Chapter

T Cell-Based Vaccines: Hope for Malaria Elimination

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Abstract

Among the numerous infectious diseases, malaria still remains the main cause of morbidity and mortality across the world. Every year more than 200 million cases are registered and death toll is of around 4,00,000. The emergence of insecticide and drug resistance has surged an alarming situation to find an effective means to tackle it. From various approaches used for reducing the damage created by malaria to the society, developing effective vaccine has gained the attention of scientific community. The large genome size (24 MB), heterogeneity of the genes, complex life cycle in two different hosts, and expression of wide range of these genes are claimed to hinder the malaria vaccine development. It requires good understanding of the host-pathogen interaction and its correlation with the sterile protection. Recently, subunit vaccine have shown certain promising responses; however, the currently in use of RTS,S vaccine has failed to generate the long-term sterile protection as well as effector memory $CD8^+$T cells. However, the success of sterile protection through vaccination has been proven long back by experimental approaches, where it could be achieved using irradiated sporozoites (RAS) in rodents and humans. Similarly, GAP (genetically attenuated parasite) and CPS (chloroquine chemoprophylaxis with *Plasmodium sporozoites*) have been shown to induce sterile immunity. Despite all the developments, generation of species and stage specific-$CD8^+$ T cell responses has been modest. In order to generate long-lasting immune response, particularly, liver-stage specific-$CD8^+$ T cells, it is indeed required to study the $CD8^+$ T cell epitope repertoire and its implications on the host immune system. In this chapter we will discuss the current status of T cell-based vaccines and the challenges associated with it.

Keywords: Malaria, subunit vaccine, $CD8^+$ T cells response, sterile immunity, memory T cells

1. Introduction: Malaria pathogenesis

Since the origin of *Homo sapience* through the continuous evolution process, we have become the most successful creature on the Earth by maintaining the symbiotic relation with other species to live freely. On the other hand, there are several tiny, single/multi-cellular organisms which may not be possible to see by naked eye have created the threat for us and we have witnessed the long-fight to fulfill the basic principle of *Survival of the fittest*. Underneath all these tiny organisms, a group of species falls under the category of an *infection*, an invasion process through which they enter into the host system, and follow the multiplication/replication process that results in the release of toxins inside the body [1]. From the list of myriad infectious diseases, malaria caused by *Plasmodium* species (a parasite of

Apicomplexa phylum) and transmitted through the bite of *Anopheles* female mosquitoes, still persists as the leading cause of morbidity and mortality [2, 3]. As per the latest World Malaria Report 2020, there are 229 million cases registered in more than 87 countries across the globe in 2019; it was 238 million in the year of 2000. Malaria is considered a life-threatening disease as the mortality rate is still higher, and estimated 409,000 death were reported in the last year [2]. Today half of the population lives under the risk of malaria infection and it has mainly affected the South-African region which is responsible for more than 94% cases in 2019 followed by South-East Asia and Western pacific region (**Figure 1**).

Despite the reduction in the malaria mortality rate (from 25% in 2000 to 10% in 2019), the children under the age of 5 years are the most vulnerable group (due to the lack of adequate acquired immunity) as 67% of total death in 2019 was reported in this group. Likewise, pregnant women are also at the higher risk as it quells the immune system [2, 4]. Athwart the other Apicomplexa parasites having the commodious range of metazoans for the infection, *Plasmodium* species have a restricted range in terms of specificity for insect and vertebrate hosts [5]. In humans, malaria is mainly caused by four *Plasmodium* species and, *P. falciparum* is responsible for the most severity as it has been accounted for more than 70% cases across the world (more prominent in African region-99.7%) in 2018 [2, 6] whereas, *P. vivax* is responsible for malaria infection in American region (75%) followed by South East Asia [2, 7, 8]. Other species, known as *P. ovale* [9] and *P. malariae* [10] are also responsible for the malaria infection, yet the complications are less. Recently, *P. knowlesi* is found to infect humans in Malaysia [11] however, the host specificity is not limited to the humans as reports of infection are also reported in monkeys [10].

The *Plasmodium* species carries the complex life cycle consist of two hosts: *Anopheles* mosquitoes (sexual cycle) and human (asexual cycle). The cycle begins with the bite of infected female *Anopheles* mosquitoes to the human during the blood meal and transfers sporozoites [12] into the skin. They remain at the inoculation site for a short time and further travel to the liver where they invade the liver hepatocytes. Inside the liver by tight regulation and signaling mechanism, they select the hepatocytes, invade them and mature into the schizonts. This phase of

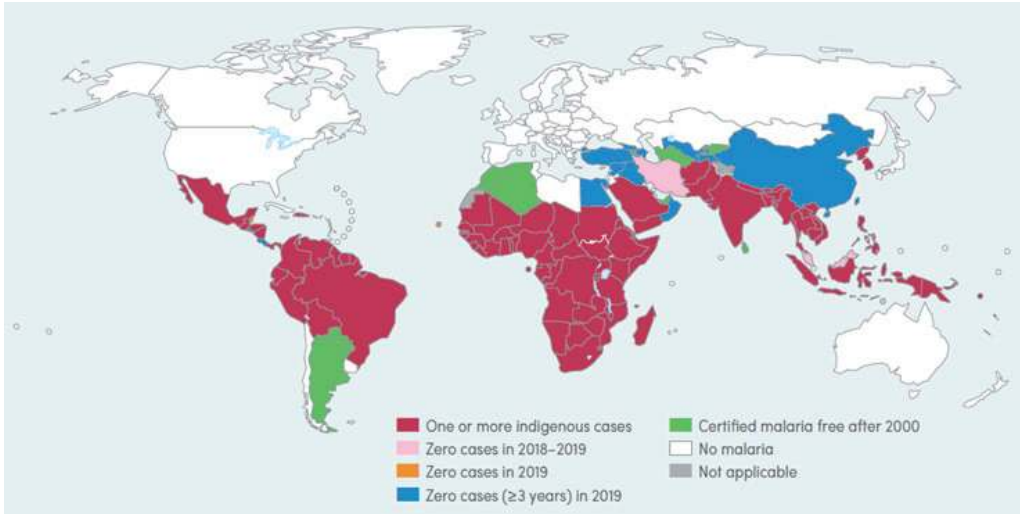

Figure 1.
Countries with indigenous cases in 2000 and their status by 2019: Countries with zero indigenous cases over at least the past 3 consecutive years are considered to have eliminated malaria. In 2019, China and El Salvador reported zero indigenous cases for the third consecutive year and have applied for WHO certification of malaria elimination; also, the Islamic Republic of Iran, Malaysia and Timor-Leste reported zero indigenous cases for the second time. Source: WHO database *(adapted with permission from [2]).*

cycle is also known as *pre-erythrocytic stage* [13]. Further, these schizonts rupture and release merozoites which come out and infect the erythrocytes (RBCs). However, *P. vivax* and *P. ovale* remain in the dormant stage in the liver for a prolonged period of time if left untreated and may relapse after several years. Once the merozoites pop-up in the blood stream, they infect the RBCs which further divide, replicate and passes through several developmental stages, ring stage-trophozoite stage-schizont formation in chronological order, and ended up in release of countless merozoites. These merozoites further infect the new RBCs and cycle continues. During this *erythrocytic stage (or blood stage)*, a number of trophozoites develop into male (micro) and female (macro) gametocytes [13]. The mature gametocytes migrate towards the dermis of skin; during the next blood meal of vector mosquitoes, they are taken-up by the mosquitoes. Formation of zygotes takes place by fertilization of micro and macro gametes in the stomach of *Anopheles* mosquito. Later, these zygotes are converted into the motile ookinetes which finally develop into the oocytes and rupture the mid-gut [13]. These oocytes evolve, unfold and materialize into sporozoites, which subsequently reach to the salivary gland of the mosquito and ready for the next cycle in human host. The phase of conversion of asexual gametocytes into mature sporozoites is also known as *sporogonic cycle* [13]. **Figure 2** depicts the basic malaria life cycle of *P. falciparum*.

