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REVIEWS

Spotlight

Structure Solves the Problem with Malaria Merozoite Vaccines

Patrick E. Duffy[1,*]

Malaria vaccines targeting merozoite invasion of erythrocytes have long held appeal but failed in clinical trials. Three structural studies of antibody–antigen complexes by Alanine *et al.*, Urusova *et al.*, and Rawlinson *et al.* define neutralizing and nonneutralizing epitopes in essential invasion proteins, leading to rational design of improved merozoite vaccines.

Malaria is a killer that targets children and pregnant women for its most dire consequences. Parents in sub-Saharan Africa live each day with the fear that their child might succumb to *Plasmodium falciparum* malaria. *Plasmodium vivax* malaria is more widespread in Latin America and Asia; it debilitates but kills less frequently than *P. falciparum*. Since the early 1900s, scientists dreamed of a malaria vaccine. Vaccine prospects brightened with the 1961 report that passive IgG transfer from African adults cleared *P. falciparum* parasitemia in infected children [1], and later that immunization with parasite preparations rich in red cell-invasive merozoites protected monkeys from *P. falciparum* infection [2]. However, decades of research culminated in a series of vaccine trials targeting merozoite antigens, like AMA1 and MSP1, that failed to show any activity.

Following these disappointments, merozoite vaccine research was revitalized in part by the approach of identifying novel antigens, such as the *P. falciparum* reticulocyte-binding protein homolog (*Pf*RH5) of merozoites that binds the essential receptor basigin on red blood cells [3], or the RON2 protein that complexes with AMA1 and alters its antigenicity to induce more potent anti-invasion antibodies [4]. A newer approach uses structural analysis of antigen–antibody complexes to identify functional and nonfunctional epitopes, thereby providing a blueprint for rational design of vaccines. A series of three papers [5–7] has now taken this latter approach to advance vaccine development against *P. falciparum* and *P. vivax* merozoites (Table 1).

Draper, Higgins, and colleagues studied the structure of antibodies bound to the *Pf*RH5 protein [5]. These scientists generated a panel of recombinant human monoclonal antibodies (mAbs) based on plasmablasts collected after vaccination with viral vectored *Pf*RH5. The ability of mAbs to inhibit parasite growth *in vitro* correlated with association rates (K_{on}), and one mAb (R5.011) that delayed but did not block merozoite invasion of red cells potentiated the neutralizing activity of other mAbs. These neutralization studies highlight an Achilles' heel of merozoite vaccines: merozoites pass quickly between red cells and provide a brief time window for antibodies to act. Indeed, mAb R5.011 potentiated the neutralizing activity of polyclonal sera against other invasion proteins including AMA1. X-ray crystallography and hydrogen–deuterium exchange mass spectrometry (HDX-MS) revealed epitopes for two neutralizing *Pf*RH5 mAbs close to the basigin binding site, providing a mechanism for neutralization. The potentiating but nonneutralizing mAb R5.011 epitope is distinct, and R5.011 binding to *Pf*RH5 did not alter the structure, K_{on}, or binding affinities of neutralizing mAbs.

A similar approach is informing development of antimerozoite vaccines for *P. vivax*. *P. vivax* invasion has unique aspects: (i) the parasite exclusively invades reticulocytes by interaction of *P. vivax* reticulocyte-binding protein 2b (*Pv*RBP2b) with the reticulocyte's transferrin receptor (CD71), and (ii) invasion requires engagement of Duffy antigen receptor for chemokines (DARC) by the parasite's Duffy-binding protein (*Pv*DBP). The latter interaction explains the low prevalence of *P. vivax* in sub-Saharan Africa where most people carry red cells that lack Duffy antigen.

Two manuscripts published simultaneously in *Nature Microbiology* dissect the structural basis for *P. vivax* neutralization with naturally acquired [6] or vaccine-induced [7] antibodies against *Pv*DBP. The DARC-binding activity of *Pv*DBP maps to a Duffy binding-like (DBL) domain referred to as Region II (PvDBPII), hence this was the focus of both study teams. Tolia and his colleagues studied naturally acquired antibodies against *Pv*DBPII, since high titers of *Pv*DBPII antibodies that prevent DARC binding are associated with clinical protection [8]. They generated 11 *Pv*DBPII-reactive mAbs based on antigen-sorted B cells from a Cambodian donor and identified corresponding epitopes for two using X-ray crystallography, HDX-MS, and mutational mapping. As seen for *Pf*RH5, the neutralizing antibodies against *Pv*DBPII target its red cell binding site which is formed by receptor-induced *Pv*DBP dimerization that creates a DARC-binding cleft between two *Pv*DBPII moieties [9]. The *Pv*DBPII antibodies engaged residues in the DARC-binding cleft and the *Pv*DBPII dimer interface, providing the mechanism for neutralization. Common naturally occurring polymorphisms in the vicinity of the epitopes did not impair antibody binding, echoing the strain-transcending properties of *Pf*RH5-neutralizing antibodies and supporting the potential for broadly effective vaccines.

