AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

MINIREVIEW

Controlled Human Malaria Infection: Applications, Advances, and Challenges

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ABSTRACT Controlled human malaria infection (CHMI) entails deliberate infection with malaria parasites either by mosquito bite or by direct injection of sporozoites or parasitized erythrocytes. When required, the resulting blood-stage infection is curtailed by the administration of antimalarial drugs. Inducing a malaria infection via inoculation with infected blood was first used as a treatment (malariotherapy) for neurosyphilis in Europe and the United States in the early 1900s. More recently, CHMI has been applied to the fields of malaria vaccine and drug development, where it is used to evaluate products in well-controlled early-phase proof-of-concept clinical studies, thus facilitating progression of only the most promising candidates for further evaluation in areas where malaria is endemic. Controlled infections have also been used to immunize against malaria infection. Historically, CHMI studies have been restricted by the need for access to insectaries housing infected mosquitoes or suitable malaria-infected individuals. Evaluation of vaccine and drug candidates has been constrained in these studies by the availability of a limited number of *Plasmodium falciparum* isolates. Recent advances have included cryopreservation of sporozoites, the manufacture of well-characterized and genetically distinct cultured malaria cell banks for blood-stage infection, and the availability of *Plasmodium vivax*-specific reagents. These advances will help to accelerate malaria vaccine and drug development by making the reagents for CHMI more widely accessible and also enabling a more rigorous evaluation with multiple parasite strains and species. Here we discuss the different applications of CHMI, recent advances in the use of CHMI, and ongoing challenges for consideration.

KEYWORDS controlled human malaria infection, drug development, experimental malaria, *Plasmodium*, vaccine development, malaria

Controlled human malaria infection (CHMI) can be undertaken either by inoculation of sporozoites via mosquito bite or by direct injection of sporozoites or *Plasmodium*-infected blood. The inoculation of sporozoites allows both liver- and blood-stage infection to develop, while induced blood-stage infection with parasitized erythrocytes results in blood-stage infection only. Blood-stage infection is truncated by antimalarial drug treatment that is initiated according to predefined study-specific criteria. Studies utilizing induced blood-stage infection typically treat infections at a predefined blood-stage parasite density (as determined by PCR) or at the onset of microscopic patency. CHMI studies involving sporozoite-initiated infections have also relied on microscopic patency as the trigger for treatment, although more recently, quantitative PCR (qPCR) has been explored as the primary test for initiating treatment in sporozoite-initiated CHMI studies (1).

Accepted manuscript posted online 18 September 2017

Citation Stanisic DI, McCarthy JS, Good MF. 2018. Controlled human malaria infection: applications, advances, and challenges. Infect Immun 86:e00479-17. https://doi.org/10.1128/IAI.00479-17.

Editor Helene L. Andrews-Polymenis, Texas A&M University Health Science Center

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TABLE 1 Applications of controlled human malaria infection

Application	Reference(s)
Drug evaluation	
Sporozoite challenge	12, 92–106
Blood-stage challenge	9–11, 95, 96, 98–103, 105–114[a]
Vaccine evaluation	
P. falciparum preerythrocytic vaccine candidate	
Sporozoite challenge	18, 20, 36–38, 40, 42, 79, 80, 115–151
Blood-stage challenge	115
P. falciparum blood-stage vaccine candidate	
Sporozoite challenge	14, 20 36, 42, 125, 126, 131, 145, 146, 151
Blood-stage challenge	13, 16, 19
P. vivax preerythrocytic vaccine candidate	
Sporozoite challenge	15, 17, 37, 149
Blood-stage challenge	Not applicable
Immunization strategy	
Chemoprophylaxis and sporozoites	7, 8, 49–51, 81, 152
Blood-stage infection and drug treatment	6
Parasite diagnostics	21, 22
Parasite biology	153
Factors influencing virulence	23–25, 154–156
Disease processes	26, 157–159
Human immune response	27–32, 84, 86, 160–209

[a]Studies registered on the Australian New Zealand Clinical Trials Registry but not yet published: ACTRN12617000244303 and ACTRN12614000781640.

