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Malaria prevention: from immunological concepts to effective vaccines and protective antibodies

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Development of a malaria vaccine remains a critical priority to decrease clinical disease and mortality and facilitate eradication. Accordingly, RTS,S, a protein-subunit vaccine, has completed phase III clinical trials and confers ~30% protection against clinical infection over 4 years. Whole-attenuated-sporozoite and viral-subunit vaccines induce between 20% and 100% protection against controlled human malaria infection, but there is limited published evidence to date for durable, high-level efficacy (>50%) against natural exposure. Importantly, fundamental scientific advances related to the potency, durability, breadth and location of immune responses will be required for improving vaccine efficacy with these and other vaccine approaches. In this Review, we focus on the current understanding of immunological mechanisms of protection from animal models and human vaccine studies, and on how these data should inform the development of next-generation vaccines. Furthermore, we introduce the concept of using passive immunization with monoclonal antibodies as a new approach to prevent and eliminate malaria.

A first guiding principle for the rational development of any vaccine is to formulate a hypothesis regarding the type of adaptive immune response required to mediate protection. For malaria, understanding the life cycle of the infection and determining the immune responses that confer protection after natural infection provide important insights for vaccine development. Malaria parasites have multiple stages of infection, thus making vaccine design more complex than for most viral and bacterial infections for which effective vaccines have been developed, because protection can occur through different immunological mechanisms depending on the specific stage of infection. Infection is initiated when, via bites by mosquitoes, sporozoites are delivered into the skin and the surrounding microvasculature of the host (Fig. 1). Minutes to hours later, sporozoites migrate to the liver and enter the hepatocytes[1]. Within an infected hepatocyte, a single *Plasmodium falciparum* sporozoite can develop into ~30,000 merozoites over a period of ~7 d. Together, this sequence defines the clinically asymptomatic preerythrocytic stage of infection. The blood stage of infection commences when the merozoites enter red blood cells (RBCs) and initiate a continuous cycle of asexual infection that can cause clinical symptoms and mortality. A small number of parasites in the blood develop into sexual-stage gametocytes, which can be taken up by the mosquito and continue the cycle of infection.

Naturally acquired immunity refers to whether the infection itself induces protection. Such protection is indeed the case with many viral infections and can provide insight into the type of immune response required for vaccine protection. Natural immunity against malaria develops against the blood stage of infection, thereby providing protection against clinical disease; however, adults remain susceptible to repeated infections, suggesting that there is little or no natural immunity developed against the preerythrocytic stages, i.e., to the parasite in the liver[2–6]. Of note, blood-stage immunity requires continuous exposure to induce and maintain protection against clinical disease and mortality, and such immunity is not sterilizing[2,3]. This immunity is mediated largely by antibodies that target *Plasmodium* antigens on the RBC surface that facilitate adhesion and allow the parasite to avoid clearance by the spleen[7,8]. However, these blood-stage surface antigens can be highly variable, and this variability limits their potential as targets for vaccine development[7,8]. Naturally acquired immunity can also target multiple merozoite surface proteins that facilitate RBC adhesion and invasion, although these molecules are often highly polymorphic[9]. Finally, data from mouse models show that, in addition to antibodies, $CD4^+$ and $CD8^+$ T cells can mediate protective blood-stage immunity[10–13].

Because repeated malaria infection (natural immunity) has limited ability to induce immunity at the preerythrocytic stage and consequently prevent blood-stage infection, investigators have sought to assess other approaches for preventing infection. Although there was some prior evidence in avian malaria that attenuated sporozoites can confer protection against live-parasite challenge[14,15], the seminal discovery by Nussenzweig and colleagues in the 1960s that radiation-attenuated sporozoites (RAS) administered to mice induces complete protection at the preerythrocytic stage of infection was the starting point for intensive investigation of this approach[16]. Importantly, these findings with rodent *Plasmodium* parasites were confirmed in human studies showing that the bites of hundreds to thousands of irradiated *P. falciparum*–infected mosquitoes protected subjects after a controlled human malaria infection (CHMI)[17,18]. RAS vaccination induces production of antibodies that can prevent parasites from reaching the liver and T cell responses that eliminate parasites from hepatocytes[19–21]. It is the cellular immune response that is likely to be necessary and sufficient to mediate durable protection by RAS. The ability of RAS vaccination to mediate protection at the preerythrocytic stage, compared with natural exposure, is likely to be due to the delivery of thousands to millions of sporozoites administered by thousands of mosquito bites or more recently by vaccination, as compared with natural exposure by occasional mosquito bites, in which ~100 sporozoites are delivered per bite[22]. Moreover, as RAS immunization does not lead to blood-stage infection, as would be the case with natural infection, there is no influence by the immunoregulatory effects that blood-stage infection can have on the development of adaptive immunity[23,24].

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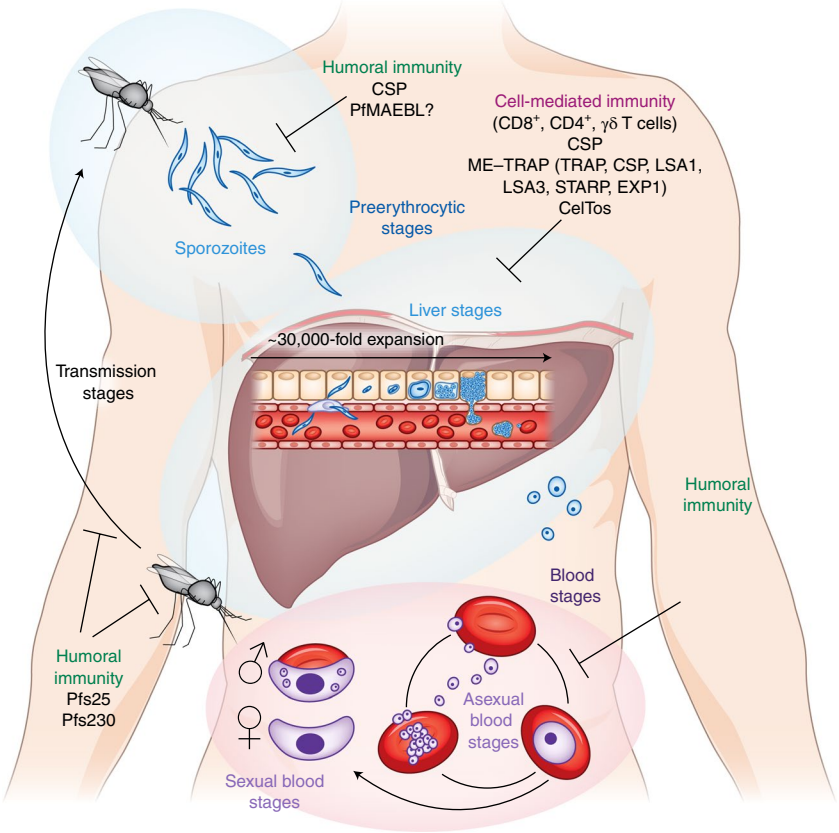

Fig. 1 | *Plasmodium falciparum* life cycle, immunological mechanisms of protection and vaccine candidates. Malaria infection is initiated when the bite of an infected mosquito delivers parasites in the form of sporozoites into the skin and local blood vessels of the host. A small number of sporozoites migrate via the blood stream to the liver. At this stage, antibodies can prevent sporozoites from reaching the liver, particularly those targeting the major circumsporozoite surface protein (CSP). After sporozoites have infected hepatocytes, liver-stage parasites can be eliminated by cell-mediated immunological mechanisms. These characteristics define the clinically asymptomatic preerythroctyic stage of infection. Vaccines for the preerythrocytic stage of infection are focused on preventing sporozoites from reaching the liver by inducing antibodies, eliminating parasites in the liver by T cells or both. In the liver, a single sporozoite can expand into ~30,000 merozoites over a period of 7 d for *P. falciparum*, after which they can emerge from the liver and establish blood-stage infection, which induces morbidity and mortality. In the blood, parasites can be cleared in the spleen and sequestered in the peripheral vasculature by using variant surface antigens. Most blood-stage vaccines focus on developing antibody responses against proteins that are involved in invasion of RBCs. Importantly, RBC invasion is a rapid process with limited time for antibodies to mediate protection. Finally, a subset of blood-stage parasites take a different developmental pathway and become sexual gametocytes, which are taken up by mosquitoes and initiate the mosquito stage of infection.

