VACCINATION AGAINST MALARIA: TARGETS, STRATEGIES AND POTENTIATION OF IMMUNITY TO BLOOD STAGE PARASITES

Andrew W. Taylor-Robinson

School of Biology, University of Leeds, Leeds, United Kingdom

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1. ABSTRACT

Malaria is the world's major parasitic disease, for which effective control measures are urgently needed. One of the difficulties hindering successful vaccine design against *Plasmodium* is an incomplete knowledge of antigens eliciting protective immunity, the precise types of immune response for which to aim, and how these can be induced. A greater appreciation of the mechanisms of protective immunity, on the one hand, and of immunopathology, on the other, should provide critical clues to how manipulation of the immune system may best be achieved. This review discusses the current state of malaria vaccine development and research to understand the factors involved in the modulation of vaccine-potentiated immunity to the pathogenic blood-borne stages of the parasite.

2. INTRODUCTION

Malaria is caused by protozoan parasites of the genus *Plasmodium*. Each year, 2-3 million children die as a result of *P. falciparum* malaria, and up to 500 million people throughout the world suffer clinical disease (1). Malaria thus ranks alongside acute respiratory infections, measles and diarrheal diseases as one of the major causes of mortality worldwide. By far the most mortality occurs in sub-Saharan Africa, but large areas of Asia, Central and South America contribute appreciably to global incidence of morbidity (2). Outbreak of disease is often linked to the movement of refugees and trans-migrants and to environmental change, including forestry, mining and irrigation projects (3). The situation has gradually worsened in recent years because of increasing resistance of the anopheline mosquitoes that transmit malaria to insecticides,

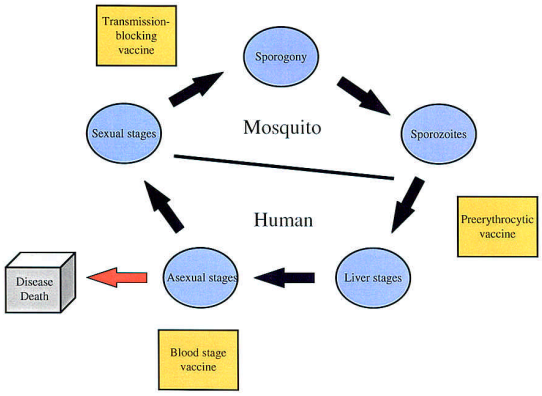

Figure 1. Schematic representation of the life cycle of the malaria parasite, *Plasmodium*, indicating where vaccination may be expected to intervene.

and of the parasites themselves to antimalarial chemotherapy. Thus, the development of an effective malaria vaccine represents a high priority intervention strategy to control both the transmission of infection and the impact of disease (4).

Unlike many acute viral diseases which produce lifelong resistance to reinfection, malaria only elicits immunity after several years' continuous exposure, during which time recurring infections and illness occur. Immunity to malaria acquired in this way is only partially effective and results in milder, sometimes asymptomatic infections in spite of harboring low blood-borne parasitemias. This immunity is short-lived unless reinforced through frequent reinfection and is therefore only acquired by so-called 'semi-immune' adults resident in malaria-endemic areas. Consequently, in order to be effective, a malaria vaccine must boost this immune steady state by potentiating the native immune mechanisms through which this has been achieved to rapidly induce extensive and long-lasting protection. In endemic areas, an effective vaccine should protect not only semi-immune persons, but also those who most frequently develop severe forms of malaria (3). More susceptible groups include pregnant women, who are selectively immunosuppressed by a $CD4^+$ T helper 2 cell-biased cytokine imbalance (5), and young children, whose maternally-derived antibodies have waned but who have not had enough exposure to develop effective anti-parasite immunity of their own (6). A prophylactic vaccine would also protect individuals resident in non-malarious areas, who have little or no specific acquired immunity, from contracting severe disease while traveling through regions of high endemicity.

The advent in recent years of molecular tools to identify, produce and study malarial antigens has encouraged researchers to attempt to construct subunit vaccines containing multiple targets at each stage of parasite development. However, both natural and vaccine-induced immunity are hampered by the capacity of blood stage parasites to vary critical antigenic determinants on the surface of the parasitized erythrocyte. An additional complication to vaccine development is the genetic heterogeneity of human populations. In a bid to overcome the dual difficulty of poor immunogenicity and parasite diversity, much experimental work is now focused on complex antigenic constructs delivered as synthetic peptides, recombinant proteins, live vectors or 'naked' DNA.

3. RATIONALE FOR DEVELOPMENT OF MALARIA VACCINES

3.1. Experimental support

There are two major experimental findings that have provided much of the support for the concept of a malaria vaccine. First, in murine and primate models and in human trials, immunization with radiation-attenuated sporozoites may induce sterile protective immunity against malaria (7-9). Second, passive transfer of immunoglobulin G (IgG) from semi-immune individuals can provide partial protection against infection (10-12). Obviously, neither strategy is practicable for application on an industrial scale suitable for commercial production, but they do indicate the feasibility of inducing immunity to malaria by vaccination.

3.2. Several parasite life cycle stages

A malaria vaccine could act at one of several distinct stages of the *Plasmodium* life cycle (figure 1). By preventing either invasion of hepatocytes by sporozoites (antibodies) or exoerythrocytic stage development within hepatocytes (T cells, cytokines and perhaps antibodies), a vaccine targetting preerythrocytic stages would preclude both the progression of disease, since clinical symptoms of malaria manifest only during the erythrocytic stage, and parasite transmission, since no gametocytes would develop (13, 14). By interfering with the asexual erythrocytic cycle (antibodies, cytokines and other cellular products), a vaccine would prevent or reduce morbidity and mortality by eliminating or lowering the parasite load (2, 4). The concept of an anti-toxic vaccine is based on the observation that adults who are frequently exposed to malaria suffer few symptoms, although they remain susceptible to infection (15). The release of merozoites and harmful byproducts of parasite replication upon rupture of the parasitized erythrocyte is thought to stimulate dysregulated production of cellular factors, principally tumor necrosis factor (TNF), by the host that contribute to, or exacerbate, malaria pathogenesis. Existing studies suggest that symptomless malaria-infected individuals may have developed antibodies that inhibit TNF-inducing parasite toxins (16). By inducing these neutralizing antibodies, or by inhibiting cytoadherence of parasitized erythrocytes implicated in cerebral malaria and other complications of the disease, an asexual erythrocytic vaccine would reduce disease severity but without affecting the rate of infection. The possible parasitological and clinical consequences of immunization against blood stage malaria are outlined in table 1. A transmission-blocking vaccine aimed at sexual stages would not protect the individual, but instead interfere with parasite development within the mosquito (figure 1). This altruistic strategy would reduce clinical disease at a population level and perhaps eradicate malaria

Table 1. Potential effects of asexual blood stage vaccines in immunized individuals

Variable Affected	Probable Effects	Other Possible Effects
Infection (parasitemia)	• Little or no change in incidence • Decreased density of asexual blood stages and, probably, gametocytes • Decreased duration	• Increased prevalence and density of other species of malaria not targeted by the vaccine[a]
Immune Response	• Increased response to asexual blood stages • No change in response to sporozoites[b]	• Decreased response to sexual stages[c]
Morbidity (disease)	• Decreased incidence • Decreased severity	• Increased incidence of complications during infections[d]
Mortality[e]	• Decreased mortality rate • Decreased case fatality rate	

[a] Supposing that infection with the species of malaria targeted by the vaccine suppresses or masks infections with other malaria parasites.[b] Unless there is suppression of the response to sporozoites by asexual blood stages, and removal of that suppression by immunization. [c] Assuming decreased production of gametocytes of the species of malaria targeted by the vaccine. [d] A malaria vaccine could theoretically induce a state of altered immunity which would result in an exaggerated and harmful immune response following natural exposure.[e] Decrease in mortality caused directly or indirectly by malaria. There is likely to be a decrease in both malaria-specific mortality and in the mortality rate from all combined causes, and the absolute reduction in the latter may be greater than in the former.

in geographically isolated areas (17). A reduced parasite load associated with decreased transmission could also enhance efficacy of vaccines targeted at other stages of the parasite's life cycle.

3.3. Vaccine multivalency

Since distinct immune mechanisms operate against different stages of the parasite's life cycle, and because most antigens are not expressed at all stages, malaria vaccine design has focused primarily on induction of immunity against single stages. However, given the improbability that a vaccine directed against a single antigen will be completely protective, a malaria vaccine will most likely need to produce different immune responses against multiple parasite antigens from several life cycle stages (2).

4. MURINE MODELS OF IMMUNOREGULATION AND PROTECTIVE IMMUNITY

4.1. Immunological paradigm

A major recent advance in immunology has been the establishment of a simple paradigm for separating mature $CD4^+$ T helper (Th) cells into two main subsets according to cytokine secretion patterns which determine their function (18). Th1 cells produce interleukin (IL)-2, interferon (IFN)-gamma and TNF-beta, and through these mediate macrophage activation and delayed-type hypersensitivity responses. Th2 cells produce IL-4, IL-5, IL-6 and IL-10, and provide help for maturation of B cells to plasma cells and for the production of antibody. Substantial evidence indicates the nature of the $CD4^+$ T cell response is similar in humans and mice (19).

