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REVIEW

Immunity to malaria: more questions than answers

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Malaria is one of the main health problems facing developing countries today. At present, preventative and treatment strategies are continuously hampered by the issues of the ever-emerging parasite resistance to newly introduced drugs, considerable costs and logistical problems. The main hope for changing this situation would be the development of effective malaria vaccines. An important part of this process is understanding the mechanisms of naturally acquired immunity to malaria. This review will highlight key aspects of immunity to malaria, about which surprisingly little is known and which will prove critical in the search for effective malaria vaccines.

Malaria, caused by infection with protozoan parasites of the genus *Plasmodium*, is a major global heath problem and is responsible for the deaths of over a million people annually, mainly children in sub-Saharan Africa[1]. There has been an understandable push to develop effective antimalaria vaccines. However, it has not been matched by a similar level of investment in understanding basic aspects of the immune response to this parasite.

In this review we highlight key aspects of acquired immunity in humans and critically review work, particularly in mouse models, on two main issues: what is the function of the immune response in pathogenesis, and why is immunity apparently short-lived. Understanding these aspects may allow the delineation of pathological versus protective responses and determine the nature of long-term immunity and thus provide more effective immunological interventions.

Immunity to malaria in humans

Humans with no previous experience of malaria almost invariably become ill on their first exposure to the parasite. They develop a febrile illness, which may become severe and, in a proportion of cases, may lead to death. In malaria-endemic areas, young children are particularly susceptible, and it has been estimated that a quarter of all childhood deaths are due to malaria[2]. However, with exposure, older children and adults develop essentially complete protection from severe illness and death, although sterile immunity is probably never achieved. Although vaccines may not be limited to mimicking natural immunity, as clear a picture as possible of the mechanisms of such immunity is an important starting point.

The picture of human immunity to malaria has been provided by two main sources: deliberately induced malaria in nonimmune people, and natural history studies in endemic populations. An important body of literature was generated by the use of induced malaria in the treatment of neurosyphilis in the early twentieth century. Those studies have informed the paradigm of how humans respond to malaria, and re-analysis of the data has provided new insights into the immune response[3]. At present, induced malaria in volunteers forms an important aspect of testing of some malaria vaccines and offers the opportunity of detailed studies of possible protective mechanisms[4].

After an infected mosquito bites through the skin, sporozoites rapidly move from the dermis to the liver, where they go through an asymptomatic stage of rapid division before the parasite reenters the bloodstream (**Fig. 1a**). In the blood, exponential expansion of parasite populations leads to febrile illness. Typically, acute infection is controlled and chronic infection is established at reasonably low parasite density, with intermittent episodes of fever associated with peaks of higher parasitemia[5]. Such peaks are of progressively lower density until the infection is eliminated, usually after many months. There is relatively rapid acquisition of immunity to the homologous parasite, demonstrated as more rapid control of successive infections at lower parasite densities and less-severe or even absent clinical illness. Although less profound in terms of parasitological indices, there is also evidence of early acquisition of heterologous immunity, particularly in terms of clinical symptoms[6].

Immunity to malaria in an endemic area is seen as both lower prevalence of 'parasitization' with age and lower rates of disease (**Fig. 1b**). However, the timing of changes in the rates of parasitization, mild disease and severe disease are different. Although it could be argued that these all reflect the same underlying process, immunity to severe malaria is essentially fully established at a time when there are no changes in the rates of mild febrile disease and when parasite rates in the population are still increasing, which suggests that there may be distinct mechanisms underlying these different expressions of immunity. The picture that emerges from human studies is that immunity to malaria infection is relatively slow to develop and incomplete, although immunity to death is acquired more quickly and may be important after a single episode[7].

