hill 1995, Recker *et al* 2008, Recker *et al* 2004).

4. Immune effector mechanisms in malaria immunity

Although both cellular and humoral immunity are thought to be involved in malaria immunity, the relative importance of each in protection against malaria is not yet well established. In particular much of the data on cellular immunity comes from animal models. However, many of these animals are poor models of human malaria. Furthermore, data from different animal species and between different strains of same species often vary considerably making it difficult to generate definitive conclusion regarding immune effector mechanisms in malaria.

4.1 CD8+ T-cells (CTL)

CD8+ T cells are also referred to as cytotoxic T-cells (CTL) because they can kill infected cells directly by various cytotoxicity mechanisms. Because hepatocytes express class 1 HLA, the receptor for CD8+ T cells, the liver stage of malaria parasites is thought to be capable of inducing CTL responses. The role of CTL in the protection against malaria was first demonstrated in the classical experiments involving the immunization of animals and human with irradiated sporozoites. Such immunization resulted in complete, though short-

lived, immunity (Clyde 1975, Nussenzweig *et al* 1967, Rieckmann *et al* 1974). Adoptive transfer of CTL and CTL depletion experiments in animals showed that although high levels of anti-sporozoite antibodies were observed in the immunized subjects, the protection observed was mediated by CTL (Schofield *et al* 1987, Suss *et al* 1988, Weiss *et al* 1988). The fact that adoptively transferred CTL pre-primed with *P. berghei* failed to protect against infection by *P. yoelii* indicates that protection by CTL is species-specific (Romero *et al* 1989). Later studies showed that CTL mediate their protection by preventing parasite development in the liver (Rodrigues 1991). Although CTL can kill parasites by perforin-mediated lysis and FAS-induced apoptosis of infected cells (Kagi *et al* 1994, Lowin *et al* 1994), depletion of FasL and perforin did not affect CD8+ mediated-protection against *P yoelii* infection in mice suggesting that the protection is probably cytokine-mediated (Morrot and Zavala 2004). Indeed, The importance of the cytokine pathway in which IFN-γ stimulates the host cell to kill the parasites through nitric oxide (NO) production has been demonstrated in mice (Schofield *et al* 1987).

Indirect evidence for CTL protection against malaria in humans is borne in the association between some class 1 HLA alleles and protection against malaria (Hill *et al* 1991). Over 30 peptides on the sporozoites and liver stage antigens of malaria parasites have now been identified as epitopes for human CTL (Aidoo and Udhayakumar 2000, Aidoo *et al* 1995, Bottius *et al* 1996) Some of these epitopes exhibit extensive polymorphism generated by non-synonymous mutations, an indication that they are under some sort of selection possibly by host immunity (Hughes and Hughes 1995, Lockyer *et al* 1989, Schofield 1989).

4.2 Cytokine response to malaria – Role of innate immunity and CD4+ T-cells

Malaria disease is characterised by production of a wide range of cytokines. Studies suggest that these come from both the innate arm and the adaptive arm of the immune system. Because parasites multiply very rapidly, it is likely that the innate arm mediates early cytokines responses against malaria. An early interferon gamma (IFN-γ) response has been shown to be important in protecting against development of severe disease symptoms (Cabantous *et al* 2005, D'Ombrain *et al* 2008, Perlaza *et al* 2011) as has the ability of one's cells to produce TNF-α or INF-γ upon stimulation with live parasites in vitro (Robinson *et al* 2009). Natural killer cells (NK) have been implicated as the source of early proinflammatory responses such as IFN-γ and TNF-α against malaria parasites (Artavanis-Tsakonas and Riley 2002, Korbel *et al* 2005) while other studies point to γδT–cells and αβT-cells (D'Ombrain *et al* 2008, Horowitz *et al* 2010). However, the relative contribution of each type of cells is debatable. Activation of innate immunity depends on broad recognition of pathogens. This recognition is driven by receptors that recognise pathogen associated molecular patterns (PAMPs). Among the best characterised of these pattern recognition receptors are Toll-like receptors (TLR), of which ten have been described in man so far. Upon recognition of PAMPs, TRLs induce a signalling cascade leading to secretion of proinflammatory cytokines, chemokines, and interferons. Malaria parasite glycophosphoinostol (GPI) has been shown to interact with TLR2 and to some extent TLR4 (Franklin *et al* 2009, Gowda 2007, Krishnegowda *et al* 2005). While some studies suggest that haemozoin, a product of haemoglobin digestion by malaria parasites interacts with TLR9 (Coban *et al* 2005), other studies suggest that haemozoin is immunologically inert and it's the parasite DNA that it complexes with that interact with TRLs to induce a proinflammatory response (Parroche *et al* 2007). It is also possible that malaria parasites can induce the innate immune system through interaction between other non-TLR receptors and AT-rich parasite DNA fragments (Sharma *et al* 2011).

