Review: Controlled Human Blood Stage Malaria Infection: Current Status and Potential Applications

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Abstract. Controlled human malaria infection by blood stage parasite (BSP) inoculation is an alternative to the well-established model of infection with Plasmodium falciparum sporozoites delivered by mosquito bites. The BSP model has been utilized less frequently, but its use is increasing. Advantages of BSP challenge include greater ease of administration, better standardization of the infecting dose per volunteer, and good inter-study reproducibility of in vivo parasite dynamics. Recently, a surprising reduction in clinical symptoms at microscopic patency in the BSP model has been identified, which has an undefined and intriguing pathophysiologic basis, but may make this approach more acceptable to volunteers. We summarize clinical, parasitologic, and immunologic data from all BSP challenges to date, explore differences between the BSP and sporozoite models, and propose future applications for BSP challenge.

INTRODUCTION

The role of controlled human malaria infection (CHMI) in testing efficacy of candidate vaccines and drugs against Plasmodium falciparum malaria is well-established.1,2 A recent review has focused on the widely studied sporozoite (Spz) challenge model,3 whereby persons are exposed to the bites of typically five malaria-infected mosquitoes.4 An alternative model, involving inoculation of donor blood-stage parasites (BSP), is used less frequently. Both methods were historically employed in malaria therapy for neurosyphilis with comparable clinical and parasitologic outcomes.5

CONTROLLED HUMAN BLOOD STAGE MALARIA INFECTION

The P. falciparum BSP model developed at the Queensland Institute for Medical Research in 1997 uses a cryopreserved stock of erythrocytes from a parasitemic donor.6 Intravenous injection of a sterile suspension of thawed leukocyte-depleted erythrocytes, combined with monitoring of parasitemia post-inoculation by highly-sensitive quantitative polymerase chain reaction (qPCR), enables accurate calculation of in vivo parasite multiplication rates (PMRs), a surrogate end-point for vaccine efficacy testing.4,6,9 The inoculum, estimated from the pre-freeze donor parasitemia, is retrospectively quantified by testing viability of the thawed stock.7 Volunteers in BSP CHMIs were typically treated at a pre-defined parasite density predicted to prevent clinical malaria,4,9,10 although more recent studies have used the onset of microscopic patent parasitemia as a treatment endpoint.5,11 The former approach generally avoids symptoms, with the ethical advantage of reducing volunteer discomfort, and the latter approach mirrors the more established Spz model and enables assessment of additional endpoints such as the microscopic pre-patent period.

Fifty-nine volunteers have been infected in this manner by using the same frozen starting material,4,6,9,12 extending the safety database for this parasite stock. There is an extremely low risk of blood-borne virus transmission because of stringent screening of the original donor, and requirements for seropositivity for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) in recipients (because of donor seropositivity).4,11 Accumulated experience with BSP challenge over 14 years is summarized in Table 1. In studies that measure it, the microscopic pre-patent period is highly reproducible, suggesting overall maintenance of parasite growth and invasion characteristics despite prolonged storage. However post-thaw viability of the parasite stock varies widely (10–100%). This finding does not appear to be related to duration of storage, but may be caused by sensitivity of the parasites to freeze-thaw procedures and storage conditions,6 and the current lack of an agreed or standardized viability assay.

Blood-stage vaccine assessment. Although BSP challenge bypasses the pre-erythrocytic stages of infection, it has a role in testing blood-stage vaccine efficacy.6,9,11 The uniform inoculum simplifies PMR calculations and enables estimation of PMR with a greater degree of confidence (Figure 1A), and the low starting parasite load should enable detection of subtle effects on PMR with greater sensitivity than Spz challenge.11 Contrasts between the models are summarized in Table 2. A major advantage is that BSPs (similar to cryopreserved Spzs) can be transported to areas without mosquito culture and infection facilities, and could therefore be used for vaccine efficacy testing in centers lacking a challenge suite. However, only two blood-stage (BS) vaccines have been tested by BSP challenge to date.6,9 Although the small number of BSP challenge trials conducted reflects the relative paucity of Phase IIa CHMI trials of blood stage vaccines, there are several additional explanations (Table 2). These include negative impacts on volunteer acceptability from the perceived risks associated with receipt of a blood product, and the limitations on future blood donation imposed by blood transfusion services; the absence of regulatory approval in some regions (e.g., the United States); and the negative impact on volunteer eligibility arising from the requirement for recipient EBV and CMV seropositivity.6

In the two BS vaccine efficacy studies conducted by BSP challenge, vaccination did not reduce overall PMR6,9 or delay time to microscopic patent parasitemia.6 A promising association between in vitro growth inhibition and in vivo PMR needs to be replicated in other studies. However, in the absence of an efficacious BS vaccine capable of significantly reducing...
### Table 1
Overview of published blood stage parasite controlled human malaria infection trials