4.2. Dichotomy of antimalarial immune response

While probably more complex than initially appreciated, immunity to most infectious agents can now be broadly categorized into a predominant protective response of either type (20). In contrast, previous work by ourselves and others has firmly established that protective immunity to blood stage *P. chabaudi* involves both Th1 and Th2 cells, with a temporal shift in predominance from Th1- to Th2-regulated immune reactivity (21-26). This murine malaria is a well-characterized model of immunity and immunopathology of *P. falciparum*, in which individual facets of immunoregulation *in vivo* may be dissected (4, 27). Ongoing research involves characterization of immune responses that promote protection, identification of asexual antigens which are targets of such immunity, and investigation of the adjuvant capacity of cytokines to potentiate the native response to vaccine candidate antigens following immunization (28).

5. MODULATION OF INFECTION BY CYTOKINE ADMINISTRATION AND ABLATION

Resolution or exacerbation of blood stage malaria infection cannot be linked definitely to given Th1- or Th2-type cytokine profiles, but rather may be associated with the production of individual cytokines. Both Th1- and Th2-type cytokines are activated during infection irrespective of the outcome of infection (28). As an early predominant IL-10 response or a diminished acute phase IL-12 response may expedite a lethal outcome of blood stage malaria infection regardless of the host-parasite match (28), prevention or reversal of these cytokine secretion profiles, respectively, may form the basis of a protective immune response capable of reducing parasite density to sub-clinical levels.

5.1. IL-12

The effects of exogenous IL-12 on host resistance to blood stage malaria has been investigated in two independent studies (28, 29). By treating for 5 consecutive

days susceptible A/J mice starting from the day of infection with *P. chabaudi*, significant decreases in peak primary parasitemia were observed at all doses of murine recombinant (r) IL-12 (25-300 ng), but only treatment with 100 ng (approximately 5 µg/kg body weight) led to increased survival (>75%) (29). Mice given higher doses of IL-12, however, appeared to be more feverish, suffer greater cachexia and died earlier than control mice treated with saline. Likewise, administration of rIL-12 to resistant C57BL/6 mice reduced parasitemia but the 100 ng dose caused 40% mortality. Our experiments also indicated that prophylactic IL-12 reduces acute parasitemia and increases survival of NIH mice to primary infection with the virulent parasite clones *P. chabaudi* 7/F1 and *P. yoelii* 17XL to which they would otherwise succumb (28). A similar regimen of rIL-12 for 5 successive days enabled 100% and 80% of mice challenged with *P. chabaudi* 7/F1 or *P. yoelii* 17XL, respectively, to survive infections (over a 90 day monitoring period) which proved uniformly lethal to controls not given IL-12. The degree of protection conferred by inoculation of this cytokine varied not only with the dose but also the timing of its delivery. The findings of these two studies (28, 29) suggest the potential therapeutic value of treatment with IL-12, but stress the importance of determining the appropriate regimen to avoid toxicity associated with the induction of high levels of TNF-alpha. Ongoing work is assessing the role of IL-12 in protection from the opposite angle by examining the outcome of usually non-lethal *P. chabaudi adami* and *P. yoelii* 17XNL blood stage infections in the absence of IL-12 (30). Reconstitution of IL-12 knockout mice with this cytokine at different times post infection should determine how much and at what time IL-12 is required to engender protective immunity.

One approach to harness the ability of IL-12 to drive $CD4^+$ T cells towards the Th1 subset but avoiding immunopathology due to dysregulation of proinflammatory cytokines is to combine treatment with low doses of IL-12 with standard chemotherapeutic regimens. In A/J mice infected with *P. chabaudi*, such combined therapy may have the effect of reducing parasite density, preventing severe anemia and thereby increasing host survival (31). As this immunochemotherapeutic approach is effective even against established infections in mice, if IL-12 proves safe for use in humans its value may lie not just in prophylactic immunization of non-immune travelers to malarious areas but for treatment of patients with malaria. Current therapeutic strategies for combating acute malaria attacks rely on the use of drugs such as chloroquine which are now severely limited in effectiveness due to the widespread emergence of multidrug-resistant strains of *P. falciparum* and *P. vivax* (32). The use of IL-12 at low, non-toxic doses to augment chloroquine in reducing parasite load is of tremendous potential benefit for antimalarial therapy in humans but further studies are needed to define more fully the immune-boosting activity of IL-12 against erythrocytic stages of malaria. It is already known that administration of rIL-12 to mice and monkeys can induce sterile immunity to challenge infection with *P. yoelii* 17XNL and *P. cynomolgi* sporozoites, respectively (33, 34), by killing liver stage parasites via mediation of IFN-gamma and nitric oxide (NO). As IL-12 has a longer half life than does IFN-gamma *in vivo*, it may be of greater clinical potential for targeting both infected hepatocytes and erythrocytes. The immunotherapeutic potential of IL-12 has clear implications for its use as a vaccine adjuvant. Not only could IL-12 inoculation boost CMI against liver and blood stages, but it may also influence humoral immunity by inducing isotype switching through both IFN-gamma-dependent and -independent mechanisms and stimulating enhanced antibody secretion in switched B cells (35). This may go some way to explain the contribution of this pleiotropic cytokine to curing of established blood-borne infections of low level parasitemia (31), to which CMI may be expected to make a lesser impact.

5.2. IL-10

Since rapid induction of high levels of IL-10 occurs during lethal but not non-lethal murine malaria infections (28), the judicious administration of monoclonal antibodies to IL-10 may provide a rationale for reducing mortality from blood stage malaria. To explore this possibility, we are performing experiments to determine the effect of ablating IL-10 production at specific times during the course of otherwise lethal infections of *P. chabaudi* 7/F1 and *P. yoelii* 17XL in NIH mice. Preliminary results show that treatment of *P. yoelii* 17XL-infected mice with anti-IL-10 daily for 4 days beginning 2 days prior to infection reduced mortality from 100% to 20-25%. However, a similar regimen starting either 1 or 4 days post infection did not increase infection survival rate. None of these regimens lowered mortality from *P. chabaudi* 7/F1 infection, but instead the earlier the anti-IL-10 treatment the greater the pathology observed and the shorter the time to death. The starkly differing effect of IL-10 ablation on the outcome of infection with these two malaria species confirms the complexity of the cytokine network involved in resistance to blood stage malaria (27). Our data support a role for IL-10 in normally ameliorating pathology during *P. chabaudi* infection previously demonstrated in IL-10 knockout mice in which a failure to downregulate production of proinflammatory cytokines caused fatal pathology (36). In contrast, for *P. yoelii* 17XL, not only were no immunopathology-related deaths observed upon anti-IL-10 treatment, but an early absence of this antiinflammatory cytokine enabled effective reduction of acute parasitemia through CMI, so leading to survival of infection. Delaying the start of treatment until after initiation of infection failed to have a similar effect on host survival, although mortality was in this instance due to a highly restrictive cross-regulation by IL-10 (37) downregulating IFN-gamma, TNF-alpha and NO too early for these innate mediators to suppress acute phase infection. A lack of pathology in *P. yoelii*-infected IL-10 knockout mice (38) mirrors our observation, and together suggest that the etiology of pathology of *P. chabaudi* and *P. yoelii* infections are very different.

It is apparent that the timing of IL-10 neutralization is critical for effective control of parasite density: too much IL-10 too early prevents CMI through IFN-gamma and TNF-alpha to curb a rapid escalation of parasitemia; too little IL-10 too late enables exacerbation of

disease through pathology associated with excess production of these Th1-type cytokines. This is reminiscent of the dynamic balance between the immunosuppressive and anti-parasitic roles of NO during acute blood stage malaria, which also varies dramatically depending on the exact time of infection (39). Given this dual role of IL-10 in promoting protection and pathology in different infections, this presents a great practical difficulty to identifying how anti-IL-10 treatment could be used to potentiate immunity whilst avoiding immunopathology. Additional work is needed in experimental malaria models to dissect the central function of IL-10 in influencing the balance between Th1- and Th2-type effector arms of the immune response. Without understanding precisely what, why and how the Th1-to-Th2 switch in immunity to blood stage malaria is controlled, the use of IL-10 or anti-IL-10 to potentiate a blood stage malaria vaccine seems too great a risk to consider at present.

