



Commentary

The Use of Filter-Purified Merozoites to Assess Anti-Merozoite Immunity



Sergei Biryukov, José A. Stoute*

The Department of Microbiology and Immunology, The Pennsylvania State University, College of Medicine, 500 University Drive, Hershey, PA 17033, United States

The Department of Medicine, Division of Infectious Diseases and Epidemiology, The Pennsylvania State University, College of Medicine, 500 University Drive, Hershey, PA 17033, United States

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The role of complement during the invasion of red blood cells by *Plasmodium* merozoites is an enticing field of study that has great potential to impact not just our overall understanding of how the parasite interacts with the human host but also guide future efforts in the development of efficacious malarial vaccines. The complexity of parasite and host interactions during the invasion of red blood cells (RBCs) by the merozoites and the subsequent growth within the newly invaded host cell has led to conflicting results in the literature. We have recently reported the enhancing effects of anti-merozoite antibodies and complement interactions at promoting red blood cell invasion and growth by merozoites. We have observed this enhancement using both a traditional invasion assay and the *Plasmodium berghei* ANKA model of experimental malaria (Biryukov et al., 2016). Our results are in contradiction to those reported by Boyle et al. (Boyle et al., 2015) who found that in the presence of complement, anti-merozoite antibodies are effective in preventing RBC invasion. We attributed these discrepancies in part to the fact that Boyle et al. use filter-purified merozoites as opposed to naturally egressed merozoites and argue that filter-purified merozoites are defective and more susceptible to complement attack. In their letter to the editor, Boyle and Beeson argue that the discrepancy between our results and theirs cannot be attributed to defective merozoites. We would like to thank Boyle and Beeson for taking the time to read our article and for providing stimulating discussion. We take this opportunity to reply to their comments.

Boyle and Beeson argue that a recent report by Sack et al. (Sack et al., 2015) using late arresting genetically attenuated parasites (LAGAP) supports the role of antibodies and complement in controlling parasitemia. In this study, the authors report that immunization with LAGAP induces antibodies against blood stage parasites. Use of cobra venom factor (CVF) resulted in decreased efficacy of immunization.

Importantly, CVF is a complement activator which will result in widespread C3 activation and opsonization with C3b. In our hands, CVF results in increased parasitemia and we suspect this effect is most likely due to complement activation and not to complement depletion. In order to study the role of complement activation it is best to use genetically-modified animals that are deficient in complement factors, as we did in our study. Further, the passive transfer experiments were done with serum, not with purified antibody, using relatively high amounts per animal (300 μ l on day 0, 3, and 5 post-challenge). Use of purified antibody and titration experiments would have been more informative as to the role of antibodies in this system. As mentioned by Boyle and Beeson, and as discussed in our paper (Biryukov et al., 2016), Taylor et al. (Taylor et al., 2001) reported that C1q-deficient mice had a minimal increase in parasitemia upon secondary challenge with *P. chabaudi*. We have not tested the *P. chabaudi* system. One possible explanation is that complement activation does play a role in suppressing parasitemia in that system. However, an alternative explanation is that C1q may act as a signaling ligand for phagocytosis by macrophages (Galvan et al., 2012) and that this finding may not be related to downstream complement activation.

Boyle and Beeson state that other studies have failed to see an increase in invasion when using serum and cite three reports (Campbell et al., 1979; Chulay et al., 1981; Kennedy et al., 2015). The studies by Campbell et al. and Chulay et al. were carried out with *P. falciparum* grown in human RBCs exposed to serum from immune and non-immune *Aotus* monkeys. In these cases, there is a confounding effect of incompatibility between *Aotus* serum and human RBCs that makes interpretation difficult. Kennedy et al. did not measure parasitemia directly but measured parasite LDH as a surrogate. In addition, they allowed the parasites to grow for 32–36 h as opposed to overnight cultures in our studies.

Finally, Boyle and Beeson argue that if antibodies against MSP1 were to increase invasion, trials of MSP1-based vaccines would have observed increased parasitemias in vaccinated individuals. We feel this is a misinterpretation of our findings. The final outcome of a vaccine, whether efficacy (inhibition of the pathogen), enhancement, or neither, will depend on the repertoire of antibodies that are produced and on their target antigens. Our studies have demonstrated that it is possible for anti-merozoite antibodies to enhance RBC invasion and this mechanism can counteract the efficacy of inhibitory antibodies. Thus, if a vaccine is producing both inhibitory antibodies and antibodies that enhance invasion, the net effect could be efficacy (inhibition), no efficacy, or an enhancing effect, depending on the relative amounts of each of the types of antibodies produced. Hence, the lack of enhancement effect in

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* Corresponding author at: 500 University Drive, Rm C6860, MC H036, Hershey, PA 17033, United States.

E-mail address: jstoute@psu.edu (J.A. Stoute).

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vaccine trials, does not exclude the possibility that some antibodies actually enhance.

The use of filter-purified merozoites has been championed by Boyle and Beeson and the vast majority of the published studies utilizing this merozoite purification method have come from their laboratory or close collaborators. By their own admission, their assay is “more sensitive than standard growth inhibition assays” (Boyle et al., 2010) which could be interpreted as saying that the merozoites are more susceptible to inhibition than naturally egressed merozoites (Figure 3C and 3D, (Boyle et al., 2010)). We propose that the increased susceptibility to inhibition by antibodies is due to damage from excessive manipulation during the purification process. In addition to filtration, depending on the protocol used by Boyle and Beeson, this could also include an additional purification step through magnetic columns to remove hemozoin. The damage to the merozoites is reflected in the fact that an extremely high ratio of purified merozoites to RBCs (~6:1) is needed to attain a parasitemia of ~1.8% after 24/48 h (Figure S5B, (Boyle et al., 2010)). Assuming that 16 merozoites egress from one schizont, this merozoite to RBC ratio is equivalent to incubating target RBCs with a starting parasitemia of ~27.3% (3 infected RBCs for 8 uninfected RBCs). By contrast, our invasion assays can easily achieve a parasitemia of 8% or higher with a starting parasitemia of 0.5 to 1%.

We hypothesize that filtration is also causing stripping of merozoite surface proteins. Recent work has shown that merozoites can recruit the soluble complement regulator factor H (Kennedy et al., 2015; Rosa et al., 2015). We are concerned that filtration could also strip factor H or other complement regulators off merozoites making them more susceptible to complement. However, we are aware that the studies of factor H used filter-purified merozoites. Therefore, if stripping does occur, it does not lead to 100% removal of surface antigens.

Despite its shortcomings, we believe that the use of filter-purified merozoites can be a useful tool in the understanding of host-parasite interactions. However, we need to understand its limitations. In our hands (Biryukov et al., 2016), filter-purified merozoites are not equivalent to naturally egressed merozoites that are allowed to invade within

minutes. Therefore, in our opinion, any invasion data that utilizes filter-purified merozoites should be interpreted with caution.

Sincerely,

José A. Stoute

Sergei Biryukov

The authors declare no conflict of interest.

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