*Pv*DBPII-based vaccines have recently advanced to the clinic [10]. Draper,

Table 1. Summary of Studies That Characterize Human Monoclonal Antibodies against Malaria Merozoite Invasion Proteins and Their Corresponding Epitopes

Parasite	Merozoite antigen	B cell source	mAb screening	Antigen-specific mAb generated	mAb activity in parasite neutralization assays					Epitopes defined by structure studies			Refs
					Number tested	Neutralizing	Nonneutralizing	Potentiating	Antagonistic	Total	Binding site	Other	
Plasmodium falciparum	*Pf*RH5	Plasmablasts from UK subjects after *Pf*RH5 vaccine (chimpanzee adenovirus prime; poxvirus boost)	ELISA to *Drosophila* S2 cell-expressed *Pf*RH5FL	17	17	7 high; 3 low	7	1	1	3	2	1	[5]
Plasmodium vivax	PvDBP (region II)	PvDBPII-specific memory B cells from Cambodian donor with high plasma activity that blocked PvDBPII binding to DARC	ELISA to *Escherichia coli*-expressed PvDBPII	11	2	2	0	n.d[a]	n.d.	2	2	0	[6,11]
		Plasmablasts from UK subjects after PvDBPII vaccine (chimpanzee adenovirus prime; poxvirus boost)	ELISA to *Drosophila* S2 cell-expressed PvDBPII; Dot blot capture of native PvDBP from parasite culture supernatants	10	10	1 high; 6 intermediate; 3 low	0	0	5	1	0	1	[7]

[a]n.d., no data.

Higgins, and their colleagues again developed mAbs from a clinical trial, this time of viral vectored *Pv*DBPII vaccine, and screened these for their ability to block binding of *Pv*DBP to DARC, and to block parasite invasion of reticulocytes [10]. Two of ten *Pv*DBPII-specific mAbs showed strain-transcending activity against the *Pv*DBP–DARC binding interaction, and one of these (DB9) showed high growth-inhibitory activity against 10/11 Thai clinical isolates. The crystal structure of DB9 Fab fragment with *Pv*DBPII identified the epitope in subdomain 3, and this was confirmed by DB9 reactivity to recombinant subdomain 3, indicating that the epitope is distant from the *Pv*DBP DARC-binding site [9]. Superimposing the DB9:*Pv*DBPII structure on the known structure of the *Pv*DBPII dimer suggested that DB9 orientation could impair the *Pv*DBPII approach toward the reticulocyte membrane to engage DARC.

These elegant studies provide a map to improve upon merozoite vaccines, by defining epitopes for neutralizing, nonneutralizing, potentiating, and antagonistic antibodies (Table 1). General principles emerge: neutralizing antibody can target conserved regions to offer strain-transcending activity; neutralizing antibody can contact antigen at the red cell binding site or at other sites; human mAbs often have distinct epitopes to those of rodent mAb, suggesting that human mAbs should be prioritized to inform vaccine design. Expanding the panels of mAbs and epitopes in future will refine our understanding of elements within specific antigens that enhance or impair protective responses. Now on to the tasks of designing and testing improved immunogens.

Malaria vaccines work. The RTSS vaccine that targets sporozoites has been confirmed in Phase III trials to

reduce clinical *P. falciparum* malaria in children, and newer whole-sporozoite vaccines have shown efficacy to prevent *P. falciparum* infection of adults in early field trials. The addition of antimerozoite vaccines that prevent clinical disease will be important new interventions as our existing tools for malaria control, including drugs and insecticides, lose their activity to increasingly resistant parasites and mosquitoes.