5.3. TGF-beta

IL-10 and IL-12 are 2 cytokines which play key roles in polarization of mature $CD4^+$ T cells into Th subsets and as such are attractive to the vaccinologist wishing to manipulate the immune system to optimize the host environment into which a vaccine is to be delivered. Another cytokine worth investigating as a vaccine-potentiating immunomodulatory expedient is transforming growth factor (TGF)-beta, produced by a range of cells including macrophages and T cells and which has both pro- and anti-inflammatory properties, depending on its environment and concentration (40). Importantly, TGF-beta suppresses both production of TNF-alpha and NO from macrophages and IFN-gamma and TNF-alpha secretion by NK cells (41, 42). It has been proposed that these effects may be mediated via enhanced secretion of IL-10 by macrophages, leading to an immune response skewing in favor of a Th2-type profile (43). The role of TGF-beta in influencing the outcome of blood stage malaria infection has recently been examined in mice (44). A causal association between the failure to produce TGF-beta and the severity of malaria infection was demonstrated by treatment of infected mice with neutralizing antibody to TGF-beta, which exacerbated the virulence of *P. berghei* and converted a resolving *P. chabaudi* infection into a lethal challenge. Parasitemia rose more rapidly in anti-TGF-beta-treated mice but this may not have been responsible for the increased pathology as levels of peak parasitemias were unaltered. Administration of rTGF-beta to *P. berghei*-infected mice slowed the rate of parasite proliferation and prolonged survival, and was accompanied by a significant decrease in serum TNF-alpha and increase in IL-10. Thus, TGF-beta seems to induce protective immunity via CMI early in infection, leading to slower growth of parasitized erythrocytes, but may subsequently downregulate pathogenic inflammatory responses during chronic parasitemia.

This bimodal activity defines TGF-beta as a likely major immunoregulatory molecule associated with successful control of blood stage malaria, maintaining the balance between the protective and pathological effects of other proinflammatory cytokines during infection. If this proposal is correct, it may be predicted that severe malaria in humans is linked to a reduced capacity of an individual to produce TGF-beta, in which case inoculation of rTGF-beta, either alone or in combination with a vaccine, may form the basis of a therapeutic strategy. As TGF-beta upregulates IL-10 production without downregulating IFN-gamma in murine malaria infections (44), this may explain the observation of raised levels of both cytokines in acute bouts of *P. falciparum* in humans (45). What little is known about TGF-beta production during human malaria provides a confused picture: mononuclear cells co-cultured with *P. falciparum*-infected erythrocytes secrete high levels of TGF-beta (46); but serum TGF-beta is lower in patients with acute *P. falciparum* than in non-infected persons (47). Again, these studies may not be conflicting, as the timing of sampling may largely influence the recorded circulatory levels of a cytokine that plays a pivotal role between protective immunity and immunopathology. As for IL-10, further studies are clearly required to unravel what function TGF-beta performs in the Th1-to-Th2 switch in response to blood stage malaria.

6. TARGET BLOOD STAGE ANTIGENS FOR MALARIA VACCINE DEVELOPMENT

The erythrocytic stages of the parasite are responsible for the pathology induced by malaria and present a particular challenge for vaccine development. Except for brief extracellular periods when reinvasion occurs, the blood stage parasite resides in a membranous vacuole within an erythrocyte, which lacks class I and class II major histocompatibility complex (MHC) antigens. However, asexual antigens have the potential to induce protection in primates against *P. falciparum* challenge (48), indicating the feasibility of developing blood stage vaccines. With regard to individuals living in endemic regions, vaccine-induced immunity does not necessarily have to be sterile. Rather, controlled malaria infections could serve the purpose of repeatedly boosting immunity. A number of different stage-specific asexual antigens are considered as potential vaccine candidates (table 2), of which three are undergoing analysis in our laboratory using known homologues identified in *P. chabaudi* (49-51). Using this established murine model, the merit as adjuvants of the cytokines IL-10, IL-12 and TGF-beta to potentiate vaccination with immunogenic peptides of the asexual antigens described below is currently being evaluated (28).

6.1. MSP-1

The merozoite surface protein-1 (MSP-1) is made during schizont maturation and presents as a complex of processed fragments on the merozoite surface (52). Antibodies against the C-terminal region of MSP-1 correlate with protection from clinical episodes of malaria (53-55), suggesting that either recognition of MSP-1 plays a part in acquisition of immunity or it is simply a marker of cumulative parasite exposure. Additional experimental evidence of partial protection by immunization of affinity-purified native MSP-1 against *P. falciparum* and *P. yoelii* challenge in primate and murine systems, respectively (56, 57), indicates an important role. Induction of immunity depended on the adjuvant and was mediated by serum

Table 2. Antigens of asexual blood stages of *Plasmodium falciparum* under consideration as vaccine components

Antigen	Mol wt (kDa)	Location	Function	Antigenic Diversity
PfEMP-1	250-300	Erythrocyte surface	Cytoadherence	Extensive
Pf332	750	Erythrocyte surface	Unknown	Unknown
Rosettin	22	Erythrocyte surface	Rosetting	Unknown
MSP-1	185-220	Merozoite surface	Unknown	Extensive
MSP-2	45	Merozoite surface	Unknown	Extensive
SERP	113 or 126	Parasitophorous vacuole	Proteinase	Minor
GLURP	220	Parasitophorous vacuole	Unknown	Yes
RESA/Pf155	155	Dense granules, erythrocyte skeleton	Unknown	Minor
AMA-1	80	Rhoptries	Unknown	Unknown
HRP-2	65-85	Secreted	Unknown	No

antibodies, which are thought to act against merozoites as they are released at rupture of mature parasitized erythrocytes (58).

6.2. RESA/Pf155

Ring-infected erythrocyte surface antigen (RESA/Pf155) (59, 60) is present in dense granules in the apical region of merozoites. It is released into the parasitophorous vacuole during merozoite invasion and translocated to the erythrocyte membrane where it associates with spectrin in the membrane skeleton. It is a polypeptide containing 2 immunodominant regions of tandemly repeated sequences which include both B and T cell epitopes (61). The central repeat region contains 2 repeats with the sequence DDEHVEEPTVA and 5 degenerated variants of this sequence. The second repeat region is located C-terminally and contains 5 copies of EENVEHDA and 28 copies of EENV (62). Antibodies reactive with epitopes within these repeat regions are involved in parasite-neutralizing effector mechanisms (63, 64). These may involve direct interference with merozoite invasion or antibody-dependent cellular inhibition of parasite growth. Furthermore, immunization of *Aotus* monkeys with a recombinant RESA protein induced partial protection against *P. falciparum* challenge in two trials (65, 66) but not in another (67). Immunoepidemiological studies show an age-related pattern of production of antibodies to RESA in Gambian children (68, 69).

6.3. PfEMP-1

Parasitization of the erythrocyte leads to changes to the cell surface, involving both modified host structures (70) and the expression of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) (71). PfEMP-1 is the molecule primarily involved in binding to the vascular endothelium, and would thus seem an ideal target for immune attack. Unfortunately, the parasite has developed an extraordinary capacity for phenotypic variation of PfEMP-1, presumably as a means of immune evasion (72). There is strong evidence in favor of antigenic variation in the *P. chabaudi*/mouse system (73), thus making it amenable to analysis of host-parasite interactions in the context of immune evasion. A number of *P. chabaudi var* gene products, encoded by a large gene family, with homology to variants of PfEMP-1 have been characterized recently and are the subject of ongoing research (74).

7. VACCINE DELIVERY SYSTEMS

Despite the fact that immunization of humans with radiation-attenuated sporozoites of *P. falciparum* confers sterile protective immunity (9), malaria vaccines are not likely to be based on killed or attenuated organisms, primarily because it is not feasible to produce parasites in sufficient quantity at a reasonable cost. Development of malaria vaccines is therefore focused on construction of subunit vaccines consisting of selected antigens involved in inducing protection. Subunit vaccines may consist of purified native proteins, synthetic peptides or recombinant proteins representing complete gene products or a number of selected epitopes. The subunit vaccine could be delivered as a pure protein with or without an adjuvant or by live delivery vehicles. Indeed, the advent of recombinant DNA technology enables expression and delivery of an antigen by live, attenuated microorganisms or by the DNA encoding the antigen.

For induction of efficient immune responses to antigens of *P. falciparum*, a subunit vaccine should comprise both B cell and T cell epitopes. The elicited responses must be protective in nature and directed against conserved antigenic regions. To be efficacious, a vaccine should also be immunogenic among genetically heterogeneous populations and be capable of inducing immunity that can be recalled by natural malaria infection. Considerable efforts have been made towards this goal over the last three decades. Table 3 summarizes chronologically some important advances made in the design of vaccine prototypes against human malaria.

