Controlled Human Malaria Infection

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Background. Since 1986, investigators at Walter Reed Army Institute of Research (WRAIR) have been using controlled human malaria challenge (CHMI) in malaria-naive adults in order to define the protective efficacy of a malaria vaccine and thus guide programmatic decisions on vaccine candidates. Adapting this model to the dengue field could provide similar evidential support for a vaccine or therapeutic product.

Methods. After completing a vaccine regimen, volunteers are bitten by 5 malaria-infected female Anopheles mosquitoes in a controlled environment. Volunteers are then monitored daily for peripheral parasitemia in a hotel setting with 24-hour access to a nurse and physician. If a single verified parasite is detected, effective antimalarials are promptly administered.

Results. The vast majority of the over 1000 volunteers having participated in CHMI clinical studies have done so at US military research centers. Numerous pre-erythrocytic and erythrocytic vaccine candidates have been evaluated safely and without any related serious adverse events using this model, including the soon-to-be licensed RTS,S malaria vaccine.

Conclusion. The lessons learned from over 25 years of experience in consistent, careful preparation and execution of the CHMI model at WRAIR can provide a foundation from which the dengue field can begin to develop a rigorous and safe “CHDI” model.

Keywords. malaria; challenge; efficacy; clinical trial; mosquito.

The burden of malaria puts approximately 3.3 billion people at risk of disease, largely affecting children under 5 as well as pregnant women, with an estimated 219 million cases and 660,000 deaths in 2010 [1]. Control of malaria requires a multipronged approach ranging from personal protective measures and insecticide-treated bed nets, vector control programs, administration of effective safe antimalarials and, hopefully, an effective malaria vaccine to provide protective immunity. As malaria drug resistance increases, it is crucial that the scientific community maintains a pipeline of new drugs and potential vaccine candidates. Although the definitive assessment of an experimental product occurs in phase 3 and 4 trials conducted in endemic areas in host populations affected by the disease, challenges to the execution of said trials are many: large numbers of subjects are required for enrollment and lengthy follow-up; disease transmission and exposure may change or vary during the study period; genetic heterogeneity exists among populations that may influence host responses; and financial investments are substantial both for infrastructure, training, and execution of studies, as well as planning for affordable provision of the tested product if success is demonstrated. For a select number of diseases—malaria, as well as influenza, shigella dysentery, dengue, and others—effectiveness of a product can be initially evaluated by conducting small live organism challenge studies in naive adult volunteers. A recent meeting at University of Oxford entitled “Controlled Human infections Studies in the Development of Vaccine and Therapeutics” was held for the purpose of promoting further collaborations in establishing and conducting such challenge studies using bacterial, parasitic, and viral pathogens [2]. These studies can provide valuable insights into potential efficacy and immunogenicity and be an up/down
programmatic decision point for the product allowing for evidence-based channeling of financial and personnel resources. In this article, we discuss how the malaria challenge model has furthered scientific advancement of malaria vaccines and the lessons learned from over 25 years of experience with this model at the Walter Reed Army Institute of Research (WRAIR).

