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Malaria vaccines: if at first you don't succeed...

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The Roll Back Malaria campaign vowed to halve the global burden of malaria in ten years but, midway into that campaign, few new malaria control tools have been introduced, and many established methods appear to be failing with effective chemotherapy being perhaps the most problematic. It has been repeatedly argued that the discovery and implementation of a safe and effective vaccine against malaria is a major priority in the control of the disease. Indeed, many malaria control experts believe that sustainable reductions in malaria control will be nigh on impossible in the absence of such a vaccine. While most would agree that we are still some way from being able to introduce a vaccine, steady progress is being made. We review here some new approaches and developments in vaccine research that were discussed at the Molecular Approaches to Malaria conference held 1–5 February 2004 in Lorne, Australia.

In 1998, the Roll Back Malaria (RBM) campaign set itself the goal of halving the global burden of malaria by 2010 by combining advocacy for malaria control with the implementation of control methods of established efficacy [i.e. case management (diagnosis and chemotherapy) and integrated vector control (insecticide-treated bednets and residual house spraying)]. However, with the midpoint of the RBM campaign upon us, the emergence and/or resurgence of malaria in many parts of the world might have resulted in an estimated net increase, rather than decrease, of malaria cases [1,2], suggesting that these control methods alone are not enough to achieve worldwide reductions in the burden of malaria. Vaccination has been viewed as the magic bullet in the public health arsenal, with the successful smallpox and current polio eradication campaigns highlighting their potential for a dramatic impact on human health. An antimalarial vaccine has long been a public health priority but, despite extensive research, a safe, effective and affordable antimalarial vaccine remains elusive.

Several candidate vaccines have been developed and tested, with varying efficacy against malaria (Figure 1). The empirical approaches to vaccine design – pathogen inactivation and attenuation – that have proved so successful for some viral diseases have traditionally been viewed as impractical for malaria. However, this viewpoint might now be changing, with the case being made for attempting to develop a commercial attenuated sporozoite vaccine [3], and for the concept of protection by a challenge with ultra-low dose blood-stage parasites [4]. Whether these approaches will be any more successful than the more traditional subunit vaccine approach for malaria remains to be seen.

The acquisition of effective antimalarial immunity in endemic populations keeps alive the hope that a vaccine is possible. However, because blood-stage parasites are ubiquitous in all age groups in highly endemic settings, 'immunity' in this context implies 'immune to disease' and not 'immune to infection'. The natural state of immunity to malaria is one of premunition, whereby immune effector mechanisms maintain low densities of parasites, and chronic asymptomatic infection appears necessary for long-term maintenance of immune memory and effective clinical immunity [5]. Furthermore, the host immune response is implicated in the aetiology of severe complications of malaria, driven by an uncontrolled cycle of antiparasite inflammatory responses emanating from both the innate and adaptive arms of the immune system [6]. The potential for vaccines to induce severe (even fatal) pathology despite very effective control of parasite replication was recently demonstrated in a study of a murine malaria vaccine [7]. Thus, an ideal malaria vaccine must elicit an immune response that overcomes the evasive and immunomodulatory defences of the parasite, while maintaining a balance between antiparasitic effector mechanisms and immune-mediated pathology.

Fulfilling these requirements will be a major challenge, but the pool of candidate antigens has been expanded in recent years and novel vaccine designs such as prime-boost strategies [8] are showing promise. The four years since the first Molecular Approaches to Malaria (MAM) meeting, held 2–5 February 2000 in Lorne, Australia, have seen remarkable progress in our understanding of the parasite, vector and human genome and proteome, promising a significant shift in approaches to vaccine development.

