during pregnancy. In this context, it would be valuable to extend the above studies using hnRNPUL2-deficient mice, specifically myeloid-cell-specific hnRNPUL2 mice.

In summary, the study of Chen et al. highlights a role of the hnRNPUL2-NLRP3 axis, mediated by dysbiosis of *P. merdae* with the reduction of the FMN metabolite, in the excessive trigger of macrophage pyroptosis as part of the sepsis-induced immune dysfunction during pregnancy. An important point to be considered in the future is whether FMN treatment or supplementation could be used as an additional therapeutic strategy for sepsis in pregnant women. Moreover, since NLRP3 is also implicated in the pathogenesis of many autoinflammatory and autoimmune diseases, it is tempting to speculate that FMN might also be a promising pharmacological approach to treat other inflammatory diseases.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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X marks the shot against malaria

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The development of a transmission-blocking vaccine (TBV) against malaria is hampered by poor understanding of functional antibody responses. In this issue of *Immunity*, Fabra-Garcia et al., Ivanochko et al., and Tang et al. isolate human monoclonal antibodies against the two most promising TBV candidates, Pfs48/45 and Pfs230, and map the epitopes responsible for potent transmission-reducing activity.

Plasmodium falciparum malaria remains a significant public health problem, predominantly affecting children in sub-Saharan Africa. Every year, over 200 million cases of malaria are reported, and over 600,000 people die from the disease.[1] Current malaria treatment and prevention interventions seem insufficient to further reduce malaria morbidity and mortality, highlighting the need for additional strategies to ultimately achieve malaria eradication. A highly efficacious malaria vaccine could be a breakthrough in the fight against malaria. While the recombinant protein-based RTS,S/AS01 vaccine has received endorsement from the World Health Organization for "broad use" in areas endemic for malaria,[2] this vaccine showed low to modest long-term efficacy in preventing malaria, and it is clear that more effective malaria vaccines are urgently needed. Most malaria vaccine strategies focus on inhibiting parasite development in the human host. A promising complementary approach to combat malaria incidence is through transmission-blocking vaccines (TBVs).[3] These vaccines do not protect the vaccinated person against infection or disease but elicit antibodies that target and prevent parasite development in the mosquito, thus breaking the cycle of transmission.

