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# Current Status of Circumsporozoite Protein in Malaria Vaccine Development

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The life cycle of *Plasmodium* reveals several points where researchers have focused their vaccine-development efforts to disrupt the development of malarial parasite. During the past few years various vaccine candidate antigens have been identified but none has received the attention that surrounds the circumsporozoite protein, which covers the infective sporozoite. This certainly relates to the remarkable observation that immunization of host with radiation-attenuated *Plasmodium* sporozoites protects host against challenge with infectious sporozoites. This set the stage for an intensive search for the mechanism of protection and the development of a vaccine that can reproduce the immunological response. Considerable progress has been made but a complete picture of factors involved in the protective response(s) induced by injection of irradiated sporozoites has proven to be quite complex. Along with antibodies, helper and cytotoxic T cells, various cytokines and factors like nitric oxides have been shown to be involved in protection. The relative importance of each factor has not been established and the complete response has not yet been duplicated. Various groups around the world are working to develop vaccines that produce immunity to circulating sporozoites and to parasites developing in the liver. Here we review the role of circumsporozoite protein (CS), a predominant surface antigen of malaria sporozoite in the development of malaria vaccines.

**Key Words: Circumsporozoite protein, Malaria, *Plasmodium*, Sporozoite, Vaccine**

## Introduction

Malaria, caused by species of *Plasmodium* genus, is by far the world's most important tropical disease found in more than 90 countries, inhabited by more than 2 billion people, representing 40% of world's population (WHO 1995) . Malaria is one of the leading causes of morbidity and mortality in the tropics. It represents 2.3% of the overall global disease burden and kills more people than any other communicable disease except tuberculosis. This treatable disease, once in decline, has now reached endemic proportions in 101 countries and territories and continues to spread unchecked. Worldwide, the number of malaria cases is rising at a rate of 5% annually. Widespread and increasing resistance of the parasite to antimalarial drugs, development of resistance of Anopheles mosquito vectors to commonly used insecticides, an inadequate infrastructure for delivery of control measures, population growth and movement of non immune populations to malarious areas have all contributed

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**Abbreviations**: *CS*, Circumsporozoite; *CTL*, Cytotoxic T Lymphocyte; *MAP*, Multiple antigen peptide; *TRAP*, Thrombospondin related anonymous protein; *IL-1*, Interleukin-1; *IL-6* Interleukin-6; *IL-12*, Interleukin-12; *TNFα*, Tumor necrosis factor alpha; *IFN-γ*, Interferon gamma

the persistence and in many cases worsening of the malaria problem (WHO 1995). An effective vaccine against malaria would represent a large step in turning this trend.

Four different species of *Plasmodium* viz. *P. falciparum, P. vivax, P. malariae* and *P.ovale* infect humans leading to malaria. *P. falciparum* species causes the most severe form of human malaria. Worldwide prevalence of the disease is estimated to be in the order of 300-500 million clinical cases each year. About half the malaria cases are caused by *P. falciparum* resulting in 2 million deaths annually (WHO 1995). Resistance of *P. falciparum* has spread through Asia, Africa and South America. With the realization that the eradication of malaria does not appear to be achievable in the near future, control of the disease has focussed on minimization of morbidity and mortality. The complexity of the parasite life cycle, imperfect tools to measure the degree of efficacy generated by various vaccine constructs and the limited understanding of the factors that determine the outcome of an infection have hampered the progress.