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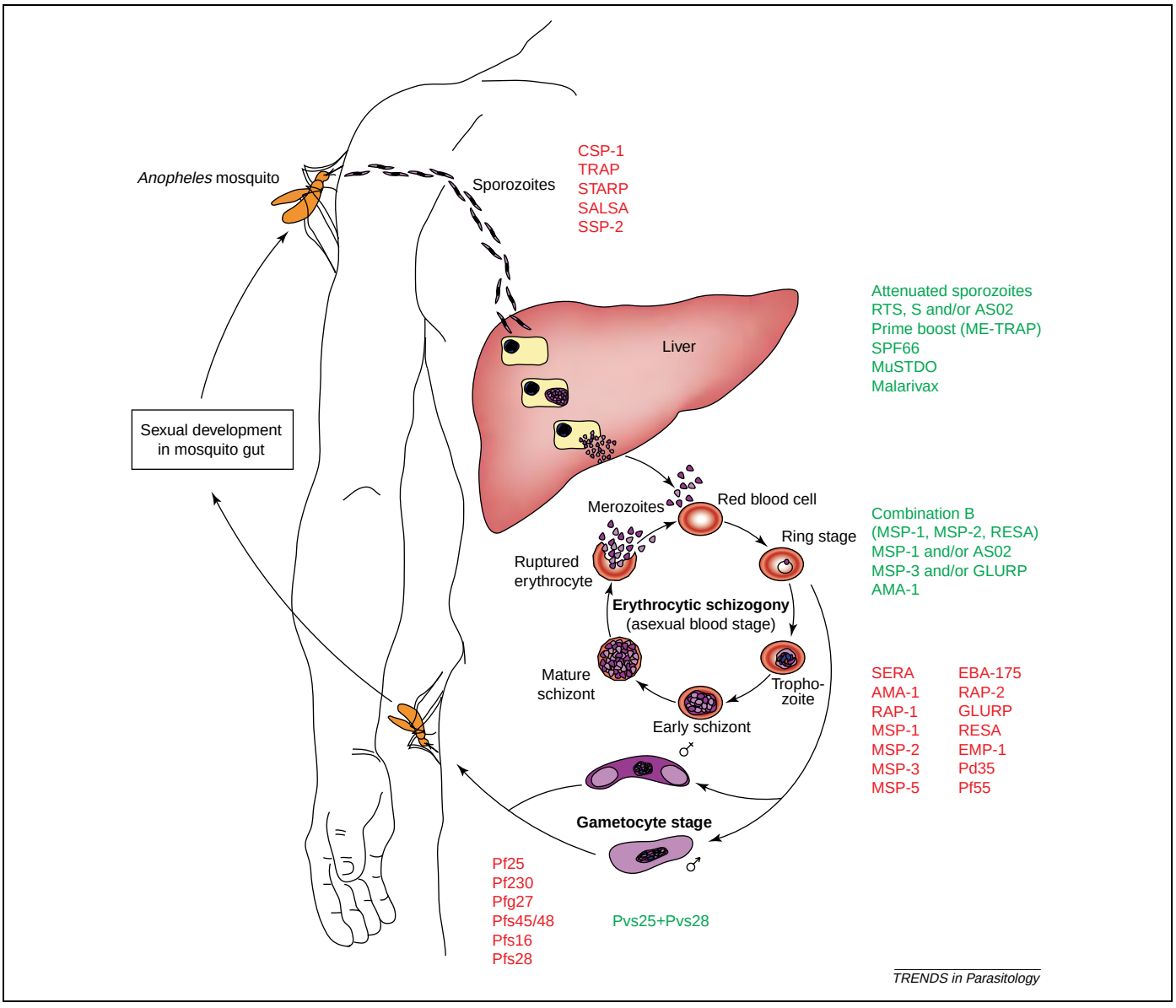


Figure 1. Antigens developed for malaria vaccines. Life cycle of *Plasmodium falciparum* showing individual antigens that are being, or have been, evaluated as vaccine candidates (in red), and vaccine constructs that are currently being, or have been, evaluated in clinical trials (in green), for each stage of the life cycle. Abbreviations: AMA, apical membrane antigen; CSP, circumsporozoite surface protein; EBA, erythrocyte-binding antigen; EMP, erythrocyte membrane protein; GLURP, glutamate-rich protein; ME–TRAP, multiple epitope–thrombospondin-related adhesive protein; MSP, merozoite surface protein; *Pf, Plasmodium falciparum* protein; *Pv, Plasmodium vivax* protein; RAP, rhoptry-associated protein; RESA, ring-infected erythrocyte surface antigen; SALSA, sporozoite- and liver-stage antigen; SERA, serine-repeat antigen; SPf66, synthetic *P. falciparum* 66; SSP, sporozoite surface protein; STARP, sporozoite threonine- and asparagine-rich protein; TRAP, thrombospondin-related adhesive protein.

Seeking a needle in a database haystack

One possible explanation for the limited success of malaria vaccine development is that we have not selected the most ideal target antigens to incorporate into the malaria vaccine constructs. Although the leading vaccine candidates, or their orthologues in other *Plasmodium* spp., have been shown to induce significant protection in model systems where their immune responses have been well characterized in terms of immunogenicity and immune effector mechanisms [9–11], very few of the many stage-specific malaria antigens that have been characterized have been used in malaria vaccine development [12,13]. Some of our favourite antigens are not particularly highly immunogenic and, even after years of exposure, individuals residing in malaria-endemic settings demonstrate relatively weak cellular and humoral responses to conserved epitopes of, for example, merozoite surface protein-1 (MSP-1) and circumsporozoite surface protein (CSP) (for a review, see Ref. [5]). Moreover, most of the malaria antigens under study are polymorphic and/or clonally variable with transient expression during specific stages of the life cycle, raising concerns over the potential for cross-protection. Broadening the list of possible vaccine candidates could allow us to overcome some of these issues.

In 2002, *Nature* published a series of articles that provided the blueprint for future vaccine development in the context of improved genomic and proteomic information. *Plasmodium* genomics (otherwise known as PlasmodiOMICS) was established by the publication of a reference sequence for the entire *Plasmodium falciparum* genome [14], the genome sequence of the rodent malaria

parasite *Plasmodium yoelii yoelii* [15] and the launch of the *Plasmodium* genome database [16], providing bioinformatics tools essential for genomic analyses. Microarray analyses, on several platforms [e.g. glass slide complementary DNA (cDNA) arrays, glass slide oligonucleotide arrays and Affymetrix™ photolithographic arrays] have supplemented the sequence data with genome-wide transcript profiling studies, mapping gene expression patterns throughout the asexual intraerythrocytic developmental cycle of *P. falciparum*, and in sporozoites and gametocytes [17–19]. Concomitant with the genomic and expression data, proteomic analyses (protein identification) have been carried out for four stages (sporozoites, merozoites, trophozoites and gametocytes) of the *P. falciparum* life cycle by high-throughput multidimensional protein-identification technology (MudPIT) [20]. In addition, a large-scale mass spectrometric proteome was constructed identifying conserved secreted and membrane-associated stage-specific proteins [21]. These resources will be invaluable for identifying new target antigens for vaccine research [22,23], but perhaps the most exciting results to emerge from these studies is the recognition that most *Plasmodium* genes are expressed in a rigorously regulated cycle, allowing candidate vaccine antigens to be identified based on the known expression patterns of antigens that already show some promise.