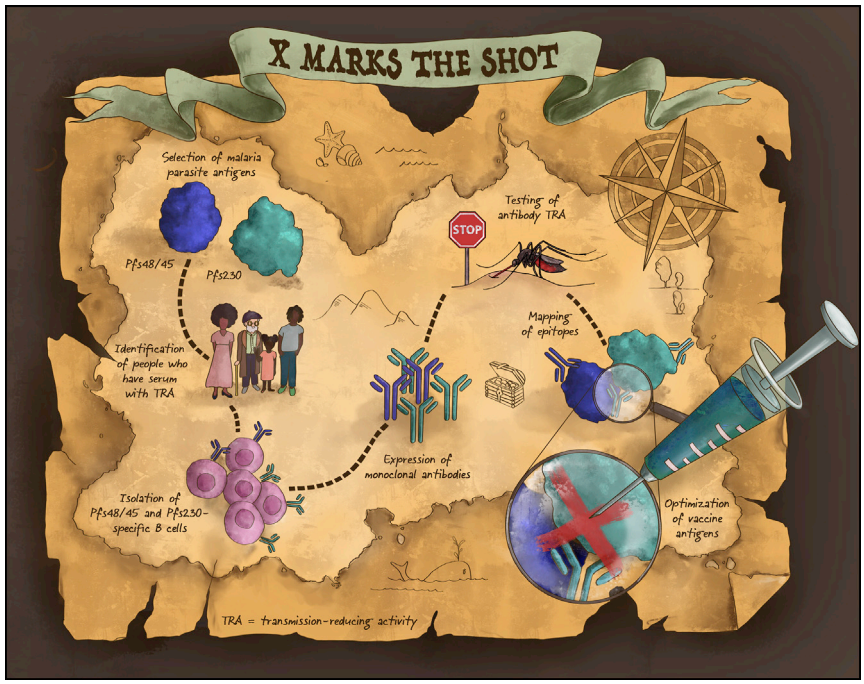

Figure 1. A treasure map showing the route toward a better malaria vaccine

Transmission-blocking vaccines against malaria aim to elicit antibodies that prevent development of the *Plasmodium falciparum* parasite in the mosquito host. The three studies discussed in this Preview analyze human antibody responses that can block parasite transmission. Monoclonal antibodies were isolated from people with prior exposure to the two most promising vaccine candidates, Pfs48/45 and Pfs230, either through infection or immunization. The transmission-reducing activity (TRA) of these antibodies was tested and followed by epitope mapping to identify which sites on the two antigens are targeted by antibodies with highest TRA. The results of these studies will enable optimized design of these vaccine targets to continue the path toward a vaccine able to completely block transmission of the malaria parasite.

Shortly after ingestion by a mosquito, *P. falciparum* male and female gametes undergo fertilization, a critical step for parasite development in the mosquito host. This process is dependent on the presence of various surface proteins, including Pfs48/45 and Pfs230.[4] Pfs48/45 consists of three domains and is attached to the parasite cell surface. Pfs230 is a large secretory protein with 14 domains that is anchored to the parasite through its interaction with Pfs48/45. Antibodies against both proteins have long been known to have transmission-reducing activity (TRA).[5,6] As a result, these two antigens have emerged as the two most promising TBV candidates, and TBVs based on these antigens have recently entered phase 1/2 clinical trials. However, the epitopes targeted by antibodies with TRA are not well characterized, and the human antibody response against these antigens has not been mapped in detail. Three new studies therefore sought to determine the binding sites of large panels of human monoclonal antibodies (mAbs) against Pfs48/45 and Pfs230 with varying degrees of TRA. These studies have yielded antigenic maps of both proteins that will enable improved design of malaria TBV antigens (Figure 1).

Amanda Fabra-Garcia and colleagues[7] aimed to determine the binding sites of Pfs48/45-specific mAbs derived from two naturally exposed humans whose serum had high transmission-reducing activity. Single B cells were isolated using fluorescently labeled full-length Pfs48/45, and the corresponding antibody heavy and light chain variable regions were sequenced. Recombinant antibodies were then produced, and reactivity against Pfs48/45 was confirmed. Out of 81 antibodies tested, the authors found that 27% bound domain (D) 1, 44% bound D2, and 19% bound D3. For the remaining 10% of mAbs, domain specificity could not be determined. The functional potencies of these mAbs were then tested in a membrane feeding assay. In this assay, *Anopheles stephensi* mosquitoes were fed blood containing *P. falciparum* transmission-stage parasites with or without mAbs. After about a week, the number of parasites that have developed inside the mosquitoes was quantified. Using this assay, it was observed that mAbs targeting D1 and D3 were better at preventing transmission than antibodies binding D2. The most potent mAbs were directed against D3, suggesting that this may be the preferential domain to target with a Pfs48/45-based TBV.

To identify the epitopes bound by anti-D3 mAbs with potent *P. falciparum* TRA, the authors used a structural biology approach. Prior to this study, only a single epitope on D3 had been identified as the target of anti-Pfs48/45 mAbs with TRA, the most potent of which is antibody TB31F.[8] Using X-ray crystallography, the authors solved the structures of D3 in complex with four mAbs. The binding sites of two of these mAbs had considerable overlap with that of TB31F, and this epitope was designated Ia. Examination of the two other mAbs that bound to non-TB31F epitopes led to the identification of the novel epitope Ib. Both epitopes Ia and Ib are relatively conserved in *P. falciparum* field isolates, with only three and four known single amino acid polymorphisms in the global parasite population, respectively. The binding affinity of one mAb was significantly reduced by a rare mutation in epitope Ia, but binding of the other mAbs was not impacted by the presence of mutations. Collectively, these data suggest that domain 3 of Pfs48/45 contains two relatively conserved, non-overlapping epitopes that can elicit antibodies with strong TRA. However, a large proportion of the human antibody response to Pfs48/45 is directed against domain 2 and is non-functional. Pfs48/45-based vaccines may therefore induce more potent antibody responses if domain 2 is not present and the immune response is instead directed to the conserved epitopes Ia and Ib on domain 3.