The life cycle of malaria infection and the analysis of immunity to malaria has led to the development of several vaccine approaches (summarized in Table 1 and Fig. 1). RTS,S, the most advanced malaria subunit-vaccine candidate, aims to induce antibodies that target the circumsporozoite protein (CSP), the most abundant protein on the surface of the sporozoite, thereby preventing them from reaching the liver[25]. The ability of T cell immunity induced by RAS immunization to provide protection in the liver has provided a rationale for whole-sporozoite and viral-vaccine approaches to protect the host by eliminating infection in the liver. Over the past several years, a variety of whole-sporozoite vaccine approaches have been based on the data from RAS vaccines[18]. These include the PfSPZ Vaccine, a preparation of RAS that are sterile and cryopreserved, as well as the related approaches of genetically attenuated parasites (GAPs), which self-attenuate at various stages in the liver, and chemotherapy with *Plasmodium* sporozoites (CPS), in which live sporozoites are administered via mosquito bites or as purified PfSPZ (also termed Cvac), thus allowing for full liver-stage infection before chemoprophylaxis is provided to control blood-stage infection[26–28]. Subunit vaccines based on DNA and viral vectors have also been developed to induce cell-mediated immunity and eliminate infection in the liver. Finally, whole-parasite vaccines, protein-subunit vaccines and, more recently, viral-vector vaccines targeting the blood stage of infection have been developed. Most efficacy studies in humans have been performed with preerythrocytic vaccines to target the asymptomatic stage of infection and either prevent infection or decrease clinical disease. Overall, although both scientific and clinical progress have been made with a variety of vaccines, none of the current approaches are sufficiently effective for long-term sterile protection.

In this Review, we focus on how the magnitude, quality, breadth and location of immune responses affect protection at different stages of *P. falciparum* infection. On the basis of these data, we highlight the specific scientific hurdles that must be overcome to improve malaria-vaccine efficacy with current approaches. This article is not meant to provide an exhaustive review of all malaria-vaccine trials or to encompass the field of transmission-blocking vaccines (reviewed elsewhere[29]) but instead is intended to highlight the basic immunological mechanisms that can be harnessed for vaccine-mediated protection.

Cellular mechanisms of protection against preerythrocytic stages

The major focus on cell-mediated immunity for vaccines at the preerythrocytic stage (liver stage) is based on long-standing data

Table 1 | Malaria vaccines currently in clinical trials

Vaccine	Target	Type of immunity	Stage	Reference
Preerythrocytic/asymptomatic-stage vaccines				
RTS,S/AS01	CSP	Antibodies > $CD4^+$ T cells	Phase IV rollout	105
RTS,S/AS01 (fractional dose)	CSP	Antibodies > $CD4^+$ T cells	Phase 2b	120
ChAd63/MVA ME-TRAP	TRAP, CSP, LSA1, LSA3, STARP, EXP1, pb9	$CD8^+$ T cells	Phase 2b	55–57
PfSPZ	Whole sporozoites	T cells ($CD8^+$, $CD4^+$, γδ) > antibodies	Phase 1/2b	40–42,44,150
CVac	Whole sporozoites	T cells ($CD8^+$, $CD4^+$, γδ) > antibodies	Phase 1	27,28,72–74
GAP	Whole sporozoites	T cells ($CD8^+$, $CD4^+$, γδ) > antibodies	Phase 1	81
PfCelTOS FMP012	PfCelTOS,	T cells, antibodies	Phase 1a	151
CSVAC	CSP	T cells, antibodies	Phase 1a	152
R21	CSP	Antibodies > $CD4^+$ T cells	Phase 1b	153
R21/ME-TRAP	TRAP, CSP, LSA1, LSA3, STARP, EXP1, pb9	Antibodies, $CD4^+$ and $CD8^+$ T cells		
Blood-stage vaccines				
GMZ2	GLURP MSP3	Antibodies	Phase 2b	154
pfAMA1-DiCO	AMA1	Antibodies	Phase 1b	155
P27A	P27A	Antibodies	Phase 1b	156
MSP3	MSP3	Antibodies	Phase 2b	157
SE36	SERA5	Antibodies	Phase 1b	158
PfPEBS		Antibodies	Phase 1a	
CHAd63RH5/MVARH5	RH5	Antibodies > T cells	Phase 1a	141
PRIMVAC	Var2CSA	Antibodies	Phase 1b	159
PAMVAC	Var2CSA	Antibodies	Phase 1b	159
Transmission-blocking vaccines				
Pfs25 VLP	Pfs25	Antibodies	Phase 1a	
Pfs25-EPA/Pfs230-EPA	Pfs25, Pfs230	Antibodies	Phase 1a	
ChAd63 Pfs25/MVA-Pfs25	Pfs25	Antibodies	Phase 1a	160

Based on Malaria Vaccine Rainbow Tables (World Health Organization, http://www.who.int/immunization/research/development/Rainbow_tables/en/).

from mice and nonhuman primates after RAS immunization[20,21,30]. These data show that $CD8^+$ T cells are potent effectors capable of clustering around and eliminating infected hepatocytes[31–33] (Fig. 2). Although most evidence shows that the cytokine IFN-γ is the key mediator of protection, some reports have shown an IFN-γ-independent mechanism[34,35]. $CD4^+$ T cells are also required for RAS-mediated immunity in some mouse models of malaria infection[19,36]. $CD4^+$ T cell responses can mediate protective immunity through a variety of mechanisms, such as increasing the survival and proliferation of $CD8^+$ T cells[37] (Fig. 2) or acting as direct effectors through production of IFN-γ (refs [36,38,39]).

The current vaccine approaches to induce cell-mediated immunity against the liver stage are with whole-sporozoite and subunit DNA or viral-vector vaccines. Results from several human clinical studies have shown that immunization with the PfSPZ Vaccine confers up to 100% short-term sterile protection after CHMI with homologous parasites and ~60% sterile protection for 1 year after CHMI with homologous parasites or a single heterologous strain in adults in the United States with no prior malaria exposure[40–43]. However, in PfSPZ Vaccine studies in adults in Mali who had substantial prior or ongoing malaria exposure, using the same dose and regimen as those used in some of the US trials, the protection at 6 months was found to be ~30% by proportional analysis and ~50% by time-to-event analysis[44]. In that study, ~100% of subjects in the placebo group were infected over the 6 months after the final immunization, thus demonstrating the difficulties that a malaria vaccine may need to overcome in the setting of such high-level exposure. These data highlight how prior and ongoing malaria exposure may limit optimal immunity and protection with this single-strain vaccine against intense heterogeneous parasite exposure. In terms of defining the immunological correlates of protection after immunization with the PfSPZ Vaccine in humans, it is notable that PfSPZ-specific $CD8^+$ T cells are not consistently induced in the blood of many of the vaccinated subjects that are protected. Moreover, $CD8^+$ T cell responses in the blood are highest after the first immunization, and no additional boosting occurs after subsequent immunizations[42]. These data suggest that $CD8^+$ T cells are primed in secondary lymphoid organs, such as the spleen, after the primary immunization and immediately migrate to the liver[45,46]. Indeed, nonhuman-primate studies have shown that PfSPZ-specific CD8 T cell responses can be three to four log units higher in the liver than the blood after intravenous immunization[40,41]. Thus, understanding the role of $CD8^+$ T cell responses in vaccine-elicited protection may be limited from blood analysis[40,41]. In contrast to the poor induction of circulating $CD8^+$ T cells, immunization of humans with the PfSPZ Vaccine, CPS or Cvac in adults in the United States or Europe consistently induces a high frequency of multifunctional cytokine or cytolytic $CD4^+$ T cells in the blood, and these effects have been correlated with protection in some of the studies[27,28,40–42,47].