The plethora of malaria genomic and proteomic data generated in recent years is accessible via an integrated *Plasmodium* genome database (http://PlasmoDB.org), providing powerful bioinformatics tools that facilitate mining of the database in response to specific queries. PlasmoDB integrates sequence information with other genomic-scale data emerging from the *Plasmodium* research community, including gene expression analyses, microarray projects and proteomics studies [24]. New strategies, computational models and approaches are constantly being developed to facilitate and empower genomic and proteomic analyses [24–26] although, of course, the use of database-based approaches will always be limited by the ability to define relevant research questions and parameters [27].

A sample vaccine candidate query in PlasmoDB is represented in Table 1, based on three specific attributes: (i) secreted proteins, identified by the presence of a signal peptide (651 hits) [this query could also be expanded to include proteins with putative transmembrane domains or glycosylphosphatidylinositol (GPI) anchors]; (ii) proteins with a phylogenetic profile indicating conservation in *P. yoelii*, a malaria species of rodent, but not in the human genome (2260 hits) (this query could also be expanded to include other *Plasmodium* spp., or to exclude proteins with putative orthologues in mouse); and (iii) a transcript expression profile targeting genes that are most abundant in the late schizogony (merozoite release) stage of the life cycle (247 hits) (this query could be modified to further expand or restrict the desired window of transcript timing, abundance or regulation, to include data from multiple expression profile datasets, or to include proteomics). The intersection of these three simple queries yields a list of 26 hits that includes MSP-1, apical membrane antigen-1 (AMA-1), serine-rich antigen and 23 additional genes, including many previously unexamined hypothetical proteins. Thus, one simple interrogation of the database yields a significant number of novel, and potentially important, vaccine candidates (Table 1). These genes require experimental analysis at the bench and could potentially identify novel targets to bolster the current vaccine candidate list.

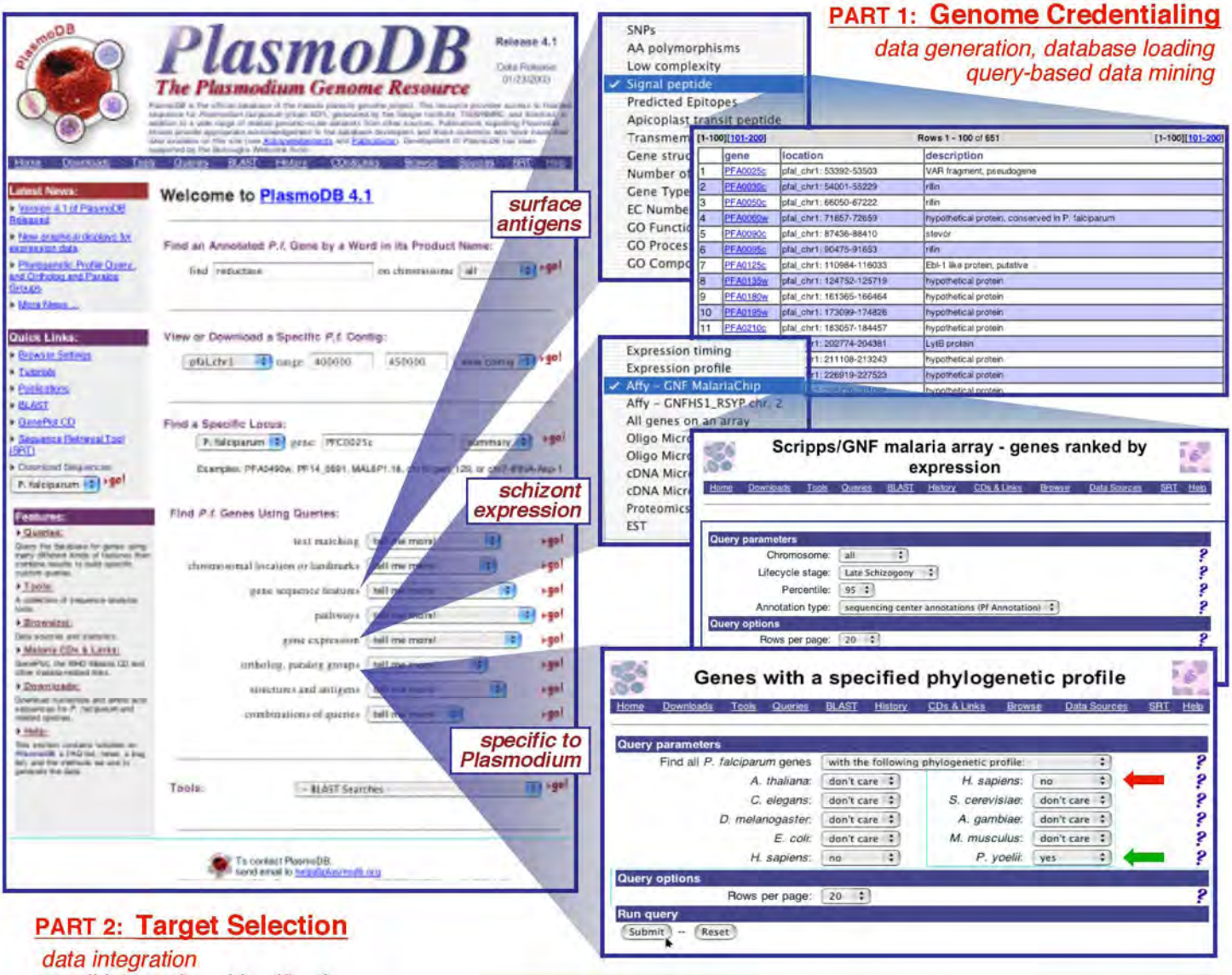
Back to the future: new hope from old ideas