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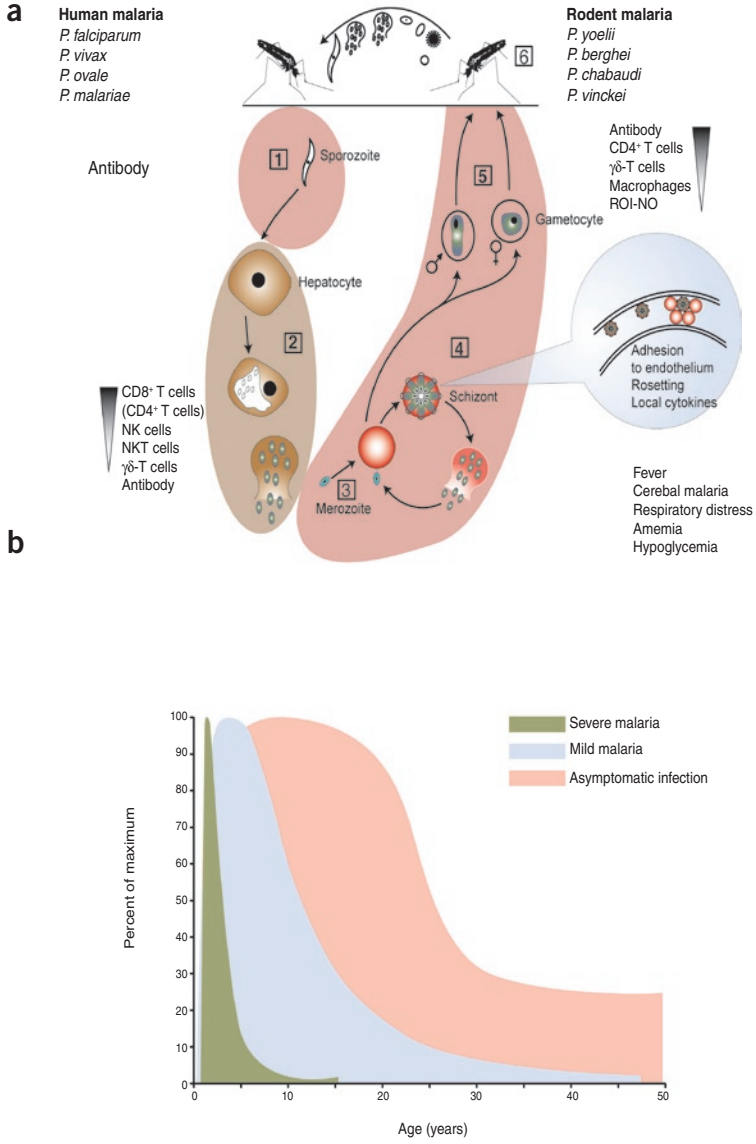

Figure 1 The life cycle of the malaria parasite *Plasmodium falciparum* and acquisition of immunity in an area of endemic transmission. (**a**) Possible immune mechanisms at various stages of the plasmodium life cycle in the mammalian host. Top left and right, various plasmodia that infect humans and experimental rodent models. Sporozoites are injected through the dermis by a female anopheles mosquito, enter the bloodstream (pink shaded area, left) and migrate to the liver, then enter hepatocytes (brown shaded area) and undergo an amplification phase lasting between 2 and 9 days. Merozoites released after rupture of the infected hepatocyte invade RBCs and initiate the asexual cycle in RBCs (pink shaded area, right); each cycle of invasion and replication is between approximately 24 and 72 hours, depending on the species of plasmodium. Sexual stages (male and female gametocytes) are formed during the erythrocytic cycle; this stage continues the life cycle in the mosquito after a blood meal. Parasite stages in the liver are clinically silent. Fever and severe malaria are associated with the parasite cycle in the blood, as well as adherence of infected RBCs to blood vessel endothelium and to each other (rosetting; inset in gray). Numbers indicate effector mechanisms thought to be effective against plasmodium in the mammalian host and in blocking transmission to the mosquito: 1, antibodies to sporozoites neutralize sporozoites and/or block invasion of hepatocytes; 2, IFN-γ and $CD8^+$ T cells ($CD4^+$ dependent), natural killer (NK) cells, natural killer T (NKT) and γδ T cells kill intrahepatic parasites; 3, antibodies to merozoites opsonize merozoites for uptake and/or inhibit invasion of RBCs; 4, antibodies to infected RBCs surface opsonize infected RBCs for phagocytosis and/or block the adhesion of infected RBCs to endothelium; TNF and IFN-γ activate macrophages to phagocytose and/or kill infected RBCs and merozoites; antibodies to glycosylphosphatidylinositol neutralize parasite toxins and prevent the induction of excessive inflammation; 5, antibodies to infected RBCs prevent the sequestration of gametocytes, which prevents the sequestration and maturation of gametocytes; and 6, antibody and complement taken up in the blood meal mediate the lysis of gametocytes and prevent fertilization and further development of the parasite in the mosquito. ROI, reactive oxygen intermediate; NO, nitrous oxide. (**b**) Population indices of immunity in an endemic area of *P. falciparum* transmission (adapted from ref. 96). Change over time of various indices of malaria in a population living in an endemic area of *P. falciparum* transmission: asymptomatic infection (pink), mild disease (febrile episodes caused by malaria; blue) and severe or life-threatening disease (green). The data are normalized and are presented as the percent of maximum cases for each population index.