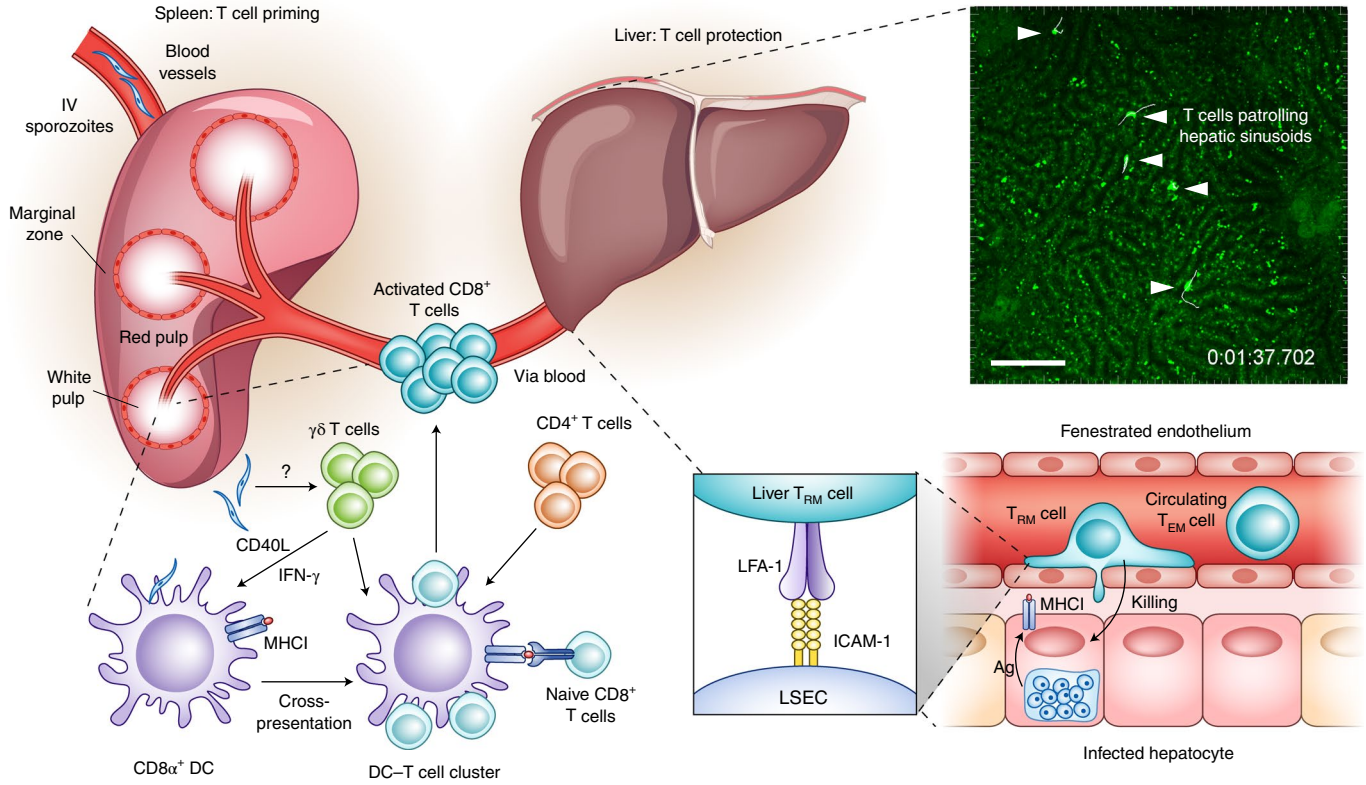

Fig. 2 | Cellular mechanisms of protection in the liver after RAS vaccination. CD8+ T cells are primed by dendritic cells (DCs) that have taken up sporozoites in the spleen after intravenous immunization with RAS vaccines. The magnitude and durability of CD8+ T cell responses may be influenced by CD4+ T cells or γδ T cells during the priming phase. CD8+ memory T cells circulate, and a proportion of them become resident in the liver (T_{RM} cells), as a result of reexposure to antigen (Ag) or local inflammation. T_{RM} cells in the liver migrate in the sinusoids in an LFA-1-dependent manner until they encounter infected hepatocytes. Clusters of CD8+ T cells can form around infected hepatocytes, thus leading to parasite elimination. Inset shows a two-photon image of sporozoite-specific CD8+ T_{RM} cells in the liver patrolling the hepatic sinusoids, which are dark in contrast with the autofluorescent hepatocytes 4 weeks after immunization with RAS. Micrograph reprinted with permission from ref. [66], AAAS. Scale bar, 50 μm.

A remarkable and provocative finding from both preclinical mouse and human trials is the potential role of γδ T cells, notably those expressing Vγ9Vδ2 chains, in protection by sporozoite vaccines or natural infection[40,41,47]. An older study in mice suggested that γδ T cells may have some protective activity after RAS immunization[38]. More recently, in two separate studies, the frequency of Vγ9Vδ2 T cells before vaccination with the PfSPZ Vaccine has been found to correlate with protection in adults in the United States and Mali[40,41]. In agreement with these findings, depletion of γδ at the time of RAS immunization has been found to limit the induction of T cell immunity and subsequent protection in mice; however, their ablation immediately before challenge has no effect on protection[48]. Together, these data highlight a potentially important role for Vγ9Vδ2 T cells in priming T cell responses with sporozoite vaccines. Indeed, because γδ T cells directly respond to certain phosphoantigens expressed by sporozoites and become activated and secrete IFN-γ, they may provide 'adjuvant'-like effects that enhance antigen presentation by dendritic cells at the initiation of the response[49] (Fig. 2). Importantly, the frequency of Vγ9Vδ2 T cells increases with age but decreases with malaria exposure, thus leading to the possibility that whole-sporozoite vaccines might be less effective in malaria-exposed younger children or infants if such cells have a critical role in T cell priming with the PfSPZ Vaccine[50,51].

Subunit vaccines may also generate T cell responses that protect against liver stages. For example whereas RTS,S with the AS01 adjuvant mediates most of its effects through antibodies (as discussed below) there is some evidence that protection may be associated with CD4+ T cells targeting T cell epitopes included in the C terminus of CSP[52]. The most directed subunit-vaccine approach to elicit T cells to control liver-stage infections is heterologous prime-boost immunization using DNA and recombinant viral vaccines. Although such approaches can protect mice[53], in humans they have achieved only ~20% short-term protection after CHMI, and there is no evidence of sustained protection in field studies[54–57]. The limited protection in humans with these viral vaccines might be due to the lack of antigenic breadth or to a failure to achieve high frequencies of CD8+ T cells in the liver. Changes in vaccine delivery and defining new protective antigens will be critical for improving viral-vector vaccines.

Magnitude: how many CD8+ T cells are needed for protection?