Deliberate human malaria infection with malaria parasites was initially used as a treatment (malariotherapy), for neurosyphilis in the early 1900s (reviewed in references 2 and 3). It was also used in the 1990s in limited and contentious studies as a potential treatment for HIV infection (4). From the 1940s, its utility as a tool to evaluate candidate antimalarial drugs was recognized when it was employed to assess their efficacy in healthy, nonimmune males by inoculation of *Plasmodium*-infected blood or mosquitoes (5). Since then, it has been increasingly recognized that CHMI offers a well-controlled and safe framework to undertake *in vivo* assessment of the efficacy of malaria vaccine candidates and drugs (Table 1). Researchers are also investigating the use of CHMI to immunize against malaria infection, with protection observed following multiple cycles of infection (sporozoite or blood-stage initiated) and drug treatment (see, e.g., references 6 to 8) (Table 1). Cumulative experience with CHMI over the past decades, as well as recent advances in methodologies and reagents, has resulted in the development of well-characterized experimental systems that are becoming more widely accessible to researchers working in malaria vaccine and drug development. Below, we describe the primary applications of CHMI and recent advances and highlight some of the challenges to be considered.

USES OF CONTROLLED HUMAN MALARIA INFECTION

CHMI is a valuable tool that can be used to evaluate novel antimalarial drugs (see, e.g., references 9 to 12) (Table 1), malaria vaccine candidates (see, e.g., references 7 and 13 to 20) (Table 1), and diagnostic tools (see, e.g., references 21 and 22) (Table 1). Substudies undertaken within the framework of CHMI studies for drug or vaccine evaluation have also enabled an examination of parasite biology, e.g., factors influencing virulence/disease processes (see, e.g., references 23 to 26) (Table 1) and malaria-specific human immune responses (see, e.g., references 27 to 31) (Table 1), including identification of possible immune correlates of protection (19, 31, 32). Recently, vaccine development efforts have focused on using CHMI to induce protective immunity by

truncating malaria infection at low parasitemia with drug treatment (see, e.g., references 6 to 8) (Table 1). Following multiple cycles of infection and drug treatment, protection against malaria infection has been demonstrated (6–8). Below, we discuss the main applications of CHMI: its role in drug and vaccine evaluation and as an immunization strategy against malaria infection.

Drug evaluation. Increasing levels of antimalarial drug resistance, including resistance against artemisinin-containing drugs, emphasize the urgent need for the development of new antimalarials. Following preclinical and phase I studies, phase II clinical studies are required to identify the correct dosing regimen to enable cure. CHMI in malaria-naive individuals offers a well-controlled environment to rapidly assess the efficacy of drugs with unknown therapeutic activity and to obtain pharmacokinetic and pharmacodynamics data (9–11). An additional advantage of this approach is that, providing study participants are screened appropriately, antimalarial immunity will not affect parasite clearance rates, which could lead to an overestimation of drug efficacy (33). The use of rapid and sensitive qPCR assays for parasite quantification ensures that rescue treatment with fast-acting antimalarial drugs can be administered promptly (11). Both mosquito bite- and blood-stage-initiated infections have been used in this way to evaluate antimalarial drugs (see, e.g., references 9, 12, and 34) (Table 1). While sporozoite-initiated infection is obviously required to assess the causal prophylactic activity of drugs (12, 34), one of the major advantages of induced blood-stage malaria infection for assessing parasite clearance by blood schizonticidal drugs is the standardization and precise quantification of the number of parasites initiating the blood-stage infection in each study participant.