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References

1. Cohen, S. *et al.* (1961) Gamma-globulin and acquired immunity to human malaria. *Nature* 192, 733–737
2. Siddiqui, W.A. (1977) An effective immunization of experimental monkeys against a human malaria parasite, *Plasmodium falciparum*. *Science* 197, 388–389
3. Crosnier, C. *et al.* (2011) Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 480, 534–537
4. Srinivasan, P. *et al.* (2017) A malaria vaccine protects *Aotus* monkeys against virulent *Plasmodium falciparum* infection. *NPJ Vaccines* 2, art. no. 14.
5. Alanine, D.G.W. *et al.* (2019) Human antibodies that slow erythrocyte invasion potentiate malaria-neutralizing antibodies. *Cell* 178, 216–228.e21
6. Urusova, D. *et al.* (2019) Structural basis for neutralization of *Plasmodium vivax* by naturally acquired human antibodies that target DBP. *Nat. Microbiol.* 4, 1486–1496
7. Rawlinson, T.A. *et al.* (2019) Structural basis for inhibition of *Plasmodium vivax* invasion by a broadly neutralizing vaccine-induced human antibody. *Nat. Microbiol.* 4, 1497–1507
8. Cole-Tobian, J.L. *et al.* (2009) Strain-specific duffy binding protein antibodies correlate with protection against infection with homologous compared to heterologous *Plasmodium vivax* strains in Papua New Guinean children. *Infect. Immun.* 77, 4009–4017
9. Batchelor, J.D. *et al.* (2011) Dimerization of *Plasmodium vivax* DBP is induced upon receptor binding and drives recognition of DARC. *Nat. Struct. Mol. Biol.* 18, 908–914
10. Payne, R.O. *et al.* (2017) Human vaccination against *Plasmodium vivax* Duffy-binding protein induces strain-transcending antibodies. *JCI Insight* 2, e93683
11. Carias, L.L. *et al.* (2019) Identification and characterization of functional human monoclonal antibodies to *Plasmodium vivax* Duffy-binding protein. *J. Immunol.* 202, 2648–2660

Spotlight

Killing of *Plasmodium vivax* by Primaquine and Tafenoquine

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Primaquine administration results in H_2O_2 accumulation in bone marrow, where gametocytes and asexual parasites are therefore killed. This finding, by Camarda *et al.*, supports the theory that the nonperipheral blood origin of recurrent *Plasmodium vivax* malaria is both hypnozoites (relapse source) and merozoites (recrudescence source), not hypnozoites only.

It is invariably assumed that the reason why patients who have been given primaquine (PQ) or tafenoquine (TQ) together with a blood schizontocidal drug often do not suffer any *Plasmodium vivax* malarial recurrences is that PQ and TQ kill hypnozoites (a term coined by me in 1978). The explanation for this good treatment outcome may not be so straightforward, however (see Box 1 for some of the remaining questions).

PQ's antimalarial mechanism has hitherto been unclear, but Camarda *et al.* [1] recently concluded that a two-step biochemical relay process is involved. PQ is first converted into hydroxylated metabolites via the CPR/CYP2D6 metabolic complex [1,2]. Thereafter, spontaneous oxidation of metabolites produces quinoneimine forms, with simultaneous generation of hydrogen peroxide (H_2O_2). The quinoneimines are then reduced back to the hydroxyl forms, perpetuating a catalytic cycle which results in H_2O_2 accumulation. Camarda *et al.* believe that it is the build-up of cytotoxic amounts of H_2O_2 that efficiently kills plasmodial parasites at sites of metabolic transformation. This will happen in the liver and bone marrow; perhaps elsewhere too, a possibility that remains to be clarified. These insights from *Plasmodium falciparum* infection [1] should improve our interpretation of biological and epidemiological aspects of *P. vivax* malaria, and lead to rational drug design.

Of special interest and significance is that the PQ-associated findings for bone marrow [1] correlate with the latest (8-year-old) explanation for recurrence of *P. vivax* malaria [3,4]. See Box 1 in reference [5] for the definitions of 'recurrence', 'recrudescence', and 'relapse', the meanings of which are central to understanding the discussion here. The recurrence hypothesis [3,4] is that (in addition to recrudescences thought to be initiated by parasites in the bloodstream) both hepatic hypnozoites and merozoites in organs and tissues are sources of clinical and parasitemic *P. vivax* malarial recurrences, as opposed to hypnozoites in the liver only. There is no logical reason why recurrent *P. vivax* malaria should necessarily have only one nonbloodstream origin (namely, hypnozoites). The PQ research results [1] and the bimodal *P. vivax* malarial recurrence concept [3,4] are directly related because the new information reported for PQ gives