A critical metric that should facilitate the development of vaccines for infections, such as malaria, which require T cells at specific tissue sites, is to define the frequency of antigen-specific cells required for protection. A major hurdle to inducing protective immunity in the liver by T cell immunity is based on the requirement to have a sufficient frequency of cells to cover all focal areas of infection in a large organ such as the liver[58,59]. To determine the number of CD8+ T cells required for durable liver-stage protection against malaria challenge in mice, one study has used primed T cells in mice against a well-characterized protective T cell epitope in CSP, by using peptide-pulsed dendritic cells and recombinant *Listeria* to induce high numbers of cells[60]. Strikingly, the mice were protected only if more than 10% of total circulating CD8+ T cells were specific for CSP. Current prime-boost approaches in humans with subunit vaccines or whole-sporozoite vaccines do not elicit this number of T cells in

blood either as a response to specific antigens or throughout the total sporozoite response, respectively[40,55]. The most advanced viral-vector-vaccine approach in humans against malaria is with chimpanzee adenovirus 63 (ChAd63) and modified Vaccinia Ankara (MVA) encoding the thrombospondin-related adhesive protein (TRAP) conjugated to a multiepitope (ME) string of other parasite antigens (TRAP–ME). This prime-boost immunization approach induces TRAP–ME-specific T cells in the blood at a peak frequency of ~0.24% of peripheral blood mononuclear cells several weeks after the boost, and it confers ~20% sterile short-term protection by CHMI in malaria-naive individuals[55]. However, frequencies of ~10% of CD8$^+$ T cells in the blood specific for a single epitope can be seen in humans after human cytomegalovirus (CMV) infection[61]. The potency and durability of CMV-specific CD8 T cell responses to natural infection in humans has provided the fundamental basis for developing CMV-based vaccine vectors encoding specific antigens for HIV and tuberculosis. Data from nonhuman-primate simian immunodeficiency virus and tuberculosis vaccine and infection models show that rhesus CMV vectors induce high-frequency T cell responses in the blood and other tissues, including the liver[62,63]. These data suggest that this is platform may potentially be useful for malaria vaccines, if protective T cell epitopes can be defined and if these vectors are able to induce immunity in the majority of individuals who have been exposed to human CMV.

Location: tissue-resident memory T cells in the liver are critical for vaccine protection

Because the liver is the site where parasites must be eliminated to prevent blood-stage malaria infection, vaccines must be able to efficiently generate T cell responses that will be induced in, or migrate to, the liver. Recently, substantial analysis has been performed on the roles of tissue-resident memory cells (T_{RM} cells), a highly specialized population of nonrecirculating cells that act as sentinels and effectors in the tissue sites and are capable of mediating protection against specific pathogens (reviewed in ref. [64]). T_{RM} cells induced in the liver have been shown to be powerful mediators of protection against malaria[59,65] (Fig. 2). Liver T_{RM} cells have several unique properties compared with those of other T_{RM} cells; in particular, they have a distinct patrolling behavior that allows them to scan the liver sinusoids for pathogens[59,66]; however, they avoid entering the general circulation by expressing high levels of the adhesion molecule LFA-1 (ref. [66]). Therefore, the prevention of preerythrocytic-stage malaria infection is likely to require vaccine strategies that favor a high frequency of T_{RM} cells in the liver.

A major advance for inducing high frequencies of sporozoite-specific CD8$^+$ T cells in the liver has come from comparing immunization with the PfSPZ Vaccine by subcutaneous and intravenous routes in nonhuman primates. Strikingly, intravenous immunization induces high frequencies (~3%) of sporozoite-specific T cells in the liver several months after the final immunization, whereas the subcutaneous route results in nearly undetectable responses[40,41,67]. This study provided the basis for the first clinical trial assessing the safety, immunogenicity and protection by intravenous immunization as a preventive vaccine in humans. The ability of intravenous immunization to confer ~80% short-term protection by CHMI, in contrast to a prior observation that subcutaneous immunization with the same dose and regimen had limited immunity and protection, provides clear evidence for how the intravenous route is critical for protection against malaria with this vaccine[41,67]. Subsequently, another conceptually similar vaccine approach is the so-called 'prime and trap' strategy in mice. In this protocol, CD8$^+$ T cells are primed with an intravenously delivered antigen targeted to dendritic cells, and are immunized 1 day later with a hepatotropic adeno-associated virus to draw the T cells into the liver and trap them there[59]. This immunization regimen induces large numbers of liver T_{RM} cells, which are capable of conferring high-level protection in mice; however, this effect was lost when the liver T_{RM} were depleted with an anti-CXCR3 antibody[59]. On the basis of these data, clinical trials are now in progress with intravenously delivered viral vectors to achieve a similar boost in liver T_{RM} cells. A critical question for vaccine development is the longevity of T_{RM} cell populations in the liver. In some organs, notably the skin, T_{RM} cell populations appear stable over long periods of time[68]; however, in the lung, T_{RM} cell populations decline rapidly and are replaced from circulating T cell populations[69,70]. These data suggest that long-lived protective responses might require a sufficient number of T_{RM} cells at the time of infection and an additional reservoir of circulating T cells in secondary lymphoid organs to replenish the T_{RM} cell population. Because there is no current evidence that malaria infection boosts preerythrocytic immunity[3], maintaining a protective threshold of T_{RM} cells in the liver might require periodic boosting.

Antigenic breadth is a key determinant of protection: lessons from whole-parasite vaccines

A potential advantage of whole-sporozoite vaccines versus subunit vaccines is that they are composed of as many as ~2,000 proteins[71] and may induce responses of greater antigenic breadth. However, because RAS vaccines are fully attenuated, they arrest early and do not undergo the full liver-stage expansion that would increase the breadth of immunity. Thus, to maximize the breadth of the immune response by allowing the sporozoites to expand through the liver; CPS studies were performed with live sporozoites administered by mosquito bites in the presence of chloroquine chemoprophylaxis[28,72]. This approach allows for full expansion of sporozoites in the liver stage and limited blood-stage infection that is eliminated by the drug. Malaria-naive subjects immunized by CPS had complete protection (ten of ten subjects) against CHMI after three exposures to 15 infectious mosquito bites[28]. This result provides a striking contrast with protection by RAS vaccination, which has been found to require up to 1,000 mosquito bites[18]. These data highlight the greater efficiency of CPS for protection after CHMI with a parasite homologous to the vaccine. More recently, complete protection in CHMI has been obtained in a dose-dependent manner by using Cvac, which are cryopreserved live sporozoites administered intravenously with chemoprophylaxis[27]. These CPS and Cvac studies in adults in the United States and Europe have provided an important proof of concept for the greater efficiency of this approach compared with RAS, per mosquito bite or sporozoite, against homologous CHMI, by using the natural biology of liver-stage expansion to enhance immunity and protection. However, whereas CPS confers complete protection against homologous challenge strains during CHMI, protection against two different heterologous strains has been found to be limited[73,74].

The failure of CPS to protect against heterologous parasites is somewhat surprising, given the basic premise that full infection in the liver would increase the magnitude and breadth of T cell responses, as compared with those to RAS[26,75]. One explanation is that the heterologous parasite strains used for CHMI in these CPS studies (NF135.C10 and NF166.C8) are more infectious than the related NF54 and 3D7 strains used in most CHMI studies[73,74]. Alternatively, data from an infection and treatment approach in mice show an induction of both T cell and antibody responses to blood-stage antigens, which may contribute to protection[26,76]. However, no differences have been observed in parasite growth between human subjects receiving CPS immunization and control individuals after direct blood-stage challenge[77], thus suggesting that this vaccine approach confers protection exclusively through the liver stage. An alternative hypothesis is that the blood-stage parasites used for the CHMI might contain antigenically diverse parasites, whereas the parasites that emerge from the liver after CPS immunization may be more homogenous, because the variant surface antigens of *Plasmodium* blood stages are reset during the

sexual stages[78]. Thus, during CPS vaccination, each individual will probably have been repeatedly exposed to the same surface antigens, including at challenge, and these antigens might be the target of potent blood-stage immunity. This hypothesis may account for the strong strain-specific protection induced by CPS and the lack of protection against blood-stage infection in the previous human study. In future studies, fully delineating the mechanisms of protection by CPS or Cvac will be critical. Because Cvac uses purified PfSPZ administered through an intravenous route, it can deliver far greater numbers of sporozoites per immunization than the CPS approach using mosquito bites. Thus, future studies with Cvac using much higher doses of PfSPZ with different drug treatments that can eliminate parasites in the liver or early blood stages should clarify the likely importance of T cells in the liver for mediating protection. Finally, establishing whether Cvac can confer high-level and durable protection against heterogeneous parasites in field studies will be the ultimate test of whether this approach is superior to RAS.