Vaccine evaluation. Following the demonstration of safety and immunogenicity in a phase I study, undertaking phase IIa studies in areas where malaria is not endemic using CHMI enables the generation of proof-of-concept efficacy data prior to transitioning a vaccine candidate into costly phase IIb field trials in areas of endemicity (35). The efficacy of malaria vaccine candidates has been assessed using sporozoite or induced blood-stage malaria infection prior to field testing (see, e.g., references 8, 13, 18, 36, and 37) (Table 1) and for further optimization of the immunization regimen following suboptimal efficacy of a vaccine candidate in the field (38). For the former, if good efficacy is not demonstrated in phase IIa trials, this may halt progression of the vaccine candidate. For some candidates, however, one might predict an improvement in vaccine-induced protection in areas of endemicity where boosting of the vaccine-specific immune response may occur following natural exposure, or alternatively, the vaccine may augment preexisting naturally acquired immune responses. This has not yet been observed for any malaria vaccine candidate that has progressed into trials in areas where malaria is endemic, including the licensed malaria vaccine RTS,S/AS01 (39). In CHMI studies in malaria-naive humans, the endpoints for evaluation of vaccine efficacy are life cycle stage specific. For preerythrocytic vaccine candidates, the traditional study endpoint is detection of a patent blood-stage infection by microscopy. Where blood-stage infection does develop and with the use of sensitive qPCR methods, the resulting blood-stage parasitemia data can also be analyzed to obtain information on additional parasite parameters, e.g., reduction in liver load (40, 41). For blood-stage vaccine candidates, the primary assessment is the parasite multiplication rate (PMR). This can be derived from analysis of qPCR-based parasitemia data from an adequate number of time points and is used to detect differences between vaccinees and control subjects.

Historically, experimental sporozoite-initiated infection has been more widely used to evaluate vaccines in phase IIa trials and has been used predominantly for testing preerythrocytic vaccine candidates (see, e.g., references 8, 18, and 37) (Table 1), although it has been used for a small number of studies involving blood-stage vaccine candidates (14, 42). The obvious benefit of using the sporozoite-initiated infection model is that it mimics the natural route of infection; however, as it is not possible to control the number of sporozoites being inoculated by a mosquito, the challenge dose

can be highly variable (43, 44). While the use of cryopreserved, purified sporozoites delivered by needle and syringe may result in a more reproducible inoculum, further work is required to optimize this system (45).

Induced blood-stage malaria infection has also been used for evaluation of blood-stage vaccine candidates in phase IIa studies (13, 16, 19), and although a direct comparison of both CHMI models to test blood-stage vaccine efficacy has not yet been undertaken, it offers a number of advantages in malaria-naive individuals compared with sporozoite-initiated infections (46). First, being able to precisely enumerate the number of parasites initiating the blood-stage infection allows modeling of the PMR with greater accuracy, thus providing greater power to detect partial efficacy of blood-stage vaccines (19). Second, initiating a blood-stage infection with fewer parasites in the inoculum than the theoretical number of merozoites released from an infected hepatocyte can result in a prolongation of the period when submicroscopic parasitemia can be observed and measured before drug treatment is required (46, 47). Not only does this increase the number of time points at which to collect parasitemia data and thus enable a more accurate modeling of PMR, it also increases the time over which a vaccine-induced immune response can operate (and thus prevents the premature abandonment of a partially effective vaccine that could be further optimized). Similarly to the case for the sporozoite-initiated infection model, there are a number of potential shortcomings that should be considered. Viability of the injected parasites can be determined only retrospectively, making it difficult to standardize the number of viable parasites in the inoculum. Parasite viability has been shown to vary across different studies and sites (47), and this can be influenced by storage conditions and the time between thawing of the parasites and inoculation of the volunteers (46). Additionally, by circumventing the liver, induced blood-stage challenge will not detect effects on preerythrocytic parasite stages and thus may underestimate the efficacy of vaccines containing antigens that are shared between liver and blood stages.