7.1. Particulate carriers

Vaccine delivery systems can be broadly categorized as either particulate carriers, live vehicles or encoded vaccines. Particulate vaccine carriers, such as virus-like particles, liposomes and immunostimulatory complexes (ISCOMs), aim at enhancing immunogenicity to such an extent that less of the immunogen is needed to achieve the desired response (75). One viral vector for heterologous gene expression that is worthy of note is based on the RNA bacteriophage MS2 which enables display of short peptides expressed from chimeras of the gene encoding the MS2 coat protein and inserted foreign DNA (76). We have employed this novel vaccine strategy to produce chimeras containing the putatively protective epitope T1 from the immunodominant liver stage antigen-1 (LSA-1) of *P. falciparum*, which contains known human and murine MHC class I binding motifs (77, 78). Recognition of LSA-1 T1 was recently suggested from field work in a malaria-endemic region of Papua New

Table 3. Approaches to vaccine design applied to antigens of *Plasmodium falciparum*

Type	Description	Immune Responses	Examples (year)
I Whole Organisms			
1. Inactivated	No replication	Humoral	None
2. Attenuated	Abbreviated growth	Humoral and CMI ($CD4^+$ and $CD8^+$)	Irradiated sporozoites (1973)
II Subunit Vaccines			
1. Synthetic peptides	Immunodominant B and T cell epitopes as linear peptides	B and $CD4^+$ T cells	$CSP(NANP)_3$ + tetanus toxoid (1987); SPf66 (1988)
2. Multiple-antigen peptides (MAP)	Linear peptides coupled to a core of lysine residues	B and $CD4^+$ T cells	CSP (1991)
3. Recombinant proteins	Genes encoding antigens expressed in prokaryotes or eukaryotes	B and $CD4^+$ T cells	CSP (1987); MSP-1 (1990); RESA/Pf155 (1991)
4. Live vectors	Genes encoding antigens expressed and delivered by attenuated viral or bacterial vehicles		
4.1 Recombinant virus	Vaccinia, Adenovirus	MHC class I-restricted CTL, antibodies	NYVAC-Pf7 (1996)
4.2 Recombinant bacteria	Salmonella, BCG	Mucosal and systemic MHC class I-restricted CTL	CSP (1991); MSP-1 (1998)
5. Nucleic acids	DNA vectors	MHC class I-restricted CTL, $CD4^+$ T cells, antibodies	Pf332 (1997); CSP, LSA-1, TRAP (all 1998)
	RNA vectors	Unknown	None

Guinea to be a surrogate marker of acquired resistance, responsiveness to which was associated with a lack of parasitemia (79), hence the prime importance of T1 as a malaria vaccine candidate. The immunogenicity of the native MS2 capsid and the recombinant construct was investigated in BALB/c ($H\text{-}2^d$) mice as the T1 peptide contains a putative MHC class I H-2kd binding epitope (78). The native protein appeared to elicit both humoral and cellular immune responses, observed as a predominance of Th2-type cytokines but with a mixed profile of immunoglobulin isotypes (80). In contrast, the LSA-1 chimera stimulated a Th1-type-polarized response, observed as upregulation of IL-12, IFN-gamma and TNF-alpha and downregulation of IL-4 and IL-10. In particular, substantial levels of IFN-gamma were observed (seen as a 233-fold increase over the control) (80), a finding which corroborates naturally acquired resistance to liver stage malaria (14). These results validate RNA phage capsid display of immunogenic determinants as a basis for the development of novel peptide vaccines, and indicate that further evaluation of MS2 coat protein as a vector for immunization of malaria epitopes is merited.

7.2. Live vehicles

Live vaccine vehicles, for example pox viruses, bacillus Calmette-Guerin (BCG) and attenuated strains of *Salmonella*, preclude the need to produce and purify protective immunogens as these are expressed by the vector (81). The most widely studied live virus vehicles are recombinant vaccinia viruses, which tolerate insertions of large segments of foreign DNA and replicate within the cytoplasm of the host cell. Recombinant vaccinia expressing *P. falciparum* circumsporozoite protein (CSP)-derived sequences induce specific cytotoxic T lymphocytes (CTL) and antibodies that inhibit liver stage parasites (82). NYVAC, a highly attenuated strain of vaccinia, is also being utilized to develop a multistage, multiantigen vaccine for malaria by including genes encoding 7 *P. falciparum* antigens derived from sporozoite, liver, blood and ookinete stages of the parasite (83). This vaccine formulation, designated NYVAC-Pf7, elicited high titer specific antibodies in rhesus monkeys. In subsequent phase I and II trials in humans, the vaccine was safe and well tolerated but variably immunogenic, most vaccinees presenting a delayed time to onset of parasitemia but only 1 of 35 did not develop blood-borne infection (84).

Among the bacteria considered for live recombinant vaccine vehicles, one the most studied is *Salmonella*. Attenuated strains utilized for developing vaccines against CSP from *P. berghei* (85) and *P. yoelii* (86) have demonstrated the capacity of recombinant *Salmonella* to induce specific $CD8^+$ CTL and to protect against sporozoite challenge. This approach was recently evaluated in humans immunized orally with *S. typhi* expressing *P. falciparum* CSP, to which 3 of 10 volunteers mounted an anti-CSP humoral response and 1 produced CSP-specific $CD8^+$ T cells (87). *S. typhimurium* has also been used for the expression of blood stage antigens such as serine repeat protein, histidine-rich protein II and RESA/Pf155 (88, 89), to each of which immunized mice produced specific antibodies. However, due to its propensity to induce specific CTL (90), the potential of this delivery system for malaria vaccines may be restricted to preerythrocytic stages of the parasite. Indeed, our own laboratory is currently exploiting this feature of mutated strains of *S. typhimurium* for efficient potentiation (91) of the native immune response to regions of LSA-1 (79).

7.3. Encoded vaccines

The most recent strategy of vaccination, genetic immunization, utilizes the DNA or RNA encoding the immunogen. These polynucleotides are directly introduced or transfected into tissues of the host individual which then express the requisite gene product, thereby obviating the necessity for production design and formulation processes tailored to each immunogen (92). Although almost all vaccination studies to date have used pure plasmid DNA, naked mRNA theoretically offers an attractive alternative as it does not present a potential risk of integratation into the genome. However, because of its instability and the insufficient time it allows for antigen synthesis *in vivo*, the best use of mRNA may lie in the development of direct gene transfer as an *in vitro* methodology. Vectors to be used for DNA vaccination should permit a high level of expression of the foreign gene in mammalian cells, but without replicating or integrating into host chromosomes. They must also be stable and amplify to high copy number in *Escherichia coli* to enable large scale DNA preparations to give high yields of the immunogen. Most plasmids used for vaccination possess the basic attributes of vectors developed for *in vitro* expression of genes in transfected cell lines (93). Notably, these include an origin of replication suitable for producing high yields of plasmid in *E. coli*, an antibiotic resistance gene to confer antibiotic-selected growth in *E. coli*, and strong enhancer/promoter and mRNA transcript termination/polyadenylation sequences for directing expression in mammalian cells.

Although immunization of naked plasmid DNA not associated with any vector can lead to strong and persistent antibody-dependent and -independent immune responses (94), a novel way to enhance the efficacy of DNA vaccines by using attenuated *S. typhimurium* as a delivery vehicle for plasmid-mediated immunization has recently been reported (95). The concept is that DNA is carried into antigen-presenting cells by the attenuated *Salmonella*, which then die due to their lethal mutation, releasing multiple copies of the DNA vaccine construct inside the host phagocyte (96). This overcomes possible problems associated with low levels of expression of MHC class I and costimulatory molecules by myocytes transfected after intramuscular injection. In this case, transfer of antigen from transfected myocytes to professional antigen-presenting cells such as bone marrow-derived dendritic cells must take place, most probably by secretion or excretion from transfected cells, to facilitate activation of both B and T cells and generation of vaccine-induced humoral or CMI (97).

8. MODULATION OF THE IMMUNE RESPONSE BY DNA VACCINATION

8.1. Route of inoculation

The route of inoculation plays a role in determining the nature of the immune response following vaccination, most clearly exemplified by the new wave of naked DNA vaccines. Intramuscular gene vaccination of mice has been associated with induction of antigen-specific Th1-type responses with secretion of high levels of IFN-gamma and stimulation of production of antibodies of the IgG2a isotype (98). In contrast, intradermal vaccination with plasmid DNA generally induces a Th2-type response dominated by IL-4 and IL-5 and IgG1 and IgE antibodies (98, 99). This generalization does not always hold true and a number of factors appear to influence the polarization of $CD4^+$ T cell subset responsiveness. These include the adjuvanticity of DNA (100), cytokines encoded within the same (101) or a different plasmid that is co-injected (102), whether the antigen is secreted, cytoplasmic or membrane-bound (98, 103, 104), the dose of antigen (105, 106) as well as its identity and the genetic background of the host.

8.2. Cellular localization

The type of immune response elicited to a pathogen may be manipulated to promote protection by directing the cellular localization of the target antigen. The localization of a DNA-expressed antigen within a transfected cell may be determined by including various targetting sequences in the plasmid vector. Inclusion of transmembrane regions or secretion signals in a DNA construct in order for cell surface expression or secretion of the antigen, or the lack of such sequences for cytosolic retention of the antigen, are strategies recently undertaken to enhance and/or modulate immune responses induced by *P. falciparum* and other human pathogens (103, 104, 107, 108). Profuse and prolonged secretion of an antigen from the DNA plasmid-transfected cell has been associated with high and sustained antibody levels (109, 110). In contrast, antigen retention in the cytosol may be appropriate for induction of CTL-based immunity, although long lasting high titer antibody responses can also be obtained with such protein localization (98, 108, 111). The cellular location of an antigen expressed following immunization of a DNA plasmid could also influence activation of a particular subset of Th cell, presumably by favoring the type of cell involved in antigen uptake, processing and presentation (103, 104, 112).