A second approach for enhancing the antigenic breadth of sporozoite vaccines compared with RAS is to use GAPs that arrest at a defined point in the late liver stage[71,79]. In mouse models, late-arresting GAPs induce a higher frequency of $CD8^+$ T cell responses than RAS vaccination and protect animals at lower immunizing doses than early-arresting parasites[75]. Thus, late-acting GAP vaccines offer the potential advantage of having more robust immunogenicity and breadth than RAS vaccines, but would not require drug treatment as with Cvac. However, in the first human study with a GAP vaccine containing two gene deletions, breakthrough infection was observed in one of six subjects[80]. A more recent study using a GAP vaccine bearing three gene deletions that arrests early in the liver stage, which was administered by mosquito bites to humans, had no breakthrough infection[81]. Ongoing trials with this and other first-generation GAP vaccines that have been purified and administered intravenously will generate important comparative data for assessing PfSPZ vaccines and provide a benchmark for subsequent studies with late-acting GAP vaccines.

In conclusion, given the complex genetic diversity of *P. falciparum*, it is essential that all sporozoite approaches demonstrate protection against heterogeneous strains in field studies at different sites. We speculate that improving the efficacy of sporozoite vaccines will require using multiple strains from geographically diverse areas to enable cross-strain protection.

Defining the targets of protective cell-mediated immunity

Although whole-sporozoite vaccines provide greater and more consistent protective efficacy than viral-vectored vaccines against CHMI, the protective epitopes have not been clearly delineated. An understanding of these epitopes would provide insight into how sporozoite vaccines mediate protection and should improve vaccine development for viral-vaccine and other subunit-vaccine approaches. Importantly, not all antigen-specific $CD8^+$ T cell responses induced by sporozoite immunization are protective[82], probably because target proteins must enter the cytosol of the host cell to be presented on major histocompatibility complex (MHC) class I, thereby limiting the number of potential protective antigens[32,83]. However the mechanisms mediating export of protein into host hepatocytes are unclear, and Pexel motifs, which define a proteolytic-cleavage site that is critical for this process in infected erythrocytes, have been found to be dispensable for host-cell targeting in the liver stages[32,83]. Two antigens, CSP and TRAP, have been confirmed as targets of protective immunity in rodent models, although protection depends on the mouse strain carrying an MHC molecule capable of presenting antigen-derived peptides[82,84,85]. Notably, transgenic mice that express CSP, and are thus tolerant to this antigen, are still protected by multiple immunizations with RAS[86]. Similarly, RAS immunization can protect against challenge with genetically engineered parasites expressing antigenically distinct CSP molecules[87]. Both of these studies have shown that anti-CSP T cell responses are not absolutely required for protection. The low frequency of CSP-specific T cells in humans after RAS or PfSPZ vaccination that are protected after CHMI also suggests that additional T cell epitopes are important for mediating protection[41,88].

Several studies have determined the breadth of protective antigen responses in mice and humans receiving whole-sporozoite vaccines. In humans immunized with RAS through mosquito bites, T cell responses to 27 candidate antigens have been assessed in the blood in protected and unprotected subjects. The results led to the identification of the *Plasmodium* cell traversal protein CelTOS as a vaccine candidate, because this antigen was found to elicit elevated T cell responses in protected individuals[88]. However, follow-up studies using this antigen in mice and humans have shown limited efficacy[89]. More recently, T cell responses to the antigens CSP, LSA1 and TRAP have been measured in protected subjects who received the PfSPZ Vaccine, although these responses were very low (<50 IFN-γ spot-forming cells per million peripheral blood mononuclear cells)[41]. Future efforts using large blood volumes from protected and nonprotected individuals across different MHC types, with peptides encompassing the liver-stage proteome, might aid in identifying protective epitopes. On the basis of the current data, protection by whole-sporozoite vaccines is likely to be mediated by either low-level T cell responses to many different antigens or higher responses to specific antigens that have not yet been identified.

Another approach, evaluated in mice, is to identify potential antigens on the basis of either their expression profiles or whether they are the targets of immune responses in humans[90,91]. In these screens, antigens are formulated as subunit vaccines for an initial assessment of their ability to induce protective immunity, thus leading to the identification of some promising candidates[90–92]. Finally, mass spectrometry techniques could be used to identify parasite epitopes presented by MHC class I molecules on the surfaces of infected hepatocytes. This approach has been used to identify targets of antiviral and antitumor immunity[93]. However, for malaria, this approach currently remains an elusive goal, because the proportion of infected cells in hepatocyte cultures is too low to allow sufficient material to be obtained. However, continued advances in the sensitivity of mass spectrometry techniques and parasite culture may make this approach feasible in the near future.

Antibody-mediated protection

Most approved vaccines mediate protection against viral and bacterial pathogens through the induction of antibody responses[94]. Such protection is usually long lasting and is achieved by a variety of vaccine formulations without a requirement for additional boosting. In understanding antibody-mediated immunity, it is critical to define the protective threshold and an antibody-titer 'inflection point', i.e., the level at which antibody titers stabilize after the initial antibody spike induced by immunization. After the inflection point, antibody titers generally decline much more slowly[95]. For current effective vaccines against bacterial and viral infections, the protective threshold is low and well below the inflection point of the vaccine; consequently, protection is long lived[96]. As such, improving vaccines that prevent sporozoites from reaching the liver, or that block the asexual cycle in the blood, would require raising the inflection point and decreasing the rate of decay of the antibody responses. These requirements could be achieved through improvements in vaccine delivery or regimen and adjuvant approaches that favor more durable immunity. Alternatively, lowering the protective threshold by increasing the potency and breadth of the antibodies induced by vaccination may also be required. In this section, we examine these issues by focusing on antibody responses against the preerythrocytic antigen CSP and the blood-stage antigens AMA-1 and Rh5. These antigens have the most extensive structural and mechanistic immunological data from both mouse and human studies.

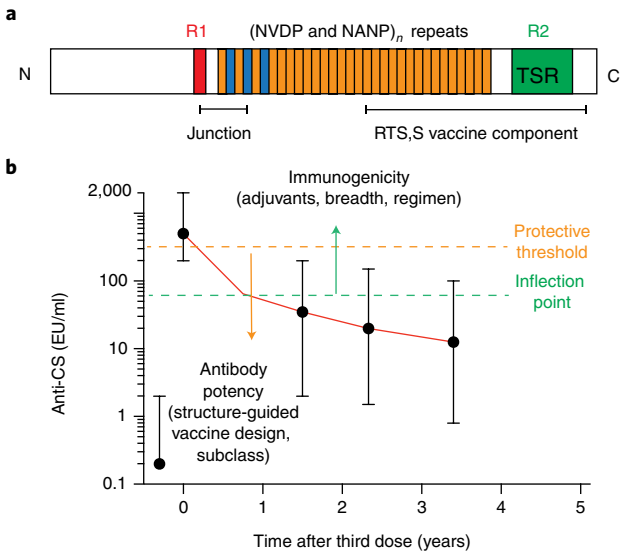

Fig. 3 | Antibody responses after RTS,S vaccination. a, Schematic of the structure of CSP, with region 1 (R1) in red, NANP repeats in orange, NVDP repeats in blue and the region 2 (R2) thrombospondin repeats (TSR) in green. The partial NANP repeat and C-terminal domain included within the RTS,S vaccine that are fused to hepatitis B–based virus-like particle are marked (RTS,S vaccine component). **b**, After three doses of RTS,S at 4-week intervals, 5- to 17-month-old subjects have high titers of antibody specific for CSP (200–500 EU depending on age; data from ref. [106]). This titer declines within 1 year to ~10% of the peak titer, which is below the estimated protective threshold of 121 EU/ml. Antibody titers continue to decline but more slowly, with a half-life of ~600 d. Improving immunity and protection by a subunit vaccine designed to induce antibodies would require decreasing the protective-antibody threshold by generating antibodies with greater potency, increasing the inflection point above the protective threshold by using different adjuvants, or altering the vaccine regimen or modifying the immunogen and adjuvant to elicit a more efficient GC reaction, memory B cells and LLPCs.