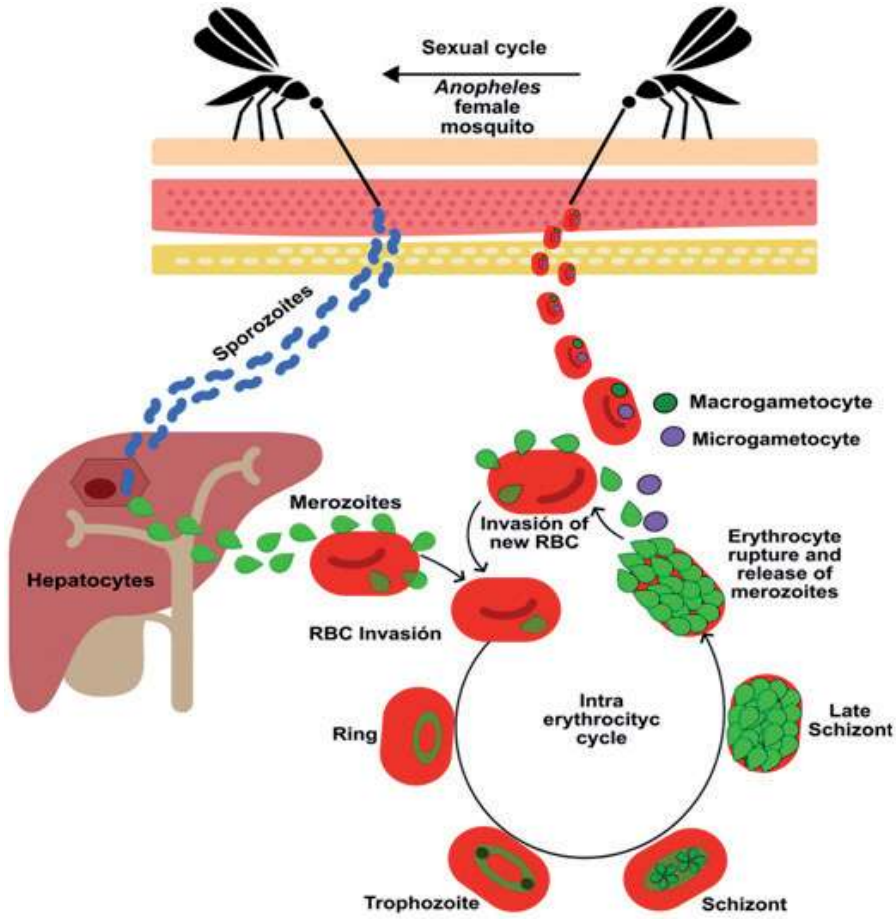

Figure 2.
The P. falciparum *life-cycle. An infected female* Anopheles *mosquito inoculates Spz as it bites a host, they then travel in the host's bloodstream and infect the hepatocytes. Merozoites are released and then invade erythrocytes, where they mature through various stages (ring, trophozoite and schizont stages) and undergo asexual multiplication (~10 or lower) every 48 h, releasing new merozoites which perpetuate the asexual cycle. Some of them enter the sexual cycle by becoming female and male gametocytes which are ingested by the mosquito when it bites an infected host, thereby starting the cycle all over again (adapted with permission from [14]).*

2. Malaria elimination and eradication: Grasping at straws

It was 1897 when Ronald Ross has identified the parasites responsible for the malaria infection and later on the development in the field of science and technology has opened up several areas to work upon and eliminate the malaria infection [15]. The basic understanding of epidemiology and entomology, host-pathogen interaction, surveillance and numerous studies have provided the information about the usage of two main prophylactic methods to curtail down the morbidity and mortality spread by malaria infection: prevention of infection and diseases by controlling the mosquitoes and usage of antimalarial therapy, respectively. As per the recommendation by WHO, insecticides-treated nets (ITNs) have been used to prevent the insect from reaching out the humans. Nonetheless, the results were discouraging as less than 2% children were protected in African region through the usage of ITNs [16]. Further, to surpass the limitation of ITNs, spraying of various insecticides (such as DDT, permethrin, deltamethrin, pyrethriods) have come into the practice, although it has only cover the tip of the iceberg [17]. To strengthen the above approaches WHO has launched the global malaria eradication program in early 1955 [18] and DDT & chloroquine have been recommended for the ITNs and prevention and treatment of infection, respectively [19]. Despite significant reduction in several developing and underdeveloped countries, the reports of *Plasmodium* resistance against the chloroquine and *Anopheles* resistance towards the DDT forced WHO to abandon the program in 1972 [20, 21]. Thereupon, several other approaches have been implemented for vector controls. However, the reports of insecticide resistance against almost all the *Anopheles* vector and resistance against 1-out-of 5 publicly used nets in more than 68 countries have created alarming situation [22]. Further, out of these 68 countries, more than 50 countries have shown the resistance against more than two or more insecticides which has added the fuel to the fire [22, 23]. To tackle the situation of resistance, noninsecticidal approach of bacterial endosymbionts [24], long-lasting insecticides nets (LLINs) and LLINs treated hammocks [25] are under development.

Likewise, the drug resistance against the frontline antimalarial drug quinine has forced the mankind to use the other drugs and combination. However, the reports of resistance against the artemisinin in Greater Mekong Subregion (GMK) in a short span of its launch [26] and unavailability of other options for artemisinin combination therapy (ACT) have invoked the bleak condition for the treatments [27]. To prevail the condition of drug resistance, scientist have opted for mass drug administration (MDA) approach in which antimalarial drugs have been recommended to the specific group of people without getting into the details of their illness. It has been further known as *targeted malaria elimination* (TME) [28, 29]. The results of this approach found to be satisfactory, however proper planning and management are the key factors for the success of MDA approach [15].

Apart from the well-known approaches, usage of antibiotics and their role to fight against the various diseases is widely accepted and therefore, azithromycin, doxycycline and clindamycin have been tested against the malaria. These antibiotics were found to be effective and also reached to the clinical phases; yet the delayed response is the major drawback due to which they cannot be utilized in mild-to-severe and severe conditions of malaria [30]. Besides, a range of antibiotics named quinoloes, tetracycline, trimethoprim, erythromycin and others are in the early stage of development as they are showing promising results which kill the parasites [31], yet it has a long way to go.

Alongside the approaches used to control the malaria infection, human body also consists of a defense mechanism which respond according to the nature of threat (humoral and cell mediated immunity). It is believed that people living in

malaria endemic areas naturally develop immunity due to the continuous exposure of malaria infection. However, age, gender, geographical location, time (months to several years) and numerous other factors are considered to play a vital role in long-term protection [32–34] against malaria. Despite the characteristics of developing protection against the malaria infection naturally, the poorly understood mechanism and the long-term protection are controversial. Therefore, it is of utmost priority to gain the sterile protection against the malaria infection.

2.1 Approach of vaccine development: A ray of hope

Number of experimental approaches have been adopted to understand the *Plasmodium* species and provided us the immense knowledge about the host-pathogen interaction, and how a parasite can escape and invade the immune system for its survival. It has been well documented that during the malaria infection, the protective response vis-a-vie humoral (antibody against malaria) and cell mediated ($CD4^+$ and $CD8^+$ T cells) immunity is dampened [35]. Hence, to achieve the sterile protection against malaria infection, activation/boosting up the wings or full immune system is the fundamental target for any malaria biologist. In this direction, Nussenzwig and colleagues more than 53 years ago have used the approach of killed parasites of *P. berghei* to immunize the mice; however, they failed to generate the immune response. Therefore, approach of partial inactivation of sporozoites by X-irradiation has been used by them to achieve the protective immunity [36]. The observation of precipitation at one end of sporozoites and elevated level of the antibody (in serum) of the induced animals was correlated with the protective immunity. Further, they have observed the high level of antibody after each repetitive dose.

After successful immunization strategy of X-irradiation in non-human host, Clyde *et al*., 1972 used the same approach for *P. falciparum* sporozoites and injected to the healthy volunteers frequently up-to 84 days, and on day 98 they were challenged with infectious sporozoites [37]. Out of three volunteers, one failed to develop the malaria infection and on day 327 anti-sporozoite antibodies were found. The result of this study has opened a new avenue for the vaccine development (usage of attenuated sporozoites) against the malaria. Later, they have extended the experiment by using two strains: *P. falciparum* and *P. vivax* and injected attenuated sporozoites [38]. The species-specific antibody formation and impact of the geographical location on the species were noticed. Also, the protection of immunized volunteers has found maximum for 3 and 6 months for the infectious sporozoites challenge of *P. falciparum* and *P. vivax*, respectively. Besides, they did not find any significant changes in the serum level of IgG and IgM [38]. In all, these data reveal the role of attenuated sporozoites in generating immune response specific to malaria, yet the long-term and species-specific immunity was remained to be unriddled. Regardless of the promising results of attenuated sporozoites approach, the tasks of rearing mosquitoes, maintaining the infection cycle and isolation of sporozoites are time-consuming, laborious and challenging. Besides, skilled-personnel for appropriate functioning and injecting the live sporozoites are the pre-requisites for the successful vaccination. On the other side, the sustainability and higher-efficacy for the long term protection is found to be limited alongside the development of naturally acquired immunity against malaria [39, 40].