Mechanisms of immunity

Immune attack could theoretically be directed at any point in the life cycle from the time of entry of the sporozoite (**Fig. 1a**). However, longitudinal studies in exposed populations suggest that immune responses to the pre-erythrocytic stages probably have limited involvement[8]. Certainly when the liver stage is bypassed by direct injection of blood-stage parasites, immune adults are still able to limit parasite population expansion and avoid symptoms[9]. This is in contrast to deliberate immunization with either whole irradiated sporozoites[10] or vaccines based on pre-erythrocytic antigens, which can induce considerable immunity and indeed form the basis of the most advanced malaria vaccine available at present[11]. The mechanism by which these vaccines may act is indicated by studies in mice in which elimination of pre-erythrocytic parasites requires mainly $CD8^+$ effector cells producing interferon-γ (IFN-γ) that kill parasites in infected hepatocytes[12]. Antibodies to the sporozoites are thought to have a lesser function.

For erythrocytic stages, potential targets for an immune response are free merozoites or intraerythrocytic parasites. Given that HLA class I or II molecules are absent from the surface of the parasite or the infected red blood cell (RBC), it is usually assumed that humoral responses are key in blood-stage immunity. In mouse models, B cells and antibodies are important in eliminating parasites[13], with some contribution from 'parasiticidal' mediators released from macrophages or similar cells of the innate system and probably activated by T cells[14]. Most information on the function of human immune responses to blood-stage parasites has been provided by longitudinal studies in endemic populations, supported by classical passive-transfer experiments[15,16].

The mechanisms by which antibody is effective include blockade of the invasion of RBCs by merozoites[17], antibody-dependent cellular killing mediated by cytophilic antibodies[18] and binding of antibody to parasite-induced molecules on the RBC surface, leading to greater clearance of infected RBCs[19]. However, the relative importance of each of these mechanisms is still a matter of debate. Some human studies point to an important function for antibody-dependent cellular killing[20], and work with mice expressing a human Fcγ receptor (FcγR) supports this view[21]. However, the few studies of mouse models lacking FcγR or complement suggest that parasite can be eliminated without opsonization[22,23]. Perhaps the 'take-home' message is that there is no single measure of a protective antibody response for a complex pathogen.

Many putative 'target' antigens on the infected RBC surface and antigens either on the merozoite surface or released during merozoite invasion have been identified as being potentially protective. Although the picture emerging from such studies has not always been consistent, and identification of immunological markers for protection has been difficult, studies suggest that responses to many antigens are involved in protection[24]. In this context, it is important to note that only about 1% of the antigens encoded by the parasite have been studied so far.

Mouse models have been very informative about potential protective mechanisms and provide the only system in which regulation of such responses can be delineated. However, rodent parasites are not natural mouse pathogens, and much of the knowledge has been gained from infections initiated by unnatural routes. The importance of $CD8^+$ T cells in immunity to pre-erythrocytic stages was first determined in studies in which mice were immunized with large numbers of attenuated sporozoites and challenged with live sporozoites injected intravenously, which bypasses the natural intradermal route of inoculation by the mosquito. Sporozoites injected in this way can enter the liver within seconds and, because of the large numbers injected, may be taken up by antigen-presenting cells, thus stimulating a substantial host response. However, they may be less available for elimination by antibodies than the small numbers of sporozoites inoculated into the dermis (as few as ten per mosquito), which may take minutes, if not hours, to enter the liver[25]. The immune response may also be affected by the contents of the mosquito bite itself[26], and retention of parasites in the dermis most probably affects the site of antigen presentation