HISTORY OF THE MALARIA CHALLENGE MODEL

Deliberate administration to humans of all species of malaria was widely practiced in the early part of the 20th century as a treatment for neurosyphilis, both by inoculation of parasitized red blood cells taken from a malaria-infected individual and by mosquito bite (whereby the mosquito became infected by feeding on a person with peripheral gametocytemia) [3, 4]. In the 1970s, using these techniques, Clyde and Rieckmann both were able to separately demonstrate protection from Plasmodium falciparum malaria in malaria-naive adult volunteers previously administered irradiated sporozoites by bites of female Anopheles mosquitoes [5–9]. The success of these vaccine trials provided the impetus to find a way to reproducibly culture P. falciparum in vitro to avoid the complications of obtaining malaria samples for challenge from infected patients. These goals were achieved in the following years: In 1976, Trager and Jensen published their seminal paper on in vitro culture methods for P. falciparum, and in 1980 Vanderberg and Gwadz published on the infection of female Anopheles mosquitoes feeding on these in vitro cultures driven to gametocytemia [10, 11]. This quickly led to a collaboration between the US Army, Navy, and National Institutes of Health (NIH), and, in 1986, this group successfully executed the first human malaria challenge study using female Anopheles mosquitoes infected by feeding on P. falciparum cultures [12]. Six malaria-naive adults developed peripheral parasitemia and malaria signs/symptoms approximately 11 days after bites from 5 Plasmodium-infected mosquitoes. Since that initial study, more than 1000 volunteers have been challenged using these procedures, with the majority of these (approximately 800) challenged by the US military at WRAIR and the Naval Medical Research Center (NMRC). Six other centers are capable of conducting challenges: University of Oxford in the United Kingdom, University of Maryland, Raboud University Nijmegen Medical Center, Johns Hopkins University School of Public Health, and Seattle Biomed. Although methods of P. falciparum challenge are generally similar among all institutions, in order to harmonize the results and allow for additional institutions to establish challenge centers, the World Health Organization (WHO) held 2 consensus meetings and subsequently developed guidelines for conducting a challenge [13]. The P. falciparum sporozoite challenge model now has the collective term ‘controlled human malaria infection’ or CHMI. Several institutions have also developed methods for P. vivax CHMI (at WRAIR in collaboration with Armed Forces Research Institute of Medical Sciences [AFRIMS], Bangkok, Thailand, and the Instituto de Immunología, Cali, Columbia [14]), as well as a blood stage challenge [15, 16] model using a small dose of P. falciparum infected red blood cells inoculated into volunteers (University of Oxford, United Kingdom, and the Queensland Institute of Medical Research [QIMR] Australia).

Antimalarial vaccine development requires significant investment of time and resources both in terms of antigen selection, development, and manufacture, as well as repeated preclinical testing in mice and nonhuman primates; however, preclinical efficacy testing may not predict human responses due to differences in immune systems or factors intrinsic to murine Plasmodium parasites. In addition, there has not yet been any immune correlate of protection identified for malaria, humoral or cellular, which could allow for determination of a successful vaccine candidate. Conducting a CHMI study can provide an initial assessment of the efficacy of a vaccine or product in a matter of weeks, thus informing programs of the desirability of going further to field studies and/or pediatric populations.

CONDUCT OF CHMI

In the WRAIR P. falciparum CHMI model, parasites, usually 3D7, NF54, or 7G8 strains, are thawed and expanded in normal human erythrocytes using standard culture medium containing 6% human erythrocytes and 10% normal human serum. All blood products used for malaria and mosquito culturing are obtained from commercial blood suppliers approved by the Food and Drug Administration (FDA) and have already been commercially tested for human immunodeficiency virus (HIV), hepatitis B, C, HTLV I/II, and syphilis. The mosquitoes are laboratory-born and -reared Anopheles stephensi. This species is relatively easy to maintain in the laboratory for up to 21 days after infection with malaria, feeds readily on humans, and is able to transmit malaria parasite to volunteers via infectious bite [11, 12]. Mosquitoes are infected with P. falciparum by allowing them to feed through membranes on cultures containing sexual stage gametocytes. One week after infection, a subset of mosquitoes are removed from each container of infected mosquitoes and dissected to estimate the malaria infection rate. Approximately 14–16 days after infection, a second sample of mosquitoes is removed from each carton and dissected to look for sporozoites in their salivary glands. At 17–24 days after infection, mosquitoes are selected and placed in mesh-covered cartons, 5 per carton. Vaccinated volunteers place their nondominant arm over the carton for 5 minutes to allow the mosquitoes infected with wild type P. falciparum to bite. The 5 mosquitoes are dissected to confirm they took a blood meal and were adequately infected as assessed by the scoring of the salivary glands for the presence of the sporozoites.
according to the scale below [11]. If required, additional mosquitoes are allowed to feed until a total of 5 infected mosquitoes with a minimum +2 salivary gland score have taken a blood meal. The salivary gland score is as follows:

- 0 = no sporozoites observed.
- +1 = 1–10 sporozoites observed.
- +2 = 11–100 sporozoites observed.
- +3 = 101–1000 sporozoites observed.
- +4 = >1000 sporozoites observed.

Healthy, malaria-naive adult volunteers, aged 18–50 years, from the locally diverse community of the greater Metropolitan Washington, DC, are recruited, screened, and enrolled as either a vaccine recipient or as one of the 6 unimmunized infectivity control volunteers challenged at the same time to verify adequacy of the challenge. Every CHMI vaccine efficacy study since 1987 has been conducted under a greater-than-minimal risk human use protocol approved by the WRAIR Institutional Review Board (IRB), with informed consent obtained in accordance with FDA Code of Federal Regulation (CFR) Title 21 Part 50, Department of Defense (DoD) regulations, and the principles of the Belmont Report, prior to initiating any screening and/or study procedures. The informed consent form specifically outlines the risks of P. falciparum infection and the measures followed by the study team to ensure the safety and care of the volunteer during the challenge period.