The role of antibodies in preerythrocytic immunity was first established when the passive transfer of sera from RAS-immunized mice was demonstrated to delay patency in *Plasmodium berghei* infection[97]. More recently, these results have been substantiated through the transfer of sera from PfSPZ Vaccine– or CPS-immunized human volunteers to *P. falciparum*–challenged, immunodeficient mice carrying human hepatocytes resulting in a significant decrease in liver parasite burden[40,98–100]. Inhibitory monoclonal antibodies isolated from RAS-immunized mice have been found to target the central-repeat region of CSP, consisting of ~40 NANP amino acid repeats interspersed with approximately four NVDP amino acid repeats mainly concentrated near the N terminus (Fig. 3a), which thus became a leading candidate for subunit-vaccine approaches such as RTS,S[101]. The RTS,S vaccine contains 18 NANP repeats as well as a truncated C-terminal domain, which has conserved epitopes for inducing CD4 T cell responses that may provide help to B cells[25]. RTS,S does not contain any portion of the N-terminal domain, junction region or the NVDP repeats, some of which have recently been shown to be targets of protective antibodies[102–104]. In phase III clinical trials, RTS,S has been found to confer up to ~50% protection against clinical infection in the first year after three vaccinations and ~30% protection over a 4-year follow up in infants 5–17 months of age who received an additional booster vaccine[105]. Protection is correlated with anti-CSP titers, which are initially very high (~200 ELISA units (EU)/ml in infants and ~500 EU/ml in older children) but decline in the first 4 months to ~10% of the peak (the inflection point). This level is well below the estimated protective threshold of 121 EU/ml (Fig. 3b)[106], thus providing an explanation for why protection wanes over time. The protective cutoff appears to be one to two orders of magnitude greater than the antibody titers required to protect against tetanus, measles and probably many other viral or bacterial infections[94]. Such high titers are required because antibodies must block every single parasite within the minutes to hours before they reach the liver, thus limiting the time for an anamnestic response. Finally, antibodies targeting other antigens expressed at the preerythrocytic stage, such as *Pf*MAEBL, have been demonstrated to inhibit sporozoite invasion of human hepatocytes in vitro, although whether such antibodies can protect in vivo remains unclear[99]. Overall, antibodies against additional antigens may possibly have inhibitory activity that can be additive or synergistic to those against CSP[107].

Given the number of parasites released from the liver and the kinetics of infection of RBCs, blocking blood-stage infection will also require a high titer of antibodies[108,109]. In blood-stage infection, antibodies do not need to neutralize every parasite but ideally would decrease the replication rate to <1. In addition, there may be an opportunity for recall responses to contribute to protection. A major problem with current blood-stage vaccines (Fig. 1 and Table 1) to date is that most target merozoite surface antigens, which are typically polymorphic and are exposed to antibody for only a few seconds as they move from one RBC to the next[110,111]. Accordingly, although a blood-stage vaccine targeting the merozoite surface antigen AMA1 was found to be able to elicit titers in excess of 100 µg/ml, it induced only around 20% protection over the study period of 8 months. However, there was an estimated 64% protection against parasites with homologous AMA1 sequences, though the sample size was small[109].

Sustaining high titers of antibodies: generating affinity-matured long-lived plasma cells

On the basis of existing preclinical and clinical data, subunit vaccines against preerythrocytic and blood stages of infection will require sustained high antibody titers to protect. Long-term antibody responses are maintained by populations of long-lived plasma cells (LLPCs), a pool of nondividing cells in the bone marrow that continuously produce high-affinity antibodies[112,113]. As such, successful vaccination will require the development of vaccine strategies that increase the formation of these rare LLPCs to sustain antibodies at the required infection point. LLPCs emerge from germinal center (GC) responses, as do memory B cells that have been activated by follicular helper T cells, thereby facilitating somatic hypermutation and affinity maturation[114]. However, not all B cells enter GCs after encountering antigen, and some can develop into short-lived plasmablasts, which secrete generally low-affinity antibody as an initial response against invading pathogens[114]. Notably, short-lived plasmablasts do not survive for more than a few days, and so antibody levels from this type of response decay rapidly[114]. Inducing such short-lived plasmablast responses by vaccination or infection can therefore limit durable antibody immunity. Thus, vaccines for malaria must improve the generation of LLPCs and memory B cells.

The induction and maintenance of antibodies are controlled by the immunogen formulation, vaccine regimen and immune adjuvants. RTS,S is given with the AS01B adjuvant, containing monophosphoryl lipid A and the saponin QS-21 administered in liposomes[25]. Although antibody titers after RTS,S and AS01B vaccination are initially high, they diminish over time, thereby limiting the long-term protective efficacy. The half-life of the antibody response to CSP after RTS,S vaccination in human volunteers is approximately 600 days, even after the inflection point (Fig. 3b), a decay much faster than that seen in response to other subunit vaccines such as tetanus vaccine (half-life of 14 years) and diphtheria