Subsequent to the innate responses that mediate early resistance to malaria infection, the adaptive immunity takes over with CD4+ T-cells becoming the main producers of cytokines. Traditionally mature CD4+ T-cells are placed in two groups that are associated with distinct cytokine profiles. Production of interferon alpha/gamma (INF-α/γ), lymphotoxin-α (TNF-β), interleukin-12 (IL-12) defines type 1 helper cells (Th1) and is associated with a strong cell-mediated immunity while production of IL-4, 5, 6, 9, 10 and 13 define type 2 (Th2) which are associated with antibody production. However, because some T-cells and non-T-cells can produce both Th1 and Th2 cytokines, it may be more appropriate to talk of a type 1 (TR1) or a type 2 response (TR2) (Clerici and Shearer 1994). In malaria, the TR1/TR2 dichotomy is most evident in the mouse-*P. chabaudi* model (Langhorne *et al* 1989, Taylor-Robinson and Phillips 1993). In this model, TR1 dominates the early response of mice to acute *P. chabaudi* infection and parasite killing is mediated by INF-γ, tumour necrosis factor (TNF-α) and nitric oxide (NO) secreted by activated Th1 CD4+, macrophages, and natural killer cells. In *P. berghei* and *P. yoelii* models, TR1 response induced through sporozoites vaccination have been shown to provide strong protection against challenge infections (Oliveira *et al* 2008, Purcell *et al* 2008). On the other hand, a shift towards TR2 leads to less symptomatic chronic infections (Clerici and Shearer 1994). Along with inhibiting both INF-γ and TNF-α, type 2 cytokines also stimulate B-cells to secrete of antibodies (Fell and Smith 1998, Pretolani and Goldman 1997, Taylor-Robinson 1995). The dual anti-parasite/ pathogenetic nature of TR1 is also evident in *P. berghei* infections (Hirunpetcharat *et al* 1999, Rudin *et al* 1997). Other murine-malaria models display variable tendencies towards either type of responses during acute and chronic infections (Taylor-Robinson and Smith 1999).

The distinction between type 1 and 2 responses is less clear in human malaria. Increased IFN-γ is associated with the resolution of parasitaemia in acute malaria episodes (Winkler *et al* 1998) and a delay in re–infection (Luty *et al* 1999) while reduced levels accompany hyper-parasitaemia in children (Winkler *et al* 1999). Similarly, levels of type 1 response were lower among Malawian malaria patients than among patients of other disease, with a reverse trend being observed for the type 2 responses (Jason *et al* 2001). INF-γ levels were found to be higher in pregnant women who did not have placental malaria than in those who did (Moore *et al* 1999). These observations argue for a possible anti-parasite role of TR1 in humans. Furthermore CD4+ secreted IL-2 and TNF–α are associated with the protection provided by the experimental vaccine RTS'S (Lumsden *et al* 2011). On the other hand, IL-10 and IL-4, both type 2 cytokines, have been associated with protection against malarial anaemia (Biemba *et al* 2000, Kurtzhal *et al* 1998). Although reduced secretion of INF-γ by immune T-cells in response to malaria led to the conclusion that reduced pathology in immune individuals may be attributable to down-regulation of TR1 cytokines (Chizzolini *et al* 1990), Winkler et al (1999) observed a striking increase in type 1 cytokines in immune adults (Winkler *et al* 1999). It is likely that efficient immunity to malaria requires a balance in between TR1 and TR2.