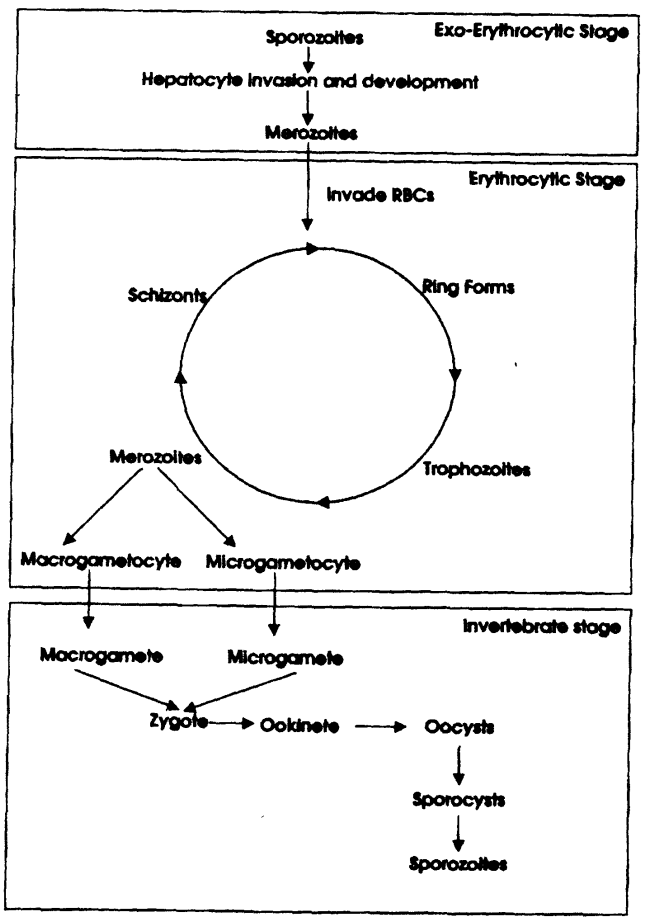


**Figure 1** Schematic representation of the life cycle of Plasmodium parasite

## Life Cycle of *Plasmodium*

The life cycle of the malaria parasite is complex, with several stages that are morphologically and antigenically distinct. *Plasmodium* species require two types of hosts. An invertebrate (mosquito) and a vertebrate (reptile, bird or mammal) host to complete its life cycle. The sexual stages of the life cycle are maintained in the invertebrate host while asexual stages take place in the vertebrate host.

### *Vertebrate Stages*

Malaria is transmitted to man when the sporozoite stage of the *Plasmodium* parasite is inoculated into the blood stream during blood feeding by infected female mosquitoes. The sporozoite is 10-15 μm long by 1 μm in diameter and has a pellicle composed of a thin outer membrane, a double inner membrane and a layer of subpellicular microtubules. After being injected into the blood stream sporozoites rapidly invade hepatocytes (figure 1). Once inside the host cell, a sporozoite metamorphoses into a feeding trophozoite. The trophozoite feeds on the host cell cytoplasm, matures and initiates schizogony. Within a few days, each sporozoite is able to generate tens of thousands of merozoites. This transformation of sporozoites into merozoites in the liver constitutes the asymptomatic pre-erythrocytic phase of malaria. Infected liver cells burst and release merozoites into the circulation (figure 1). Once in circulation, these merozoites rapidly invade erythrocytes, where they undergo further transformation and multiplication. Inside erythrocytes, the merozoite again transforms into a trophozoite. These trophozoites ingest host cell cytoplasm forming a large food vacuole giving an appearance of a ring of cytoplasm with the nucleus conspicuously displayed at one edge. As the trophozoite grows its food vacuole becomes less noticeable and the parasite rapidly develops into a schizont. Once this development is complete, the host cell ruptures releasing parasites as well as the metabolic waste into the circulation. The metabolic waste thus released

is one of the factors responsible for the characteristic symptoms of malaria. This process constitutes the asexual, erythrocytic phase of the life cycle. After an indeterminate number of asexual generations, some merozoites enter erythrocytes and undergo gametogenesis, and become macro and microgametocytes.