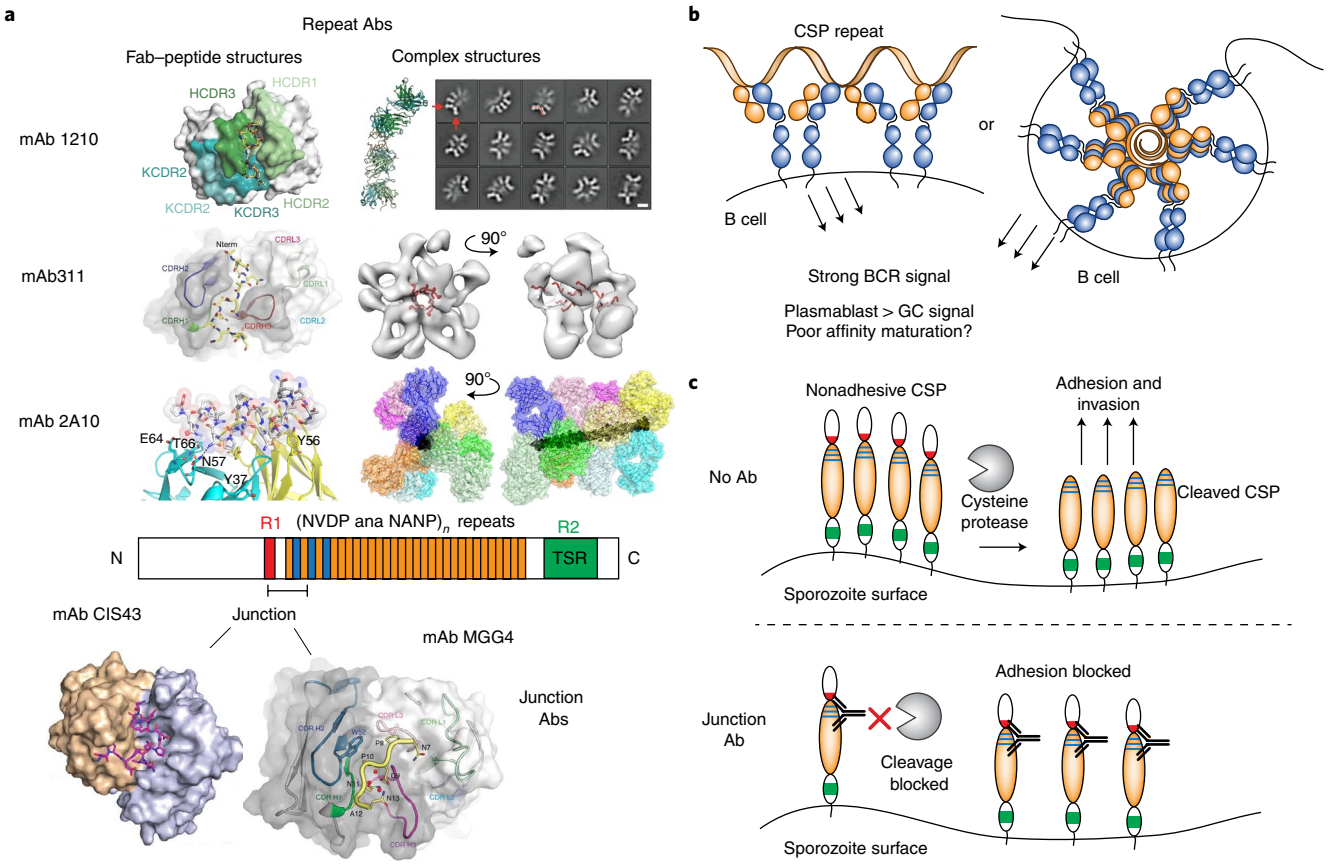

Fig. 4 | Antibody binding to *P. falciparum* CSP: implications for immune responses and protection. a, Structures derived by X-ray crystallography, cryo-EM and modeling approaches of repeat- and junction-binding antibodies (data from refs [102,103,116–118]). For repeat-binding structures, Fab–peptide structures and structures and models of the whole complex are shown. For junction antibodies, Fab–peptide structures are shown. Ab, antibody; mAb, monoclonal antibody. Images reprinted with permission from ref. [118], AAAS; ref. [117], under Creative Commons license CC BY 4.0; ref. [116], under Creative Commons license CC BY 4.0; ref. [102], Springer Nature America, Inc.; and ref. [103], Springer Nature America, Inc. **b**, Cross-linking of multiple BCRs by the NANP repeats may lead to B cell responses dominated by short-lived plasmablasts or inefficient GC reactions. **c**, A possible mechanism of enhanced protection by junction-binding antibodies. (i) A cleavage event at region 1 in CSP allows for the exposure of the thrombospondin repeats in the C-terminal domain to increase the adhesiveness of the CSP molecule. (ii) Anti-junction antibodies may prevent this cleavage from occurring by blocking adhesion in the liver and subsequent invasion of hepatocytes.

vaccine (half-life of 27 years). One explanation for the decreasing memory responses to RTS,S and AS01B may relate to the repeat region of CSP not being optimal for engaging B cells and thereby favoring the formation of short-lived plasmablasts. Repeating antigens such as bacterial polysaccharides often induce T cell–independent responses associated with poor B cell memory[115]. Several recent studies have used isothermal titration calorimetry to show that the CSP repeat can be bound by six to eight antibodies, thus raising the possibility that it might cross-link multiple B cell receptors (BCRs) in a manner akin to polysaccharide antigens[102,116,117] (Fig. 4a). In CD28-deficient mice, which are unable to provide CD4 T cell help, there is indeed a T cell–independent component to the CSP-specific B cell response. However, T cell–dependent affinity maturation in GCs does occur in the immune response to this antigen[23,116]. Interestingly, the process of affinity maturation favors not only mutations that increase binding to CSP but also mutations that enhance Fab–Fab interactions stabilizing the multivalent complex of multiple antibodies bound to a single CSP molecule[118]. Mutations that increase Fab–Fab interactions also enhance BCR signaling, thus providing functional evidence of cross-linking of multiple BCRs on the B cell surface by CSP[118] (Fig. 4b). In contrast, there is also evidence that the GC reaction after sporozoite immunization may be inefficient. Direct sequencing of CSP-specific B cells after Cvac immunization has revealed low numbers of mutations, which in many instances do not increase affinity[119]. On the basis of these data together with support from mathematical modeling, the low levels of affinity maturation have been proposed to be due to the complexity of the repeat antigen; consequently, many mutations are required for affinity maturation to occur, and the GC reaction accordingly becomes dominated by clones with high-affinity germline (unmutated) BCRs[119]. An alternative explanation for the low levels of affinity maturation observed is that high-avidity cross-linking of specific BCRs by the CSP repeat may compensate for low-affinity interactions, and B cells consequently receive a signal to exit the GC prematurely (Fig. 4b). Overall, the structure of CSP itself is likely to favor the formation of low-affinity short-lived plasmablasts over a durable high-affinity LLPC response.

Beyond the specific features of the GC reaction to the repeat region of CSP, there is evidence that B cell responses are generally impaired in malaria infection, a finding with implications for vaccine delivery. One consistent finding across several human clinical studies and vaccine platforms is the lower immunogenicity of vaccines in previously exposed individuals and young children and infants than in malaria-naive adults in the United States or Europe[41,44,55–57,120,121]. Several potential mechanisms may explain these observations. First, malaria exposure leads to the induction of

Box 1 | Impediments and solutions to the development of highly effective malaria vaccines

Hurdles that may limit the development of a highly effective and durable malaria vaccine include the following:

Durability. Maintaining durable antibody and tissue-resident T cell immunity is challenging at thresholds sufficient to mediate protection. At present, there is little evidence that malaria infection boosts antibody or T cell immunity at the preerythrocytic stage. Thus, current malaria preerythrocytic vaccine approaches will probably require booster immunizations to sustain high-level, durable protection.

Breadth. Parasite diversity is a major challenge for T cell immunity and antibodies, for the preerythrocytic and blood stages, respectively. Whole-sporozoite- or viral-vector-vaccine approaches to eliminate parasites in the liver may require multiple strains or additional antigens, respectively, to develop sufficient breadth for protection. For whole-sporozoite approaches, GAP vaccines that arrest at later stages in the liver, as compared with RAS-based vaccines, may improve both the magnitude and breadth of immunity.

Immunological dysregulation due to malaria exposure. Prior or ongoing malaria infection causes immunological dysregulation, which can negatively influence the priming of adaptive immune responses depending on the vaccine platform. Preexisting antibody responses may also limit the potency of vaccination by binding to the vaccine and preventing immunological priming. A consideration is whether treatment before vaccination may improve immunity and protection.

Age. The age of the subject can strongly influence the potency of both antibody and T cell responses, and this variable may depend on the specific vaccine formulation. Vaccines should be tested across all age groups to establish how age may influence immunity. Different vaccines may possibly be optimal at certain ages.

circulating antibodies that may limit vaccine responses by binding to antigens contained in the vaccine. For example, the presence of maternal anti-CSP in infants (6–11 weeks of age) might explain their lower antibody responses to RTS,S compared with those in older children[105,121]. Second, blood-stage infection is immunosuppressive, by inducing potent IFN-γ and tumor necrosis factor responses that in mouse models can suppress the formation of GCs[23,122]. Third, follicular helper T cells in humans appear to be skewed toward a $CXCR3^+$ type I helper T cell phenotype during malaria infection, which is associated with poor antibody formation[123]. Evidence that these processes may result in defective B cell memory comes from the observation that malaria-exposed individuals have large numbers of atypical memory B cells that are considered dysfunctional[124,125]. These problems might not be insurmountable; for example, antibodies to CSP isolated from individuals in endemic areas immunized with the PfSPZ Vaccine show higher concentrations of affinity maturation than cells isolated from similarly vaccinated malaria-naive individuals[102,103]. These data suggest that the vaccine antibody responses in previously exposed individuals develop from responsive memory cells[102,103].