4.3 Regulatory T cells

There is now an increasing recognition of the role played by a third population of CD4+ T-cells in malaria immunity (Walther *et al* 2009). This cells, designated regulatory T-cells (Tregs), additionally express CD25 and FOXP3 cellular markers and mediate their actions through immunomodulatory cytokines IL-10 and TGF-β. Studies in mouse models suggest

that these cytokines help reduce immunopathology by suppressing proinflammatory cytokines (Nie *et al* 2007) although if induced too early in an infection they may suppress the protective effects of the proinflammatory cytokines and allow the parasite to multiply uncontrollably (Walther *et al* 2005). In humans, TGF–β, which appears to interact with Tregs, is associated with increased risk of clinical disease and a high parasite growth in vivo (Todryk *et al* 2008). As such a fine balance between the symptom-suppressing effects of Tregs and the parasite-suppressing effect of symptom-inducing cytokines is needed for an optimal outcome following malaria infection (Berretta *et al* 2011).

5. Humoral responses in malaria

5.1 Evidence for involvement in protection against malaria

There is no doubt that humoral responses are important in protection against malaria. Direct evidence for this comes from passive transfer experiments both in animal models (Groux and Gysin 1990) and humans. In a series of experiments carried out in the early 1960s by Cohen, Macgregor, and Carrington intra-muscular administration of purified IgG from malaria immune African adults into Gambian and East African children suffering from clinical malaria caused a marked drop in parasitaemia within five days. IgG from Europeans without prior exposure to malaria did not show this parasitocidal effect; indicating that the antibodies from Africans were malaria-specific (Cohen *et al* 1961, McGregor 1963). Similar results were obtained in more recent transfer experiments that used African immune serum to treat Thai malaria patients (Druilhe and al 1997, Sabchareon *et al* 1991). In addition, Edozien et al (1962) showed that antibodies that protected against malaria could be obtained from cord blood thus demonstrating that the passively acquired maternal immunity against malaria in infant, is at least in part, antibody–mediated (Edozien *et al* 1962).

Strong, albeit indirect, evidence for the protective efficacy of antimalarial antibodies comes from classical longitudinal studies where a person's history of malaria disease during a follow-up period is assessed for association with levels of antibodies to various malaria antigens at the beginning of the follow-up period. Using this approach a number of antibody responses to various antigens, some of which listed in figure 2, have been shown in to be associated with protection against malaria. Two shortcomings of this approach are the assumptions that all those who did not get an malaria episode during the follow-up are immune and that the levels of antibodies measured at the beginning of the follow-up period last through the period and any failure to see protection reflects lack of protection by the antibodies. (Bejon *et al* 2010, Kinyanjui *et al* 2009). However, the presence of variation of exposure even within limited geographic region (Bejon *et al* 2010) means that some people may fail to get an episode during follow-up simply because they were not exposed rather than because they are immune. In addition, humoral responses to malaria have been found to be short-lived (Kinyanjui *et al* 2003) as such even people with protective levels at the beginning of a follow-up might have non-protective levels by the time they encounters the next infection.

5.2 Mechanisms by which antibodies protect against malaria

In vitro, antibodies from immune individuals have been shown to inhibit sporozoites invasion of hepatocytes (Dent *et al* 2008, Fidock *et al* 1997, Pasquetto *et al* 1997), prevent

merozoites invasion of red blood cells (Haynes *et al* 2002, Tham *et al* 2009, Vande Waa *et al* 1984), depress parasite growth (Crompton *et al* 2010, Dent *et al* 2008, McCallum *et al* 2008, Wilson *et al* 2010), and promote parasite phagocytosis by macrophages (Druilhe and Khusmith 1987, Groux *et al* 1990). Furthermore, immune serum can disrupt rosettes (Carlson *et al* 1994, Vigan-Womas *et al* 2010) and the binding of infected erythrocytes to endothelial cell ligands (Iqbal *et al* 1993, Ricke *et al* 2000, Udeinya *et al* 1983), two phenomena which, as earlier indicated, have been implicated in the pathogenesis of severe malaria.