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>No. volunteers</th>
<th>Primary objective</th>
<th>Center</th>
<th>No. parasites inoculated (median) (range)</th>
<th>Viability (%)</th>
<th>Treatment indication</th>
<th>Symptomatic at diagnosis</th>
<th>Parasite density at treatment parasites/mL (median)</th>
<th>Microscopic patency</th>
<th>Pre-patent period (median)</th>
<th>PMR (median)</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng and others</td>
<td>1997</td>
<td>5†</td>
<td>Pilot</td>
<td>QIMR</td>
<td>3,000 (300–6,000)</td>
<td>100</td>
<td>3/5: MP, 2/5: PD (&gt;1,000 p/mL)</td>
<td>Treated at day 8 without symptoms</td>
<td>3,178 (peak)††</td>
<td>3/3‡</td>
<td>8</td>
<td>12.5</td>
<td>NA</td>
</tr>
<tr>
<td>Lawrence and others</td>
<td>2000</td>
<td>17</td>
<td>Vaccine impact on PMR</td>
<td>QIMR</td>
<td>127 (114–140)</td>
<td>38</td>
<td>3/5: MP, PD (&gt;1,000 p/mL)</td>
<td>Treated at day 8 without symptoms</td>
<td>913–3,000 (range)#</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Combination B</td>
</tr>
<tr>
<td>Pinto and others</td>
<td>2002</td>
<td>5</td>
<td>Protection with repeated challenge</td>
<td>QIMR</td>
<td>30</td>
<td>62</td>
<td>4/5: MP, PD (&gt;1,000 p/mL)</td>
<td>1/5: Sympt.</td>
<td>Not stated (≥10,000)</td>
<td>4/5‡</td>
<td>8</td>
<td>21.5</td>
<td>NA</td>
</tr>
<tr>
<td>Sanderson and others</td>
<td>2008</td>
<td>5</td>
<td>Pilot</td>
<td>CCVTM</td>
<td>1,800</td>
<td>82</td>
<td>4/5: MP, PD (&gt;1,000 p/mL)</td>
<td>Treated at day 8 without symptoms</td>
<td>3,178 (peak)††</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Duncan and others</td>
<td>2011</td>
<td>8</td>
<td>Relationship of PMR to GIA</td>
<td>CCVTM</td>
<td>250</td>
<td>8</td>
<td>4/5: MP, PD (&gt;1,000 p/mL)</td>
<td>1/5: Sympt.</td>
<td>4,108</td>
<td>3/3‡</td>
<td>8</td>
<td>NA</td>
<td>Combination B</td>
</tr>
<tr>
<td>McCarthy and others</td>
<td>2011</td>
<td>19</td>
<td>Kinetics of parasite clearance by antimalarial drugs</td>
<td>QIMR</td>
<td>30</td>
<td>25</td>
<td>4/5: MP, PD (&gt;1,000 p/mL)</td>
<td>NA</td>
<td>4,108</td>
<td>3/3‡</td>
<td>8</td>
<td>NA</td>
<td>Combination B</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>59</td>
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*PMR = parasite multiplication rate per 48 hours; GIA = in vitro assay of growth inhibitory activity; QIMR = Queensland Institute for Medical Research, Brisbane, Queensland, Australia; CCVTM = Center for Clinical Vaccinology and Tropical Medicine, Oxford, United Kingdom; MP = microscopic patency; PD = parasite density threshold; p/mL = parasites/mL; Sympt = treatment on symptoms and parasitemia (by quantitative polymerase chain reaction) before microscopic patency; NA = not available; AMA1 = apical membrane antigen 1.

† Two of five participants who had had malaria received their own autologous blood stage parasites, 1 of 5 malaria-naïve participants received the blood stage parasite stock and was treated at blood film patency, and 2 of 5 malaria-naïve participants were treated with 500–1,000 parasites/mL before symptoms or microscopic patency.

‡ The denominator (3) includes only persons given a diagnosis by blood film microscopy (although 2 were not malaria naïve and received their own autologous blood stage parasites).

§ Dr J. McCarthy (unpublished data).

¶ Day of diagnosis data not provided.

# Individual values and central tendency not provided.
PMR, possibly to a level approaching that estimated for semi-immune persons, we cannot assess whether efficacy in the BSP model predicts efficacy against clinical malaria in the field. Without such a vaccine, the relationship between reductions in PMR, as measured by qPCR, and delay in time to a clinically-relevant outcome, such as microscopic patency also remains experimentally unconfirmed. The absence of detection of a significant vaccine effect(s) in Phase IIa BSP challenge studies to date may also be seen as a limitation to the more widespread acceptance of this model in BS vaccine efficacy assessment, although this criticism could also be leveled at the Spz model. No blood stage vaccine candidate has yet provided convincing evidence of BS efficacy in Spz CHMI although this is a more stringent model for BS vaccine efficacy assessment given the larger parasite load. Promising evidence for protective efficacy of vaccines targeting the leading blood-stage candidate antigen (apical membrane antigen 1) suggests a potential added contribution from responses acting at the pre-erythrocytic stage(s), resulting in significant reductions in liver-to-blood inocula, which would not be detected by BSP challenge. Ultimately, determination of the relative advantages of one model over the other in determining BS vaccine efficacy awaits the development of efficacious BS vaccines.