The prospects for developing an antimalarial vaccine have not always been seen as a cause for doom and gloom. A safe and protective antimalarial vaccine incorporating attenuated (irradiated) *P. falciparum* sporozoites was first administered to humans in 1973 [28], founded on the pioneering rodent work of Ruth Nussenzweig and Jerry

Table 1. PlasmoDB vaccine candidate query[a]

Parameters	Sequencing center annotation	No. of hits	Example hits			
			No.	Gene	Location	Description
Query 1: Genes whose proteins contain a predicted signal peptide	Pf	651	1	PFA0025c	Pfal_chr1:53392–53503	Var fragment, pseudogene
			2	PFA0030c	Pfal_chr1:54001–55229	Rifin
			4	PFA0060w	Pfal_chr1:71857–72659	Hypothetical protein, conserved in Pf
Query 2: Genes with (or without) a specific phylogenetic profile (orthologues)	Pf Exclude *Homo sapiens* Include Py	2260	1	PF11_0274	Chr11:1027592–1028394	Hypothetical protein
			5	MAL6P1.56	Chr6:272734–274282	ST kinase, putative
			6	PF10_0154	Chr10:633681–635285	Ribonucleotide reductase, small subunit, putative
			1	PFB120w	Pfal_chr2:127994–128314	Hypothetical protein
Query 3: Genes ranked by expression using the Pf TSRI/GNF malaria array	Pf 95% or above in late schizogony	247	3	PFB0300c	Pfal_chr2:273689–274507	MSP-2
			6	PF14_0598	Chr14:2558046–2559295	Glyceraldehyde-3-phosphate dehydrogenase
			1	PFA0210c	Pfal_chr1: 183057–184457	Hypothetical protein
History: Intersect the three malaria vaccine candidate queries	Query 1 Query 2 Query 3	26	12	PFI1475W	Pfal_chr9: 1201802–1206964	MSP-1
			15	PF11_0344	Chr11:1290767–1292635	AMA-1

[a]Searching for secreted proteins, expressed during late schizogony, with homology to rodent malaria and with minimal homology to human proteins gives 26 hits. The other parameters not listed in table [including orthologue group and group size (phylogenetic analysis query), average intensity and percentile (gene expression using Pf TSRI/GNF malaria array query] are available on: http://www.PlasmoDB.org. This table is adapted from Ref. [27]. Abbreviations: AMA, apical membrane antigen; GNF, Genomics Institute, Novartis Research Foundation (http://www.gnf.org/); MSP, merozoite surface protein; Pf, *Plasmodium falciparum*; Py, *Plasmodium yoelii*; ST kinase, serine–threonine kinase; TSRI, The Scripps Research Institute (http://www.scripps.edu).


PART 1: Genome Credentialing
data generation, database loading
query-based data mining
PlasmoDB
The Plasmodium Genome Resource
Release 4.1
Welcome to PlasmoDB 4.1
surface antigens
schizont expression
specific to Plasmodium
SNPs
AA polymorphisms
Low complexity
Signal peptide
Predicted Epitopes
Apicoplast transit peptide
Expression timing
Expression profile
Affy - GNF MalariaChip
Affy - GNFHS1_RSYP.chr_2
All genes on an array
Scripps/GNF malaria array - genes ranked by expression
Genes with a specified phylogenetic profile
Query parameters
Query options
Run query
Submit
Reset
PART 2: Target Selection
data integration
candidate antigen identification
Query result: rows 1 - 20
location query
phylogenetic query
expression query
MSP1
AMA1
vaccine antigens MSP1, AMA1
... and numerous hypothetical
proteins for further analysis