Beginning approximately 5 days after mosquito challenge, volunteers are evaluated daily by a nurse and clinical investigator to assess for any malaria signs and/or symptoms and have Giemsa-stained peripheral thick blood films obtained and examined for the presence of malaria parasites. During the time of expected patency, days 9–19 post-challenge, the volunteers are required to spend their nights in a predetermined hotel to allow for timely assessment by trained and experienced physicians of any potential symptoms of malaria 24 hours a day. The infection is treated with FDA-approved standard doses of an antimalarial early, that is, as soon as ≥2 parasites can be identified on thick film. Vital signs are taken and volunteers are asked specific health status questions: presence of fever, malaise/fatigue, chills/rigor, headache, myalgia, arthralgia, nausea, vomiting, diarrhea, abdominal pain, lower back pain, or any other symptoms. Once a positive film is identified and treatment is initiated, daily blood films continue to be obtained until 3 consecutive films are negative, then the volunteer can leave the hotel and any scheduled follow-ups can occur in the clinic.

CHMI is usually administered 2–4 weeks after the last vaccination in order that humoral and/or cellular immune responses can be mounted. Given that the prepatent period of falciparum malaria is 7–14 days, even with vaccines that may induce some delay in parasitemia but not sterile protection, efficacy can usually be determined in the group by the end of 28 days. In approximately 500 volunteers challenged at WRAIR from 1993 to 2007, the prepatent period ranged from 7 to 25 days (combining controls and vaccines). Subjects who do not develop malaria by day 28 do not receive malaria treatment. This allows a true estimation of efficacy and allows for rechallenge without confounding factors such as immunomodulation by the antimalarial. Finally, all P. falciparum challenge strains have been sensitive to either chloroquine (NF54 or 3D7) or mefloquine (7G8), and there has never been an instance of P. falciparum recurrence after treatment with these medications.

**PREDICTABILITY**

CHMI demonstrates its greatest utility in its ability to predict efficacy of malaria vaccines in the field. This would not necessarily be expected given that the 2 populations tested are at opposite ends of the immunological spectrum: semi-immune and malaria-naive adults. The first subunit vaccine in which protection was demonstrated using CHMI was with a DNA subunit vaccine based on the repeat region of circumsporozoite protein (CSP): R32tet32 [17]. Immunogenicity of this candidate was then evaluated in Kenyan adults [18], but no efficacy studies were conducted. In the early 1990s R32ToxA, formulated in alum, was tested in US adults using CHMI [19], and then evaluated in Thailand and Kenya [20, 21]. In the US study, 1 of 8 volunteers challenged using the 5-bite CHMI model was protected. This low rate of efficacy was reflected in both the Thailand and Kenya studies whereby attack rates were essentially identical in vaccinees and controls. The evolution of GlaxoSmithKine’s RTS,S/AS01 vaccine, the first malaria vaccine planned for licensure, has been well reviewed in several publications [22–24]. The initial study of RTS,S in the AS02A adjuvant system conducted at WRAIR in 1996 by Stoute et al [25], in which 86% efficacy was demonstrated in vaccinees, led to further refinement of RTS,S regimen using the CHMI. In the following four CHMI studies of RTS,S in malaria-naive adults at WRAIR, different vaccination timing/spacing, antigen formulation, and adjuvant systems were evaluated; all gave a similar range of efficacy within 32%–57% [26–29]. Subsequent field studies in adults in Gambia and Kenya [30, 31] demonstrated a similar range of efficacy (30%–47%) to what was found in the CHMI. The first pediatric study of over 2000 children aged 1–4 years done in Mozambique found an efficacy of approximately 30% against first infection and 57% against severe malaria [32]. In the largest malaria vaccine trial to date, in 6000 children 5–17 months, a 50% efficacy against clinical disease was demonstrated with a lower efficacy of 31% in infants 6–12 weeks of age, all consistent with what was demonstrated with CHMI albeit with a different endpoint (disease vs incident infection [parasitemia]) [33].