### *Invertebrate Stages*

When a mosquito imbibes erythrocytes containing gametocytes, the enclosing erythrocytic membrane opens and the gametocytes are released in the insect gut. The macrogametocyte matures to the macrogamete (female gamete) and its nucleus shifts towards the periphery. In contrast the microgametocyte (male gamete) undergoes rapid division forming 6 to 8 daughter nuclei each of which is associated with the elements of a developing axoneme resulting in the formation of microgametes. The life span of these microgametes is short since they contain little more than the nuclear chromatin and the flagellum covered by a membrane. One of these microgametes fertilizes the macrogamete resulting in the formation of a diploid zygote that develops into an ookinete. The ookinete then penetrates the peritrophic membrane in the mosquito's gut and migrates to the hemocoel side of the gut, where it begins its transformation into an oocyst. The exact point of reduction division is still somewhat of a mystery but it is now known that both haploid and diploid products are found in a single oocyst indicating that the encysting ookinete is diploid. Oocyst undergoes mitotic divisions giving rise to a large number of haploid nucleated masses called spheroblasts. These spheroblasts in turn divide repeatedly to form thousands of sporozoites. The sporozoites are released from the oocyst into the hemocoel and they migrate throughout the mosquito's body with some contacting the salivary gland. Those that contact the salivary gland enter its channels and can pass into a new host at the next feeding. Sporozoite development takes from 10 days to two weeks depending upon the species of *Plasmodium* and the temperature. Once infected a mosquito remains infected for life, capable of transmitting malaria to every susceptible vertebrate it bites. The life cycle of Anopheles gambiae, however, averages only about two weeks, restricting the number of infectious blood meals that it takes and the percentage of parasites reaching full maturity.

## A Vaccine against Malaria: Available Options

The genome of *P. falciparum* probably contains about 5000 genes and only a subset of these is expressed at any one developmental stage. Expression of these protective stage-specific proteins has formed the foundation for three distinct approaches to vaccine development:

1. Vaccines targeted against sporozoites and intrahepatocytic form (pre-erythrocytic stages) of the parasite
2. Vaccines targeted against selected antigens on the merozoite and parasite proteins found on the surface of the erythrocyte
3. Transmission blocking vaccines targeted against gametocytes, gametes or later stages in the mosquitoes

Only a fully effective pre-erythrocytic vaccine can disrupt the development of the parasites to erythrocytic stages and thus prevent the disease. Erythrocytic stage vaccines are designed to prevent diseases by reducing or completely inhibiting growth of the parasite in the blood. Attacking infected erythrocyte, a cell without major histocompatibility complex molecules on its surface can be done only by antibodies that recognize exposed parasite antigens or by directing cellular products like cytokines and free radicals to the infected cell. In contrast to pre-erythrocytic and erythrocytic stage vaccines, a transmission blocking vaccine would not have any impact on the clinical manifestations of malaria in an individual. However, if administered successfully to a community, it would have an impact on the level malaria transmission the area.

## Circumsporozoite Protein: A Pre-erythrocytic Vaccine Candidate

When injected into humans and other animals, irradiated sporozoites of the malaria parasite *Plasmodium* provide protection against further challenge with viable sporozoites (Nussenzweig et al. 1967, Clyde et al. 1973). The irradiated sporozoites are able to invade hepatocytes but

are unable to mature to the stage that infects erythrocytes. Clinical symptoms of the disease and transmission of malaria does not occur. This protection is mediated by cellular effector mechanisms that destroy the liver stages, and at least in part by antibodies (Potocnjak et al. 1980). Despite the success of experimental irradiated sporozoite vaccines in human volunteers, limitations and practical difficulties in producing sufficient numbers of sporozoites or infected mosquitoes for irradiation has rendered this approach almost non-practical for being used in the field. In spite of being impractical in the field, this model has provided the foundation for more than 20 years of work by various groups to elucidate the mechanisms of the immune response and to identify the target antigen(s) that can induce comparable immunity. Immunogenic epitopes of various sporozoite surface proteins have been identified, produced either by recombinant DNA technology or by chemical synthesis, and evaluated for their potency as an effective vaccine candidate.

Out of all the studies performed CS protein has received more attention than all the pre-erythrocytic antigens put together. The circumsporozoite protein expressed in sporozoites and intrahepatocytic stages was one of the first malaria antigens whose structure was identified after the cloning of the respective gene (Ozaki et al. 1983, Dame et al. 1984, McCutchan et al. 1985). As discussed below, its role in protective immunity has been demonstrated in animal models of malaria by various approaches, including the use of synthetic constructs, recombinant proteins, immunization with DNA and microbial vectors expressing the entire circumsporozoite protein or selected B and T-cell epitopes. Several of these approaches resulted in complete resistance to challenge in some of the immunized animals thus providing the opportunity to investigate the underlying immune mechanism.