9. POTENTIAL BENEFITS OF APPLYING DNA VACCINE TECHNOLOGY TO MALARIA

9.1. Multivalency

One potentially useful feature of DNA vaccines is that they can be designed to express several antigens simultaneously, either by encoding multiple antigens or epitopes on a single plasmid or by immunizing with a mixture of plasmids each encoding a single antigen (113, 114). This feature seems particularly relevant for a malaria vaccine for two reasons. First, because it is unlikely that a vaccine directed against a single antigen expressed during one stage of the *Plasmodium* life cycle will remove all parasites, a vaccine will probably need to target several antigens from different life cycle stages to be completely protective (115). A DNA vaccine against malaria could therefore be designed to express antigens from sporozoite, preerythrocytic and erythrocytic stages, and thereby raise immune responses against multiple parasite stages. Ideally, this vaccine should induce T cell responses that eliminate infected hepatocytes, and protective antibodies and $CD4^+$ T cells to prevent multiplication of erythrocytic parasites that escape from the liver. Second, relatively few target epitopes for T cells have been identified within malaria antigens.

These epitopes may vary in sequence among different parasite isolates and the variants often do not cross-react (116). Thus, to elicit effective immunity against multiple wild isolates with variant epitopes, immunization with a vaccine encoding many different antigens may be required. DNA vaccines appear to be well suited for this purpose.

Different strategies for the development of a DNA-based vaccine against malaria are the subject of current investigation in the *P. yoelii* mouse model and in *P. falciparum*-infected monkeys through delivery of genes that encode potentially protective antigens. The first DNA vaccine studied encoded a single antigen, *P. yoelii* CSP (PyCSP), the major surface antigen of the *P. yoelii* sporozoite (117). Immunization induced both antigen-specific CTL and antibody responses and provided protection against sporozoite challenge in some inbred strains of mouse (117, 118). The genetic restriction of protection was subsequently circumvented by a bivalent vaccine encoding both PyCSP and PyHEP17, a protein expressed on the surface of infected hepatocytes and erythrocytes (119), which broadened the protective response to other strains of mice unable to respond to PyCSP vaccination alone (118). Promising results have also been obtained using DNA vaccines encoding *P. yoelii* blood stage antigens (PyMSP-1) in mice, and *P. falciparum* antigens in rhesus monkeys (114, 115, 120). The latter has provided a foundation for ongoing clinical trials in humans with a multi-gene *P. falciparum* DNA vaccine, initial results of which suggest both safety and, through induction of CTL, immunogenicity (121, 122).

9.2. Cytokine adjuvants

With regard to using cytokines as vaccine adjuvants, plasmid DNA offers the attraction of the capacity to encode one or more cytokine genes which may then be expressed together with the immunogen following codelivery (101, 102). This molecular adjuvant approach is already showing promise in experimental models of hepatitis B and HIV-1 (123, 124), in which the magnitude and nature of the immune response to DNA vaccines can be differentially regulated by co-injection of different cytokine genes. Application of this strategy to multistage, multiantigen malaria vaccine design could potentially influence the differentiation of Th cells at the site of inoculation and hence drive the localized immune response in an antigen-specific, tissue-targeted manner. Such a process would greatly improve the prophylactic and therapeutic efficacy of any candidate vaccine.

10. PERSPECTIVE

At the dawn of the new millennium, malaria still presents a major threat to the well-being of mankind in much the same way as it has since prehistory. Although the prospect of a commercially available vaccine to combat the disease is not immediate, in recent years significant progress has been made towards this goal through research aimed at determining points of susceptibility in the *Plasmodium* life cycle and targeting antigens which induce a protective response as immunogens in prototype vaccines. In order to understand the mechanisms by which protective immunity is elicited, both by exposure to natural infection and artificial induction through immunization with preparations of vaccine candidate antigens which ideally would provide a similar level of resistance more rapidly, experimental animal models provide a means for dissection and manipulation of the immune response *in vivo*. The work of our laboratory and others has made considerable strides in recognizing the involvement of $CD4^+$ T cells in polarizing immunity to malaria and of Th1- and Th2-type mediators in regulating the balance between protective and pathological facets of responsiveness to infection. Application of this knowledge to subunit vaccine development should provide vital clues to the way in which immunity may be driven by cytokines towards a favorable response and provide the basis for the use of certain cytokines, or possibly anti-cytokine antibodies or antagonists, as adjuvants for delivery of specified malarial antigenic determinants as vaccines. Presently, we are investigating both virus and bacteria as possible vaccine vectors (80, 91). The advent of nucleic acid vaccines provides an exciting opportunity to explore the suitability of cytokine adjuvants as tools to optimize the delivery of immunogens and to assess by what route of inoculation, and the mechanism by which, potentiation of vaccine-induced protective immunity may best be achieved. Co-delivery of cytokine genes along with a DNA vaccine construct encoding malarial antigen may represent the ultimate vehicle for modulation or enhancement of the peptide-specific immune response. The most appropriate use of a vaccine as a weapon in the war against malaria may not be in isolation in an attempt to eradicate from the world the *Plasmodium* parasites responsible for the disease, but rather in conjunction with drugs (31) and anti-mosquito measures to work towards reducing the impact of malaria on the demography and socioeconomic conditions of those countries in which the disease is endemic.

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12. REFERENCES

1. Engers H D & T Godal: Malaria vaccine development: current status. *Parasitol Today* 14, 56-64 (1998)

2. Miller L & S Hoffman: Research towards vaccines against malaria. *Science* 284, 520-524 (1998)

3. World Health Organization: Malaria control among refugees and displaced populations. *CTD/MAL* 96, 6- 7 (1996)

4. Taylor-Robinson A W: Immunoregulation of malarial infection: balancing the vices and virtues. *Int J Parasitol* 28, 135-148 (1998)

5. Wegmann T G, H Lin, L Guilbert & T R Mosmann: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 14, 353-356 (1993)

6. Marsh K: Immunology of human malaria. In: Bruce-Chwatt's essential malariology, 3rd edn. Eds: Gilles H M, Warrell D A, Edward Arnold, London, U.K., 60-77 (1993)

7. Nussenzweig R S, J Vanderberg, H Most & C Orton: Protective immunity produced by the injection of X- irradiated sporozoites of *Plasmodium berghei*. *Nature* 216, 160-162 (1967)

8. Gwadz R W, A H Cochrane, V Nussenzweig & R S Nussenzweig: Preliminary studies on vaccination of rhesus monkeys with irradiated sporozoites of *Plasmodium knowlesi* and characterization of surface antigens of these parasites. *Bull WHO* 57, suppl. 1, 165-173 (1979)

9. Clyde D F, V C McCarthy, R M Miller & R B Hornick: Specificity of protection of man immunized against sporozoite-induced falciparum malaria. *Am J Med Sci* 266, 398-401 (1973)

10. Cohen S, I A McGregor & S C Carrington: Gamma-globulin and acquired immunity to human malaria.*Nature* 192, 733-737 (1961)

11. McGregor I A, S Carrington & S Cohen: Treatment of East African *Plasmodium falciparum* malaria with West African human gammaglobulin. *Trans Roy Soc Trop Med Hyg* 57, 170-175 (1963)

12. Sabchareon A, T Burnouf, D Ouattara, P Attanath, H Bouharoun-Tayoun, P Chantavanich, C Foucault, T Chongsuphajaisiddhi & P Druilhe: Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. *Am J Trop Med Hyg* 45, 297-308 (1991)

13. Sinnis P & V Nussenzweig: Preventing sporozoite invasion of hepatocytes. In: Malaria vaccine development. Ed: Hoffman S L, ASM Press, Washington DC, 15-34 (1996)

14. Hoffman S L, E D Francke, M R Hollingdale & P Druilhe: Attacking the infected hepatocyte. In: Malaria vaccine development. Ed: Hoffman S L, ASM Press, Washington DC, 35-76 (1996)

15. Playfair J H L, J Taverne, C A W Bate & J B de Souza: The malaria vaccine: anti-parasite or anti-disease? *Immunol Today* 11, 25-27 (1990)

16. Bate CAW & D Kwiatkowski: Inhibitory immunoglobulin M antibodies to tumor necrosis factor- inducing toxins in patients with malaria. *Infect Immun* 62, 3086-3091 (1994)

17. Kaslow D C: Transmission-blocking vaccines: uses and current status of development. *Int J Parasitol* 27, 183-189 (1997)

18. Mosmann T R & R L Coffman: TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 7, 145-173 (1989)

19. Mosmann T R & S Sad: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17, 138-146 (1996)

20. Allen J E & R M Maizels: Th1-Th2: reliable paradigm or dangerous dogma? *Immunol Today* 18, 387-392 (1997)

21. Langhorne J, S Gillard, B Simon, S Slade & K Eichmann: Frequencies of $CD4^+$ T cells reactive with *Plasmodium chabaudi chabaudi*: distinct response kinetics for cells with Th1 and Th2 characteristics during infection. *Int Immunol* 1, 416-424 (1989)

22. Taylor-Robinson A W & R S Phillips: Functional characterization of protective $CD4^+$ T cell clones reactive to the murine malaria parasite *Plasmodium chabaudi*. *Immunology* 77, 99-105 (1992)