Increasing antibody breadth and potency

Given the challenges in increasing the magnitude and durability of malaria-specific B cell responses to CSP, another approach to improving vaccine efficacy is decreasing the protective threshold by increasing the potency and breadth of antibody responses. This goal can be achieved by altering the vaccine regimen, enhancing the effector function of antibodies or improving immunogen design. As an example of how the vaccine regimen can alter the quality of protective antibody responses, administration of RTS,S and AS01B at 0, 4 and 28 weeks, as compared with the standard regimen of 0, 4 and 8 weeks, has been found to significantly improve short-term protection[120]. Importantly, the final vaccination at 28 weeks contained 20% of the vaccine and adjuvant dose. This so-called 'split dose' regimen, compared with the traditional regimen, induces antibodies with increased somatic hypermutation[120]. Whether the differences in responses seen with this regimen are due to the increased interval before the third immunization or the split dose remains unclear. However, whereas 26 of 30 subjects who underwent CHMI 3 weeks after the last split-dose immunization were protected, only 3 of 7 were protected after being rechallenged 8 months later without receiving an additional booster dose. Thus, ongoing field trials will determine whether this split-dose regimen confers improved durable protection by the RTS.S vaccine versus the conventional regimen.

Antibody responses might also be improved by generating highly functional antibodies that can fix complement or mediate cytolytic functions through Fc-mediated binding of other cell types. Notably, the presence of complement-fixing antibodies that bind both sporozoites and merozoites has been associated with naturally acquired immunity[126,127]. Memory IgM antibodies, which are multivalent and capable of fixing complement, have also been proposed as potent first responders to blood stages[128]. Finally, IgM antibodies to CSP are produced after PfSPZ vaccination and can limit hepatocyte invasion in vitro[129].

Structure-based vaccine design

A major paradigm that has transformed vaccine development for many viral infections is 'structure-based vaccine design', in which newly discovered neutralizing monoclonal antibodies from vaccinated or infected humans are isolated and used to define new sites of vulnerability on the protein on the basis of defined structural analysis. This paradigm has led to improved vaccine design for respiratory syncytial virus and is being actively used for next-generation HIV and universal influenza vaccines[130]. With regard to malaria, whereas protective monoclonal antibodies against NANP-repeat regions of CSP were isolated almost 30 years ago and provided the basis for the RTS,S vaccine[101], relatively few non-NANP-repeat human antibodies to CSP have shown potent in vivo function[104]. The recent isolation of human monoclonal antibodies from memory B cells and plasmablasts from subjects receiving the PfSPZ Vaccine or Cvac, or from malaria-infected individuals, has defined additional neutralizing sites on CSP and improved the resolution of binding to NANP-repeat regions[102,103,117,131,132]. Two studies have independently revealed that highly potent antibodies enhance binding at the junction of the N-terminal domain and repeat region of CSP[102,103] (Fig. 4a). These results have defined a new site of neutralization on CSP referred to as the junctional epitope. Mouse antibodies that bind just upstream of this region have also been shown to have potent inhibitory activity, because they block a cysteine-protease-mediated cleavage event at a conserved site called region 1 in the CSP molecule[104,133] (Fig. 4c). In striking contrast, antibodies specific to the C terminus may not be protective[134–136]. Importantly, the N-terminal domain and some of the repeat domain, such as the junctional regions, are not present in the current RTS,S vaccine. On the basis of these new findings, next-generation protein-based vaccines targeting the preerythrocytic stage will include the junctional epitopes and potentially other regions of the N terminus to enhance the breadth and potency of the antibody response to CSP.

A similar issue of antibody breadth and potency affects protective immune responses to blood-stage infection. Immunization with Ama-1 in complex with its binding partner Ron2 induces Ama-1-targeting antibodies that are more potent than those elicited by immunization with Ama-1 alone[137], and immunization with Ama-1–Ron2 complexes confers superior protection in *Aotus* monkeys challenged with homologous parasites[138]. In the search for a less variable blood-stage antigen, attention has also been focused on the PfRh5–PfRipr–CyRPA complex, which binds Basigin on the RBC surface during invasion[139,140]. PfRh5 is highly conserved and is required for parasite viability, thus making it a major vaccine candidate[140]. The level of anti-Rh5 in the sera of vaccinated volunteers required to achieve infection blocking in an in vitro growth-inhibition assay is approximately five- to tenfold less than that for Ama1-targeting antibodies[141]. Moreover, monoclonal antibodies against PfRh5 appear capable of blocking growth (half-maximal effective concentration) at relatively low concentrations of ~10–15 μg/ml (ref. [142]). Finally, the solution of the structures of PfRh5 and its partner CyRPA in complex with blocking antibodies raises the possibility of structure-guided vaccine design for this antigen[143–145]. Structural analysis has revealed that although some antibodies to PfRh5 can bind the Basigin-binding site, the most potent antibodies to PfRh5 and CyRPA bind away from this site and might mediate their effects by disrupting the overall complex[143,145].

Passive transfer of neutralizing antibodies: a new solution for malaria prevention and elimination

Given the current and potential future challenges to developing highly effective and durable malaria vaccines (Box 1), an alternative solution for short-term protection up to 6 months is passive immunization with monoclonal antibodies[102,103,117,146]. The passive transfer of monoclonal antibodies bypasses the immunoregulatory effects of malaria infection and other obstacles to vaccination by directly providing the desired immune response. A similar passive prevention strategy is now being tested for the prevention of HIV, by using highly potent broadly neutralizing antibodies[147,148]. As a proof of principle for malaria prevention, the critical first step will be to demonstrate whether a single antibody directed against the NANP or the junctional region of CSP can protect humans after passive transfer and CHMI. This study would define the pharmacokinetics and protective threshold for the antibody. A critical question will be whether a single antibody is sufficient to mediate protection or whether additional antibodies to different sites on CSP, or antibodies to other antigens, may also be required. This approach could potentially be used for travelers, military personnel or health workers, for whom protection would be needed for up to 6 months. Antibodies could also be used to prevent seasonal infection in specific geographic areas.

The most important application of passive prevention is in the context of malaria elimination. For this application, drug treatment would be given to completely eliminate the malaria reservoir in the host, and antibodies would be administered at the same time to maintain high-level protection for at least 6 months. This approach would be efficient, because both steps can be done simultaneously and would not require the series of immunizations likely to be needed with any of the current vaccine candidates. At present, the level or duration of protection that would be required for successful elimination is unclear and would need to be determined by modeling and field studies. Finally, for longer-term protection over many years, for example, repeated immunizations could be given, or antibodies could be delivered by mRNA or adeno-associated virus, which has been shown to be protective in mice[149].

Challenges and future directions

Although the development of RTS,S, whole-sporozoite and viral-based vaccines has provided important scientific and clinical insights, none of these approaches mediate durable immunity that can facilitate eradication. Nonetheless, regarding the challenges of developing immunity to a complex pathogen with multiple modes of immune evasion, important progress has been made. Most encouragingly, the successes and failures have provided a scientific roadmap for future vaccine design. Notably, improved vaccine efficacy may be achieved by inducing higher frequencies of $CD8^+$ T_{RM} cells through intravenous administration. Achieving greater antigenic breadth will require either multiple strains of sporozoites, developing late-acting GAP sporozoite vaccines and defining conserved T cell epitopes for viral-based vaccines. Second-generation preerythrocytic subunit vaccines targeted toward preventing parasites from reaching the liver will require greater potency and breadth of antibody responses beyond RTS,S. In addition, a multistage vaccine approach targeting the preerythrocytic and blood stages of the infection may prove additive or synergistic in mediating protection. Continued progress will depend on integrating the data from translational efforts in humans with basic-science efforts to define mechanisms in experimental mouse models that can ideally lead to the prevention and elimination of malaria.

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Competing interests

The authors declare no competing interests.

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