As discussed earlier, it is possible that people living in malaria endemic can develop naturally acquired immunity after prolonged exposure although it could be restricted to blood-stage infection. However, induction of immunity to liver-stage (LS)infection among endemic population has not been thought to be possible because of lower infection load, heterogeneity of liver immunology of individuals

and characteristics which differ from blood stage [41]. Also, among the different stages of malaria infection, blocking the transmission of human-to-mosquitoes-to-human and generation of modified mosquitoes may halt the spreading of malaria are in dire straits conditions. Similarly, to target particularly the infected RBCs containing merozoites and preventing them to infect other RBCs in the blood/symptomatic stage has been very difficult. Nonetheless, the longest exposure of infected sporozoites towards the host immune system by invading hepatocytes in the liver stage (5.5 to 7 days in humans and 48 hrs in rodents) and releasing of thousands of merozoites which further continue the blood-stage or symptomatic stage of infection makes the LS most promising stage for the target of vaccine development [14, 42], though LS-vaccine has its own limitation of tedious and challenging task of sporozoites.

Taking into the above considerations, scientific community across the globe is working on multiple targets of different stages to tackle the dire condition. At present, there are mainly three types of vaccines based on the life-cycle: the liver (pre-erythrocytic) stage (LS), asexual blood (symptomatic) stage and the third one is transmission blocking vaccines (TBV).

2.1.1 History of malaria vaccine development

The successful experimental approach of using the attenuated sporozoites (*P. berghei, P. knowlesi, P. falciparum,* and *P. vivax*) in rodents and humans [43–47] including monkeys [48] to generate the sterile and protective immune responses have unfolded the newer therapeutic options. Likewise, development of humoral-transmission blocking immunity in chicken and turkeys by immunizing killed-asexual stage parasites [49], clearance of blood-stage infection in monkeys by arming the *P. knowlesi* asexual-parasites with adjuvants [50] and significant reduction in the *P. falciparum* blood-stage infection after passive transfer of immunoglobulins from continuous exposure of malaria to naïve ones in humans [51–53] and monkeys [54] have excavated the different role of immune system. As stated above, work carried out by Clyde and colleagues corroborates the generation of protective immunity; however, requirement of large number of irradiated sporozoites was the biggest hassle. During the early 1980s, the breakthrough identification of circumsporozoite protein (CSP) as a major constituent of the sporozoite coat resulted into the sequencing and cloning of this gene followed by identification of several blood-stage antigens (Ags) led the world with the hope of early malaria vaccine [55]. Regardless of the CSP identification and other target Ags, approach of blood-stage Ags, heat-killed, lysed and formalin inactivated sporozoites and sporozoites Ags in their early clinical studies have shown the moderate immune responses and unable to reach the threshold of immunity generated by irradiated sporozoites approach [55, 56].

The advancement in the field of sequencing helped in identifying the region of CSP protein having species-specific immunodominant epitope that consists of tandem repeated sequences of amino acids, Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Pro $(NANP)_3$, remain conserved and found to be present in most of the people. The outcome of this work has led the pioneers to develop the approach of *synthetic peptide*. Upon synthesizing this specific epitope region $(NANP)_3$ of CSP protein, several groups have conducted the experiments in monkeys and observed the partial or complete protection [57–59]. After having promising results in monkeys, SPf66 (a synthetic peptide based vaccine from Colombia) have been tested through independent trials in Asia and Africa; however, it was found ineffective in generating protection [60]. Despite the expected results of the SPf66, it has opened an avenue to study the immune response of other prominent vaccine candidates.

Several studies have revealed the important role of CSP protein in developing immunity during the sporozoite challenge studies and become the most prominent candidate for the anti-malaria vaccine development [61]. However, the poor immunogenicity and lesser efficacy (in clinical trials) were the major concern to proceed further for CSP Ag. Therefore, Stoute *et al*., 1997 have used a novel formulation of CSP Ag, known as RTS,S: a hybrid (fusion of CSP protein with hepatitis B surface Ag-HBs Ag) construct armed with novel adjuvants (AS02). The results were encouraging and also correlated with earlier studies [61]. This study led the foundation for the first clinical trials in Africa (in Gambia). The RTS,S/AS02 was given to the men (N = 250 & age:18–45 years) and followed up to 15 weeks. It was found to be safe and actively producing CSP specific B-and-T cell responses and also protection was not limited only to the NF54 strain of *P. falciparum* from which the vaccine has been made [62]. In subsequent studies, the involvement of $CD4^+$ and $CD8^+$ T cells, sentinels of cellular immunity against the LS infection and role of Abs specific to the sporozoites have revealed the significant role of T- and B cells against malaria infection, respectively [63].

Further, parasite-specific Ags presented by MHC I and II to the $CD8^+$ and $CD4^+$ T cells, respectively on the surface of infected hepatocytes [64–68] delineate the importance of cell mediated immunity (CMI) to target the LS infection. All the above and other experimental evidences have unveiled the prominent role of CMI in generating sterile protection. As a result, it has changed the scenario in the field of vaccine development and to generate the sterile protection via boosting up CMI through a novel approach of *plasmid DNA immunization*. In the initial stage of development, Wang *et al*., 1998 have used the plasmid DNA bearing malaria Ag and injected into the malaria-naïve individuals. It has been shown to generate the Ag-specific, $CD8^+$ cytotoxic T lymphocytes (CTLs); however, it was found to be genetically and HLA-restricted [69]. Regardless of restricted-CTL responses, this technology has driven the field and allowed to explore different suitable options to overcome the issue of poor immunogenicity of vaccine candidate(s) against malaria infection. To boost up the T-cell specific heterologous immune response, McConeky *et al*., 2003 have used the novel non-replicating *viral vector* (recombinant modified vaccinia virus Ankara-MVA) approach. It has shown certain level of efficacy in terms of delayed parasitemia after the challenge with sporozoites. It induced five to ten fold elevated IFN-γ producing T-cells (specific-to-Ag) compared to the plasmid DNA or recombinant MVA strategy, and the protection was independent of antibody response [70] creating the base for the preventive and therapeutic vaccine development. Thereafter, experimental results have confirmed that homologous or heterologous priming using viral vector approach could induce the CMI [71]. By using this approach, ME-TRAP, CSVAC (encodes *Pf*CSP alongside a truncated C-terminal lacking 14 amino acids of CSP GPI anchor moiety), combination of *Pf*TRAP with liver stage antigen 1 (LSA1) & LSA2 were developed and tested for their efficacy [42]. The enhanced T-cell based response is the major advantage of this approach. Nonetheless, the efficacy of different candidate(s) found to be low in malaria endemic regions. Inclusion of more target specific immunogen(s) of *Plasmodium* and or combination with RTS,S may improve the overall efficacy.

The technological advancement in the field has helped the scientific community to understand host-pathogen interaction in detail. As a result of it, numerous new and improvised approaches come into practice for the successful vaccine development. To review the status of vaccine candidates and accelerate the development of second generation malaria vaccines, several scientific meetings and forums [72, 73] were organized as well as provided the literature for the same [17, 55, 74, 75]. Therefore, here we have not discussed about all the different types of vaccines.

In early 1980s, *monoclonal Abs and recombinant-DNA* approach have also been used for several antigens followed by their cloning and efficacy testing. However, the efficacy was tested only in experimental (*in-vivo*) settings and results were not up-to the mark [76]. From the beginning, most of the vaccine development has targeted LS of malaria infection. However, people have also focused on other target of life cycle known as BS (blood stage), another approach which can kill the asexual stage of parasites. During this symptomatic stage of infection, merozoites released from the LS invade the naïve RBCs, which is followed by its multiplication and infection of the surrounding RBCs. This stage of cycle persists from 1 to 3 days depending upon the *Plasmodium* species [74].

Targeting the *blood stage* for the vaccine development is equally important as malaria symptoms occurs during this stage and it has also gained interests as results of clinical studies have confirmed the clearance of parasites in African children [51, 77] and adults in Thailand [53] after the purification and passive transfer of IgG from semi-immune African adults and *P. falciparum* infected Thai-patients, respectively. In the early 1980s, trials were conducted on monkeys by using the *P. falciparum* fully developed merozoites armed with adjuvant followed by challenge with homologous strain of *P. falciparum* [78]. Except the control monkeys, all the immunized monkeys survived and generated the strong immune response. The promising findings of the study encouraged the community to focus on development of vaccine based on blood stage resulting in more than 30 blood stage vaccine candidates that registered during 2000–2015 from the targets of the merozoites surface protein (MSP) 1 and apical membrane antigen 1(AMA-1). MSP 3 and erythrocyte binding antigen 175 (EBA-175) are some of the other BS targets which were also explored to check the efficacy. The high titer Ab was observed against most of the MSP proteins; a protein express on merozoites which further invades RBCs, and Ab-mediated cellular inhibition was noticed during the clinical trial of MSP3 in malaria endemic area [79]. In sum, the results of pre-clinical and clinical studies of BS vaccine candidates suggest that they were unable to generate the strong evidence for the protection in controlled human malaria infection (CHMI) or in natural infection.

