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**Towards an effective pre-erythrocytic stage malaria vaccine: a new
site of attack on an old enemy**

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Two recent papers^{1,2} identify a novel antibody epitope on the liver-infective form of the malaria parasite, opening new doors for vaccine development.

Malaria, one of the most ancient diseases of humanity, still causes hundreds of millions of cases and around half a million deaths each year. The quest to develop an effective vaccine has been long and, with many failed attempts, new sites of vulnerability on the causative parasite are urgently required. One potential target is the sporozoite form of the parasite, which is injected into a human host through the bite of an infected mosquito, before invading the cells of the human liver. If we can reliably prevent liver cell invasion, malaria will be no more.

Two parallel approaches lead the way towards a vaccine that targets the sporozoites of *Plasmodium falciparum*. One option is a whole parasite vaccine using live sporozoites, which have been attenuated by irradiation or genetic modification, or are inoculated during treatment with anti-malarial drugs³. 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 strain⁴. More recently, vials of cryopreserved irradiated sporozoites, known as PfSPZ, have been developed by Sanaria, with repeated intravenous immunization leading to significant high-level protection in clinical studies⁵. However, while highly promising, such whole parasite-based strategies continue to face questions related to the breadth of protection against heterologous *P. falciparum* strains⁶, as well as practical challenges related to scale-up and deployment of a vaccine requiring a liquid nitrogen cold chain, intravenous administration and repeated high doses.

A second approach focuses on the circumsporozoite protein, PfCSP, as the parasite component of a protein-in-adjuvant vaccine. This is the most abundant molecule on the sporozoite surface, is essential for sporozoite development and mediates attachment to liver cells. PfCSP contains three principle regions: an N-terminal domain which binds to hepatocyte heparin sulphate proteoglycans, a central region comprising ~40 copies of repeats of sequence NANP or NVDP and a C-terminal thrombospondin repeat domain. The significant majority of antibodies raised on immunization with sporozoites target PfCSP and these prevent hepatocyte invasion⁷. 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 particle⁸. RTS,S is currently the only malaria vaccine to have been tested in a Phase III clinical trial, where it demonstrated moderate protective efficacy against clinical malaria in the first year after immunization⁹ but with protection waning substantially thereafter¹⁰. 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?

Two recent papers have tackled these questions, identifying a new, and thus far unexploited site of vulnerability on PfCSP^{1,2}. In both cases, the investigators started by exploring antibody responses in volunteers immunized with attenuated PfSPZ sporozoites. Through cloning of individual B cells, they isolated panels of sporozoite-reactive human monoclonal antibodies. These were assessed for protective efficacy in an *in vitro* hepatocyte invasion

assay and in humanized mouse models. Antibody panels were also screened for reactivity against PfCSP and its constituent domains. All of the sporozoite reactive antibodies bound to PfCSP, with the majority targeting the NANP repeats, confirming this as the immuno-dominant site on the sporozoite surface. Indeed, the most protective antibodies also bound to NANP repeats. However, the power of fine-mapping of monoclonal antibodies, together with quantitative comparisons of efficacy and binding, revealed that these most effective antibodies also preferentially bound to a previously unidentified epitope.

Lying between the N-terminal domain of PfCSP and its central NANP/NVDP repeats, is a single NPDP sequence. The most protective antibodies cloned from sporozoite-immunized volunteers bound to this 'junctional' epitope around 7-fold more tightly than to an NANP repeat². Crystal structures of monoclonal antibodies bound to peptides, and fine mapping by mutagenesis, revealed the structural basis for this selectivity^{1,2}. One of the studies also suggested why this junctional epitope might raise particularly protective antibodies. PfCSP is cleaved at a site only a few residues upstream of NPDP and modulation of this cleavage inhibits hepatocyte invasion¹¹. Antibodies that bind to the junctional epitope also prevent PfCSP cleavage². Has NPDP evolved as a spacer between the immuno-dominant repeats and the N-terminus to prevent occlusion of a crucial cleavage site by antibodies that bind 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 also have important implications for vaccine development. The current formulation of RTS,S contains 18 NANP repeats fused to the C-terminal domain of PfCSP⁸. Monoclonal antibodies which bind to the C-terminus are rarely cloned from sporozoite-immunized volunteers and are not protective in a mouse model^{1,12}, challenging the inclusion of this region of PfCSP in RTS,S. Also, discovery of the junctional epitope challenges its omission from the current vaccine construct^{1,2}, although it is noticeable that RTS,S, while lacking the full junctional epitope, does have PDP before the NANP repeats⁸. Would a modified version of RTS,S, which includes the complete junctional epitope, prove more effective in raising a protective antibody response in humans? The initial data are disappointing, as immunization of mice with a protein-conjugate containing this epitope did not elicit a polyclonal antibody response matching that of the most effective human monoclonal antibodies¹. Nevertheless, the desirable antibodies do not show major divergence from human germ-line sequences¹ and have been found in multiple sporozoite-immunized human volunteers^{1,2}, suggesting the potential for their induction by immunization. It will therefore be fascinating to see if future immunization of volunteers with variations of RTS,S, containing the junctional epitope, do significantly improved vaccine efficacy. It will also be important to know if the junctional epitope is essential for PfCSP function, or if it will be lost if put under selection pressure due to vaccination. Whatever the outcome of such future endeavours, these two studies are extremely welcome as an in-depth assessment of a human inhibitory immune response and its use to guide rational vaccine design, and have the potential to significantly impact the development of future generations of malaria vaccines.

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