However, it is not clear how well in vitro antibody activities correlate with effector mechanisms in vivo. Malaria literature is replete with reports of lack of a correlation between antimalarial antibody titres measured in vitro and malaria protection (Erunkulu *et al* 1992, Marsh *et al* 1989, Thelu *et al* 1991) This is because the majority of malaria antibodies are probably directed against cellular debris released when schizonts burst and are of little consequence with regard to protection.

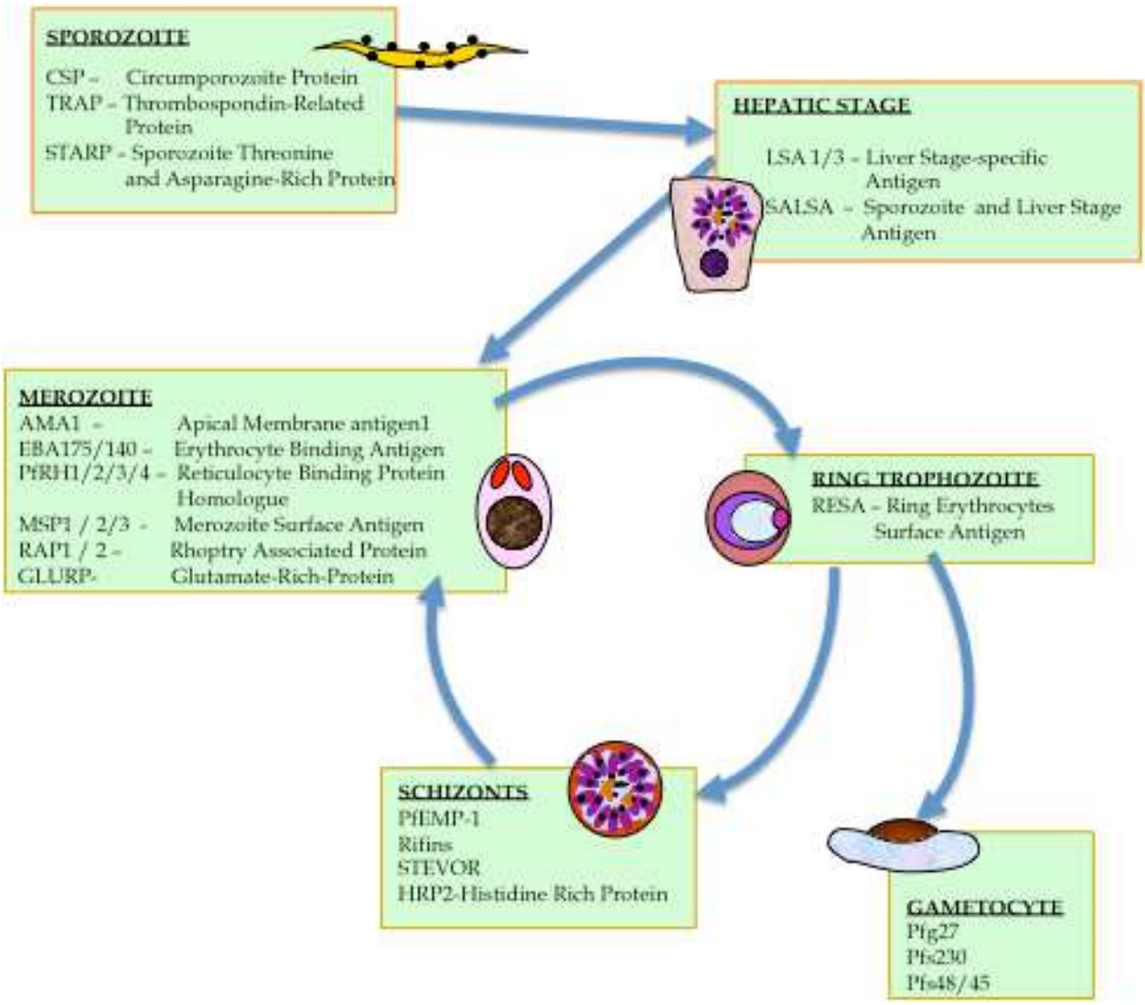

Fig. 2. Malaria antigens associated with various stages of the parasite that are thought to be targets for immune responses.

Under a variety of in vitro situations, malaria antibodies are often ineffective against parasites in the absence of effector cells and may even promote parasite growth (Galamo *et al* 2009, Shi *et al* 1999). Despite exhibiting potent anti-parasitic activity in vivo, the antibodies used in the transfer experiments in Thailand showed no activity in vitro except in presence of monocytes (Bouharoun-Tayoun *et al* 1990, Sabchareon *et al* 1991). Conversely, antibodies that do not protect in vivo were unable to interact with monocytes in vitro (Groux and Gysin 1990). Thus it has been suggested that the ability of antibodies to cooperate with effector cells may be more important than their quantity (Bouharoun Tayoun and Druilhe 1992). It has been noted that humoral responses to malaria show pronounced skewing towards cytophilic antibodies IgG1 and IgG3 subclasses, unlike responses to other pathogens where IgG1 and IgG2 dominate (Ferrante and Rzepczyk 1997). This bias has been reported severally in responses against ring-infected erythrocyte surface antigen (RESA) (Beck *et al* 1995, Dubois *et al* 1993), merozoites surface antigens (MSA1/2) (Rzepczyk *et al* 1997, Taylor *et al* 1995) and schizont antigens (Nguer *et al* 1997, Piper *et al* 1999, Thelu *et al* 1991).

This skew towards cytophilic antibodies, which need to bind to effector cells before they can mediate any action against antigens, could explain the failure of malaria antibodies to exert anti-parasitic activity on their own. In vitro work has shown that while cytophilic antibodies cooperate with monocytes in inhibiting parasites, non-cytophilic subclasses antagonise this cooperation (Bouharoun Tayoun and Druilhe 1992). Data from field studies indicate that young