Similarly, *in-vitro* experiments of AMA-1 have been shown to generate elevated Ab titer in two trials, but during the clinical trials of CHMI it had failed to generate the sterile protection against the homologous strain of *Plasmodium* parasites [80, 81]. Out of all the BS vaccine candidates, only GMZ2 (combination of conserved domain of MSP and glutamate-rich protein-GLURP) have shown the statistically significant efficacy in controlled trial conducted in African children [82]; however, the efficacy was around 14%. To overcome the existing issues associated with poor immunogenicity, other candidate vaccines as well as novel therapeutic options are explored and some of them are currently in pipeline. As a result of it, *P. falciparum* reticulocyte-binding protein homolog 5 (PfRH5) and AMA1-RON2 (rhoptry neck protein-2) complex are currently under experimental phases; early-stage results were found to be promising which may aid in designing the better vaccine candidate [74]. In defiance of optimistic results of BS vaccines, a narrow window period of merozoites which allow Abs to recognize and identify their epitopes during their infection cycle to RBCs (infected-to-naïve-to-infected), convoluted and complex invasion mechanism, polymorphic nature of Ags and simultaneously targeting a large number of merozoites in contrast to confined targets of LS and TBVs (transmission blocking vaccines) are the key questions which needs to be address at the earliest for the successful blood stage vaccine development.

Apart from LS and BS, researchers have also explored the third vaccine type, *transmission-blocking vaccines* (TBVs) comprising of sexual stage Ags (of gametes

and/zygotes) which generate the Abs and kill the parasites during the blood-meal of mosquitoes and result in the interruption of mosquito cycle [74]. Till date, there are four major candidates, Pfs230 and Pfs48/45 of *P. falciparum* (expressed by gametocytes in human) and Pfs25 and Pfs28 (zygote surface proteins expressed in mosquitoes) are under investigation. Due to the structural nature of these Ags (cysteine-rich armed with number of 6-cys), there has been a major concern to develop the stable recombinant protein; Pfs25 is the first recombinant protein based TBVs [83] and shown the encouraging results by equal or higher Ab titer against the target of transmission [84, 85]. Apart from this, Pfs230 another TBVs is also under clinical trials as it shows the strong lysis activity against the *P. falciparum* gametes in *in-vivo* system [86]. Both the recombinant Ag based TBVs have depicted the poor immunogenicity during their immunization strategy; to boost up the immune system, approach of administration of adjuvants and or combination of both candidates together are explored and currently it is under trials [74]. Albeit the optimistic results in the early phase, safety of individuals, to cover the larger population for the herd immunity and prolonged adaptive immune response to maintain the higher Ab titer are the major challenges which need to be keep in mind for the development of next generation TBVs. Additionally, the combinational approach of LS and TBVs or BS stage and TBVs is also need to be explored to target multiple sites at a once [74]. Currently, the leading vaccine candidates for all the three different types are illustrated in below given **Figure 3**.

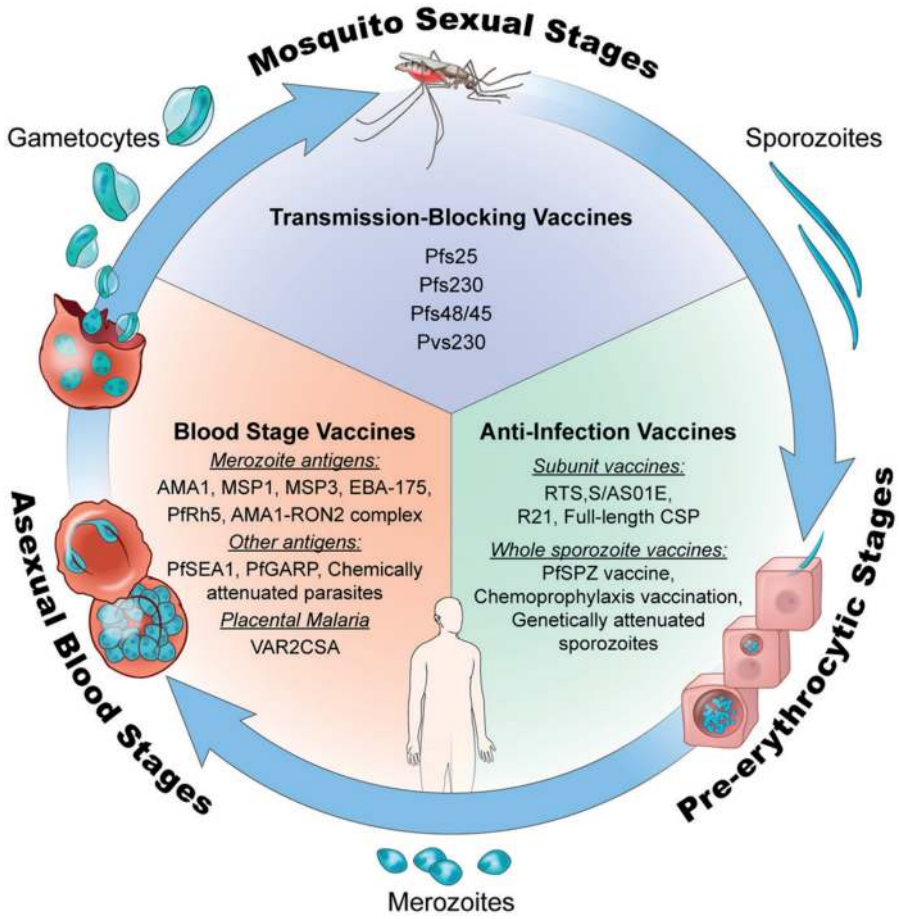

Figure 3.
Life cycle stage of Plasmodium *and vaccine candidates that target each stage (adapted with permission from [74]).*

2.1.2 RTS,S malaria vaccine: Learning lessons from gained knowledge

RTS,S (a recombinant protein based vaccine) was first developed by SmithKline Beecham Biologicals (now known as GlaxoSmithKline Vaccines) in the early 1980s, and has become the first ever licensed vaccine approved by European regulators to use against the human parasite infection in 2015 [87]. From the early experimental studies, modification has been adapted and the novel RTS,S is combined with AS01 (a liposomal formulation) or AS02 (a emulsion based formulation) adjuvant system which trigger the toll-like receptor (TLR4) mediated cytokines and appropriate co-stimulatory signaling molecules on APCs. Additionally, it also activates both wings of the immune system against the CSP and able to generate sterile protection in *Plasmodium* challenge study, led it to enter in Phase III clinical trials [14]. In a phase III study, 829 cases were treated out of 1000 malaria affected children, yet it has unable to boost up the immune system in infants [88]. In another phase III study conducted on children and infants (age group of 1–4), 56% and 65% reduction was observed in case of severe malaria, respectively [89]. The selection of CSP Ag in RTS,S vaccine is derived from the earlier reports where approach of inactivated sporozoites has generated sterile protection and provoked immune response which was mediated by CSP-specific Abs and CMI. These CSP-specific Abs withhold the penetration of sporozoites into liver hepatocytes, still they are unable to achieve the sterile protection alone and therefore, RTS,S vaccine was designed by incorporating a CSP T-cell epitopes (CMI response) alongside the B-cell epitope [90]. The prominent role of CMI in generating sterile protection against malaria infection was noticed during the phase II clinical study, in which three different adjuvant formulations (AS02, AS03 and AS04) were explored, and only formulation i.e., RTS,S/AS02 was shown to invoke the sterile immunity after the *P. falciparum* challenge [90]. Later, more focused approach was developed towards the enrichment of CMI response which further helps in designing and selection of better adjuvant system to enhance the efficacy of RTS,S vaccine [91, 92]. Thus, RTS,S/AS02 was evaluated in field trials; after prolonged study, it has been replaced with earlier adjuvant of AS01 with higher efficacy, safety and elevated immunogenicity [91, 93–95]. Once the role of T-cell started revealing in terms of protection, several assays were developed to study the phenotypic characteristics and nature & involvement of different types of T-cells in malaria infection. Further, T-cell based response against the immunization of RTS,S has been verified in CHMI & field study and further also summarized in **Table 1**.