Pathophysiology. An interesting aspect of the BSP model is the apparent lower frequency of malaria symptoms compared with Spz challenge (P = 0.005) (Figure 1B), which appears unrelated to PMR (Figure 1A), parasite density at diagnosis or peak parasitemia (Figure 2). An intriguing possibility is that reduced diversity of P. falciparum var gene expression (observed after blood stage passage), may lead to reduced switching of P. falciparum erythrocyte membrane protein 1, resulting in attenuated pathogenicity. Conversely, the reduced diversity of var gene expression observed could reflect less host immune recognition. Antibodies against candidate vaccine

Figure 1. Confidence of parasite multiplication rate (PMR) calculations and frequency of malaria symptoms at blood film diagnosis. A, Individual PMRs ± 95% confidence intervals (modeled from quantitative polymerase chain reaction [qPCR] data as described) for seven volunteers with sufficient data from a recent blood stage challenge trial and unimmunized infectivity controls from a recently conducted sporozoite challenge study (n = 12 volunteers) (Ewer KJ, and others, unpublished data). See also Sanderson and others for a similar analysis. B, Clinical symptoms from two blood-stage challenge trials conducted in Oxford (n = 13 volunteers) and unimmunized infectivity controls from a recently conducted sporozoite challenge study (n = 12 volunteers) (Ewer KJ, and others, unpublished data). Two of eight and one of five volunteers were symptomatic in the respective blood stage parasite (BSP) infection studies compared with 10 of 12 sporozoite (Spz) infection volunteers. Methods of qPCR and clinical assessment of symptoms were identical in all studies. Significance testing by Fisher’s exact test (Prism version 5.0).
antigen(s) were not increased in immunized persons after BSP exposure and were not detectable in unimmunized participants after the two reported Phase IIa challenge studies.6,9

Alternatively, the low starting parasite dose (median = 360 parasites) (Table 1), which results in a prolonged sub-patent phase of parasitemia undetectable by qPCR (3–5 days), could provide a greater window of opportunity than following Spz challenge for the acquisition of undefined anti-disease immunity (Figure 2) because in the Spz model, just one infected hepatocyte will seed the blood with ~30,000 merozoites.7 Similarly, there are indications that a larger BSP inoculum (3,000–6,000 parasites) may increase the frequency of symptoms.4,12 Further work is thus needed to explore the phenotype of the host response to cryopreserved BSPs.

Blood stage CHMI has also provided some insights into malaria immunity. Protection against repeated low-dose BSP infection followed by drug cure was associated with parasite-specific T cell responses and nitric oxide synthase activity of mononuclear cells, but not antibodies.10 However, prolonged in vivo effects of atovaquone are likely to have confounded the protection observed,10 and to date this widely cited finding has never been replicated. Similarly, in a Spz challenge study, the BSP-specific T effector memory response was associated with protection in volunteers previously exposed to Spzs under chloroquine prophylaxis,20 suggesting that a protective T cell response to BSPs may operate in vivo. An on-going BSP challenge trial will help to determine the stage-specificity of this protection (ClinicalTrials.Gov/NCT01236612).

FUTURE APPLICATIONS

One of the central unanswered questions in malaria immunology concerns the true nature of the protective immune responses in vivo in humans.21 Spz and BSP CHMIs in semi-immune volunteers have significant potential to help address this question. A comparative approach using both models to infect semi-immune volunteers may dissect stage-specific determinants of natural immunity. Moreover, direct and accurate quantification of liver-to-blood inocula and PMRs in semi-immune volunteers would validate these models and set the goal posts for future prophylactic malaria vaccines. It should be noted that such studies would not be designed to provide efficacy assessment of candidate vaccines in target populations (e.g., non-immune infants), which would continue to be addressed by traditional Phase IIb studies.

To date, only three related parasite strains have been tested in CHMI.1 A panel of diverse cryopreserved BSPs would enable assessment of cross-strain efficacy prior to large-scale Phase IIb field trials. New BSP stocks from EBV/CMV seronegative donors could also dramatically improve recruitment of volunteers in areas with low EBV/CMV seroprevalence.1 Finally, a P. vivax Spz challenge model has also been recently developed which requires a supply of infected donor blood for gametocyte propagation and mosquito infection. An alternative approach may involve directly preparing P. vivax BSP stocks from a carefully screened parasiticemic donor.

CONCLUSIONS

Interesting and unanswered questions remain about the pathogenicity and immunogenicity of the BSP inoculum, and it is highly likely this model will continue to complement Spz challenge in assessing candidate BS vaccine efficacy, as well as providing insight into mechanisms of malaria immunity in vivo in humans.

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REFERENCES