23. Taylor-Robinson A W & R S Phillips: Protective $CD4^+$ T cell lines raised against *Plasmodium chabaudi* show characteristics of either Th1 or Th2 cells. *Parasite Immunol* 15, 301-310 (1993)

24. Taylor-Robinson A W, R S Phillips, A Severn, S Moncada & F Y Liew: The role of Th1 and Th2 cells in a rodent malaria infection. *Science* 260, 1931-1934 (1993)

25. Stevenson M M & M-F Tam: Differential induction of helper T cell subsets during blood-stage *Plasmodium* chabaudi AS infection in resistant and susceptible mice. *Clin Exp Immunol* 91, 77-83 (1993)

26. Taylor-Robinson A W & E C Smith: Modulation of experimental blood stage malaria through blockade of the B7/CD28 T cell costimulatory pathway. *Immunology* 96, 498-504 (1999)

27. Taylor-Robinson A W: Regulation of immunity to malaria: valuable lessons learned from murine models. *Parasitol Today* 11, 334-342 (1995)

28. Taylor-Robinson A W & E C Smith: A role for cytokines in potentiation of malaria vaccines through immunological modulation of blood stage infection. *Immunol Rev* 171, 105-124 (1999)

29. Stevenson M M, M F Tam, S F Wolf & A Sher: IL-12-induced protection against blood-stage *Plasmodium chabaudi* AS requires IFN-gamma and TNF-alpha and occurs via a nitric oxide-dependent mechanism. *J Immunol* 155, 2545-2556 (1995)

30. Smith E. C. & A. W. Taylor-Robinson: Enhanced Th2 responses characterise control of blood stage malaria in the absence of IL-12. *Immunology* 98, suppl. 1, 94 (1999)

31. Mohan K, H Sam & M M Stevenson: Therapy with a combination of low doses of interleukin 12 and chloroquine completely cures blood-stage malaria, prevents severe anemia, and induces immunity to reinfection. *Infect Immun* 67, 513-519 (1999)

32. White N J: Treatment of malaria. *N Engl J Med* 335, 801-806 (1996)

33. Sedegah M, F Finkelman & S L Hoffman: Interleukin 12 induction of interferon gamma-dependent protection against malaria. *Proc Natl Acad Sci USA* 91, 10700-10702 (1994)

34. Hoffman S L, J M Crutcher, S K Puri, A A Ansari, F Villinger, E D Franke, P P Singh, F Finkelman, M K Gately, G P Dutta & M Sedegah: Sterile protection of monkeys against malaria after administration of interleukin-12. *Nat Med* 3, 80-83 (1997)

35. Metzger D W, R M McNutt, J T Collins, J M Buchanan, V H Van Cleave & W A Dunnick: Interleukin-12 acts as an adjuvant for humoral immunity through interferon-gamma-dependent and -independent mechanisms. *Eur J Immunol* 27, 1958-1965 (1997)

36. Linke A, R Kühn, W Müller, N Honarvar, C Li & J Langhorne: *Plasmodium chabaudi chabaudi*: differential susceptibility of gene-targeted mice deficient in IL-10 to an erythrocytic-stage infection. *Exp Parasitol* 84, 253-263 (1996)

37. Moore K W, A O'Garra, R De Waal Malefyt, P Vieira & T R Mosmann: Interleukin-10. *Annu Rev Immunol* 11, 165-190 (1993)

38. van der Hyde H C, B Pepper, J Batchelder, F Cigel & W P Weidanz: The time course of selected malarial infections in cytokine-deficient mice. *Exp Parasitol* 85, 206-213 (1997)

39. Taylor-Robinson A W & E C Smith: A dichotomous role for nitric oxide in protection against blood stage malaria infection. *Immunol Lett* 67, 1-9 (1999)

40. Wahl S M, N McCartney-Francis & S E Mergenhagen: Inflammatory and immunomodulatory roles of TGF-beta. *Immunol Today* 10, 258-261 (1989)

41. Ding A, C F Nathan, J Grayear, R Derynck, D J Stuehr & S Srimai: Macrophage deactivating factor and transforming growth factor-beta1, -beta2 and -beta3 inhibit induction of macrophage nitrogen oxide synthesis by interferon-gamma. *J Immunol* 145, 940-944 (1990)

42. Bellone G, M Aste-Amezaga, G Trinchieri & U Rodeck: Regulation of NK cell functions by TGF-beta1. *J Immunol* 155, 1066-1073 (1995)

43. Maeda H & A Shiraishi: TGF-beta contributes to the shift toward Th2-type responses through direct and IL-10-mediated pathways in tumor-bearing mice. *J Immunol* 156, 73-78 (1996)

44. Omer FM & E M Riley: Transforming growth factor beta production is inversely correlated with severity of murine malaria infection. *J Exp Med* 188, 39-48 (1998)

45. Wenisch C, B Parschalk, E Narzt, S Looareesuwan & W Graninger: Elevated serum levels of IL-10 and IFN-gamma in patients with acute *Plasmodium falciparum* malaria. *Clin Immunol Immunopathol* 74, 115-117 (1995)

46. Wahlgren M, J S Abrams, V Fernandez, M T Bejarano, M Azuma, M Torii, M Aikawa & R J Howard: Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells and secretion of cytokines (IL- 1-beta, IL-1Ralpha, IL-6, IL-8, IL-10, TGF beta, TNF alpha, G-CSF, GM-CSF). *Scand J Immunol* 42, 626-636 (1995)

47. Wenisch C, B Parschalk, H Burgmann, S Looareesuwan & W Graninger: Decreased serum levels of TGF- beta in patients with acute *Plasmodium falciparum* malaria. *J Clin Immunol* 15, 69-73 (1995)

48. Siddiqui W A, S C Kan, K Kramer, S Case, K Palmer & J F Niblack: Use of a synthetic adjuvant in effective vaccination of monkeys against malaria. *Nature* 289, 64-66 (1981)

49. Lewis A P: Cloning and analysis of the gene encoding the 230-kDa merozoite surface antigen of *Plasmodium yoelii*. *Mol Biochem Parasitol* 36, 271-282 (1989)

50. McKean P G, K P O'Dea & K N Brown: Nucleotide sequence analysis and epitope mapping of the merozoite surface protein 1 from *Plasmodium chabaudi chabaudi* AS. *Mol Biochem Parasitol* 68, 199- 209 (1993)

51. Giraldo L E, D J Grab & M F Wiser: Molecular characterization of a *Plasmodium chabaudi* erythrocyte membrane-associated protein with glutamate-rich tandem repeats. *J Eukaryot Microbiol* 45, 528-534 (1998)

52. Cooper J A: Merozoite surface antigen-1 of *Plasmodium*. *Parasitol Today* 9, 50-54 (1993)

53. Riley EM, S J Allen, J G Wheeler, M J Blackman, S Bennett, B Takacs, H J Schonfeld, A A Holder & B M Greenwood: Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced malaria morbidity. *Parasite Immunol* 14, 321-337 (1992)

54. Kramer K J & R Oberst: Antibodies to the major merozoite surface coat protein of *Plasmodium falciparum* (gp195) in a human population living in a malaria-endemic area of the Phillipines. *Am J Trop Med Hyg* 47, 429-439 (1992)

55. Egan A F, J Morris, G Barnish, S Allen, B M Greenwood, D C Kaslow, A A Holder & E M Riley: Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C- terminal fragment of the merozoite surface antigen, PfMSP-1. *J Infect Dis* 173, 765-769 (1996)

56. Siddiqui W A, L Q Tam, K J Kramer, G S Hui, S E Case, K M Yamaga, S P Chang, E B Chan & S C Kan: Merozoite surface coat precursor protein completely protects *Aotus* monkeys against *Plasmodium falciparum* malaria. *Proc Natl Acad Sci USA* 84, 3014-3018 (1987)

57. Holder A A: Preventing merozoite invasion of erythrocytes. In: Malaria vaccine development. Ed: Hoffman S L, ASM Press, Washington DC, 77-104 (1996)

58. Daly T M & C A Long: A recombinant 15-kDa carboxyl-terminal fragment of *Plasmodium yoelii yoelii* 17XL merozoite surface protein-1 induces a protective immune response in mice. *Infect Immun* 61, 2462-2467 (1993)

59. Coppel R L, A F Cowman, R F Anders, A E Bianco, R B Saint, K R Lingelbach, D J Kemp & G V Brown: Immune sera

recognize on erythrocytes *Plasmodium falciparum* antigen composed of repeated amino acid sequences. *Nature* 310, 789-792 (1984)

60. Perlmann H, P Perlmann, K Berzins, B Wåhlin, M Troye-Blomberg, M Hagstedt, I Andersson, B Hogh, E Petersen & A Bjorkman: Dissection of the human antibody response to the malaria antigen Pf155/RESA into epitope-specific components. *Immunol Rev* 112, 115-132 (1989)

61. Kabilan L, M Troye-Blomberg, H Perlmann, G Andersson, B Hogh, E Petersen, A Bjorkman & P Perlmann: T-cell epitopes in Pf155/RESA, a major candidate for a *Plasmodium falciparum* malaria vaccine. *Proc Natl Acad Sci USA* 85, 5659-5663 (1988)