In a similar approach, Danton Ivanochko et al.[9] and Wai Kwan Tang et al.[10] sought to better understand which antigenic epitopes confer potent TRA for Pfs230. Previous work showed that potent transmission-reducing antibodies only bound to domain 1 (D1) of Pfs230.[11] Both studies therefore focused on mapping antibodies to this single domain instead of the full-length 14-domain protein. Ivanochko et al. isolated Pfs230 D1-specific B cells

from two naturally exposed humans, while Tang et al. used B cells from participants of a Pfs230 D1 vaccine trial in Mali. From a total of 16 monoclonal antibodies, Ivanochko et al. identified five antibodies with potent transmission-reducing activity in the membrane feeding assay. The epitopes of three of these potent antibodies were defined using X-ray crystallography, revealing a shared conformational epitope. Furthermore, the authors showed that three antibodies lacking TRA recognized epitopes that were distinct from those bound by the potent antibodies. Tang et al. screened 63 Pfs230 D1-specific human mAbs for transmission-reducing activity using the membrane feeding assay. Of this panel, four mAbs with and four without potent TRA were selected for analysis by X-ray crystallography to determine the epitopes targeted by these antibodies. Similar to the results obtained by Ivanochko and colleagues, all mAbs with potent TRA targeted a large, contiguous surface on one side of Pfs230 D1. All non-potent mAbs bound to distinct epitopes on the opposite side of the protein. Two of these non-potent antibodies also did not bind live parasites, suggesting that their epitopes were not accessible in natively expressed full-length Pfs230.

To understand why one face of Pfs230 D1 may result in (more) potent TRA, Ivanochko et al. modeled the 3D structure of a Pfs230 fragment that spans domains 1 and 2. In this model, the epitopes bound by antibodies with no or weak TRA on D1 were blocked by D2. This computational model was confirmed experimentally by Tang et al., who solved the structure of the Pfs230 D1-D2 protein. These results explain why some antibodies that bind with high affinity to Pfs230 D1 have no TRA and do not react with the full-length protein. Importantly, all non-functional antibodies that bound the backside of Pfs230 D1, which is buried in the native full-length Pfs230, were elicited by immunization. Optimization of Pfs230 vaccine antigens can thus be achieved by shielding these non-desirable epitopes, thereby directing the antibody response to parts of the protein we now know are targeted by potent transmission-reducing antibodies. Finally, both studies tested whether antibodies with potent TRA would also bind polymorphic variants of Pfs230. In general, the epitopes bound by these antibodies were strongly conserved. While a few naturally occurring single amino acid substitutions did reduce antibody binding strength, affinity remained high (in the nanomolar range).

Collectively, these three studies provide insight into the human antibody response to two candidate antigens for a malaria TBV and provide an improved understanding of epitopes that should ideally be targeted by such a vaccine. Focusing the immune response on these specific epitopes can be achieved through various strategies, for example by (1) presenting these epitopes on an unrelated protein backbone, (2) masking undesirable epitopes using N-linked glycans, or (3) introducing specific amino acid substitutions to stabilize the protein in a conformation that better presents desirable epitopes. Indeed, stabilized versions of Pfs48/45 were recently shown to elicit antibody responses with strongly increased TRA as compared to the wild-type protein.[12] The large number of mAbs against Pfs48/45 and Pfs230 with TRA isolated in these studies will enable computational design of improved versions of these antigens. Future studies can also focus on the analysis of synergistic effects between antibodies with TRA. Tang and colleagues showed that antibodies targeting distinct epitopes on Pfs230 D1 can act synergistically. Testing the combinatorial effect of antibodies that target both vaccine candidates will help to discern whether a combination vaccine would potentially elicit superior TRA. Altogether, the results of these three studies bring us a step closer to a vaccine that can successfully block the transmission of the malaria parasite and provide renewed focus on this alternative strategy to combat malaria.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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