## Structural Features of CS Protein

CS is an essential protein for the development of sporozoites in mosquito and once sporozoites enter its vertebrate host, it also promotes the binding of sporozoites to liver cells (Cerami et al. 1992, Menard et al. 1997). The main structural and antigenic properties of CS are identical in all the species of malaria sporozoites (Ozaki et al. 1983, Dame et al. 1984, McCutchan et al. 1985, McCutchan et al. 1996). It is made up of a secretory signal sequence at its amino terminus, a central repeat region, two conserved amino acid motifs, Region I and Region II-plus, and an anchor sequence at its carboxyl terminus (figure 2) (Dame et al. 1984, Frevert et al. 1993, Sinnis et al. 1994, Sinnis 1996). The repeat domain is species specific, immunodominant and constitutes about one-half of the molecule. In *P. falciparum* the number of repeats varies from 36 to 43 and 3 to 4 additional variant NVDP repeats are also found in every isolate (Dame et al. 1984). Identical NANP determinants also exist in asexual blood stages, which are recognized by anti-sporozoite monoclonal antibodies. Such immunodominant block of repeats with homologies to other epitopes are hypothesized to act as "smokescreens" to divert the immune response from epitopes crucial for parasite survival (Anders et al. 1986) . In the non-repetitive flanking regions, CS protein from different isolates can differ both in length and by single amino acid substitutions (Caspers et al. 1989). Region II-plus, an 18 amino acid motif, constitutes the binding ligand of CS (Frevert et al. 1993, Sinnis et al. 1994). The region II-plus motif is not only conserved among the CS of all malaria parasite, it is shared with other sporozoite surface proteins like thrombospondin related anonymous protein (TRAP) and a variety of host proteins such as thrombospondin or properdin (Lawler & Hynes 1986, Goundis & Reid 1988, Robson et al. 1988). In *P. falciparum* it is represented by "EWSPCSVTCGNGIQVRIK" (Dame et al. 1984) .

With regard to function of CS during invasion, it is known that basic and hydrophobic amino acids associated with Region II-plus specifically interact

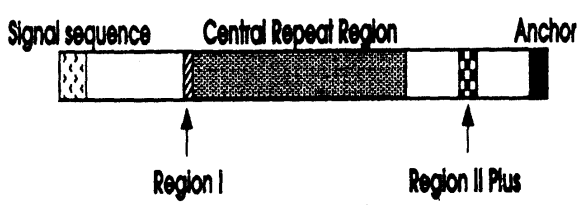


**Figure 2** Schematic representation of the structure of circumsporozoite protein

with the negatively charged glycosaminoglycans chains of heparan sulfate proteoglycans (HSPG) present on the cell surface of hepatocytes (Pancake et al. 1992, Frevert et al. 1993, Sinnis et al. 1994). Avidity of binding relates to the degree of sulfonation of the proteoglycans and hence varies in accordance with host related factors. Low-density lipoprotein receptor related protein (LRP) present on hepatocyte cell surface has also been shown to interact with the region II-plus of CS (Shakibaei & Frevert 1996). Identification of exact residue(s) involved in binding has yielded discrepant results (Rich et al. 1990, Sinnis et al. 1994, Gantt et al. 1997) . Recently, CS has also been shown to inhibit protein synthesis in mammalian cells but the exact mechanism is not fully understood (Hugel et al. 1996, Frevert et al. 1998) .

## Immune Response against CS

Both humoral and cellular immune responses along with various cytokines and factors have been shown to be involved in providing protection against malaria.