Later on, Strategic Advisory Group of Experts on Immunization (SAGE) of WHOs and Malaria Policy Advisory Committee (MPAC) have jointly recommended a pilot study for the vaccination in Africa; it has began in Malawi, Ghana and in Kenya between the April and October, 2019 [96]. Despite the approval of RTS,S vaccination, the issue of safety in children is well-documented and therefore, combination of different novel adjuvants with change in the length of CSP-specific peptides and structural information of CSP-specific Abs may further improve the efficacy of RTS,S vaccine [97]. Subsequently, a next generation vaccine was developed (R21) and accounted for the improvised version of RTS,S as it incorporates the longer portion of *Pf*CSP C-terminal armed to N-terminal of HBs Ag. The elevated CSP-specific Ab response, higher immunogenicity at the lower doses (in *in-vivo* challenge study) and formulation with AS01 adjuvant resulted into beginning of clinical trials of phase 1/2a for its efficacy [42].

The development and efficacy of RTS,S vaccine in clinical trials and field study have confirmed the role of T-cell based sterile protection. Recent advancement in the field of genetics and structural biology has driven the area and again the older approach of *whole parasite/sporozoite* vaccine has been reintroduced to fulfill

Vaccine schedule (location)	Vaccine type	No. of samples analyzed	CMI conclusion
Malaria naïve adults			
0, 1, 6 month Belgium	RTS,S/AS02	10	CSP-specific IFN-γ ELISPOTs were induced in 8/10 subjects. RTS, S-specific IFN-γ production was induced in all subjects. LPR to CSP were induced in all subjects. CSP-specific $CD8^+$ CTL responses were not detected
0, 1, 2 month Belgium	RTS,S/AS01 RTS,S/AS02 RTS,S	11 11 12	CS-specific $CD4^+$ T-cell responses (i.e. cells expressing at least 2 markers among CD40L, IL-2, TNF-α, and IFN-γ) were detected in all vaccine groups with a trend for higher responses in the RTS,S/AS01 and RTS,S/AS02 groups versus the RTS,S group
CHMI studies in malaria naïve adults			
0, 2, 6 month USA	RTS,S/Alum RTS,S/AS04	10 10	One of two protected subjects had RTS,S and CSP-specific LPR and cytotoxic T-cell activity
0, 1, 7 month USA	RTS,S/AS02 RTS,S/AS03 RTS,S/AS04 RTS,S/AS02 RTS,S/AS03 RTS,S/AS04 RTS,S/AS02 RTS,S/AS03 RTS,S/AS04	07 07 08 01 05 01 07 07 06	Highest rate of protection with RTS,S/AS02 although CMI results inconclusive Inconclusive due to small sample size IFN-γ ELISPOTs associated with level of protection, ~2 weeks after Dose 3 and on DOC. Protection most frequent for RTS,S/AS02 recipients
0, 1, 2 month USA	RTS,S/AS01 RTS,S/AS02 RTS,S/AS01 RTS,S/AS02	36 44 36 44	Association between CSP-specific $CD4^+$ T cells and protection, 2 weeks after Dose 3 and on DOC. Association between short duration IFN-γ ELISPOTs and protection. Higher frequency of CSP-specific $CD4^+$ T cells with RTS,S/AS01 vs. $RTS,S/AS02_A$ Association between CSP-specific IL-2^+ $CD4^+$ T-cell central-memory and effector-memory populations and protection
0, 1, 2 month USA	RTS,S/AS01 (group RRR) Ad35.CS.01 (dose-1) & RTS,S/AS01 (dose-2 & 3; group ARR)	25 21	No evidence of independent association between CSP-specific $CD4^+$ T cells or IFN-γ ELISPOTs and protection. No difference in protection between groups. CMI responses significantly greater in AAR group than in RRR group
Adults in the field			
0, 1, 6 month Gambia	RTS,S/AS02	20	CSP-specific LPR, short duration IFN-g ELISPOT levels were increased by vaccination. All 20 vaccine recipients responded to at least one of the CMI tests after Dose 3 whereas only 15/20 responded before vaccination. No CMI data on protection

Vaccine schedule (location)	Vaccine type	No. of samples analyzed	CMI conclusion
0, 1, 5 month Gambia	RTS,S/AS02 Rabies RTS,S/AS02 Rabies	16 16 ≤131 ≤119	Higher LPR in RTS,S/AS02 recipients than in rabies-vaccine recipients two weeks after Dose 3 An association between long duration IFN-γ-ELISPOT response and protection was seen across the total population of vaccine recipients and controls, and was not caused or confounded by vaccination with RTS,S/AS02. A significantly higher level of IFN- γ-ELISPOTs was also observed in RTS,S/AS02 vaccine recipients compared with rabies-vaccine recipients at 11 weeks after Dose 3.
Children in the field			
0, 1, 2 month Mozambique	RTS,S/AS02 HBsAg	≤63 ≤69	Significant induction of IL-2 secretion in CSP re-stimulation cultures in 24% of RTS,S vaccine recipients. IL-2 secretion was detected in CSP-re-stimulation cultures from 32% of individuals without a malaria episode whereas IL-2 secretion was detected in only 6% of individuals with malaria episodes (p = 0.053)
0, 1, 2 month Gabon	RTS,S/AS01 RTS,S/AS02	≤31 ≤32	The frequencies of IL-2$^+$ CD4$^+$T cells were higher than pre-immune levels in both RTS,S vaccine groups. CD40L$^+$ CD4$^+$ T cells were not detected. Responder rates ranged from 13–29%. No CMI data on protection
0, 1 month; 0, 1, 2 month; and 0, 1, 7 month Ghana	RTS,S/AS01 RTS,S/AS02 Rabies (0, 1, 2 month only)	≤77; ≤37; ≤73 ≤80; ≤38; ≤73 ≤45	Higher no. of IL-2$^+$ CD4$^+$T cells with compare to other marker positive CD4$^+$ T cells (and responder rate of 76% 1 month after dose 3 with 0, 1, 7 month schedule). CD40L$^+$ CD4$^+$ T cells were detected in 0, 1, 7 schedule. Highest T-cell responses were induced by a 0, 1, 7-month immunization schedule (and responder rate of 73% 1 month after dose 3 with 0, 1, 7 month schedule). RTS,S/AS01$_E$ induced higher CD4$^+$ T-cell responses than RTS,S/AS02 for the 0, 1, 7-month schedule. No CMI data on protection
0, 1, 2 month Kenya/ Tanzania	RTS,S/AS01 Rabies RTS,S/AS01 Rabies	≤182 ≤ 197 ≤80 ≤ 98	The frequency of RTS,S-induced CSP-specific (IFNγ$^-$IL-2$^-$)TNF-α$^+$ CD4$^+$ T cells was associated with protection, and CSP-specific TNF-α$^+$ CD4$^+$ T-cell responses and anti-CSP antibody responses were synergistically associated with protection Evidence that IL-2$^+$-secreting CSP-stimulated memory CD4$^+$T cells can activate NK cells to secrete IFN-γ. IFN-γ ELISPOTs may include IFN-γ-secreting activated NK cells. No CMI data on protection.

Lymphoproliferative response: LPR.

Table 1.
CMI conclusions from clinical studies (adapted and modified with permission from [90]).

the criteria of generating long-lasting sterile protection with improved efficacy. Besides, radiation attenuated sporozoites (RAS) and genetically attenuated sporozoites (GAPs) and attenuated sporozoites under the drug coverage (chemoprophylaxis and sporozoite-CPS) have recently gain the interest despite the various difficulties [98].

2.2 Whole sporozoite vaccines

It has been well-documented that malaria life cycle begins with the invasion of hepatocytes (in liver) by sporozoites; if restrained its development to the LS, the immune responses to *Plasmodium* is developed. Usage of frontline-antimalarial prophylaxis to overcome the clinical manifestation or approach of attenuated parasites to arrest the life cycle at LS are the two availed options which have been explored in numerous ways (finding of specific immunogen(s) bearing B-and-T-cell based epitopes, route of administration and dosage) to empower the humoral and CMI specific response [99]. Under the beneath of sporozoite approach, there are three different ways through which protective immune responses can be achieved [42].