children and non-immune adults have a high proportion of non-cytophilic antibodies (Wahlgren *et al* 1983), while cytophilic antibodies are associated with protection against infection (Aribot *et al* 1996, Ferreira *et al* 1996, Salimonu *et al* 1982) and better prognosis during acute malaria episodes (Sarthou *et al* 1997). Taken together, these data suggests that acquisition of immunity to malaria may involve a shift in responses from non-cytophilic to cytophilic antibodies (Bouharoun Tayoun and Druilhe 1992).

5.3 Antibody dependent cellular inhibition (ADCI)

An interesting observation in the transfer experiments was the failure of passively transferred antibodies to completely eradicate all the parasites. This may have parallels in the failure of otherwise highly immune individuals to eliminate chronic low-grade infections. One proposal is that the parasites that escaped the transferred immunity comprised "strains" of parasites against which the antibodies lacked specificity. Two arguments against this are that the antibodies from immune African adults are expected to be directed against multiple antigens, which should help overcome restriction by the strain-specificity of responses to individual antigens, and more importantly, the same antibodies were subsequently shown to be effective against the breakthrough parasites. A density dependent mechanism designated antibody dependent cellular inhibition (ADCI), has been proposed to explain the interaction between cytophilic antibody and monocytes (Druilhe and Perignon 1997). This interaction causes the monocytes to release mediators that reversibly inhibit the growth of parasite ring stages. The amount of inhibiting mediators released is proportional to the ratio of merozoites to monocytes, which explains why the drop in parasitaemia following injection of immune IgG was proportional to the initial parasitaemia. Decline of either antibody levels, or numbers of monocytes or merozoites reverses inhibition and the parasite population flares up. A further implication of the hypothesis is that since the inhibiting mediators are non-specific, this mechanism does not

select for particular parasite variants. However, the huge in drop parasitaemia seen in the transfer experiment is more consistent with a parasitocidal rather than the parasitostatic effect implied by ADCI and other antibody-mediated mechanisms cannot be excluded.