### *Humoral Immune Response: Attacking Sporozoites*

In malaria-endemic areas >90% of the sporozoite specific antibodies recognize repeat sequences (Zavala et al. 1985). Screening a large number of *P. falciparum* sporozoites from different areas of the world with antibody to the repeat region of CS protein has shown that all the isolates bear the same repeats although the non repeat regions of the protein show variations Zavala et al. 1985, Doolan et al. 1992). This showed that repeats are a potential target for development of a vaccine. Antibodies to peptides representing repeat region of *P. falciparum* CS protein recognized native CS protein and blocked sporozoite invasion of human hepatoma cells in *vitro* (Ballou et al. 1985). Studies in rodents, monkeys and humans further demonstrated that antibodies alone can protect against malaria infection. This antibody response is not only directed against the repeat, but non-repeat regions are also recognised (Sharma et al. 1986, Bhardwaj et al. 1995). Passive immunization of a monoclonal antibody, directed against the repeats of CS protein of *P. berghei,* prevented rodent malaria infection in mice Yoshida et al. 1980) . Similar results were obtained against *P. falciparum* sporozoites in chimpanzees (Nardin et al. 1982). Though repeats in *P. falciparum* CS protein are conserved, considerable diversity has been observed in the CS repeats of *P. vivax, P. knowlesi* and *P. cynomolgi* parasites (Sharma et al. 1985, Galinski et al. 1987, Qari et al. 1991).

Numerous repeat based vaccines have been designed and some of them have undergone phase I trials in humans. In one study six volunteers were injected with a recombinant fusion protein containing 32 copies of repeat NANP and were challenged with the bites of five infected mosquitoes (Ballou et al. 1987). Volunteer with the highest titer of antibodies didn't develop any parasitemia and two patients experienced lengthened pre-patency periods that correlated with the antibody titer. The antibody levels induced by these vaccines were low in most volunteers although the subject who responded well developed titers comparable to those seen in people from malaria endemic area. Though all neutralizing antibodies to sporozoites are directed against the repeats of CS protein, some antirepeat antibodies do not inhibit parasite infectivity (Jones et al. 1993) . The most likely explanation is that these antibodies differ in their binding affinities and/or fine specificities.

There are a number of seeming inconsistencies in the literature which probably reflect the incompleteness of our knowledge about the mechanism(s) involved in the protective immune response. Understanding these seeming inconsistencies may well do much to educate us about the protective response. It should also be remembered that with regard to the degree of protection more is not necessarily better. Sedegah and coworkers immunized mice with irradiated *P. yoelii* sporozoites or with a recombinant vaccinia construct encoding the full length *P. yoelii* CS protein including 19 copies of repeats (Sedegah et al. 1990). Group immunized with irradiated sporozoites was protected upon challenge with 10,000 infective sporozoites however, mice immunized with recombinant vaccinia virus were not protected even on a challenge of 200 sporozoites, though both groups of animals had generated excellent antibodies against the repeat epitope. One must ask about the difference, which may have many reasons, but one must also acknowledge that an anti-sporozoite vaccine does not need to protect against 10,000 infective sporozoites. Achieving consistent protection against 200 sporozoites might

suffice. Reducing the experimental parameters to directly compare antibody responses as opposed to comparing antibody to a single protein with the complex array of responses generated by whole sporozoites seemed useful. In another experiment, mice immunized with a monoclonal antibody directed against *P. yoelii* CS repeats were protected upon subsequent challenge with sporozoites while mice immunized with a repeat-based subunit vaccine were not protected (Charoenvit et al. 1991). The difference between the two sera with regard to reaction with sporozoites was not apparent. In a similar study four out of six saimiri monkeys were protected against a 10,000 *P. vivax* sporozoite challenge when they were infused with a monoclonal antibody directed against *P. vivax* repeats before challenged (Charoenvit et al. 1991). Monkeys immunized with a recombinant subunit vaccine containing repeats of *P. vivax* were not protected upon challenge with 10,000 *P. vivax* sporozoites (Collins et al. 1989). Again the monoclonal and the sera generated in response to vaccination were compared. While both monoclonal antibody and the antibodies generated against the subunit vaccine reacted with the sporozoites by immunofluorescence, subsequent analysis of the binding epitope revealed the fine difference. While monoclonal antibody used for passive immunization binds to a four amino acid sequence "AGDR" a component of the repeats (Charoenvit et al. 1991) , none of the animals produced detectable amounts of antibodies to this sequence even though multiple copies of this repeat were present in the subunit vaccine. This result demonstrated that even though the desired protective epitope is present in the sequence of the immunogen, a desired antibody response is not ensured.