Vanderberg [29]. The protective effects of irradiated sporozoites have been repeatedly demonstrated and, after combining data from several independent trials over the past 25 years, Hoffman *et al.* recently concluded that 24 out of 26 immunized malaria-naïve volunteers were protected against repeated challenge by bites from *P. falciparum*-infected mosquitoes, with complete protection against blood-stage infection being observed in 94% of challenges and two vaccinees being protected against challenge with a genetically distinct parasite isolate [30]. Hoffman *et al.* rightly infer that a subunit vaccine able to confer similar levels of protection would already be widely deployed (or would at least be in the late stages of clinical development) and they have recently challenged the conventional wisdom that irradiated sporozoite vaccines are impractical [3], a claim that has been received with a mixture of enthusiasm and scepticism by malariologists and vaccinologists [31]. In support of this approach, the techniques to produce infectious mosquitoes have been refined to the point where they can be reliably and routinely produced, at least in sufficient quantities for vaccine challenge experiments [8]. In addition, new techniques are coming on line to estimate precisely the parasite load of individual mosquitoes [32], which will be an important part of quality control and process monitoring. The immune effector mechanisms induced by protective irradiated-sporozoite vaccines have been characterized in great detail [11,33], indicating that immunological correlates of effective induction and maintenance of immunity might be developed to facilitate the conduct of large-scale trials and allow monitoring of the longevity of protection in immunized individuals. The logistics of scaling up production of irradiated sporozoites (or infected mosquitoes) under good manufacturing practice (GMP) guidelines and of delivering them at reasonable cost in a public health setting remain daunting. However, Sanaria Inc.™ (Gaithersburg, MD, USA) is attempting commercial development of attenuated sporozoite vaccines with a mission to 'Develop and commercialize a safe and effective malaria vaccine in seven years' (http://www.sanaria.com). It is not clear how this will be achieved at present.

One 'fly in the ointment' that has yet to be seriously considered is the problem of engendering long-lived immunity by vaccination, whether by live challenge approaches or subunit vaccines. The maximum demonstrated duration of protection following irradiated sporozoite vaccination is 10.5 months [30] and RTS,S – the only subunit, pre-erythrocytic vaccine to show any appreciable protection in field trials [which comprises a recombinant protein representing the C-terminal domains of the *P. falciparum* NF54-derived circumsporozoite protein (CSP) fused to hepatitis B virus surface antigen] had no significant effect on risk of blood-stage infection for more than two months after the final booster dose of vaccine [34].

Subunit vaccines must contend with several inherent disadvantages compared with those from whole-organism vaccines, including: (i) difficulties in retaining the correct (i.e. native) secondary and/or tertiary conformation of crucial antibody-binding sites; (ii) their inability to provide the broad range of major histocompatibility complex (MHC) class II-binding motifs that are required to induce a T-cell response in human populations with extremely heterogeneous human leukocyte antigen (HLA) haplotypes; (iii) the need for exogenous adjuvants; and (iv) their inability to induce long-term antigen persistence, which might facilitate long-term memory (premunition). In an attempt to overcome these problems, Michael Good *et al.* have been pioneering a whole-parasite approach for blood-stage vaccines, building on well-established procedures for inducing immunity to rapidly lethal rodent malarias, by repeatedly challenging human volunteers with ultra-low doses of *P. falciparum*-infected red blood cells (pRBC) (~30 pRBC per dose) [4]. The infections were detected by PCR (indicating transient parasite replication *in vivo*) and were treated immediately after PCR patency (Days 6–8), before the onset of any clinical signs or symptoms. After three rounds of (homologous strain) infection and cure, three out of four individuals infected for the fourth time remained healthy and free of parasites by microscopy and PCR for 14 days (at which point they were treated). Protection was characterized by strong $CD4^+$ and $CD8^+$ T-cell proliferative responses, interferon γ (IFN-γ) production and upregulation of nitric oxide synthase (NOS) in peripheral blood mononuclear cells with an absence of antibodies, interleukin 4 (IL-4) and IL-10.

Although this study could be viewed as highly artificial, data from recent drug trials suggests that the results could be relevant to exposure in the field. Intermittent preventive treatment (IPT) of malaria infections by administration of full curative doses of an effective antimalarial has been shown to be highly effective in reducing rates of febrile illness and severe anaemia in infants living in highly endemic areas [35]. In addition, follow-up of children who received IPT as infants has shown them to be significantly protected against clinical episodes of malaria up to the age of two years compared with those in children who did not receive IPT (D. Schellenberg, personal communication). One possible explanation for these observations is that repeated subclinical malaria infection, followed by drug treatment, has facilitated the development of antimalarial immunity.

Apart from the obvious logistic problems of administering such a vaccine, it will be crucial to address how long protection lasts in the absence of ongoing, subclinical infection. The time between drug cure and re-infection in the study by Pombo *et al.* (four weeks) [4] was too short for any significant attenuation of memory responses to be expected; it will be crucial to re-evaluate this approach with longer periods between immunization and challenge, and to determine whether long-term low-grade persistence of infection can be established to allow the development of a state of premunition. Also of interest will be whether ultra-low doses of antigen induce qualitatively different immune responses compared with those by conventional high-dose vaccines. Pombo *et al.* have proposed that low-dose antigen facilitates T-cell expansion and generation of memory cells, whereas high-dose antigen might lead to T-cell deletion and lack of effector and memory cells (Figure 2). While one might argue with some of the precepts on which this hypothesis is based [5],

data emerging from pre-clinical trials do suggest that there is no simple relationship between antigen dose and the magnitude of the immune response, and the responses elicited by prime-boost protocols vary markedly depending on immunization dose, time course and delivery system [36].