5.4 Longevity of antibody responses to malaria antigens

Among people living in endemic areas, levels of antibodies to many malaria antigens vary with the seasonality of malaria transmission, often being higher during periods of high malaria transmission than at the end of a low transmission season (Cavanagh *et al* 1998, Giha *et al* 1998, Nebie *et al* 2008). Second, levels of antibodies to malaria antigens often tend to be higher in individuals who also have malaria parasites at the time when their antibodies are measured than in those without parasites (al-Yaman *et al* 1995, Bull *et al* 2002, Kinyanjui *et al* 2004). These phenomena are typically seen in young children, probably because adults typically have much higher antibody levels that take longer to decay appreciably even in the absence of an infection [(Fruh *et al* 1991, Riley *et al* 1993, Taylor *et al* 1998). These observations and those from other longitudinal studies where malaria antibodies fell from relatively high levels to low levels within a few weeks of treatment of a clinical episode (Branch *et al* 1998, Fonjungo *et al* 1999, Fruh *et al* 1991) suggest that antibody responses to many malaria antigens are relatively short-lived. The preponderance of IgG3 subclass, which has a shorter half-life than the other IgG subclasses, might, in part, explain the brevity of antimalarial antibody responses. However, detailed kinetics studies on the decay of antimalarial antibodies suggest that even the other subclasses decline at a rate that is faster than can be explained by normal catabolic decay (Kinyanjui *et al* 2003). This brevity of circulating antibody responses might explain the rapid re-infection seen among individuals living in endemic areas after malaria treatment.

6. Mechanisms of immune evasion by malaria parasites

Like other parasites, malaria parasites are not passive partners in the interaction with the host immune system. The immune system exerts a strong selective pressure on malaria parasites. They have therefore over time evolved a number of mechanisms to evade the immune system.

Polymorphism is a common feature of many malaria antigens and is generated through recombination during fertilization or clonal antigenic variation (Anders and Smythe 1989, Borst *et al* 1995). The circumsporozoite protein (CSP) (Dame *et al* 1984, Lockyer *et al* 1989) and thrombospondin–related adhesive protein (TRAP) (Robson *et al* 1998) on the surface of sporozoites all have regions of extensive polymorphism as does the major merozoite antigens; merozoite surface proteins (MSP-2 & MSP-2) (Cooper 1993, Felger *et al* 1994), ring stage erythrocyte surface antigen (RESA) (Perlmann *et al* 1984) and the apical membrane antigen-1 (AMA-1) (Verra and Hughes 1999).

The antigens inserted by mature parasites on to the surface of the host red cells, which include PfEMP1, rifins, and STEVOR, not only exhibit extensive polymorphism between isolates from different patients, they also under go clonal variation so that each new generation of parasites exhibits different variant from the previous one (Bachmann *et al* 2011, Baruch *et al* 1995, Blythe *et al* 2004, Chen *et al* 1998, Cheng *et al* 1998, Niang *et al* 2009). In many instances, there is little immunological cross reactivity between different

polymorphic variants of the same antigens meaning that encounter with parasites bearing one variant does not generate protection against infections bearing a different variant.