2.2.1 Chemo- prophylaxis and sporozoites (CPS)

Under this approach, live sporozoites are delivered alongside the available anti-malarial drugs with the aim of targeting BS infection, generation of elevated humoral response and followed by understanding the LS infection. In this direction, the first study was conducted in mice under the cover of chloroquine (CQ) and found to be protective with more number of $CD8^{+}IFN\text{-}\gamma^{+}$ T cells [100]. In the following years, the first human trial was conducted under the cover of CQ in CHMI and found to be 100% effective after homologous challenge, and more interesting, some of the volunteers remain immune for around 2 years [101]. The study of elevated immune response depicted the major role of memory T cells producing IL-2, TNF-α and IFN-γ. It has been noticed that higher dosage in homologous CHMI enhances the protection level. However, during the heterologous challenge study, protection was found to be remained limited, raising the question for its efficacy against the diversity of *Plasmodium* genus. The invention of novel antimalarial drugs have been explored, it has found and in one clinical study that usage of mefloquine (CPS-MQ) also delineate the similar results of CPS-CQ in term of its efficacy and safety [102]. Additionally, all the volunteers (naive) having the CQ under CPS category are protected from homologous CHMI challenge [103]. Other than this, next generation anti-malaria drugs are under experimental phase.

2.2.2 Radiation attenuated sporozoites (RAS)

As mentioned earlier, mice immunized with x-ray irradiated sporozoites (*P. berghei*) were unable to develop LS infection and during the challenge study they were found to be protective in nature by homologs or *P. vinckei* challenge study. The higher doses of gamma rays have the direct impact on the LS infection. Additionally, the route of inoculation also plays a critical role in success of RAS immunization. As *in-vivo* study depicted that intravenous (IV) route is more suitable with compare to subcutaneous (SQ) or intradermal (ID) route which needs 7–10 timers higher *P. yoelii* RAS for the protective immunity [14, 104]. Moreover, a study conducted in macaques showcased the superiority of IV route in inducing and generating liver-resident *P. falciparum* specific T-cells [105]. In the clinical trials with *Pf*Spz, all the

11 volunteers (immunized with irradiated mosquito's sporozoites) were protected after the first round of homologous infection challenge whereas only 2 out 10 were able to generate the sterile immune response against the heterologous challenge [106]. It portrays the vital role of RAS approach in protection against the malaria infection, yet the issue of heterogeneity needs to be addressed. In another clinical trial of CHMI, around 64% of volunteers were protected against the homologous *P. falciparum* (3D7) challenge after 19 weeks. Further, volunteers showing no parasitemia were challenged after the final immunization (33 weeks later) with the heterologous strain (7G8) and approximately 83% have shown no parasitemia [107]. The results of *Pf*SPZ (RAS) immunization confirm the limited long-lasting immune responses, although no significant increase was observed in $CD4^+$ and $CD8^+$ T cell after second or third immunization.

2.2.3 Genetically attenuated sporozoites (GAS)

To overcome the existing hurdles of RAS, novel approach of genetic manipulation was explored in *Plasmodium* species which could prevent the transition from LS to BS infection without altering major changes. In this direction, differential gene expression study was carried out to identify the target gene(s) responsible for LS to BS transition and two genes, UIS3 and UIS4 (encodes for the protein in parasitophorousvacuole membrane), were selected for the genetic manipulation [108]. In mice having *P. berghei* infection, LS development was found to be delayed in a single knock-out gene study and gained the sterile protection upon challenge which was dependent upon $CD8^+$ T cells [42]. Subsequently, it has been extended to double-knock out study and was found to induce stronger immune response by generating IFN-γ producing memory and effector $CD8^+$ T cells that persist for a period of 6 months [109].

After the success of initial GAS study, several other genes (*p36, p36(p52) and b9*) were identified which were found to remain conserved in all the *Plasmodium* species. The different gene knock-out study in animal model demonstrated a similar immune response, yet development of parasites was also observed in some of them [42]. Subsequently, a novel gene named SAP1 (sporozoite asparagine-rich protein 1) of *P. yoelii* was discovered and found to be important for the LS development. In gene knock-out study it has conferred the delay in LS development and long-lasting protection upon challenge with wild-type sporozoites [110]. Few other genes were also explored; In a comparative study with RAS which generally arrest the early LS infection, more efficient and prominent effector and memory T-cell pool was observed, due to the GAS approach that progress towards the late LS infection and may be more diverse range of Ags were present. This lead to the first clinical trial, and *P. falciparum* double knock-out (*p52 and p36*) gene was tested for its efficacy and safety. Despite the encouraging results, 1 out of 6 volunteers showed parasitemia [111]. Therefore, further development took place in GAS and triple knock-out genes (*p52, p36 and sap1*) of *P. falciparum* strain was used. There was no breakthrough parasitemia, and higher Ab titer with elevated cytokine pool of IFN-γ, IL-2 and TNF-α were observed in all the volunteers [112]. The positive outcome led to the development of another GAS vaccine with double knock-out (B9 & SLARP), and in the early phase of 1/2a it has shown poor-to-moderate immune response as only 3 out of 25 volunteers were found to achieve the sterile protection after the mosquito-bite challenge [113].

Despite the yin and yang of GAS in clinical studies, it has been considered as more accurate and doable approach with compare to RAS. Additionally, with the novel technology of CRISPR-Cas9, various attenuated sporozoites can be generated

which might be more immunogenic in nature and aid in generating stronger immune response.

All the different approaches of vaccine development strongly suggest the correlation of T-cells and protection against malaria infection. And, therefore it may be a decisive player in enhancing the efficacy of vaccine. By keeping in mind the importance of T-cell, in the next section we have discussed the role of T-cell based immunity in all the stage of infection as well importance of it in developing T-cell based vaccines.

3. T-cell based immune response: A key player of vaccine development

From the very beginning, it has been well documented that T-cells play an important role in protection and generation of sterile immune response in malaria infection. Once the sporozoites enter into the host live and invade the hepatocytes, APCs process and present the *Plasmodium* peptides through MHC which result in the activation of CMI response (alongside humoral response), mainly $CD4^+$ and $CD8^+$ T cells. After the resolution of infection, all the immune sentinels undergo regulative apoptosis, except pool of memory cells. These Ag-experienced memory cells are crucial during the secondary infection of *Plasmodium* and specifically, liver-resident memory $CD8^+$ T cells serve as a first line of defense [114]. Although certain studies have confirmed that induction and expansion of memory $CD8^+$ T cells are not hampered by absence of helper $CD4^+$ T cells, the memory $CD8^+$ T cells developed under this condition are found to be short-lived [115]. Therefore, it infers that for long-lasting memory $CD8^+$ T cells which control the malaria infection, aid of helper $CD4^+$ T cells [116] and continuous exposure of Ag is required. Yet, the memory response against the malaria infection is bottleneck [115]. The TCR-MHC bearing peptide of *Plasmodium* interaction is also important in generating T-cell based response (memory pool) as polymorphic nature of residual anchor and supporting once of individual peptide may have an effect on TCR-MHC interaction [114]. With the development of sophisticated technology, the role of T-cells in malaria infection in all the different stages has been revealed. As a result, various advancements have been made in the vaccine development.

3.1 T-cell response: In early stage of sporozoites

During the blood-meal of *Anopheles* mosquitoes, infected sporozoites enter into the host, and dermis under the layer of skin is the first place where they get exposed and then transverse through the blood vessels and reach to the liver. Therefore, Ab-mediated humoral response is largely important for the early stage of infection. However, experimental animal studies have revealed that most of the injected sporozoites remain in dermis for about 6 hrs and very few of them are able to find the way through blood vessels [117]. Although the DCs (dendritic cells) mediated capturing mechanism of sporozoite Ags is poorly understood, it has been confirmed that several sporozoites crosses the network of lymphatic vessels and skin-draining lymph node where they can persist for around 24 hr after the infection [117]. Therefore, it may be possible that circulating DCs (cDCs) present in skin initiate the T-cell induction against the sporozoites. And, it has been well known that live sporozoites are capable enough of generating $CD8^+$ T cells response. Although the detailed mechanism is yet to be understood, arming the Ab-response by providing $CD8^+$ and $CD4^+$ T cells, CMI plays an important role in early detection and restraining the transition to LS infection.