Some vaccines with low levels of protection in CHMI have continued on to evaluation in the field with the hypothesis...
there may be improvement in protection when tested in malaria endemic areas where natural boosting may occur or, alternatively, that the 5-bite challenge model conducted in malaria-naive is too stringent. This, however, has not been borne out, and low levels of protection in CHMI seem to predict the same low efficacy in the field. Sauerwein et al reviewed and described several such studies [34]. Heterologous DNA prime/poxvirus boost regimens using antigen ME-TRAP (multi-epitope/thrombospondin) given to malaria-naive individuals at University of Oxford protected only 3 out of 74 volunteers in a CHMI [35]. Such a regimen also was not protective in Gambian adults [36]. Combination B vaccine (merozoite surface protein 1 and 2 and ring-infected erythrocyte surface antigen) in the adjuvant Montanide ISA720 was evaluated by CHMI using infected red blood cells and measuring parasite growth rates by quantitative polymerase chain reaction (PCR). In malaria-naive Australian adults there was no measurable effect of the vaccine on parasite growth rates, and when tested in children in Papua, New Guinea, despite a modest effect on parasite density, there was no effect of the vaccine on first or only episodes of *P. falciparum* malaria [37, 38].

One limitation of the predictability of current CHMI method using in vitro culture is in cross-strain or cross-species challenges. In the original irradiated sporozoite vaccine studies, because infected red blood cells were obtained from infected volunteers, these types of heterologous challenges could be easily performed [6, 7, 9]. It is only recently, 30 years later, that heterologous *P. falciparum* strain challenges using in vitro culture methods are being reattempted [39]; heterologous species challenges would not be performed until there is a vaccine candidate that transcends species-specific immunity.

**SAFETY**

Although the scientific benefits of CHMI studies are clear, malaria remains a life-threatening illness. Conduct of CHMI goes beyond provision of infected mosquitoes and requires trained staff, a consistent infection model, controlled access to malaria-exposed subjects, reliable method of diagnosis, and quick, effective treatment. With over 1000 volunteers challenged with malaria by the US Army and Navy, there have been no challenge-related serious adverse events (SAEs), no inpatient hospitalizations or cases of severe malaria, and no use of intravenous medications required.

Different institutions have adopted slightly different ways of conducting CHMI, but all rely on the principles established during the first study in 1986. Ensuring safety begins at subject recruitment and screening. Selected volunteers need to have demonstrated to the clinical staff reliability, proven comprehension and understanding of the study procedures and objectives through a “test of understanding,” as well as the requirements for participation in the study. In the study protocols, clauses are always included whereby a study investigator can remove a volunteer if he or she has not been reliable or has shown inconsistent behavior that could put the volunteer or others at risk after challenge.

WRAIR has adopted the practice of housing volunteers in hotel rooms in the local area during the expected time of patency in order to ensure constant contact with medical staff. Daily mandatory visits occur each morning for clinical assessment and preparation of peripheral blood smear. Contact is made, at minimum, with the volunteer a second time in the afternoon/evening to assess clinical condition, and blood smears are taken at any point symptoms are experienced by the volunteer or at the discretion of the study clinicians. If contact with a volunteer is lost, there runs a very serious risk of severe illness or even death. Safety of volunteers also should be assured by treating malaria infection as soon as patency can be demonstrated. Expert microscopists are trained and tested and are housed in the hotel with the volunteers and clinicians. When one unambiguous parasite is detected by trained microscopists, a second one must be found to confirm the diagnosis, and then treatment is initiated since efficacy, not modulation of parasitemia, is the endpoint. Molecular methods such as PCR are extremely sensitive compared to the “gold standard” thick blood smear evaluation by microscopy and may soon replace the need for microscopy because subjects may be treated 3–4 days earlier by detecting unambiguously the presence of parasite DNA indicative of viable parasites in the peripheral circulation. Notably, false positive PCR results as a result of DNA contamination or unvalidated and unoptimized assays runs the risk of jeopardizing the true efficacy of a vaccine intervention and potentially interferes with the interpretation of “time-to-delay” in onset of parasitemia as assessed by microscopy as measures of partial vaccine efficacy and biological effect. Quantitative PCR (qPCR) methods that measure blood stage parasite multiplication rate and estimate liver-stage burden reduction in immunized subjects are increasingly being used as adjunctive tools to assist investigators in assessing the efficacy of a vaccine intervention during CHMI studies at WRAIR for both blood and liver stage vaccine candidates [34, 40].