We all need a boost: how to induce long-term protection

Although the results from subunit malaria vaccine studies have generally been very disappointing, formulation of RTS,S with the proprietary adjuvant AS02 (previously SBAS2) has consistently induced protection in experimental and natural challenge studies (see Ref. [13] for recent review). In a trial involving 41 malaria-naïve volunteers, the overall protective efficacy against experimental challenge was 41% [95% confidence interval (CI)= 22–56%; $p=0.0006$] [37], and in a trial of 306 clinically immune Gambian adults, the protective efficacy against patent parasitemia following natural challenge was 72% (95% CI=46–85%) for two months after the last booster vaccination [34]. Unfortunately, protective efficacy fell to zero (95% CI= −52% to +34%) by five months, indicating that the protective mechanism was rather short-lived. Both $CD4^+$ and $CD8^+$ T cells from previously naïve, RTS,S/AS02-protected volunteers produced IFN-γ in response to CS peptides [38] and, in the Gambian study, the vaccine boosted lymphocyte proliferative and IFN-γ responses to CSP, CS peptides and hepatitis B surface antigen at two weeks after the last immunization [39]. The longer-term duration of these cellular responses remains to be determined.

The limited duration of protection makes the current formulation of RTS,S/AS02 of very little use for controlling malaria in endemic populations and the fact that it is <100% effective over the short-term means that it is unlikely to find a market with travellers. Elucidation of the mechanisms underlying deficient activation or maintenance of T-cell memory responses is crucial for real progress to be made with vaccine development. The antiparasitic effect of $CD8^+$ T cells is mediated exclusively by recall responses, and the protective capacity of this response depends on both the numbers and the functional properties of memory cells [5,40]. Memory $CD8^+$ T cells comprise at least two subsets with characteristic surface markers and unique functional attributes (i.e. central memory cells that reside preferentially in lymphoid organs and 'peripheral and/or effector' memory cells present in non-lymphoid organs) [41]. Central memory cells are the principal mediators of protection in systemic viral infections, in which both lymphoid and non-lymphoid tissues become infected. However, recent studies presented by F. Zavala (Johns Hopkins Bloomberg School of Public Health, MD, USA) at MAM 2004 reveal that IL-4-dependent peripheral memory $CD8^+$ T cells are required for protective immunity to malaria liver stages. Thus, in conditions of IL-4 deficiency, sporozoite-immunized mice rapidly lose both peripheral memory $CD8^+$ T cells and the capacity to inhibit parasite development in hepatocytes. By contrast, they develop and maintain a normal central memory cell subset. Zavala *et al.* postulate that, because malaria liver stages exclusively infect hepatocytes, parasite-infected cells are detected more efficiently by the peripheral memory $CD8^+$ T cells residing in the liver than by central memory cells in distant lymphoid organs. The existence of functionally distinct memory T-cell populations, with particular cytokine requirements for induction