3.2 T-cell response: In pre-erythrocytic stage (LS)

In continuation of early stage infection, once the sporozoites reach to the liver, LS infection cycle initiates and through a cascade of mechanisms, numbers of schizonts are formed between the periods of 5–6 days. *In-vivo* experimental study has confirmed that mouse $CD8^+$ T cells restrict the parasite development within the hepatocytes by recognizing MHC-I/peptide complexes presented by infected hepatocytes [118]. For this, perforation occurs in hepatocytes cell membrane is responsible which carry the perforin and granzymes (pro-apoptotic protease). It induces apoptosis and produces the reactive-oxygen species. Additional aid is also provided by liver macrophages and Kupffer cells that carry out the activity of Ag presentation as they face the sporozoites during their entry into the liver [119]. $CD4^+$ and $CD8^+$ T cells of CMI wing are crucial in generating sterile protection against malaria infection and it has been verified in several sporozoite-and subunit-based vaccine approaches, though in case of human it has been documented only in human peripheral blood as it is challenging and difficult to prove for LS infection because of ethical concern [114]. Howbeit, recent study carried out in non-human primates has shown 100-fold higher T-cell response (specific to protective $CD8^+$ T cells) in liver compared to peripheral blood [120]. Therefore, it is required to understand the detailed mechanism and role of $CD8^+$ T cells in protection. Li *et al*., 2016 have developed the human immune system bearing mice and studied the role of $CD8^+$ T cells in human malaria. They have reported the direct link between the functional activities of *Pf*CSP-specific $CD8^+$ T cells and threshold achieved of protection against the malaria infection [121]. Several other *in-vivo* experiments have also shown the correlation between parasite-specific IFN-γ producing $CD8^+$ T cells and protection achieved in clinical settings [114]. It suggests that $CD4^+$ and $CD8^+$ T cells produce IFN-γ which further enhances the activity of cytotoxic $CD8^+$ T cells by elevating the Ag presentation through MHC-I. Moreover, the enhanced IFN-γ level activates other phagocytic immune sentinels, macrophages and NK cells to perform the tightly-regulated apoptosis. Throughout the protection mechanism, pool of numerous cytokines are released including IL-12 and IL-18 that are known to activate the $CD8^+$ T cells in TCR-independent manner which can kill the parasites by a mechanism of nitric oxide (NO) production [122]. The prominent role of $CD8^+$ T cells, aid of $CD4^+$ T cells in humoral and CMI response, generation and activation of cytokines and other immune cells corroborate the significance of T-cell based vaccine development.

3.3 T-cell response: In blood stage (asexual/symptomatic stage)

The release of merozoite from schizonts confirms the beginning of erythrocytic stage, where they invade the naïve RBCs, convert into trophozoites and develop thousands of merozoites which are ready to burst from the infected RBCs (iRBCs) and target the new RBCs. Due to the lack of MHC molecules, iRBCs cannot perform the Ag presentation to T cells. However, it is believed that iRBCs bind to DCs and macrophages through the receptor-ligand (of parasite) interaction and after the maturation, most of the DCs migrate to the spleen where they present the Ag via MHC-molecules to the naïve –T cells [123]. The role of DCs in the generation of BS specific Ab is studied in animal models. Experimental evidences have shown the emerging role of novel T-cell population of $\gamma\delta^+$ and $\alpha\beta^+$ in the generation of IFN-γ responses that control the BS infection of *P. falciparum* [124]. Pombo *et al*., 2002 have studied the role of immune system by injecting low-dosage of *P. falciparum* iRBCs to the volunteers followed by challenge [125]. They have shown that iRBCs generate the $CD4^+$ and $CD8^+$ T cell specific response to the BS infection. Further

characterization of T-cells confirms that majority of them are IFN-γ producing (not IL-4 or IL-10) and there was no-significant role/production of antibodies in protection [125]. It has led the foundation to understand the role of T-cell which is now equally important for BS protection.

3.4 T-cell response: Towards the blood stage sexual parasites

During the blood-stage asexual cycle, certain number of parasites come out from the RBCs cycle and develops into male and female gametocytes. Till date very little literature is available about the T-cell response against the gametocytes. The first study conducted in 1997 where purified *P. falciparum* gametocytes were injected into malaria-naïve individuals and found that activated $CD4^+$ T cells response against the gametocytes in human PBMC is similar to the response observed in asexual stage [126], except the induction of γδ T-cells. In similar line, another study was carried out in which blood of acute (not severe) infection of *P. vivax* were fed to the *Anopheles* mosquitoes and observed the pro-and-anti-inflammatory cytokines [127]. The results of this study reveal that parasite in the blood samples with the elevated anti-inflammatory IL-10 (not IFN-γ or TNF-α) were unable to develop the infection in mosquitoes. To understand the mechanism of transmission-blocking, T-cells (from immunized spleen cells of vaccinated mice) were passively transferred to Balb/c mice via IV route followed by an infection; on day 3 post-infection, ability to transmit in mosquitoes was studied [128]. It showed the reduction in 95% of transmission, and the viability of gametocytes was found significantly lower in exflagellation compared to the controls. Although the mechanism behind triggering CMI response is unclear, authors predict that it could be cytokines or secretory products released by macrophages. This study unmasks the role of T-cells in early stage of BS infection wherein Ag-specific humoral response (Ab-based) comes latter into the picture and maybe help in the development of multi-target based vaccine.

The discovery of novel T-cell populations and their role in generating sterile protection alongside the conventional T-cells at each stage of malaria infection, aid the malaria biologist especially vaccinologists to design highly efficacious vaccine based on T-cells. Therefore, we have discussed about the different T-cells and their role in antigen diversity.

3.5 T-cell and their subtypes: Role in protective immunity against malaria

The induction of *Plasmodium* specific $CD4^+$ T cells in protection against the malaria infection naturally or in different vaccination approach is well-documented [129]. As we know, the activated $CD4^+$ T cells differentiate into several subtypes which largely depend upon on the cytokine environment. The differentiation of $CD4^+$ T cells into ***Th1*** is IFN-γ and IL-12 dependent, and the role of T-bet in Th1 differentiation is also explored. The T-bet^+Th1 IFN-γ producing cells express the MCSF (macrophage colony stimulating factor) that aid in controlling BS infection via macrophages. In addition to this, they also release IL-2 which can directly activate the NK cells which results into the direct killing of parasite infected cells. Having the multiple characteristics of Th1 cells, IFN-γ secreting Th1 cells directly/indirectly activate the potent $CD8^+$ T cytotoxic cells via NO or higher expression of MHC-I on infected hepatocytes. In CHMI study, it has been found that the cytotoxic $CD4^+$ T cells express CD107a, IFN-γ, CD38 and granzymes B, yet the mechanism of their activation is yet to be to be understood [129].

Th2, another set of $CD4^+$ T cells that express transcription factor, GATA3 are characterized by production of IL-4 and IL-5. Although the role of Th2 in malaria

infection is poorly understood, IL-4 secreting Th2 cells have a major role in B-cell class switching [130] and macrophages activity [131] in malaria infection. In different conditions of malaria infection, a population of ***T follicular helper (Tfh) cells*** is identified by the expression of chemokine receptor CXCR5, BCL-6 a transcriptional factor and programmed cell death protein 1 (PD1)-a inhibitory receptor. The *Plasmodium* specific Tfh cells mainly express the ICOS and IL-21 which helps in maturation of germinal center (GC) and aid in production of plasma and memory B cells [129]. RNA-sequencing data have confirmed that Tfh cells development is IL-6 dependent and regulated by IRF3 during the BS infection [132]. The transition of Tfh cells to any other phenotypic subsets of $CD4^+$ T cells is one of the interesting phenomena which is required to be explored as it may drive the infection on either side. There are few studies depicted the presence of ***Th17*** cells in *Plasmodium* infection that express the IL-17A. As no specific role of Th17 is found in malaria infection, IL-21 producing cells may help in GC activity and activation of $CD8^+$ T cells, further it has shown that IL-22 producing Th17 cells regulate the inflammatory mechanism in lungs and liver of malaria infected rodent system [133]. Another $CD4^+$ T cell, ***IL-27 producing CD4$^+$ T cells***, is having a dual character of pro-and-anti-inflammatory, and some of the reports have proposed their protective role in BS infection of malaria. Further it has been demonstrated that $CD4^+$ T cells can also produce the IL-17 which do not express IL-10 and IFN-γ ($CD4^+$ IFN-γ^-IL-10^-T cells) [134]. Similarly, ***IL-10 producing CD4$^+$ T cells*** and ***regulatory T cells (Treg)*** are two another sets of $CD4^+$ T cells which demarcated by their cell surface proliferative markers, their role is found to be in favor of enhancing the malaria pathogenesis, although it is yet to be defined their precise action in different stages of infection.

