

**Understanding human-derived antibodies generated by polymorphic malaria vaccine
against merozoite surface protein 2**

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There is a continuing need for an effective malaria vaccine to enhance control and elimination efforts. This has been recently highlighted in the 2017 WHO annual report indicating that in multiple areas recent advances in reducing disease burden are stalling and reversing ¹. One continuing challenge in the development of effective anti-malarial vaccine is the phenomenon of exceptional rates of genetic polymorphism and subsequent antigenic diversity in leading vaccine candidates ². Key target antigens of human immunity to malaria and leading vaccine candidate antigens (e.g., AMA1, MSP1), have evolved significant polymorphisms in order to evade immunity. This has presented a persistent challenge in malaria vaccine development as it significantly impacts on how practically efficacious a logically-designed, lab-derived vaccine is ³. Antigenic polymorphisms in protein domains of high immunogenicity have evolved to be a crucial immune evasion mechanism of the parasite. Several trials of putative blood-stage vaccines have demonstrated evidence of strain-specific efficacy, which ultimately contributes towards rendering a vaccine effective or

ineffective⁴⁻⁶. Naturally-acquired immunity to malaria is typically slow to develop and requires repeated exposure to malaria over time; this slow acquisition is attributed, in part, to the requirement for the development of a sufficiently broad repertoire of antibodies against diverse strains^{7,8}. Current vaccine strategies to overcome antigenic diversity and vaccine-escape include the incorporation of multiple alleles, developing antigens with modified sequences to enhance effective immunogenicity, or targeting conserved or less diverse regions and epitopes³. Knowledge regarding the specificity and cross-reactivity of naturally acquired or vaccine-induced human immune responses to vaccine candidates is still limited. The conditions and approaches that favor the development of cross reactive responses that may limit immune escape through antigenic diversity are largely unknown.

In this issue of the *Journal of Infectious Disease*, Feng,Boyle *et al.* use the well characterized merozoite surface protein; MSP2, as a model to better understand the specificity and function of human-derived antibodies generated by vaccines compared with naturally acquired responses, determine whether an MSP2 combination-vaccine successfully generated functional antibodies to multiple *P. falciparum* strains, and evaluate the overall specificity of vaccine-induced antibodies. Using a cohort of samples from a phase 1 bivalent vaccine trial with MSP2, Feng, Boyle *et al.* provide direct evidence that vaccination with a multivalent vaccine induces cross-reactive and functional antibodies to the polymorphic malaria antigen, MSP2, in humans. This vaccine-induced response contrasted with the highly strain-specific nature of naturally-acquired antibodies that enable immune escape.

They show that vaccine-induced cytophilic antibodies are functional by examining mediation of opsonic phagocytosis, with a significant increase observed in activity following vaccination in sera from patients receiving the active vaccine, but not with placebo controls. Furthermore, they demonstrate an increase in the ability of post-vaccination serum to

activate complement via the classical complement pathway. Using competition ELISA, they show that the majority of antibody responses in vaccine recipients were highly cross-reactive to two distinct alleles of MSP2, in contrast with antibodies derived from naturally-exposed individuals.

Using an MSP2–peptide array and pools of “high responders” from vaccines, they epitope mapped vaccine-induced antibodies and show that these antibodies predominately target the conserved epitopes at the C-terminal region of MSP2. Among naturally-exposed individuals, antibodies targeting this region were also observed, but at significantly lower levels than those observed when examining strain-specific regions, or seen when seen using vaccine-derived samples. Finally, using a C-terminal targeted monoclonal antibody, they confirm that this region of MSP2 is the target of functional antibodies, and definitively demonstrate that anti-MSP2 vaccine-induced antibodies can promote opsonic phagocytosis against multiple *P. falciparum* strains.

Taken together, these studies provide strong evidence that vaccination with a bivalent vaccine can induce cross-reactive and functional antibodies in humans to the (typically-polymorphic) malaria vaccine antigen MSP2. This contrasts with the highly allele/strain-specific nature of naturally acquired antibodies. This study provides new insights into how vaccine-induced responses in humans can differ from responses induced with naturally acquired immunity. This is clearly an important insight which has implications on future strategic decisions for malaria vaccines targeting not only the blood stages, but also the pre-erythrocytic and transmission stages. In this case, MSP2 vaccine-induced antibodies are cross-reactive and functional at the C terminus of MSP2. The scope of this study does not encompass investigation of the specific mechanisms mediating the preferential induction of cross-reactive antibodies following vaccination. The authors reason that this could be due to a differential antigenic load of conserved versus polymorphic epitopes when different alleles

are used as part of a bivalent moiety, protein conformation differences in antigens when present in a vaccine when compared to natural infection, adjuvant driven or due to the administration route of the vaccine. Studies on influenza responses in mice have found that the route of administration and form of antigen can modulate immunodominance of the antibody response specific antigen regions⁹. Extensive further examination and characterization of the mechanism of differential immunity are required if this phenomenon is to be exploited in vaccine development to overcome the confounding factors, i.e., antigenic diversity, parasite polymorphisms and pathogen-escape.

An important priority in future malaria vaccine development is not just to identify the antigenic diversity circulating within the target parasite population, but also to understand the contribution of genetic/allelic diversity to targeted epitopes within each candidate antigen. Although there are indeed multiple diverse alleles of many candidate antigens circulating within distinct populations, not all polymorphisms will mediate antigenic escape; hence, these should be individually identified and characterized to enable logical vaccine design. Rigorous investigation of available candidates, as well as the examination of novel (relatively) conserved antigenic targets, are essential to develop a framework for the selection and prioritization of antigens for further development within a development pipeline. This study makes important observations and provides key insights into addressing these issues in the immediate future.

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