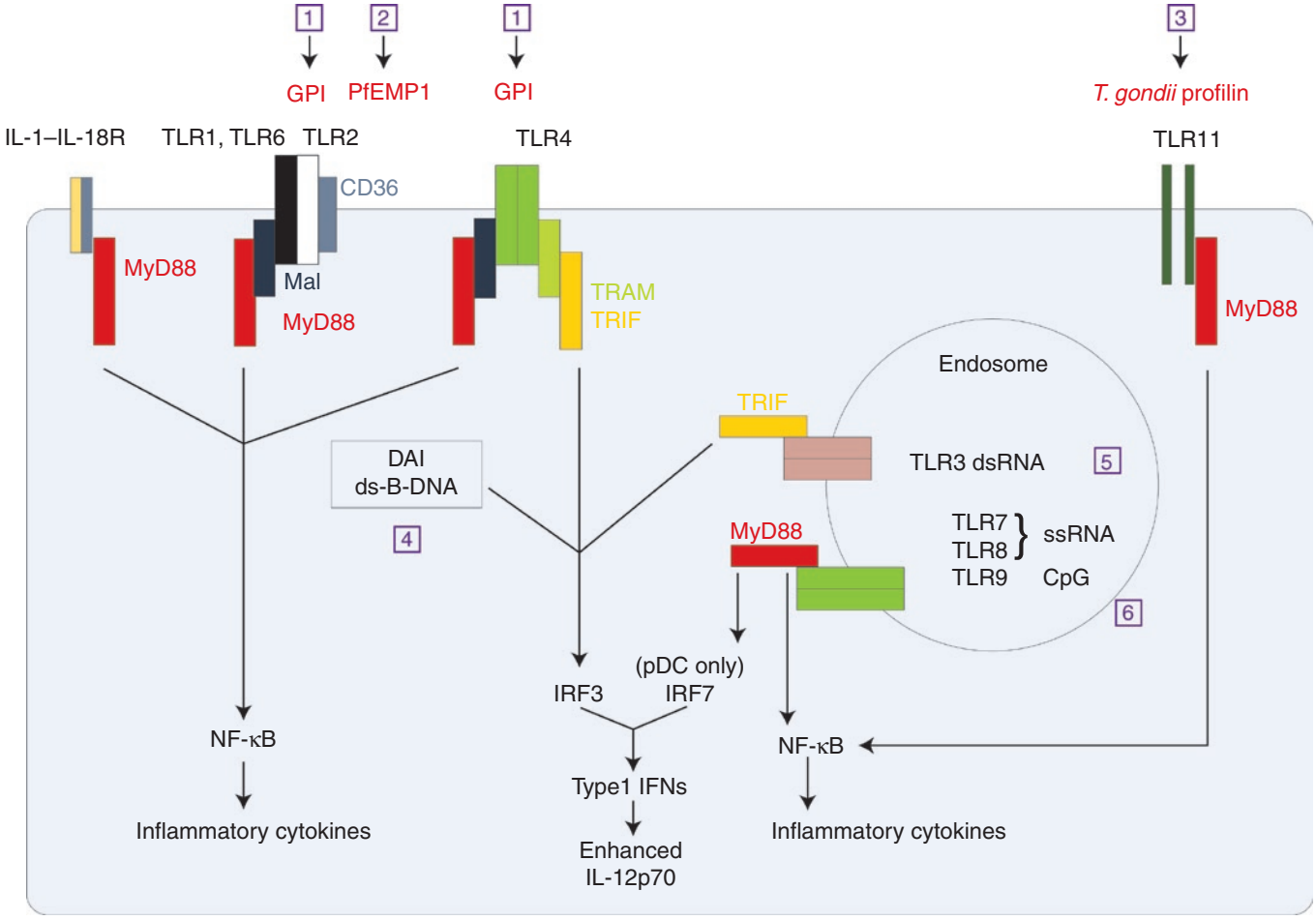

Figure 2 Interactions of plasmodium with host PRRs. The TLRs presented here have been demonstrated to interact with parasite-derived or parasite-associated molecules or could be considered potential PRRs for plasmodium. 1, many membrane proteins of plasmodium are anchored to the membrane by glycosylphosphatidylinositol (GPI) and are shed after cleavage of the molecule from the cell surface. Glycosylphosphatidylinositol is recognized by TLR2 and TLR4 (ref. 57). 2, CD36 binds to the cysteine-rich interdomain region region of PfEMP1, a variant molecule expressed on the surface of infected RBCs[53]. 3, profilin from another apicomplexan parasite related to plasmodium, *Toxoplasma gondii*, is recognized by TLR11 in mice, and this interaction is crucial for the induction of an effective $CD4^+$ T cell response[97]. 4, DAI is a sensor in the cytoplasm of cells that recognizes double-stranded B DNA (ds-B-DNA), mediating the induction of type 1 interferon and other molecules in innate immune responses[98]. 5, double-stranded RNA (dsRNA) has not been described in plasmodium, but reports from genome projects suggest its existence. Although not investigated, single-stranded RNA (ssRNA) from the parasite may be present in accessible form in phagosomes of DCs and macrophages, where phagocytosed parasites are degraded. 6, contaminants in hemozoin have been shown to activate DCs through TLR9 (ref. 60). PRRs such as Nod proteins and dectins are not presented here, as the ligands for these are not thought to be present in plasmodium. MyD88 transduces signals from TLR2, TLR5, TLR7-TLR8, TLR 9 and TLR11 as well as IL-1–IL-18 receptors, either through the transcription factor NF-κB, resulting in the transcription of genes encoding proinflammatory cytokines such as TNF, IL6, IL8 and IL1β and reactive oxygen intermediates, or, in plasmacytoid DCs (pDCs), or through the transcription factor IRF7, leading to the transcription of genes encoding type 1 interferons (IFNs)[99]. Ligation of TLR3 can lead to activation of the transcription factor IRF3 through TRIF, a transcription factor linked to IFN-β[100]. Also, signaling by TLR7-TLR9 leads to IFN-α induction[101]. Mal can act as an adaptor in the MyD88-dependent pathway from TLR2 and TLR4 (ref. 102).

as well as the locality and type of responses initiated. This does not detract from the possibility of using irradiated sporozoites[10] or genetically attenuated sporozoites[27] as vaccines but instead indicates that caution should be taken in concluding which mechanism of immunity may be more important.