CHMI: an immunization strategy. The use of whole parasites as a vaccine approach is advantageous, due to the broad array of antigens presented to the immune system. A number of research groups are focused on developing whole-parasite vaccines utilizing the CHMI model, which involves either sporozoite-induced malaria infection or induced blood-stage malaria infection. Different variations of CHMI are being examined, with the regimen consisting of multiple rounds of infection and drug treatment (see, e.g., references 6 to 8) (Table 1). The protective efficacy of a strategy using blood-stage CHMI has been examined in humans (6). Multiple low doses of *Plasmodium falciparum*-parasitized red blood cells were administered intravenously to malaria-naive volunteers, with each infection truncated with Malarone (atovaquone-proguanil) prior to patency (6). While parasite-specific antibodies were not detected, robust cellular immune responses were induced and protection was observed in 3 out of 4 volunteers, although it could not be excluded that residual antimalarial drug may have contributed to this protection (48). Preerythrocytic vaccine approaches utilizing live sporozoites are more advanced than blood-stage vaccine approaches, and multiple studies have examined the protective efficacy of this approach (see, e.g., references 7 and 8) (Table 1). The chemoprophylaxis and sporozoite (CPS) approach involves administering multiple mosquito-bite induced infections under chemoprophylaxis. Induction of long-lived sterile protection against homologous challenge has been demonstrated (7, 49). Drugs targeting blood stages are used in this approach to enable full liver-stage development. Although low levels of blood-stage parasitemia are observed following each infection, the protection is dependent on immune responses against the preerythrocytic stage (50). Only chloroquine and mefloquine have been utilized in humans in this model so far (51). To further advance this immunization strategy, inoculation of sporozoites by needle and syringe (discussed below) and a regimen that enables drug treatment to be administered concurrently with the parasite inoculum (sporozoite or blood stage) are critical to being able to successfully deploy this in areas where malaria is endemic. Recently, direct venous inoculation of aseptic, purified,

nonirradiated *P. falciparum* sporozoites under chloroquine cover (PfSPZ-CVac) was shown to induce sterile protective immunity against homologous challenge (8). Further approaches for vaccination include the administration of genetically attenuated sporozoites that arrest in the liver and do not progress to a blood-stage infection (52) or of blood-stage parasites which have reduced ability to replicate in the blood (53). An immunization regimen using these genetically attenuated parasites may not need administration of antimalarial drugs.

RECENT ADVANCES IN CONTROLLED HUMAN MALARIA INFECTION

Development of cryopreserved, purified sporozoites for CHMI. The traditional CHMI model involved administering bites of *Plasmodium*-infected insectary-raised mosquitoes to study participants and was standardized over decades (54), with the bites of three aseptically reared or five laboratory-reared mosquitoes consistently infecting malaria-naive individuals (55). Mosquito bite-initiated CHMI requires insectary access, entomological expertise, secure transportation of infected mosquitoes to the clinical trial site, and precise timing of mosquito rearing and infection in relation to the vaccination and challenge regimen (55). The number of sporozoites injected into each participant in mosquito bite-initiated CHMI is highly variable, and it has been shown that the number of sporozoites counted in each salivary gland/number of mosquito bites is a poor predictor of the number of sporozoites actually injected (44). A methodology has been developed by Sanaria Inc. to produce aseptic, purified, cryopreserved *P. falciparum* sporozoites that are manufactured in compliance with regulatory standards and are infective *in vivo* (56). These sporozoites are injected with a needle and syringe, and different routes of inoculation have been examined and optimized (45, 56–58). Although this artificial method of administration is clearly different from a mosquito bite and bypasses the "skin-stage," it enables a consistent sporozoite inoculum for this CHMI model to be utilized in numerous research centers around the world both for challenge and potentially for the preerythrocytic vaccine approach described above. Being able to standardize and define the number of sporozoites injected is advantageous in terms of sporozoite dose estimation for vaccine studies and enabling direct comparisons of CHMI sporozoite challenge studies between and within different clinical sites (59).

Access to *P. falciparum* material for CHMI. An important constraint to the ability to conduct CHMI is access to well-characterized malaria parasites with a known drug sensitivity profile to ensure that the most appropriate antimalarial drug treatment can be initiated when required. These parasites must also meet relevant region-specific regulatory standards so that they are suitable for administration to humans in clinical studies.

Historically, CHMI via mosquito bite or injection of sporozoites has been restricted to institutions with the capacity to rear and maintain *Plasmodium*-infected mosquitoes (36, 60–63). As outlined above, the manufacture of aseptic, purified, cryopreserved *P. falciparum* sporozoites enables this model to now be employed in numerous centers around the world (see, e.g., references 57, 59, and 64).