Some of the proteins in the parasite have not evolved a high level of polymorphism instead parasites escapes immune responses directed against these proteins through functional redundancy. In other words, the parasites are able to utilise more than one protein to achieve the same function. This is particularly the case for proteins involved in essential functions such as invasion of the red cell. The erythrocyte binding ligand (EBL) and the reticulocyte binding homologues (RH) protein families, (Adams *et al* 2001, Reiling *et al* 2010, Triglia *et al* 2009) both involved in invasion, consist of four and five closely related, but not identical, proteins respectively. Blocking invasion mediated by one of these protein results in a shift toward invasion pathways mediated by the other proteins (Lobo *et al* 2006, Lopaticki *et al* 2011, Persson *et al* 2008).

There is some evidence to suggest that apart from escaping immune recognition, malaria parasites can actively subvert the immune system and may direct it towards less effective responses. Some malaria antigens such as glycosylphosphatidylinositol (GPI) contain sections of tandem amino acid repeats in their structure. Such polymeric structures can cross-link B-cell antigen receptors and induce T-cell independent antibody production that is characterised by IgM dominance and poor affinity maturation and memory cells induction (Garcia de Vinuesa *et al* 1999). Besides being short-lived and ineffective, T-cell independent responses can also thwart protective responses to adjacent critical epitopes through epitopic inhibition (Schofield 1991). Disruption of splenic architecture, which prevents the formation of germinal centres and that could also lead to T-cell independent responses, has been observed in animal malaria model (Achtman *et al* 2003). Malaria parasites have been shown to prevent the maturation of dendritic cells through binding of infected red cells to dendritic cells using parasite generated variant surface antigens (Urban *et al* 1999, Urban *et al* 2001). However, other studies suggest that dendritic cell inhibition could also be mediated through other interactions not involving surface antigens (Elliott *et al* 2007). Furthermore, studies in mouse models suggest that the parasite –induced defect in maturation does not necessarily affect dendritic cells ability to induce protection in vivo (Pouniotis *et al* 2004). Recently it has been suggested that malaria parasite use the activation of regulatory T cells that suppress antiparasite cytokines production as a way of escaping the immune system. Mice whose T regulatory cells are depleted can survive infection with a lethal strain of *P. yoelii* and exhibit increased T-cell responsiveness to parasite antigens compared to normal mice (Hisaeda *et al* 2004).

7. Conclusions

Although research has contributed significantly in our understanding of immunity towards malaria, there are still considerable gaps in our knowledge. Closing these gaps in order to facilitate the development of malaria vaccines remains one of the major goals of tropical diseases research. Two areas are key to developing a comprehensive picture of the interaction between the malaria parasites and the immune system: first there is need to standardise current methods for studying immunity to malaria in order to address the numerous inconsistencies concerning the efficacy of various response to malaria often encountered in the literature. Second there is need to take advantage of the advances in molecular biology in studying immunity to malaria for example use of high throughput

sequencing and DNA and protein microarray technology to facilitate the simultaneous study of the large range of responses evoked by a malaria infection or the development transgenic animal and parasite models that approximate better to malaria infections in human.

8. References

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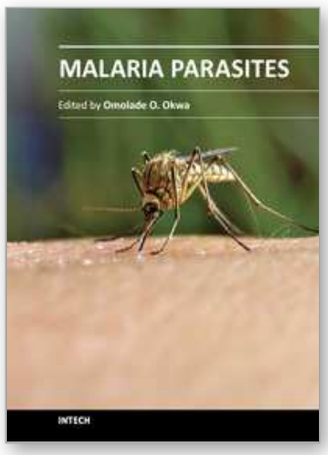

Malaria Parasites

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Malaria is a global disease in the world today but most common in the poorest countries of the world, with 90% of deaths occurring in sub-Saharan Africa. This book provides information on global efforts made by scientist which cuts across the continents of the world. Concerted efforts such as symbiont based malaria control; new applications in avian malaria studies; development of humanized mice to study P.falciparium (the most virulent species of malaria parasite); and current issues in laboratory diagnosis will support the prompt treatment of malaria. Research is ultimately gaining more grounds in the quest to provide vaccine for the prevention of malaria. The book features research aimed to bring a lasting solution to the malaria problem and what we should be doing now to face malaria, which is definitely useful for health policies in the twenty first century.

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