62. Favaloro J M, R L Coppel, L M Corcoran, S J Foote, G V Brown, R F Anders & D J Kemp: Structure of the RESA gene of *Plasmodium falciparum*. *Nucleic Acids Res* 14, 8265-8277 (1986)

63. Berzins K, H Perlmann, B Wåhlin, J Carlsson, M Wahlgren, R Udomsangpetch, A Bjorkman, M E Patarroyo & P Perlmann: Rabbit and human antibodies to a repeated amino acid sequence of a *Plasmodium falciparum* antigen, Pf155, react with the native protein and inhibit merozoite invasion. *Proc Natl Acad Sci USA* 83, 1065-1069 (1986)

64. Wåhlin B, A Sjölander, N Ahlborg, R Udomsangpetch, A Scherf, D Mattei, K Berzins & P Perlmann: Involvement of Pf155/RESA and cross-reactive antigens in *Plasmodium falciparum* merozoite invasion *in vitro*. *Infect Immun* 60, 443-449 (1992)

65. Collins W E, R F Anders, M Pappaioanou, G H Campbell, G V Brown, D J Kemp, R L Coppel, J C Skinner, P M Andrysiak, J M Favaloro, L M Corcoran, J R Broderson, G F Mitchell & C C Campbell: Immunization of *Aotus* monkeys with recombinant proteins of an erythrocyte surface antigen of *Plasmodium falciparum*. *Nature* 323, 259-262 (1986)

66. Berzins K, H Perlmann, B Wåhlin, H P Ekre, B Hogh, E Petersen, B Wellde, M Schoenbechler, J Williams, J Chulay & P Perlmann: Passive immunization of *Aotus* monkeys with human antibodies to the *Plasmodium falciparum* antigen Pf155/RESA. *Infect Immun* 59, 1500-1506 (1991)

67. Collins W E, R F Anders, T K Ruebush, D J Kemp, G C Woodrow, G H Campbell, G V Brown, D O Irving, N Goss, V K Filipski, R L Coppel, J R Broderson, L M Thomas, D Pye, J C Skinner, C Wilson, P S Stanfill & P M Procell: Immunization of owl monkeys with the ring-infected erythrocyte surface antigen of *Plasmodium falciparum. Am J Trop Med Hyg* 44, 34-41 (1991)

68. Riley E M, S J Allen, M Troye-Blomberg, S Bennett, H Perlmann, G Andersson, L Smedman, P Perlmann & B M Greenwood: Association between immune recognition of the malaria vaccine candidate Pf155/RESA and resistance to clinical disease: a prospective study in a malaria-endemic region of West Africa. *Trans Roy Soc Trop Med Hyg* 85, 436-443 (1991)

69. Berzins K & P Perlmann: Malaria vaccines: attacking infected erythrocytes. In: Malaria vaccine development. Ed: Hoffman S L, ASM Press, Washington DC, 105-143 (1996)

70. Crandall I & I W Sherman: Cytoadherence-related neoantigens on *Plasmodium falciparum* (human malaria)-infected human erythrocytes result from the exposure of normally cryptic regions of the band 3 protein. *Parasitology* 108, 257-267 (1994)

71. Baruch D I, B L Pasloske, H B Singh, X Bi, X C Ma, M Feldman, T F Taraschi & R J Howard: Cloning the *P. falciparum* gene encoding PfEMP-1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell* 82, 77-87 (1995)

72. Su X-Z, V M Heatwole, S P Wertheimer, F Guinet, J A Herrfeldt, D S Peterson, J A Ravetch & T E Wellems: The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell* 82, 89-100 (1995)

73. McLean S A, C D Pearson & R S Phillips: *Plasmodium chabaudi*: evidence of antigenic variation during recrudescent parasitaemias in mice. *Exp Parasitol* 54, 296-302 (1982)

74. Phillips R S, L R Brannan, P Balmer & P Neuville: Antigenic variation during malaria infection - the contribution from the murine parasite *Plasmodium chabaudi*. *Parasite Immunol* 19, 427-434 (1997)

75. Morein B & K L Bengtsson: Immunomodulation by iscoms, immune stimulating complexes. *Methods* 19, 94-102 (1999)

76. Mastico R A, S J Talbot & P G Stockley: Multiple presentation of foreign peptides on the surface of an RNA-free spherical bacteriophage capsid. *J Gen Virol* 74, 541-548 (1993)

77. Sidney J, H M Grey, S Southwood, E Celis, P A Wentworth, M F del Guercio, R T Kubo, R W Chesnut & A Sette: Definition of an HLA-A3-like supermotif demonstrates the overlapping peptide-binding repertoires of common HLA molecules. *Human Immunol* 45, 79-93 (1996)

78. Falk K, O Rötzschke, S Stevanovic´, G Jung & H-G Rammensee: Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351, 290-296 (1991)

79. Connelly M, C L King, K Bucci, S Walters, B Genton, M P Alpers, M Hollingdale & J W Kazura: T-cell immunity to peptide epitopes of liver-stage antigen 1 in an area of Papua New Guinea in which malaria is holoendemic. *Infect Immun* 65, 5082-5087 (1997)

80. Heal K G, H R Hill, P G Stockley, M R Hollingdale & A W Taylor-Robinson: Expression and immunogenicity of a liver stage malaria epitope presented as a foreign peptide on the surface of RNA- free MS2 bacteriophage capsids. *Vaccine* 18, 251-258 (1999)

81. Dougan G, C E Hormaeche & D J Maskell: Live oral *Salmonella* vaccines: potential use of attenuated strains as carriers of heterologous antigens to the immune system. *Parasite Immunol* 9, 151-160 (1987)

82. Li S, M Rodrigues, D Rodriguez, J R Rodriguez, M Esteban, P Palese, R S Nussenzweig & F Zavala: Priming with recombinant influenza virus followed by administration of recombinant vaccinia virus induces $CD8^+$ T-cell-mediated protective immunity against malaria. *Proc Natl Acad Sci USA* 90, 5214- 5218 (1993)

83. Tine J A, D E Lanar, D M Smith, B T Wellde, P Schultheiss, L A Ware, E B Kauffman, R A Wirtz, C de Taisne, G S Hui, S P Chang, P Church, M R Hollingdale, D C Kaslow, S Hoffman, K P Guito, W R Ballou, J C Sadoff & E Paoletti: NYVAC-Pf7: a poxvirus-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. *Infect Immun* 64, 3833-3844 (1996)

84. Ockenhouse C F, P-F Sun, D E Lanar, B T Wellde, B T Hall, K Kester, J A Stoute, A Magill, U Krzych, L Farley, R A Wirtz, J C Sadoff, D C Kaslow, S Kumar, L W P Church, J M Crutcher, B Wizel, S Hoffman, A Lalvani, A V S Hill, J A Tine, K P Guito, C de Taisne, R Anders, T Horii , E Paoletti & W R Ballou: Phase I/IIa safety, immunogenicity and efficacy trial of NYVAC-Pf7, a pox-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. *J Infect Dis* 177, 1664-1673 (1998)

85. Sadoff J C, W R Ballou, L S Baron, W R Majarian, R N Brey, W T Hockmeyer, J F Young, S J Cryz, J Ou, G H Lowell & J D Chulay: Oral *Salmonella typhimurium* vaccine expressing circumsporozoite protein protects against malaria. *Science* 240, 336-338 (1988)

86. Flynn J L, W R Weiss, K A Norris, H S Seifert, S Kumar & M So: Generation of a cytotoxic T- lymphocyte response using a *Salmonella* antigen-delivery system. *Mol Microbiol* 4, 2111-2118 (1990)

87. González C, D Hone, F R Noriega, C O Tacket, J R Davis, G Losonsky, J P Nataro, S Hoffman, A Malik, E Nardin, M B Sztein, D G Heppner, T R Fouts, A Isibasi & M M Levine: *Salmonella typhi* vaccine strain CVD 908 expressing the circumsporozoite protein of *Plasmodium falciparum*: strain construction and safety and immunogenicity in humans. *J Infect Dis* 169, 927-931 (1994)

88. Schorr J, B Knapp, E Hundt, H A Kupper & E Amann: Surface expression of malarial antigens in *Salmonella typhimurium*: induction of serum antibody response upon oral vaccination of mice. *Vaccine* 9, 675-681 (1991)

89. Haddad D, S Liljeqvist, S Kumar, M Hansson, S Ståhl, H Perlmann, P Perlmann & Berzins K: Surface display compared to periplasmic expression of a malarial antigen in *Salmonella typhimurium* and its implications for immunogenicity. *FEMS Immunol Med Microbiol* 12, 175-185 (1995)

90. Chatfield S N, M Roberts, G Dougan, C Hormaeche & C M A Khan: The development of oral vaccines against parasitic diseases utilizing live attenuated Salmonella. *Parasitology* 110, suppl., S17-S24 (1995)