Blood-stage CHMI was originally developed in Australia at the Queensland Institute of Medical Research, using cryopreserved stocks of erythrocytes from two parasitemic donors who were deliberately infected with *P. falciparum* 3D7 via mosquito bite (65). This material has now been administered intravenously to >300 volunteers in numerous studies with diverse endpoints. Until recently, the use of material suitable for induced blood-stage malaria infection relied entirely upon obtaining ethical approval to collect, cryopreserve, and store a large volume of blood from suitable malaria-infected donors (either deliberately infected individuals or malaria-infected returned travelers). Following rigorous testing and ethical approval, these *ex vivo* banks could be used in clinical trials. They are, however, a finite resource, and if the donor is not of the "universal" blood group O Rh D-negative blood type, then this limits potential recipients to those with a compatible blood type. Recently, we developed an alternative approach to generating suitable blood-

stage parasites for CHMI studies (66). It involves culturing large volumes of defined *P. falciparum* isolates in blood group O Rh D-negative blood followed by cryopreservation, characterization, and rigorous testing to ensure suitability for use in clinical studies. Parasites from the different cultured *P. falciparum* blood-stage cell banks have been used to successfully infect malaria-naive human volunteers (23). The availability of CHMI reagents to multiple research centers introduces new challenges, and these are discussed further below.

***Plasmodium vivax* and CHMI.** While this review has focused largely on the use of *P. falciparum* CHMI, the recent development of *P. vivax*-specific material will accelerate the progress of vaccines and drugs specifically targeting this parasite (67, 68).

For sporozoite-initiated CHMI, published studies have utilized *P. vivax* sporozoites that were generated by feeding gametocyte-infected blood from residents of areas in Colombia and Thailand where malaria is endemic to laboratory-reared mosquitoes (15, 17, 68–70). Due to the research facilities and capacity established in Colombia, the infected mosquitoes were used in studies at the same site, whereas the mosquitoes infected in Thailand were transported in secure containers to the United States and maintained in the insectary at WRAIR until required for challenge. These *P. vivax*-infected mosquitoes were used to successfully infect malaria-naive and semi-immune individuals (68–70) and in CHMI trials evaluating *P. vivax* preerythrocytic vaccine candidates (15, 17). Due to inherent issues with long-term *in vitro* culture of *P. vivax*, there is a requirement for fresh gametocytes from infected patients to infect mosquitoes. Currently, gametocyte infection of mosquitoes is undertaken in an area where malaria is endemic, although the mosquitoes can subsequently be shipped to other centers for the challenge component of a vaccine/drug evaluation study as described above (15). The use of a different *P. vivax* isolate for each study will be reflected in parasite parameters, e.g., differential drug sensitivities, parasite multiplication rates, and prepatent periods, and this will limit comparisons between different studies (71). Until cryopreserved sporozoites are developed for *P. vivax*, this will be an ongoing limitation. A further complication of *P. vivax* sporozoite-initiated CHMI studies is the possibility of hypnozoite formation and infection relapse. Primaquine should be administered at the conclusion of the study to clear any latent liver stages. However, participants in these studies must be assessed prior to enrollment for possible exclusion based on glucose-6-phosphate dehydrogenase (G6PD) deficiency (to avoid primaquine-induced hemolysis) and for CYPD26 polymorphisms which may affect the conversion of primaquine to its active metabolite (72, 73).

There have been four *P. vivax* blood-stage CHMI studies to date, two of which have been published (67, 71, 74). These studies were undertaken using cryopreserved blood from returned travelers (67, 71), and due to the current unavailability of a long-term *in vitro P. vivax* culture, it is conceivable that *ex vivo* banks from returned travelers or deliberately infected individuals will be the only source of material for *P. vivax* blood-stage CHMI for the foreseeable future.

CHALLENGES FOR CONTROLLED HUMAN MALARIA INFECTION STUDIES

Availability of diverse parasite strains to evaluate heterologous protection. Ultimately, a malaria vaccine must induce significant strain-transcending protective efficacy. This has proven to be a challenging proposition both in the field and in CHMI studies. When evaluated in phase III trials, the licensed malaria vaccine RTS,S/AS01 demonstrated only partial protection in the field (75). Protective efficacy was shown to be greater against *P. falciparum* infections where the parasite circumsporozoite protein genotype matched that of the vaccine strain, due to the allele-specific nature of the vaccine-induced protective immune response (76). The use of defined, genetically distinct *P. falciparum* strains in CHMI studies can therefore be seen as advantageous, as they can be used to evaluate the protective efficacy of malaria vaccine candidates against a range of diverse parasite strains prior to deployment in the field. Insight into the strain-specific nature of the protective efficacy of a vaccine candidate could also inform further optimization of the vaccine formulation prior to costly field studies (77,