Another approach that is being employed involves chemical synthesis of multiple antigen peptide (MAP) which provides a high concentration of covalently crosslinked epitopes as a vaccine (Munesinghe et al. 1991) . Unlike a conventional peptide-based vaccine, a MAP vaccine doesn't require a carrier protein and contains peptides representing B and T cells epitopes. A MAP vaccine containing *P. falciparum* repeats, along with a T epitope has been shown to elicit high levels of protective immunity which directly correlates with anti sporozoite antibody titers (Munesinghe et al. 1991). Recently another CS based vaccine RTS,S where CS is fused to hepatitis B surface antigen has undergone human trials and shown promising results (Stoute et al. 1997).

Since antibodies attack incoming sporozoites and do not recognize liver or blood stages, they achieve complete protection only when present in circulation at high titers, thus preventing all parasites from entering hepatocytes. Effectiveness of an antibody based vaccine increases with increase in the antibody levels and the degree of protection goes down when the challenge dose of sporozoites is increased or when the antibody titers are lower. Hence antibody mediated protective immunity alone is unlikely to be completely effective and other immune effector mechanism(s) need to be induced to garner complete protection.

*T-cell Response: Attacking Hepatocytes*

It is now generally accepted that the malaria parasite developing within the host hepatocyte is the major target of protective immunity directed against the pre-erythrocytic stages. Both CD8+ and CD4+ T cells recognize parasite derived peptides presented on MHC class I and class II molecules respectively on the surface of infected hepatocytes. CTL epitopes have been identified for CS protein in *P. falciparum* (Kumar et al. 1988, Hill et al. 1992) and presence of CD8+ T cell dependent CTL activity against these epitopes have been reported in population naturally exposed to malaria (Doolan et al. 1991, Hill et al. 1992, Sedegah et al. 1992). CD8+ T cells primarily mediate protection against the pre-erythrocytic stages as *in vivo* depletion of CD8+ T cells abrogates protection while adoptive transfer of CD8+ cells to naïve mice confers protection (Egan et al. 1987, White et al. 1996). It has been proposed that CD8+ T cells recognize a *Plasmodium* sp. Peptide class I MHC complex on the surface of infected hepatocytes and then secrete IFN-γ, that in turn induces the infected hepatocyte to produce nitric oxide, which eliminates the infected hepatocyte or inactivates the intracellular parasite (Seguin et al. 1989, Weiss et al. 1992). Inducible nitric oxide synthase expression in liver following

sporozoite challenge has been shown to be restricted to infected hepatocytes and is dependent on the persistence of irradiated parasites in the livers of immunized animals (Klotz et al. 1995, Scheller & Azad 1995). Alternatively, CTLs may destroy infected hepatocytes by crosslinking of CTL membrane ligands with apoptosis-inducing target cell receptors such as Apo-1/Fas (Hoffman et al. 1996). Recently gd T cells were shown to have activity against infected hepatocytes in the absence of ab T cells (Tsuji et al. 1994).

CD4+ T cells can also recognize parasite peptides with class II MHC molecules and eliminate hepatocytes by similar mechanisms (Tsuji et al. 1990). Moreno and colleagues have reported the production of a CD4+ T cell clone with cytotoxic activity from an individual immunized with radiation attenuated *P. falciparum* sporozoites (Moreno et al. 1991). Analysis of the pattern of secretion of certain CD4+ T cell clones suggests that a large number of cytokines are involved in the protection mechanism (Nussler et al. 1991, Renia et al. 1993). IL-1 and IL-6 have been reported to inhibit the development of intrahepatocytic parasites (Nussler et al. 1991, Pied et al. 1991). Similarly, TNFα has been shown to inhibit the development of parasites in a hepatoma cell line (Schofiled et al. 1987). Administration of recombinant IL-12 to mice (Sedegah et al. 1994) and monkeys provide 100% protection with *P. yoelii* in mice and *P. cynomolgi* in monkeys (Crutcher et al. 1995). It has been suggested that IL-12 acts by inducing T cells to produce gamma interferon, which induce hepatocytes to produce nitric oxide, that kills the developing parasite (Sedegah et al. 1994). There is also evidence that protection may require specific adhesion molecules such as CD44 on the surface of effector CD8+ T lymphocytes (Rodrigues et al. 1992).