Mouse models have also defined some key interactions between infected erythrocytes and merozoites and the immune system, but conclusions about effector mechanisms and immunopathology are often drawn from experiments in which large numbers of parasites are injected directly into the blood of mice, which bypasses the preerythrocytic cycle. As the ultimate aim is to develop effective vaccines and treatments for severe malaria, the mouse models should recapitulate the human infection as far as possible and involve the full parasite life cycle initiated by the mosquito bite.

Whatever the antigenic targets or effector mechanisms, key issues for both naturally acquired and vaccine-induced immune response are the function of the immune response in pathology, the duration of protective immunity and the establishment of immune memory; these are considered further below.

Immunity versus immunopathology

Although the description provided above of a febrile illness followed by control of parasitemia applies to most symptomatic infections, some progress to severe, life-threatening malaria. Three distinct but overlapping syndromes, severe malarial anemia, cerebral malaria and respiratory distress, are responsible for most severe cases and deaths[28]. The underlying pathogenesis of severe malaria is complex, and interpretation of data from human studies is dogged by the difficulty of distinguishing epiphenomena from events truly on the causal pathway. However, it has long been apparent that many of the features of severe malaria are similar to those of sepsis[29], and there is evidence that over-vigorous or disordered immune responses are central. Thus, although tumor necrosis factor (TNF) is protective against the parasite[30], very high serum concentrations of TNF are associated with greater severity and death[31]. Subsequently, many studies have shown an association between severe disease and enhanced amounts of proinflammatory cytokine responses, including TNF, interleukin 1β (IL-1β), IL-6, IL-10 and IFN-γ[32] as well as the chemokines CCL3 (MIP-1α) and CCL4 (MIP-1β)[33]. Studies also indicate that there may be specific cytokine profiles associated with different clinical syndromes[34]: severe malaria anemia has been associated with relatively low IL-10 responses[35], whereas respiratory distress is associated with abnormally large amounts of IL-10 (ref. 36). A low amount of CCL5 (RANTES) has been associated with severe disease and mortality[37,38].

It seems likely that inherent differences in people's control of immune responses and differences in parasite virulence each have their part in determining the response to infection. Polymorphisms in host genes such as those encoding IFN-γ, interferon-regulatory factors, TNF, IL-10 and IL-4 (refs. 39–42) have been associated with susceptibility to disease, and it seems that the balance in the regulation of pro- and anti-inflammatory cytokines may be critical in determining the extent of pathology in both humans and animal models[43–45]. Encouragingly, there is good concurrence between mouse models and human infection in some aspects of acute pathology[46,47]. Although TNF, lymphotoxin and IFN-γ are associated with pathology in the infection of mice[44,48,49] and are counterbalanced by IL-10 (ref. 44), it is not yet apparent how much of this pathology is dependent on a contribution from the acquired immune response. Mouse models would suggest that type 1 responses from both $CD4^+$ and $CD8^+$ T cells are chief participants in acute pathology[50], but neutralization of IFN-γ is sometimes only partially effective[51]. The involvement of other proinflammatory T cells, such as IL-17-producing T helper cells[52], has not yet been investigated, and there are too few studies in humans to allow any conclusions to be reached about the contribution of T cell responses.

The initial trigger for the production or overproduction of cytokines may depend on the type of interaction between host cells and the parasite. Attention has focused on the pattern-recognition receptors (PRRs) on host cells used to recognize and respond to plasmodium (**Fig. 2**). However, given the complexity of severe malarial disease, it is unlikely that a single mechanism or pathway is responsible for initiating the host response or for all the clinical syndromes of malaria infection. The infected RBC or parasite products can interact with several Toll-like receptors (TLRs) and cell surface receptors such as CD36 (ref. 53), a coreceptor for TLR2 (ref. 54), leading to seemingly opposing effects on innate and acquired immune responses. Variant parasite molecules encoded by large multigene families and expressed on the surface of infected RBCs, such as the *Plasmodium falciparum* protein PfEMP1, bind to CD36 and can both activate[55] and suppress human dendritic cells (DCs), monocytes and T cells[56]. Glycosylphosphatidylinositol 'anchors' on many plasmodium membrane proteins activate mouse and human DCs, macrophages and B cells through the TLR2-TLR1 complex and, to a lesser extent, through TLR4 (refs. 57,58), and hemazoin or, more likely, contaminating DNA activates DCs through TLR9 (refs. 59,60) to produce proinflammatory cytokines.

