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Editorial

Expanding the Toolbox in Pursuit of a Strain Transcendent Malaria Vaccine

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Plasmodium falciparum has proved a formidable adversary in the face of the traditional tools of vaccine development. To date, there are 27 malaria vaccines that have advanced to clinical trials.[1] However, promising preclinical results for a number of vaccine candidates have been followed by disappointing efficacy profiles in field-based studies.[2] Chief among the parasite's defenses to immune detection is extreme antigenic variation in several of its most immunogenic proteins, and this diversity is thought to be a primary obstacle to the development of a broadly efficacious and durable vaccine for malaria.[3,4] Indeed, parasite diversity has been shown to directly affect the efficacy of some of the most advanced malaria vaccine candidates, which in clinical trials have higher efficacy against parasites of the same strain than against nonvaccine strains.[5,6] In this edition of the *American Journal of Tropical Medicine and Hygiene*, Bailey and colleagues introduce a promising new tool to study the relevance of antigenic variation in two blood stage *P. falciparum* vaccine targets, apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1).[7] Using a protein microarray designed to reflect genetic diversity at their clinical research site, they analyze the seroreactivity of a cohort of Malian adults and children to 60 AMA1 ectodomain haplotypes and 10 $MSP1_{19}$ haplotypes of *P. falciparum*.

The ultimate aim of this line of research is to guide development of a "strain transcendent" vaccine with long-lasting efficacy across geographically diverse sites. Historic reliance on proteins generated from a small group of laboratory reference strains has no doubt limited our ability to identify and study protective immune responses that can be replicated and ideally improved upon in a vaccine format. The available data suggest that we cannot rely on targeting just a few dominant haplotypes based on the level of diversity that many of these proteins carry.[8] Although there are promising results in animal studies and early clinical trials of multivalent vaccines and allelic chimeric fusion proteins,[9–11] even a rare haplotype not covered by these vaccines could lead to vaccine failure. Considering these concerns, the platform described here appears to be a valuable new tool for investigation of naturally and vaccine-derived immune responses.

The short-term goal of diversity-covering microarrays is the identification of antigenic targets against which hosts maintain serologic reactivity across diverse genetic alleles, so-called conserved or cross-reactive epitopes. However, each identified epitope must cross several additional hurdles to warrant inclusion in a novel vaccine. First, it must be determined if natural antibodies to these epitopes protect the host from malaria. Shrewdly, *P. falciparum,* like other microbes, employs nonfunctional and highly immunogenic decoy epitopes to evade immune clearance.[12] Epitopes that are cross-reactive may have conserved structure and may therefore be critical for function. Nonetheless, structural biologic research, prospective field studies evaluating association of these epitopes with disease protection, and genetic epidemiologic investigations may all be needed to determine the immunologic relevance of identified epitopes. Second, we must determine if antibodies generated by a vaccine are as protective (or ideally more protective) than the polyclonal antibodies generated from natural *P. falciparum* exposure.[9,13] Research evaluating the performance of cross-reactive versus allele-specific antibodies in the multivalent AMA1 vaccine suggest that the cross-reactive antibodies are responsible for *in vitro* parasite growth inhibition.[14] However, in an animal-based study of VAR2CSA, two immunogens (partial versus full-length VAR2CSA) both yielded cross-reactive antibodies, but growth inhibition of parasites *in vitro* was superior with antibodies from vaccination with the full-length protein.[15] These findings suggest that generation of the desired cross-reactive antibodies may not be sufficient for efficacy, and that structural and conformational components may be important in ultimate functional activity of the vaccine. Finally, cross-reactive and functional antibodies that are elicited by vaccines must lead to protective immunity in the field. There is an increasing body of literature addressing the role of parasite-driven immune modulation in the development of naturally acquired immunity.[16–18] Individuals with previous or ongoing natural malaria exposure may develop parasite-driven induction of dysfunctional T and B cell responses that could render vaccines with optimized T and B cell epitopes less effective than in individuals without prior malaria exposure. Overall, it is clear that antigenic variation is not the parasite's only defense to escape immune detection, and ongoing investigation will be needed at the interface of this highly polymorphic organism with the host immune response to achieve the ambitious goal of a vaccine with over 75% clinical efficacy by 2030.

Bailey and colleagues have examined the role of antigenic variation in serologic reactivity to two key malaria proteins in a small region in Mali. The next steps are for researchers to expand this approach to include diverse geographic sites, multiple *P. falciparum* antigens, and assessments of how responses to multiple epitopes are associated with protection from clinical malaria. This will require the collaboration of scientists from diverse malaria-endemic areas including Asia, Africa, Oceania, and Latin America. Notwithstanding the obstacles still ahead, the design and use of high-throughput technologies such as diversity-covering protein microarrays could be an important step in overcoming antigenic diversity to design a highly efficacious malaria vaccine.

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