A new site of attack for a malaria vaccine

Simon J Draper and Matthew K Higgins

A newly discovered antibody epitope on the liver-infective form of the parasite that causes malaria opens new doors for vaccine development.

Malaria is one of the most ancient diseases. Yet, there are still hundreds of millions of clinical cases of malaria and around half a million deaths from the disease each year. The quest to develop an effective vaccine has been long and is marked by many unsuccessful attempts. Therefore, there is an urgent need to find new sites of vulnerability on *Plasmodium falciparum*, the causative parasite, that can be targeted by future vaccines. One potential source of vaccine components is the sporozoite form of the parasite, which is injected into the human host through the bite of an infected mosquito, before the parasite invades the cells of human liver. It is thought that reliable prevention of invasion into liver cells would eradicate malaria. Two recent studies by Tan *et al.*[1] and Kisalu *et al.*[2], which identify a new site of attack on the major surface protein of the sporozoite, are therefore highly welcome.

Two parallel approaches have led the way toward a vaccine that targets the sporozoites of *P. falciparum*[3]. One option is a whole-parasite vaccine using live sporozoites, which are attenuated through irradiation or genetic modification before injection or are injected in conjunction with antimalarial drugs to ensure that the parasites are cleared before they can cause disease. Early studies in the 1970s showed that immunization of humans with high numbers of sporozoites from irradiated mosquitoes led to almost complete protection against malaria challenge with a homologous parasite strain[4]. More recently, vials of cryopreserved irradiated *P. falciparum* sporozoites (PfSPZs) have been developed by Sanaria. Repeated intravenous immunization of individuals with this vaccine led to high-level protection in clinical studies[5]. However, although promising, such whole-parasite-based strategies continue to face questions related to the breadth of protection against heterologous *P. falciparum* strains[6]. There are also practical challenges related to scale-up and deployment of a vaccine requiring a liquid nitrogen cold chain, intravenous administration and repeated high doses, all of which are particularly challenging in remote areas with under-resourced healthcare systems.

A second approach is to develop a protein-based vaccine that targets sporozoites. The *P. falciparum* circumsporozoite protein (PfCSP) is the most abundant molecule on the sporozoite surface, is essential for sporozoite development and mediates their attachment to liver cells. PfCSP contains three principle regions (**Fig. 1**): an N-terminal domain that binds to hepatocyte heparin sulfate proteoglycans, a central region comprising ~40 copies of repeats of the NANP or NVDP amino acid sequence and a C-terminal thrombospondin-repeat (αTSR) domain. The majority of antibodies raised after immunization with sporozoites target PfCSP, and these can prevent invasion of hepatocytes *in vivo*[7]. These discoveries guided the development of GlaxoSmithKline's RTS,S/AS01 vaccine, in which 18 NANP repeats and the C-terminal domain of PfCSP are fused to a hepatitis B surface antigen virus-like particle[8]. RTS,S is currently the only malaria vaccine to have been tested in a phase III clinical trial, in which it demonstrated moderate protective efficacy against clinical malaria in the first year after immunization[9], but protection waned substantially thereafter[10]. Although RTS,S is the most advanced malaria vaccine to date, its composition was determined over 20 years ago, and questions remain. Why is RTS,S not more effective, and can it be rationally redesigned or reformulated to substantially improve overall vaccine efficacy and durability?

Tan *et al.*[1] and Kisalu *et al.*[2] have tackled these questions and, in the process, each identified the same new and thus far unexploited site of vulnerability on PfCSP[1,2]. In both cases, the investigators started by exploring antibody responses in volunteers who had been immunized with attenuated PfSPZ sporozoites and who were protected from the development of malaria induced by subsequent sporozoite challenge. Through cloning of individual B cells, they isolated panels of human monoclonal antibodies. These were assessed for their ability to prevent *P. falciparum* from invading human hepatocytes and to reduce parasite invasion of liver in humanized mouse models. The authors also screened panels of these antibodies for reactivity against PfCSP and its constituent domains. They found that all of the sporozoite-reactive antibodies bound to PfCSP with the majority targeting the NANP repeats, confirming this as the immunodominant site on the sporozoite surface. Indeed, the most protective antibodies also bound to the NANP repeats. However, through fine-mapping of monoclonal antibodies together with quantitative comparisons of efficacy and binding, the authors of these studies found that the most protective antibodies also preferentially bound to a previously unidentified epitope.

A single copy of the sequence NPDP lies between the N-terminal domain of PfCSP and its central NANP and NVDP repeats. Tan *et al.*[1] and Kisalu *et al.*[2] found that the most protective antibodies cloned from sporozoite-immunized volunteers bound more tightly to a 'junctional' epitope centered on the NPDP sequence than to the larger stretch of NANP repeats. Both groups of authors were also able to generate crystal structures of monoclonal antibodies bound to PfCSP peptides, and through combining these with fine-mapping by mutagenesis, they were able to reveal the molecular basis for this selectivity. Kisalu *et al.*[2] also suggest why particularly protective antibodies might

Simon J. Draper is at the Jenner Institute, University of Oxford, Oxford, UK, and Matthew K. Higgins is at the Department of Biochemistry, University of Oxford, Oxford, UK.
e-mail: matthew.higgins@bioch.ox.ac.uk or simon.draper@ndm.ox.ac.uk