Signaling through MyD88, an intracellular adaptor molecule for several TLRs and the IL-1–IL-18 receptor (**Fig. 2**), is important for the induction of some but not all of the pathology of mouse malaria infection[61–63] but is also required for controlling acute-stage parasitemia[62]. So far, no single- or multiple-TLR-knockout model replicates this phenotype[62,63], which suggests that the parasite is recognized by several PRRs, some of which are still to be identified and some of which trigger innate responses independently of MyD88.

If TLR ligation and engagement of MyD88 are the means by which severe pathology is induced, then selection for genetic polymorphisms resulting in lower host responses might be expected in populations in endemic areas. Immunogenetic studies in this field are limited. So far, polymorphisms in TLR4, TLR9 and the adaptor protein Mal (also called TIRAP) show only weak associations with various aspects of severe malarial disease[64–66], which supports the idea that many PRRs and pathways may be acting in concert to bring about a dysregulated cytokine response.

The contribution of parasite genotype to the induction of cytokine responses likely to lead to malaria pathology remains mostly uninvestigated. Different strains of rodent parasites, such as *P. yoelii* and *P. chabaudi*, give rise to infections of different virulence[45,67] and induce different host responses[68], particularly regulatory cytokines such as IL-10 or transforming growth factor-β[45]. It will be very important to determine whether different strains of human and mouse plasmodium express either different amounts or different forms of PRR ligands, which alters the magnitude or regulation of the inflammatory response.

In conclusion, there are many examples of studies that have tested associations of malaria-induced pathology with the expression of cytokines, cytokine receptors, Fc receptors, antigen sensors on antigen-presenting cells and polymorphisms of single immune-response genes. Not unexpectedly, such disjointed and limited data are not always in agreement. It is still not clear whether the pathological effects of the host response are due to quantity of proinflammatory cytokines or to the quality of a person's response to a particular clone of parasite. The only way to distinguish pathological from protective responses will be to rely initially on large-scale multicenter studies of exposed