91. Heal K G, A W Taylor-Robinson, G McConkey, C E Hormaeche, C M A Khan & M R Hollingdale: Immunogenicity of *Plasmodium falciparum* liver stage antigen-1 peptides expressed as tetanus toxin fragment C fusion proteins in attenuated *Salmonella typhimurium. Immunology* 95, suppl. 1, 106 (1998)

92. Ulmer J B, J C Sadoff & M A Liu: DNA vaccines. *Curr Opin Immunol* 8, 531-536 (1996)

93. Schorr J, P Moritz & M Schleef: Plasmid DNA preparation. Production of plasmid DNA in industrial quantities according to cGMP guidelines. In: DNA vaccines. Methods in molecular medicine. Ed. Lowrie D B, Humana Press, Totowa, Fl, 1-18 (1997)

94. Fynan E F, R G Webster, D H Fuller, J R Haynes, J C Santoro & H L Robinson: DNA vaccines: protective immunizations by parenteral, mucosal and gene-gun inoculations. *Proc Natl Acad Sci USA* 90, 11478- 11482 (1993)

95. Darji A, C A Guzman, B Gerstel, P Wachholz, K N Timmis, J Wehland, T Chakraborty & S Weiss: Oral somatic transgene vaccination using attenuated *S. typhimurium. Cell* 91, 765-775 (1997)

96. Lowrie D B: DNA vaccination exploits normal biology. *Nature Med* 4, 147-148 (1998)

97. Fu T M, J B Ulmer, M J Caulfield, R R Deck, A Friedman, S Wang, X Liu, J J Donnelly & M A Liu: Priming of cytotoxic T lymphocytes by DNA vaccines: requirement for professional antigen presenting cells and evidence for antigen transfer from myocytes. *Mol Med* 3, 362-371 (1997)

98. Feltquate D M, S Heaney, R G Webster & H L Robinson: Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. *J Immunol* 158, 2278-2284 (1997)

99. Raz E, H Tighe, Y Sato, M Corr, J A Dudler, M Roman, S L Swain, H L Spiegelberg & D A Carson: Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc Natl Acad Sci USA* 93, 5141-5145 (1996)

100. Roman M, E Martin-Orozco, J S Goodman, M D Nguyen, Y Sato, A Ronaghy, R S Kornbluth, D D Richman, D A Carson & E Raz: Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. *Nature Med* 3, 849-854 (1997)

101. Hengge U R, E F Chan, R A Foster, P S Walker & J C Vogel: Cytokine gene expression in epidermis with biological effects following injection of naked DNA. *Nature Genet* 10, 161-166 (1995)

102. Xiang Z & H Ertl: Manipulation of the immune response to a plasmid-encoded viral antigen by coinoculation with plasmids expressing cytokines. *Immunity* 2, 129-135 (1995)

103. Lewis P J, G J M Cox, S D van Drunen Littel-van den Hurk & L A Babiuk: Polynucleotide vaccines in animals:

enhancing and modulating responses. *Vaccine* 15, 861-864 (1997)

104. Haddad D, S Liljeqvist, S Ståhl, P Perlmann, K Berzins & N Ahlborg: Differential induction of immunoglobulin G subclasses by immunization with DNA vectors containing or lacking a signal sequence. *Immunol Lett* 61, 201-204 (1998)

105. Barry M & S A Johnston: Biological features of genetic immunization. *Vaccine* 15, 788-791 (1997)

106. Taylor-Robinson A W & R S Phillips: Infective dose modulates the balance between Th1- and Th2- regulated immune responses during blood stage malaria infection. *Scand J Immunol* 48, 527-534 (1998)

107. Kuhröber A, J Wild, H-P Pudollek, F V Chisari & J Reimann: DNA vaccination with plasmids encoding the intracellular (HBcAg) or secreted (HBeAg) form of the core protein of hepatitis B virus primes T cell responses to two overlapping K^b-and K^d-restricted epitopes. *Int Immunol* 9, 1203-1212 (1997)

108. Haddad D, S Liljeqvist, S Ståhl, I Andersson, P Perlmann, K Berzins & N Ahlborg: Comparative study of DNA-based immunization vectors: effect of secretion signals on the antibody responses in mice. *FEMS Immunol Med Microbiol* 18, 193-202 (1997)

109. Michel M L, H L Davis, M Schleef, M Mancini, P Tiollais & R G Whalen: DNA-mediated immunization to the hepatitis B antigen in mice: aspects of the humoral response mimic hepatitis B viral infection in humans. *Proc Natl Acad Sci USA* 92, 5307-5311 (1995)

110. Davis H L, C L Brazolot Millan & S C Watkins: Immune-mediated destruction of transfected muscle fibers after direct gene transfer with antigen-expressing plasmid DNA. *Gene Therapy* 4, 181-188 (1997)

111. Kuhröber A, H-P Pudollek, K Reifenberg, P V Chisari, H J Schlicht, J Reimann & R Schirmbeck: DNA immunization induces antibody and cytotoxic T cell responses to hepatitis B core antigen in H-2^b mice. *J Immunol* 156, 3687-3695 (1996)

112. Cardoso A I, M Blixenkrone-Moller, J Fayolle, M Liu, R A Buckland & T F Wild: Immunization with plasmid DNA encoding for the measles virus hemagglutinin and nucleoprotein leads to humoral and cell- mediated immunity. *Virology* 225, 293-299 (1996)

113. Hoffman S L, D L Doolan, M Sedegah, J C Aguiar, R Wang, A Malik, R A Gramzinski, W R Weiss, P Hobart, J A Norman, M Margalith & R C Hedstrom: Strategy for development of a pre-erythrocytic *Plasmodium falciparum* DNA vaccine for human use. *Vaccine* 15, 842-845 (1997)

114. Weiss W R, K J Ishii, R C Hedstrom, M Sedegah, M Ichino, K Barnhart, D M Klinman & S L Hoffman: A plasmid encoding murine granulocyte-macrophage colony stimulating factor increases protection conferred by a malaria DNA vaccine. *J Immunol* 161, 2325-2332 (1998)

115. Doolan D L, R C Hedstrom, M J Gardner, M Sedegah, H Wang, R A Gramzinski, M Margalith, P Hobart & S L Hoffman: DNA vaccination as an approach to malaria control: current status and strategies. *Curr Top Microbiol Immunol* 226, 37-56 (1998)

116. Good M: Towards the development of the ideal malaria vaccine. A decade of progress in a difficult field. *Med J Aust* 154, 284-289 (1991)

117. Sedegah M, R Hedstrom, P Hobart & S L Hoffman: Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein. *Proc Natl Acad Sci USA* 91, 9866-9870 (1994)

118. Doolan D L, M Sedegah, R C Hedstrom, P Hobart, Y Charoenvit & S L Hoffman: Circumventing genetic restriction of protection against malaria with multigene DNA immunization: $CD8^+$ T cell-, interferon gamma-, and nitric oxide-dependent immunity. *J Exp Med* 183, 1739-1746 (1996)

119. Charoenvit Y, S Mellouk, M Sedegah, T Toyoshima, M F Leef, P De la Vega, R L Beaudoin, M Aikawa, V Fallarme & S L Hoffman: *Plasmodium yoelii*: 17-kDa hepatic and erythrocytic stage protein is the target of an inhibitory monoclonal antibody. *Exp Parasitol* 80, 419-429 (1995)

120. Wang R, D L Doolan, Y Charoenvit, R C Hedstrom, M J Gardner, P Hobart, J Tine, M Sedegah, V Fallarme, J B Sacci Jr, M Kaur, D M Klinman, S L Hoffman & W R Weiss: Simultaneous induction of multiple antigen-specifc cytotoxic T lymphocytes in nonhuman primates by immunization with a mixture of four *Plasmodium falciparum* DNA plasmids. *Infect Immun* 66, 4193-4202 (1998)

121. Wang R, D L Doolan, T P Le, R C Hedstrom, K M Coonan, Y Charoenvit, T R Jones, P Hobart, M Margalith, J Ng, W R Weiss, M Sedegah, C de Taisne, J A Norman & S L Hoffman: Induction of antigen- specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. *Science* 282, 476-480 (1998)

122. Taylor-Robinson A W: Malarial vaccine? *Trends Microbiol* 7, 15 (1999)

123. Chow Y H, B L Chiang, Y L Lee, W K Chi, W C Lin, Y T Chen & M H Tao: Development of Th1 and Th2 populations and the nature of immune responses to hepatitis B virus DNA vaccines can be modulated by codelivery of various cytokine genes. *J Immunol* 160, 1320-1329 (1998)

124. Barouch D H, S Santra, T D Steenbeke, X X Zheng, H C Perry, M E Davies, D C Freed, A Craiu, T B Strom, J W Shiver & N L Letvin: Augmentation and suppression of immune responses to an HIV-1 DNA vaccine by plasmid cytokine/Ig administration. *J Immunol* 161, 1875-1882 (1998)

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Send correspondence to: Dr. A.W. Taylor-Robinson, School of Biology, University of Leeds, Clarendon Way, Leeds LS2 9JT, United Kingdom, Tel: (44)-113-233-2893, Fax: (44)-113-233-2882, E-mail: bgyawtr@leeds.ac.uk

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