78). It was only recently that a *P. falciparum* vaccine candidate, PfSPZ (a radiation-attenuated sporozoite vaccine), demonstrated significant protection against challenge with both homologous and heterologous *P. falciparum* strains in a CHMI study, albeit in a small number of volunteers (79, 80). The protective efficacy of this vaccine candidate is currently being tested in field sites in Africa where malaria is endemic. The CPS immunization strategy has also been shown to induce limited strain-transcending immunity (81).

Currently, only a limited number of defined *P. falciparum* strains are available for/have been used in CHMI: *P. falciparum* NF54 (an isolate of West African origin) (82), 3D7 (a clonal line derived from NF54) (65), 7G8 (a cloned line of the Brazilian IMTM22 isolate) (83), NF135.C10 (a clone derived from a Cambodian isolate) (84), and HMP02 (an isolate from Ghana) (23), with the latter available only for blood-stage challenge. Although they originate from different geographical areas, it is unknown how representative these strains are of the antigenically diverse circulating strains in all areas of malaria endemicity. For non-*P. falciparum* species, limited work has been undertaken with *P. vivax* (as indicated above). The ability to access suitable *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* isolates would also increase the value and utility of the CHMI model.

A recent perspectives paper from the U.S. Food and Drug Administration (FDA) discussed the possibility of using efficacy results from CHMI studies to support licensure of a malaria vaccine for use in travelers (85). Demonstrating breadth of protection against diverse strains would be critical for this, thus emphasizing the importance of further developing and characterizing different parasite strains for CHMI studies. This is not a straightforward undertaking. Initial considerations include having the necessary ethical approvals and the logistics of identifying suitable individuals for collection of *Plasmodium*-infected blood. The development process for the *P. falciparum* NF135.C10 clone involved four qualification criteria (84). They were that the strain (i) must consistently produce gametocytes and sporozoites (this is not relevant to isolates being developed for blood-stage CHMI), (ii) should be cloned to create a genetically homogeneous parasite population, (iii) must have sensitivity to commonly used antimalarials, and (iv) should be geographically and genetically distinct from the NF54 strain. Screening of >70 strains was required to eventually identify the NF135.C10 clone (86). For isolates being developed for blood-stage CHMI, cryopreserved *ex vivo* blood-stage parasite banks are finite resources. While cultured blood-stage parasite banks are therefore advantageous, not all *P. falciparum* isolates are easily culture adapted, and some of the non-*P. falciparum* species are not amenable to the large-scale culture that is required to manufacture blood-stage parasite banks.

Standardization of methodologies between different research centers. The standardization of methodologies and sharing of reagents are essential to enable a direct comparison of data generated across multiple study centers (63). This encompasses processes for the manufacturing and handling of the CHMI product, as well as assays that are used to determine initiation of rescue drug treatment and evaluate study efficacy endpoints, e.g., parasite detection methods and immunogenicity assays.

For parasite detection, Giemsa-stained thick blood smear microscopy has traditionally been the "gold standard" for CHMI studies, and in sporozoite-initiated CHMI, drug treatment is initiated as soon as parasites are detected to minimize adverse events and potential complications. Standardized reading of blood smears is essential for comparison of trial endpoints across different research centers (54). Nucleic acid tests (NATs) such as quantitative PCR (qPCR) and quantitative reverse transcriptase PCR (qRT-PCR) are also being increasingly used in both vaccine and drug evaluation CHMI studies (87). DNA-based NATs can also detect transiently circulating, dead parasites in the peripheral blood, resulting in a short period of false-positive results, while RNA-based NATs can have greater sensitivity. For many of the sporozoite-initiated CHMI studies, qPCR is only used for retrospective analysis to estimate PMR and liver load through statistical modeling (88, 89). For mosquito-bite initiated CHMI studies, it has been shown that the