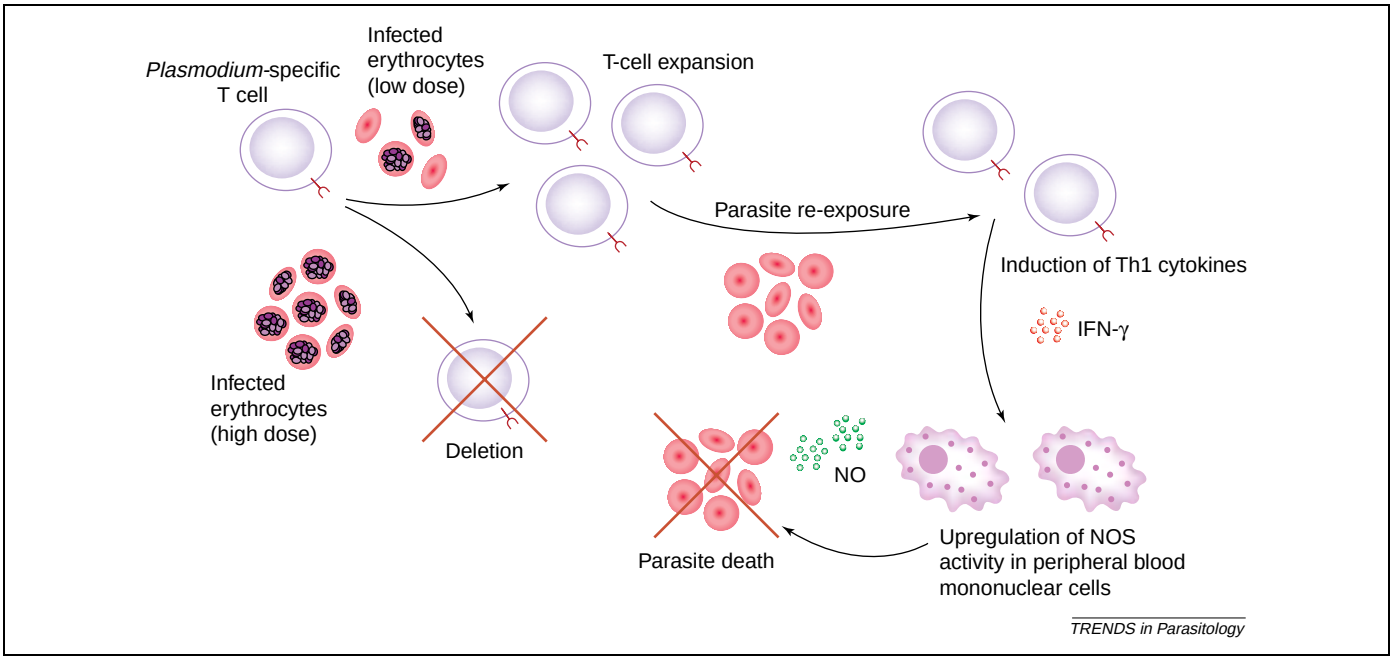


Figure 2. Proposed effects of low-dose versus high-dose antigen on induction of cell-mediated immunity to malaria. Pombo *et al.* [4] have proposed that low-dose antigen facilitates T-cell expansion and generation of memory cells, whereas high-dose antigen could lead to T-cell deletion via apoptosis, and thus fail to prime and/or maintain sufficient effector and memory cells. On subsequent exposure to high-dose challenge, individuals primed by low-dose antigen might be better able to mount an effective immune response than those primed by high-dose antigen. Figure is adapted from Ref. [4]. Abbreviations: IFN-γ; interferon γ; NO, nitric oxide; NOS, nitric oxide synthase; Th, T helper cell.

and maintenance, are important considerations for the design of the next generation of vaccines and the selection of appropriate immunomodulators. These considerations are also significant for the evaluation of T-cell responses induced by natural exposure or vaccination, which needs to include differential analysis of memory subsets.

The magnitude of memory T-cell populations is probably a crucial determinant of protection. The *in vivo* expansion of $CD8^+$ T cells is, however, a challenging task, made more difficult by memory cells themselves. Studies using murine models of *P. yoelii* [42] and *Listeria monocytogenes* [43] have shown that the inability of memory populations to expand despite continued antigen stimulation is the result of antigen-presenting dendritic cells being disabled (i.e. unable to process and present antigen) or killed, when primed T cells recognize antigen on their surfaces; as a consequence, antigen is no longer available to prime naïve cells. This mechanism probably explains, at least in part, the low frequency of primed $CD8^+$ T cells found in individuals living in malaria-endemic areas who are exposed for decades to the bites of *Plasmodium*-infected mosquitoes [44]. While this is probably an important self-regulatory mechanism to safeguard against adverse effects from excessive proliferation and activation of $CD8^+$ T-cells [45], it also represents a serious limitation for the establishment of robust protective immunity. Indeed, vaccination studies in mice and humans have encountered the limitations imposed by this self-regulation. Thus, primary $CD8^+$ T-cell responses can be easily induced with a variety of vaccine constructs, but the magnitude of these responses is modest, and repeated immunization with the same vector does not expand the magnitude of an established response. It is, however, encouraging that heterologous prime-boost immunization protocols, using recombinant pox viruses or adenovirus as boosters, do appear to overcome this self-regulatory mechanism [36,46]. A better understanding of this unique property of these viral vectors should help in the design of new immunization strategies.

Where do we go from here?

Novel vaccine targets, and a willingness to 'think outside the box' regarding attenuated vaccines, are potential ways forward for malaria vaccines and, given the limited success with the current batch of subunit vaccines, it will be important to follow-up on these new leads. However, given the very short-lived protection achieved with the current generation of malaria vaccines, exploration of issues to do with immunological memory is equally important. As the notion of immunological memory rapidly evolves from a purely functional concept towards a molecular definition, it should capture the interest of both malariologists and basic immunologists, and it warrants intensive collaborative study. It might be the key to unlocking the potential of vaccines to finally make an impact on the worldwide burden of malaria.