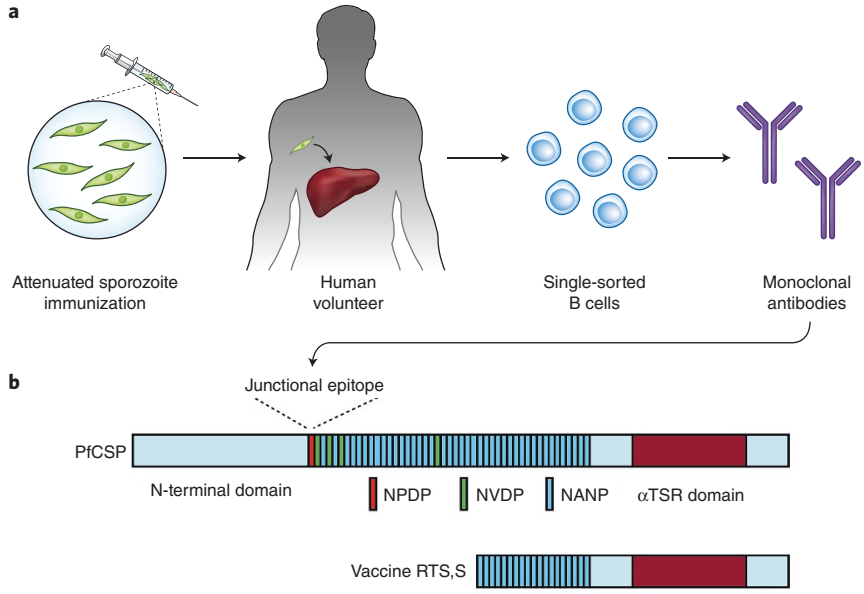

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Figure 1 Identification of a new epitope on the malaria-causing parasite for vaccine development. The sporozoite is the liver-infective form of the malaria parasite. Human volunteers were immunized with attenuated sporozoites as part of a malaria vaccine trial. The antibodies that were generated in response within these volunteers were obtained. The antibodies that were best at preventing liver invasion and development of malaria bound to a new site, the junctional epitope, on the major surface protein of the sporozoite, PfCSP. The current leading malaria vaccine, RTS,S, contains part of the PfCSP protein but lacks the junctional epitope, opening a new route for vaccine improvement.

be raised in response to the junctional epitope. PfCSP is cleaved at a site only a few residues upstream of the newly identified epitope, and modulation of this cleavage is known to inhibit hepatocyte invasion[11]. Kisalu *et al.*[2] show that antibodies that bind to the junctional epitope also prevent PfCSP cleavage. This leads to the suggestion that the protective function of antibodies that target the junctional epitope might be mediated by their ability to prevent liver cell invasion through blocking this essential cleavage event. This does, however, raise questions about the reason why the NPDP sequence has evolved. Is it a spacer that has developed between the immunodominant NANP repeats and the N-terminus to prevent occlusion of the crucial cleavage site by antibodies that bind to these NANP repeats? Or is NPDP itself functionally important, and have the NANP repeats evolved as a decoy to lure the immune response away from this important target?

These findings have important implications for vaccine development. The current formulation of the most advanced malaria vaccine, RTS,S, contains 18 NANP repeats fused to the C-terminal domain of PfCSP[8]. Monoclonal antibodies that bind to the C-terminus are rarely cloned from sporozoite-immunized volunteers and are not protective in a mouse model[1,12], challenging the rationale for the inclusion of this region of PfCSP in RTS,S. Also, discovery of the junctional epitope by Tan *et al.*[1] and Kisalu *et al.*[2] suggests that this sequence should be included in future vaccine constructs. However, it is notable that RTS,S, although lacking the full junctional epitope, does include the sequence PDP immediately before the NANP repeats[8]. Would a modified version of RTS,S that includes the complete junctional epitope prove more effective in raising a protective antibody response in humans? The initial data in mice from Tan *et al.*[1] are disappointing in regard to potential vaccine development using this epitope, as immunization of mice with a protein conjugate containing the junctional epitope did not elicit a polyclonal antibody response that matched the most protective antibodies that were isolated from immunized individuals. Nevertheless, Tan *et al.*[1] discovered that these desirable antibodies do not show major divergence from human germline sequences, and both studies have identified them in multiple sporozoite-immunized human volunteers[1,2]. In combination, these findings suggest that the desirable and protective antibodies, with their preferential binding to the junctional epitope, will be readily induced through vaccination in humans.

It will be fascinating to see whether vaccine efficacy is significantly improved following future immunization of volunteers with variations of RTS,S containing the junctional epitope. It will also be important to know whether the junctional epitope is essential for PfCSP function or whether it will be lost if put under selection pressure due to vaccination. Whatever the outcome of such future endeavors, these two studies are extremely welcome as an in-depth assessment of a human inhibitory immune response. Their findings may be used to guide rational vaccine design and have the potential to significantly impact the development of future generations of malaria vaccines.

COMPETING INTERESTS
The authors declare no competing interests.

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