These observations have led to the development of a class of subunit vaccines designed to induce protective T cell responses against *Plasmodium* sp. infected hepatocytes. Sadoff et al constructed an oral *Salmonella typhimurium* vaccine expressing *P. berghei* CS protein and showed remarkable protection in a mouse model (Sadoff et al. 1988). Similarly, Lanar et al produced a vaccinia virus based recombinant vaccine that conferred protection against rodent malaria (Lanar et al. 1996). Protection did not correlate with CS repeat-specific antibody responses and it was abrogated by in vivo CD8+ T-cell depletion. Unfortunately a recombinant *S. typhimurium* and vaccinia based vaccines expressing *P. falciparum* CS protein have only been partially successful (Gonzalez et al. 1994, Tine et al. 1996).

In 1994 Sedegah et al reported a malaria DNA vaccine in a rodent model. Balb/c mice were immunized with a plasmid encoding *P. yoelii* CS protein DNA under the control of a CMV promoter. Mice were protected against a challenge by *P. yoelii* sporozoites and the protection was mediated by CD8+T cells (Sedegah et al. 1994). A high level of anti CS antibody response was also generated but they had only moderate biological activity as assessed by the in vitro inhibition of sporozoite invasion and liver stage development assays. The success in the *P. yoelii* rodent model provided the basis for transition into the development of a *P. falciparum* DNA vaccine designed to induce protective CD8+ T cell responses against *P. falciparum* infected human hepatocytes. The gene coding for *P. falciparum* CS protein was cloned in a CMV promoter based vector containing the first 78 amino acids of a tissue plasminogen activator protein (TPA) designed to maximize the secretion of encoded gene (Luke et al. 1997) . The vaccine was tested in 20 malaria naïve human volunteers. Eleven out of twenty volunteers had antigen-specific, genetically restricted cytotoxic T lymphocytes against CS of *P. falciparum* (Wang et al. 1998).

## Problems Facing a CS Based Malaria Vaccine

### *Polymorphism in Important Antigenic Epitopes*

In *P. falciparum* CS protein two CTL epitopes have been identified in a 23 amino acid motif (KPKDELDYANDIEKKICKMEKCS) located

towards the carboxyl terminus of the protein (Kumar et al. 1988, Romero et al. 1989, Malik et al. 1991, Hill et al. 1992) . The protein shows substantial amount of amino acid polymorphism in its immunodominant T cell epitopes (Good et al. 1988, Guttinger et al. 1988, Hill et al. 1992, Udhayakumar et al. 1994, Zevering et al. 1994, Gilbert et al. 1998). Studies examining cross-reactivity to natural variants of CS epitopes have shown that polymorphism appears to affect T-cell cross reactivity, suggesting that T cell epitope variation could be a potential problem for vaccine based on such epitopes (de la Cruz et al. 1988). De la Cruz et al first studied the features of polymorphism in CS (de la Cruz et al. 1987,1988,1989). They concluded that (a) there is a correlation between the small number of T-epitopes in the protein and the regions of polymorphism and (b) there is an unusual ratio of non-synonymous to synonymous mutations resulting in a change at the protein level. They proceeded to speculate that (a) the ratio of non-synonymous to synonymous mutation was an indicator (not proof) of positive selection for variation at the site and (b) that variation may be driven by immune selection. Homoplasy in the CTL epitopes of CS as shown by McCutchan et al.(1992) could be an outcome of a balancing act resulting in CTL epitopes reverting back to its original phenotype. Recently this region has been shown to be involved in the receptor-ligand interaction essential to parasite invasion of the host (Rathore & McCutchan 2000). The residues recognized as CTL epitopes seems to be providing a proper structural conformation to the protein for its interaction with hepatocyte receptors (Rathore & McCutchan 2000). As the region involving CTL epitopes is playing dual roles, one affecting efficiency of targeting and (or) invasion of the host by the parasite and one affecting the potential of the host to repel parasite invasion, the results of molecular change must be considered to potentially alter at least two potentially opposing forces.