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References

1 Lindsay, S.W. and Martens, W.J. (1998) Malaria in the African highlands: past, present and future. *Bull. World Health Organ.* 76, 33–45
2 Schellenberg, D. *et al*. (2004) The changing epidemiology of malaria in Ifakara Town, southern Tanzania. *Trop. Med. Int. Health* 9, 68–76
3 Luke, T.C. and Hoffman, S.L. (2003) Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. *J. Exp. Biol.* 206, 3803–3808
4 Pombo, D.J. *et al*. (2002) Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. *Lancet* 360, 610–617
5 Struik, S. and Riley, E.M. (2004) Does malaria suffer from lack of memory? *Immunol. Rev.* 201, 268–290
6 Artavanis-Tsakonas, K. *et al*. (2003) The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. *Clin. Exp. Immunol.* 133, 145–152
7 Makobongo, M.O. *et al*. (2003) The purine salvage enzyme hypoxanthine guanine xanthine phosphoribosyl transferase is a major target antigen for cell-mediated immunity to malaria. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2628–2633
8 McConkey, S.J. *et al*. (2003) Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat. Med.* 9, 729–735
9 Good, M.F. (1991) Towards the development of the ideal malaria vaccine. A decade of progress in a difficult field. *Med. J. Aust.* 154, 284–289
10 Hoffman, S. (1996) *Malaria vaccine development*, Blackwell Publishing
11 Tsuji, M. and Zavala, F. (2003) T cells as mediators of protective immunity against liver stages of *Plasmodium*. *Trends Parasitol.* 19, 88–93
12 Andre, F.E. (2003) Vaccinology: past achievements, present roadblocks and future promises. *Vaccine* 21, 593–595
13 Moorthy, V.S. *et al*. (2004) Malaria vaccine developments. *Lancet* 363, 150–156
14 Gardner, M.J. *et al*. (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 419, 498–511
15 Carlton, J.M. *et al*. (2002) Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. *Nature* 419, 512–519
16 Kissinger, J.C. *et al*. (2002) The *Plasmodium* genome database. *Nature* 419, 490–492
17 Ben Mamoun, C. *et al*. (2001) Co-ordinated programme of gene expression during asexual intraerythrocytic development of the human malaria parasite *Plasmodium falciparum* revealed by microarray analysis. *Mol. Microbiol.* 39, 26–36
18 Bozdech, Z. *et al*. (2003) Expression profiling of the schizont and trophozoite stages of *Plasmodium falciparum* with a long-oligonucleotide microarray. *Genome Biol.* 4, R9
19 Le Roch, K.G. *et al*. (2003) Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science* 301, 1503–1508
20 Florens, L. *et al*. (2002) A proteomic view of the *Plasmodium falciparum* life cycle. *Nature* 419, 520–526
21 Lasonder, E. *et al*. (2002) Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. *Nature* 419, 537–542
22 Doolan, D.L. *et al*. (2003) Utilization of genomic sequence information to develop malaria vaccines. *J. Exp. Biol.* 206, 3789–3802
23 Doolan, D.L. *et al*. (2003) Identification of *Plasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9952–9957
24 Bahl, A. *et al*. (2003) PlasmoDB: the *Plasmodium* genome resource. A database integrating experimental and computational data. *Nucleic Acids Res.* 31, 212–215
25 Li, L. *et al*. (2003) Gene discovery in the apicomplexa as revealed by EST sequencing and assembly of a comparative gene database. *Genome Res.* 13, 443–454

26 Li, L. *et al*. (2003) OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res.* 13, 2178–2189
27 Kissinger, J.C. and Roos, D.S. (2004) Getting the most out of bioinformatics resources. In *Malaria Parasites: Genomes and Molecular Biology* (Waters, A.P. and Janse, C.J. eds), Caister Academic Press
28 Clyde, D.F. *et al*. (1973) Immunization of man against sporozoite-induced *falciparum* malaria. *Am. J. Med. Sci.* 266, 169–177
29 Nussenzweig, R.S. *et al*. (1967) Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. *Nature* 216, 160–162
30 Hoffman, S.L. *et al*. (2002) Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. *J. Infect. Dis.* 185, 1155–1164
31 Butler, D. (2003) Mosquito production mooted as fast track to malaria vaccine. *Nature* 425, 437
32 Bell, A.S. and Ranford-Cartwright, L.C. (2004) A real-time PCR assay for quantifying *Plasmodium falciparum* infections in the mosquito vector. *Int. J. Parasitol.* 34, 795–802
33 Doolan, D.L. and Hoffman, S.L. (2000) The complexity of protective immunity against liver-stage malaria. *J. Immunol.* 165, 1453–1462
34 Bojang, K.A. *et al*. (2001) Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet* 358, 1927–1934
35 Schellenberg, D. *et al*. (2001) Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. *Lancet* 357, 1471–1477
36 Dunachie, S.J. and Hill, A.V. (2003) Prime-boost strategies for malaria vaccine development. *J. Exp. Biol.* 206, 3771–3779
37 Kester, K.E. *et al*. (2001) Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. *J. Infect. Dis.* 183, 640–647
38 Sun, P. *et al*. (2003) Protective immunity induced with malaria vaccine, RTS,S, is linked to *Plasmodium falciparum* circumsporozoite protein-specific CD4(+) and CD8(+) T cells producing IFN-gamma. *J. Immunol.* 171, 6961–6967
39 Pinder, M. *et al*. (2004) Cellular immunity induced by the recombinant *Plasmodium falciparum* malaria vaccine, RTS,S/AS02, in semi-immune adults in The Gambia. *Clin. Exp. Immunol.* 135, 286–293
40 Morrot, A. and Zavala, F. (2004) Effector and memory CD8+ T cells as seen in immunity to malaria. *Immunol. Rev.* 201, 291–303
41 Wherry, E.J. *et al*. (2003) Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat. Immunol.* 4, 225–234
42 Hafalla, J.C. *et al*. (2003) Early self-regulatory mechanisms control the magnitude of CD8+ T cell responses against liver stages of murine malaria. *J. Immunol.* 171, 964–970
43 Wong, P. and Pamer, E.G. (2003) Feedback regulation of pathogen-specific T cell priming. *Immunity* 18, 499–511
44 Moorthy, V.S. *et al*. (2003) Safety and immunogenicity of DNA/modified vaccinia virus ankara malaria vaccination in African adults. *J. Infect. Dis.* 188, 1239–1244
45 Crispe, I.N. (2003) Hepatic T cells and liver tolerance. *Nat. Rev. Immunol.* 3, 51–62
46 Zavala, F. *et al*. (2001) A striking property of recombinant poxviruses: efficient inducers of *in vivo* expansion of primed CD8(+) T cells. *Virology* 280, 155–159