use of PCR assays allows for quantitative measurement of parasitemia on average 3.5 days earlier than microscopy and increases the statistical power of CHMI to evaluate vaccine and drug efficacy (1). It has been suggested that using qPCR as a primary endpoint in the sporozoite-initiated CHMI has a number of advantages, including shortening the duration of parasitemia (prepatent period), which has the potential to reduce the number of clinical symptoms in the volunteers (1). It has been shown that this can be implemented without negatively impacting the evaluation of the protective efficacy of preerythrocytic vaccines (1). Studies involving induced blood-stage malaria infection have used qPCR as the primary outcome variable (90) or in combination with microscopy (13). For a NAT to be used to define efficacy outcomes in CHMI, the assay requires validation prior to use in challenge studies (85). A standardized and validated NAT, including the blood collection schedule, should also be employed across multiple study sites to facilitate comparison of study results, and this is particularly pertinent to modeling of the PMR (87).

Establishment of the CHMI models in areas where malaria is endemic. It has been suggested that early-phase and challenge studies utilizing CHMI models should be established in multiple sites in areas of malaria endemicity to increase the international capacity to conduct studies that would eventually support product licensure (91). There are a number of advantages to conducting these studies in areas of endemicity, including the following: capacity building in developing countries, study participants having the same genetic background as the eventual target population, and the possibility of examining the effect of prior malaria exposure and immunity on vaccine efficacy and thus potentially having a longer time period in which to observe vaccine efficacy before initiation of drug treatment due to the presence of preexisting immunity.

As outlined above, mosquito bite-initiated CHMI for *P. vivax* has been established in Colombia (69), for practical and logistical reasons. More recently, CHMI using *P. falciparum* cryopreserved sporozoites has been established in different sites in Africa (57, 64). The advantages of using cryopreserved parasites (sporozoites or blood-stage parasites) are pertinent to establishing this research capacity in areas of endemicity (and to CHMI generally): they can be transported and stored in a liquid nitrogen vapor phase, and the administration of a predefined number of parasites would be associated with a reduction in site-to-site and trial-to-trial variation if standardized procedures are used.

There are many additional factors that must be considered when conducting CHMI studies in malaria-exposed individuals in areas of malaria endemicity. These include the following: ensuring that there are adequate clinical and laboratory resources with appropriately trained staff, dealing with a multitiered system of ethical review, defining appropriate levels of monetary compensation for study participation, ensuring comprehension of the research by participants, accounting for possible confounding of efficacy data by hemoglobinopathies, and grouping participants according to prior exposure (there are no validated assays for this), as naturally acquired immunity may impact PMR (57). Considerable research will be required to characterize the interaction between injected parasites (sporozoites and blood-stage parasites) and preexisting naturally acquired immune responses, as this will need to be factored into CHMI study design in areas of endemicity. The possibility of natural transmission of the challenge parasite strain to local areas also needs to be considered in relation to gametocyte appearance in the blood and drug treatment initiation time points.

CONCLUSION

CHMI is a versatile clinical tool which can be employed in different ways: as an immunization strategy, to assess antimalarial drug and vaccine efficacy, and to elucidate aspects of the human immune response to the malaria parasite, disease processes, and malaria parasite biology. Recent advances and development of CHMI-specific reagents that are easily transferable between different research centers highlight the potential of this model to accelerate malaria vaccine and drug development as well as

a greater understanding of host-parasite interactions. There are a number of key scientific gaps which need to be addressed to enable a more comprehensive use of this model, including development and validation of non-*P. falciparum* human malaria parasite species for sporozoite and blood-stage CHMI and a greater availability of geographically and genetically distinct *Plasmodium* species and strains for vaccine and drug evaluation. The development of aseptic, purified cryopreserved sporozoites for different *Plasmodium* species and strains would increase the international capability to use the CHMI model for vaccine and drug efficacy testing. The establishment and greater utilization of this model in multiple research centers worldwide introduces new challenges and emphasizes the need for greater harmonization and standardization of CHMI-specific processes to enable direct comparison of data across these sites.

ACKNOWLEDGMENT

We acknowledge grant support from the Australian National Health and Medical Research Council.

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