### *Genetic Restriction to T Cell Responses to Specific Parasite Epitopes*

Another potential problem with the vaccine is that its usefulness could be genetically restricted due to the paucity of T-cell epitopes in the protein. The growing body of evidence suggests that the host response to malaria is influenced by complex genetics (Hill et al. 1991). Role of major histocompatibility complex in the host response has been examined in both animal models and human populations. Malaria acts as a significant selective pressure on human populations (Weatherall 1996). Despite the difficulties associated with mapping complex traits in human populations, some progress has been made towards the mapping of malaria resistance genes. Linkage analyses have been conducted in different African populations to examine the role of host genetics in both mild and severe malaria. A large case study was performed on West African children to investigate the impact of HLA class I and class II molecules on severe malaria (Hill et al. 1991). The results from this study suggested an association of HLA-B53 (Class I) and DRB1*1302-DQB1*0501 with protection against severe malaria (Hill et al. 1991).

A closer examination of the effects of genetic restriction on infection by sporozoites has been done in inbred mice. Several congenic lines of mice have been demonstrated to be differentially susceptible to malaria. Sedegah et al. (1994) reported malaria DNA vaccine in a rodent model. BALB/c mice were immunized with a plasmid encoding *P. yoelii* CS protein DNA under the control of a CMV promoter. 56% of mice were protected against a challenge by *P. yoelii* sporozoites and the protection was mediated by CD8+ T cells (Sedegah et al. 1994). Unfortunately, this protection was genetically restricted as this vaccine showed poor protection (20%) in four different strains of mice (Doolan et al. 1996). BALB/c mice are susceptible to infection with *P. yoelii* while BALB.D2, which differs with BALB/c at the mtv-7 locus on chromosome 1 is more resistant (Swardson et al. 1997). It has been suggested that a mtv-7 linked gene regulates these phenotypes during a malarial infection and perhaps accounts for the difference in susceptibility of the congenic lines. This genetic restriction suggests that a vaccine based on the CS protein alone or any other single antigen will

be inadequate to protect an outbred human population and that a multivalent vaccine will be required. Recently, a multistage and multivalent vaccine against *P. falciparum* has been shown to strongly inhibit sporozoite invasion of hepatoma cells *in vitro* (Shi et al. 1999). The vaccine encodes for 12 B cells, 6 T cell proliferative and 3 cytotoxic T lymphocyte epitopes derived from 9 stage specific *P. falciparum* antigens corresponding to sporozoite, liver and blood stages of the life cycle (Shi et al. 1999). Vaccine elicited antibodies strongly inhibited sporozoite invasion of hepatoma cells and growth of blood stage parasites in the presence of monocytes.

## Conclusions

In the last twenty-five years significant progress has been made towards the development of a CS-based vaccine for combating malaria. A complex combination of different immune effector mechanisms involving both cytotoxic and humoral immune response along with various cytokines have been shown to be involved in protection. Genetic restriction of T cell responses and polymorphism of important T cell epitopes in the protein are serious obstacles in the development of an highly effective CS-based vaccine capable of providing a sustainable protection in multiple epidemiological settings. These first generation vaccines, through far from perfect, have provided an important foundation for the next generation of vaccines.

## Future Directions

Though CS is important, in the future additional parasite factors involved in irradiated sporozoite induced sterile immunity would need to be identified. This requires an increase in understanding of the biology of the parasite and the host immune mechanisms. A better understanding of mechanism of protection would lead to an improvement of current vaccine systems and the development of new and more effective vaccines which can duplicate the sterile immunity induced by the irradiated sporozoites.Though attacking only infected hepatocytes may not provide complete protection CS-based liver stage protection will be an essential part of an ideal malaria vaccination protocol.

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