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Commentary

Evaluating Complement-Mediated Humoral Immunity to *P. falciparum* Blood Stages

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Understanding mechanisms of immunity that protect from malaria is essential for the development of highly effective vaccines. The merozoite form of the parasite, which invades red blood cells, is an important target of protective immunity (Beeson et al., 2016). Biryukov et al. bring to our attention the potential ability of complement factors to enhance *P. falciparum* merozoite invasion *in vitro* (Biryukov et al., 2016). They proposed that i) complement activation at the merozoite surface enhanced the ability of merozoites to bind and invaded RBCs and ii) that antibodies to merozoite surface protein 1 (MSP1) C-terminal region further enhanced this process. These findings were primarily demonstrated with a murine monoclonal antibody (mAb) to MSP1-19, and some assays were performed with samples from a MSP1-42 phase I vaccine trial in malaria-naïve adults. We wish to add further discussion to the potential relevance of these findings, and how they differ from previously published studies to further understand anti-malarial immunity and pathogenesis.

We previously reported that there is no difference in merozoite invasion efficiency in normal, complement active sera compared to heat inactivated, complement inactive human sera in the absence of immune antibodies, even at 80% serum concentrations (Boyle et al., 2015). Further, we reported that complement active serum significantly increases the inhibitory capacity of naturally acquired and vaccine-induced human and rabbit antibodies targeting the merozoite (Boyle et al., 2015). We showed that many antibodies required complement factors for invasion inhibitory activity, and we demonstrated the importance of this mechanism for naturally acquired and vaccine induced human and rabbit antibodies against specific merozoite proteins including the abundant merozoite surface proteins, MSP1 and MSP2 (Boyle et al., 2015). Recent results from a mouse model of malaria supports this role for complement-dependent invasion inhibition and reported that antibody-mediated protection from *P. yoelii* infection malaria after immunization with a genetically attenuated parasite vaccine was partly mediated by antibody interactions with complement (Sack et al., 2015). Consistent with these findings are previous studies in *P. chabaudi* models that demonstrate that C1q-deficient animals were significantly more susceptible to secondary challenge with the same parasite stain, highlighting the central role of C1q and classical complement activation in acquired immune responses (Taylor et al., 2001).

We disagree with the suggestion of Biryukov et al. that the discrepancy between their and our findings is due to heighted sensitivity of isolated merozoites to complement activation. As we have previously shown, isolated merozoites are structurally intact, invade at similar efficiencies to standard routine culture, and do not require serum factors to invade nor show enhanced invasion in serum (Boyle et al., 2010). Since publication, numerous other studies have used this method for isolating viable merozoites, and increased sensitivity/decreased viability has not been reported. It is possible that the impact of complement on invasion is different in standard growth assays as used by Biryukov et al. compared to Boyle et al. (2010), or depending on parasite strains used. However, we and others have previously included normal sera in growth assays using a number of different parasite lines; reduced or comparable growth has been consistently reported, but not enhanced invasion compared to heat inactivated sera ((Campbell et al., 1979; Chulay et al., 1981; Kennedy et al., 2015) and unpublished data Boyle, Beeson et al.). It seems more likely that the major difference between the findings is the type of antibodies used; naturally acquired and vaccine induce human and rabbit polyclonal antibodies against different merozoite antigens by Boyle et al. compared to murine mAb or vaccine induced human antibodies to the C terminal of MSP1 in the case of Biryukov et al.

While Biryukov et al. propose that mouse monoclonal antibodies to MSP1-19 augment the enhancement effect of normal sera, the reported effect was only moderate when considering absolute parasitamias, and there appears to be large variability in the effect as seen across experimental replicates (data presented in the supplementary materials within Biryukov et al., 2016). Data was presented showing a reduced level of inhibition mediated by sera from MSP1-42 vaccinated malaria-naïve human subjects in C3/C4 supplemented serum compared to C3/C4

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depleted serum. However, in non-vaccinated controls, there was no difference in invasion between complement active and complement inactivated sera, as would be expected if complement activated sera significantly enhanced invasion and if MSP1-42 C-terminal antibodies augmented this effect (Biryukov et al., 2016). Further, while Biryukov et al. support their findings with *in vivo* animal models using *P. berghei*, complement levels in mice can be very low, and it is unclear how generally findings in mice and with mouse antibodies can be applied to human immunity. Perhaps the phenomena of enhanced invasion in the presence of mAb MSP1-1-19 can be attributed to a specific function of the epitope that this antibody targets and the species of origin of this antibody, rather than a more general function of antibodies that target merozoites.

A further consideration is that if antibodies to MSP1 in humans enhanced invasion, it might be expected that an MSP1-based vaccine would increase the risk of parasitemia or malaria incidence. However, in a phase 2 trial of MSP1-42 (a vaccine designed to generate antibodies), vaccination resulted in increased antibody titres, but had no efficacy and did not increase the risk of malaria or parasitemia (Ogutu et al., 2009). Furthermore, we found that the ability of acquired human antibodies to fix and activate complement on the surface of merozoites, was associated with a very strong reduction in the risk of malaria and high density *P. falciparum* parasitemia (Boyle et al., 2015), the opposite of the effect that would be seen if complement was important for enhancing invasion *in vivo*. We believe there is strong and growing evidence to support a key role in antibody-dependent complement activation in inhibition of merozoite invasion, and protection from malaria. We agree with the authors that antibody-complement activity needs to be measured as part of vaccine candidate assessments *in vivo*, to assess antibody function with complement, in contrast to currently established assays *in vitro* that use complement-free conditions.

Conflict of Interest

The authors declare no conflicts of interest.

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