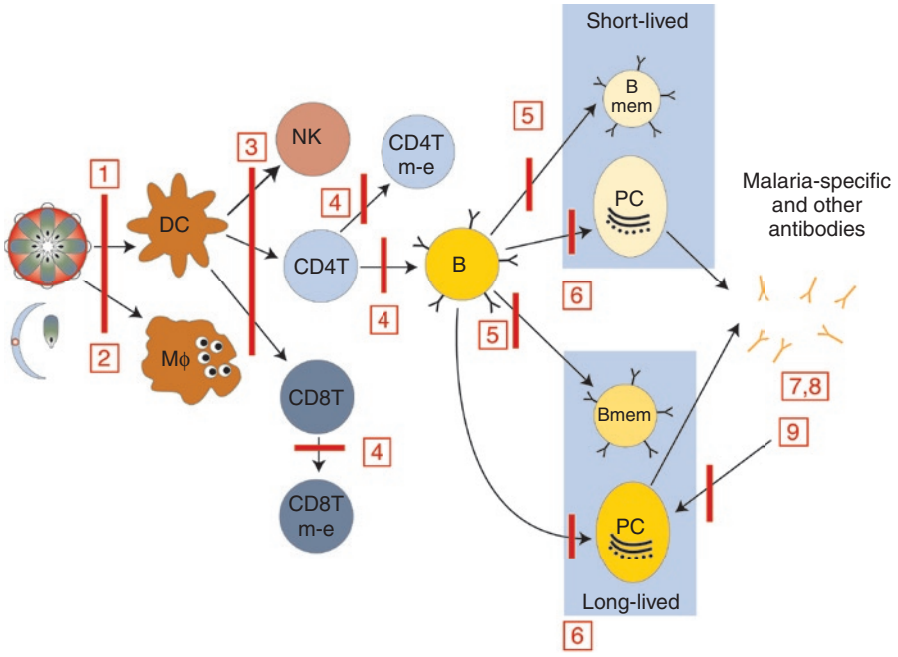

Figure 3 Possible mechanisms of interference in B cell and T cell activation and the generation of immunological memory by plasmodium. Red vertical bars indicate points in the B cell and T cell response at which parasites have been shown to or could inhibit, suppress or change host immune responses. Numbers indicate possible mechanisms of interference in the immune response: 1, interaction between infected RBC–parasite and DC can inhibit DC maturation[56]; 2, hemozoin can inhibit macrophage (Mϕ)–monocyte function[103]; 3, IL-10 from DCs and macrophages modulated by infected RBCs inhibit $CD4^+$ T cell (CD4T) activation[104]; 4, $CD4^+$ T cells produce IL-10 and transforming growth factor-β, which inhibits the generation of central and memory-effector (m-e) cells[105]; 5, infected RBCs induce apoptosis and/or depletion of memory B cells (Bmem)[93]; 6, the niche for plasma cells of many different specificities may be limiting; 7, the *P. falciparum* genome encodes over 5,300 predicted proteins, most of which are also highly diverse. Most responses induced in response to many polymorphic targets may not be protective and act as a 'smoke-screen', and hypergammaglobulinemia, a common feature of *P. falciparum* infection, may accelerate the catabolism of immunoglobulin molecules. 8, antigenic variation of proteins on the infected RBCs may be an effective mechanism for immune escape; 9, circulating immune complexes and low-affinity immunoglobulin molecules can trigger apoptosis of long-lived plasma cells (PC) through FcγRIIB[94]. CD8T, $CD8^+$ T cell.

populations using immunogenetic studies of polymorphisms (such as those of the MalariaGEN research network) and RNA or proteomic profiling of relevant host cells to identify potential susceptibility and/or resistance markers. These should be considered in conjunction with the genotype and virulence of the infecting parasite. Obviously such studies are difficult, but with defined groups, improved technology and good data analysis, it is likely that they will provide combinations of responses that are either pathogenic or protective. These should then form the basis of more refined mechanistic studies.

Immune memory in malaria

Immunity to malaria develops relatively slowly, is not sterile and is often said to wane quickly when immune adults leave malaria-endemic regions, which suggests that continued exposure to malarial antigens is required not only for the generation of memory cells and effector cells but also for their persistence. However, there is no agreement on what components of immunity to malaria are lost without exposure, and this loss is often identified only by the fact that such people do experience symptomatic infections. Immunity to severe disease is complete after only a few infections for children living in areas of high transmission but not for those living in areas of low transmission[7], which suggests that frequent exposure to malaria is needed to maintain immunity; otherwise, immunity to malaria is short-lived. However, when malaria remerged in Madagascar after a long period of complete control, people present 30 years earlier during malaria exposure were much more resistant to clinical disease than were younger subjects, which suggests that once established, key aspects of immunity may in fact be long-lived[69]. It is apparent that in young children in the process of establishing immunity, immune responses, particularly antibody responses to defined antigens, are often extremely short-lived[70] and may fail to 'boost' on exposure, even after an initial good response[71], which suggests that there may be defects in establishing functional immune memory.

Rapid boosting of antibody responses to various antigens after reinfection does take place, which indicates the presence of memory B cells. Therefore, it seems likely that people exposed to malaria do accumulate memory B cells specific for malaria antigens with exposure. However, only two studies have investigated malaria-specific memory B cells in malaria-exposed people. One has reported that anti–*P. falciparum* memory B cells are present in adults for over 8 years without evident re-exposure[72]. In contrast, a subsequent study has reported the presence of serum antibody but only very low frequencies of malaria-specific memory B cells in children exposed to *P. falciparum*[73]. Obviously, more studies of memory B cell responses to human malaria infection are needed before any conclusions can be drawn. In the mean time, mouse malaria models may be able to indicate possible correlates of protective immunity and methods by which these can be delineated to show defects (if any) in the human response to plasmodium antigens.

With few well defined markers for the various subsets of memory $CD4^+$ T cells, memory B cells and long- versus short-lived plasma cells, the identification of malaria-specific cells has been difficult, and this has hampered studies even with mouse models of malaria. However, the availability of T cell receptor–transgenic mice specific for both liver- and blood-stage antigens has now enabled investigators to study more specific issues about the mechanisms and nature of T cell–mediated responses[74,75]. For example, the formation of both central and effector $CD8^+$ memory T cells requires priming by DCs in the skin-draining lymph nodes[76] as well as $CD4^+$ T cell help[77]. These $CD8^+$ memory T cells are long-lived (up to 6 months), which is unexpected, given the short-lived nature of the immunity induced by irradiated sporozites[78] in humans, and by the most promising liver-stage–based vaccine, RTS,S[11].

The pre-erythrocytic stage of infection lasts only a few days; therefore, for specific $CD4^+$ and $CD8^+$ T cells to be protective at this stage, memory cells must be maintained as effector cells at relatively high frequencies until the next mosquito bite, or they must be able to differentiate very rapidly into effector-killer cells. It is likely that is there is a population shift from the mostly protective effector phenotype to the less protective memory phenotype, coupled with time from infection and antigen decay[79,80]. This may explain why immunological memory is not always correlated with immunity.