As $CD4^+$ T cells are important in generating strong immune response by activating humoral and CMI response, the role of ***CD8$^+$ T cells*** in malaria pathogenesis, various subunit and whole sporozoites approaches and providing protection against the infection (more defined for LS infection) makes them the most desirable target for vaccine development. Among the different types of $CD8^+$ T cells, role of liver-resident $CD8^+$ T cells is indispensible alongside the effector and memory $CD8^+$ T cells during the re-infection. Earlier studies depict the role of CD $11c^+$ DCs in priming the $CD8^+$ T cells during the LS infection [116]. Later, newly identified population of monocytes ($CD11b^+CSF1R^+CD207^+F4/80^+CD11c^+$) also found to be important in priming of $CD8^+$ T cells, which strongly support the licensing activity of $CD8^+$ T cells in the LS infection [129]. The mechanism of $CD8^+$ T cell function in interrupting LS infection is found that Fas/FasL and granzymes are the two basic armed required for the effector function of $CD8^+$ T cells which mainly produce TNF-α, IFN-γ and perforin [129].

Recently, a novel population of ***γδ T cells***, is identified which comprises of less than 5% in adult human and its role in anti-malaria immunity is yet to be revealed. The earlier studies have confirmed the proliferation of them during the *P. falciparum* or *P. vivax* infection that correlate with protection [135]. Characterization of γδ T cells shows that during the phase of mild-to-acute infection, $V\gamma9^+V\delta2^+\gamma\delta$ T cells population increase which may further curtail down during the repetitive exposure of infection [129]. The reports of rodent model used for malaria infection revealed that γδ T cells do behave like other T-cells and they are found to be present in various tissues. Additionally, they also secrete IL-21, a required cytokines for Tfh cells [136] and as a result, it can function as a bridge for innate and adaptive immunity. Their role is also found to be important in RAS immunization for effective function of $CD8^+$ T cells via production of numerous cytokines. In an experimental study of *P. chabaudi*, during the BS infection a specific type of γδ T cells ($V\gamma6.3^+\gamma\delta$ T cells) have been shown to undergo the clonal expansion and showcase the specific

phenotypic profile (with different transcriptional factors) which directly play a role in protection. Thus, it may aid in better understanding the pathogenesis and designing more efficient vaccine. In all, studying the different types of T cells and their role in individual stage of malaria infection will help in vaccine development. Despite the knowledge of T-cell mediated immunity, recently polymorphic natures of *Plasmodium* Ag(s) have shown the effect on T-cell response. Therefore, it is equally important to understand the impact of antigenic nature in generating sterile protection.

3.6 T-cell response: Effect of *Plasmodium* Ag(s) diversity

It has been studied that due to the polymorphic nature of Ag(s), effector function of $CD8^+$ T cells and MHC-I peptide presentation become less prominent; as a result, during the secondary infection, it overcomes the immune system and establish LS infection [137]. Thus, with the help of sequencing technology conserved and variable epitopes of CSP and other potential target Ag(s) were found and reported [114]. Similar results were observed during RTS,S clinical trials, in which higher efficacy was observed in those children's whose parasitic infection matches the T-cell based Th2R and Th3R epitope as compared the mismatched one [138]. It has been found in another gene based vaccine study that $CD8^+$ T cells based response is restricted to HLA-I of AMA1 Ag of 3D7 strain but failed to act against the AMA1 Ag of 7G8 strain that has the difference of only single amino acid [139]. It has been strongly supported by the earlier report of CSP where single amino acid replacement have an impact on T-cell based immune response [140]. Thus, antigenic polymorphic nature and substitution of single/multiple amino acids in the epitopes may result in less interaction between T-cell peptide and MHC molecule causing the failure in generation of protective immunity.

3.7 T cells and vaccine development

The first licensed malaria vaccine RTS,S was designed by incorporating mainly B cell epitopes that induced humoral response. The Ab-mediated response may aid the induction of T-cells and evidence have shown the $CD4^+$ T cells response, yet it is unable to understand that after RTS,S vaccination why immunity is remain short-lived. It may possible that incorporation of appropriate $CD8^+$ T cells based epitope which further enhance the sterile immunity. Additionally, it is also been interesting to study the role of B cells in direct/indirect activation of $CD8^+$ T cells. On the other hand, different approaches under the roof of whole sporozoite vaccine (CPS, RAS and GAS) depicted the prominent role of $CD4^+$ and $CD8^+$ T cells in protective responses against malaria infection in rodents, whereas in humans (under CHMI study) it has been limited to peripheral blood only. Additionally, among different subsets of T-cells which play a crucial role in providing protection and signaling pathway is yet to be fully understood. As mentioned earlier, injection of attenuated sporozoites through RAS approach have shown the antimalarial immunity against LS infection. Therefore, multiple antigenic targets of LS may help in generating strong liver-resident memory T cells. These WSV approaches have their own limitation and to prevail it, another approach of viral vector bearing Ag delivery has been explored and have shown the promising results [141]. After successful pilot study, to elicit the stronger T-cell based response several other options such as usage of nanoparticle based delivery system [142] and adeno-associated virus have been tried.

As T-cell mediated immunity is having prominent role in generating sterile protection, RAS and subunit vaccine approach which can induce stage-specific T-cell

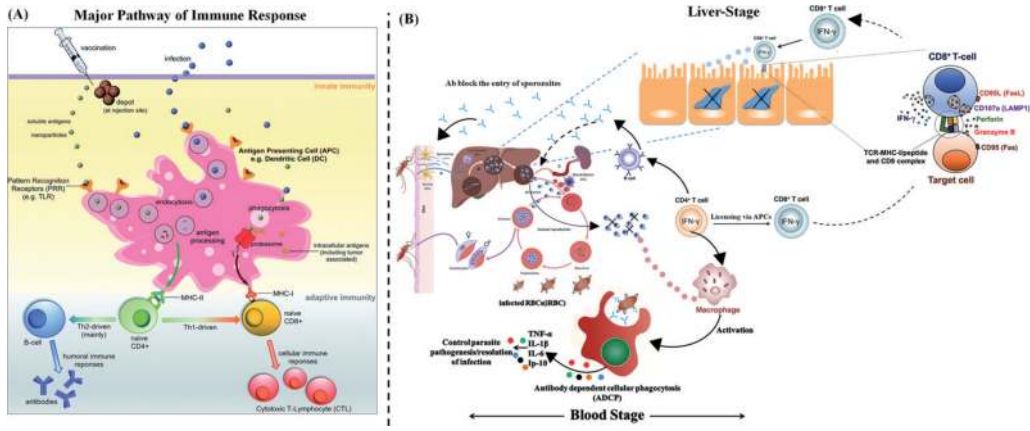

Figure 4.
(A) The major pathway of immune response and (B) at different stages of malaria infection how different immune cells can target malaria infected cells (adapted and modified from immunopaedia.org and [143–146]).

immune response and restrain the LS infection should be considered. Recently, all the experimental evidence have demonstrated that instead of using single epitope based Ag, multiple-and-stage-specific epitopes of different Ag(s) can protect and generate stage-specific sterile response, yet it has to be verified in animal settings before going for the clinical trials. Also, poor immunogenicity of stage-specific Ag(s) required screening the whole genome database and available RNA-seq. data which can predict and identify (with matched HLA-typing) B-and-T-cell based epitopes to activate the immune response in a controlled manner. To overcome the current challenges mainly in T-cell based vaccine development below-listed novel approaches may aid in better results. **Figure 4** depicted the major pathway of immune response and how they can be triggered by T-cell based vaccine.

- Reverse vaccinology
- Structural vaccinology
- Immunoinformatics
- High-throughput identification for immune protection
- Understanding the humoral and CMI response
- Selection of appropriate platform for vaccine development and delivery

4. Conclusion

Malaria still persists as a major global challenge and to fight against it several options have been explored. Despite the advancement in the field of technology, drug and insecticide resistance followed by recent threat of delayed clearance of parasite against the frontline of antimalarial(s) have created herculean situation. On the other hand, RTS,S is the only licensed vaccine against the malaria infection, which is also unable to reach the expectation in generating sterile protection. Significant role of T-cells (mainly $CD4^+$ and $CD8^+$ T-cells) is found to be a key-player against the malaria infection. To elicit the T-cell based response, novel approaches of vaccine development have been adopted and some of them

are currently in pipeline of clinical trials. The older approach of WSV has recently gained the interest because of their potency to induce T-cell responses. The changes in RTS,S vaccine design via incorporating T-cell epitopes later on together with B-cells strongly support that finding of T-cell based multiple-epitope which can accelerate the immune response and aid Ab formation having immunogenicity in nature. By using the recent reverse vaccinology, structural based finding epitopes and predicted immunogen may aid in providing additional support in T-cell induction which are protective in nature.

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Conflict of interest

The authors declare no conflict of interest.

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