Recall responses of $CD4^+$ T cells to several blood-stage malaria

antigens have been documented extensively, all of which suggest that memory CD4$^+$ T cells are induced by natural infection. However, the frequencies of responding cells and the prevalence of exposed people with measurable malaria-specific CD4$^+$ T cells are often low, and longitudinal studies showing whether these T cells are long- or short-lived and whether there is a requirement for persistent infection and/or antigen are lacking. It has been difficult to demonstrate the function of CD4$^+$ T cells in natural human infection. However, the association between loss of CD4$^+$ T cells and lower concentrations of malaria-specific antibodies in malaria-infected people positive for infection with human immunodeficiency virus[81] would suggest that CD4$^+$ T cell help is necessary not only for the induction but also for the maintenance of protective antibody. The type of memory CD4$^+$ T cell response may also be important for contributing to or regulating pathology. For example, the cytokines produced by a persistent T helper type 1 response may also contribute to malarial disease. The importance of CD4$^+$ T cells in blood-stage malaria infection is most likely to be determined from the mouse models, in which the requirement for memory CD4$^+$ T cells in immunity to reinfection, for rapid secondary antibody responses and for regulating immunopathology can be tested directly. The findings from such studies will be valuable for the design of relevant T cell assays that can be used in evaluating vaccines.

There are many levels at which parasite material or infected RBCs could interfere with the generation of effector-memory T cells and antibody responses (**Fig. 3**). The ability of infected RBC–modulated DCs to activate T cells is still controversial; studies have shown both activation[82–84] and suppression[56,85] of mouse and human DCs. However, high doses of infected RBCs induce apoptosis of DCs and hence decrease the ability of DCs to activate CD4$^+$ T cells[56,84,86]. The large amounts of antigen during an acute malaria infection, therefore, could lead to inappropriate priming and excessive apoptosis[84] and thus may not allow the efficient generation of memory T cells. In fact, a large of proportion of malaria-specific CD4$^+$ T cells have been reported to be rapidly deleted after acute primary infection[87]. Such contraction may be a normal homeostatic mechanism regulating the immune response; however, the extent of apoptosis and loss of antigen-experienced T cells may depend on the parasite load (antigen dose) and the strength of T cell receptor and PRR signals, which in some cases lead to defective T cell memory. In viral infections such as lymphocytic choriomeningitis virus in mice, CD4$^+$ T cell memory wanes, whereas T helper type 1 memory CD4$^+$ T cells produced in humans in response to vaccinia virus or dengue virus can linger for decades[88]. Protection is associated with the development of T helper type 1 cells producing TNF or IL-2 and IFN-γ, not IFN-γ alone[89]. The development of efficient T helper memory therefore could also depend on the ability of the pathogen (or the vaccine) to induce the correct cytokines necessary for long-lived T helper memory responses. These aspects of T cell memory, particularly for CD4$^+$ T cells, have not been addressed in malaria infection.

Memory CD4$^+$ and CD8$^+$ T cells require major histocompatibility complex class II and class I and IL-7 for maintenance[90]. Although lower expression of major histocompatibility comple class II is found on many circulating lymphocytes during *P. falciparum* infection, it is not clear whether production of IL-7 or indeed of any of the other cytokines necessary for the maintenance of T cell memory is also affected. It is also possible, although it has not been investigated, that *P. falciparum* parasites induce exhaustion of CD4$^+$ T cells in a way similar to that reported for infection with human immunodeficiency virus or hepatitis C virus, in which a large proportion of T cells are dysfunctional and have higher expression of the negative regulatory molecule PD-1 and low expression of CD127 (exhausted T cells)[91,92]. As for memory B cells, ablation of preexisting memory B cells by *P. yoelii* infection has been reported[93]. That observation may be explained by a study showing that crosslinking of the inhibitory receptor FCγRIIB by immune complexes can induce apoptosis of plasma cells[94]. Collectively, these data suggests that chronic plasmodium infection (and indeed other infections) may interfere with normal immune response, resulting in short-lived immunity. However, it is apparent that more studies of the effect of chronic infection on the development of immune responses to both parasite and other antigens are needed.

This review has summarized two areas of key research questions in malaria immunology. Although there have been some considerable advances in understanding the host response to plasmodium, it is not yet known what to measure as a correlate for immunity, what mechanisms regulate immune pathology in semi-immune people, what (if any) defects contribute to the relatively ineffective immunity in children and why immunity to *P. falciparum* infection can apparently be short-lived. It is perhaps not unexpected then that of the 47 new vaccine candidates, only one, RTS,S[95], seems likely to reach phase III clinical trials by 2012. To increase the chances of developing effective vaccines and other potential immunotherapies, and to understand the mechanisms of disease pathogenesis, it may help to go back to the basics and simply ask how people become immune to malaria.

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