The 2 strains of falciparum used in WRAIR CHMI (NF54 and 3D7) remain exquisitely sensitive to chloroquine, administered orally over 3 days by directly observed therapy (DOT). Medications for symptomatic relief, particularly antipyretics, are administered as needed during CHMI. As was discussed for prepatent periods above, because the main objective is protective efficacy of the vaccine, ad hoc measures such as presence or absence of fever, fever clearance time, and reduction in severity of symptoms are not endpoints. Any concerns that antipyretics may also mask symptoms and thus influence the calculation of vaccine efficacy are mitigated by the performance of daily blood smears. Time to parasitemia will not be affected by antipyretics, so the daily blood smears will reveal an
ineffective vaccine, often times before symptoms are even felt. Safety of the volunteers is thus predicated on developing and following algorithms for mosquito challenge, clinical monitoring, diagnosis, and treatment. A well-trained staff that can consistently and rigorously execute these algorithms will best ensure that a clinical trial utilizing CHMI will appropriately and safely determine efficacy of a vaccine product.

As a note, due to limited resources, the requirement for entomology expertise in insectary operations to maintain mosquito colonies, and parasitologists to culture and feed parasites in order to maintain the high standards required for malaria challenge centers, there have been efforts to substitute the traditional and proven benefit of mosquito challenge for “challenge-in-a vial,” which uses frozen and cryopreserved sporozoites [41]. Such parasites upon thaw would be administered by needle and syringe attempting to emulate mosquito-delivered infection. In our opinion, such a method is not yet ready for the malaria vaccine community to adopt as the uncertainties in delivery (subcutaneous, intramuscular, intravenous) may not fully mimic the biological properties of how sporozoites, when injected subcutaneously in a milieu of mosquito saliva, traverse cell layers and tissue spaces, nor may it replicate the precise immune functions associated with sporozoite neutralization by antibodies within such tissue spaces prior to entry into the circulation in its journey to the liver. Nevertheless, for evaluation of novel antimalarial compounds, when the only consideration is consistent presence of parasitemia, such a model may have utility and could be used in many institutions where the human mosquito challenge model is unavailable.

**RECOMMENDATIONS FOR THE DENGUE FIELD**

As steps are taken to move the dengue human infection model forward, the experience gained from years of malaria vaccine studies may provide useful guidance. The most crucial aspect of the challenge model is the development of standardized, well-controlled procedures to assure the safety of the volunteers. At WRAIR considerable effort by investigators and research coordinators has gone into creating and maintaining consistent CHMI procedures all of which are documented in Standard Operating Procedures (SOPs). This allows for the same type and timing of clinic visits, the same clinic flow and daily vital sign monitoring, symptom questionnaires and grading scales, and the same organization of hotel set-up for medication storage, biohazard disposal, volunteer study document access, and provision of emergency equipment. Similarly, appropriate setting for patient follow-up after infection, requirements for daily evaluation, and algorithms for treatment will have to be established and documented for a dengue infection model. Although there are no effective antiviral agents for dengue, well-documented diagnosis, evaluation, and treatment algorithms have been established that reduce morbidity and lower mortality of even severe dengue to <1%. These will have to be critical components of a dengue infection model. In WRAIR CHMI studies, not more than 10% of volunteers report symptoms in the 3 days before diagnosis, highlighting the sensitivity of malarial diagnostics. The method of dengue detection in naive individuals must be sensitive and, optimally, in the future, could be sensitive enough to detect infection prior to symptoms. Additionally, as is with malaria, stringent, reproducible conditions must be followed for development of dengue infection strains and administration to the volunteers to ensure 100% infection on challenge day.

WRAIR follows standard operating procedures for mosquito production, culture of *Plasmodia*, and infection of mosquitoes outlined in Biological Masters File (BMF) 5855, prepared by the Division of Entomology, WRAIR, and kept with the FDA. Currently all CHMI studies at WRAIR are submitted to FDA and conducted under IND (Investigational New Drug application) in which the BMF is referenced, ensuring the conduct of the challenge is highly regulated in order to both better protect volunteers as well as to allow for comparisons of results between studies. The new CHMI guidelines, sponsored by WHO, also now provide consistent guidance for CHMI among institutions [13]. As the dengue infection model evolves, investigators and institutions should seek to also integrate such rigorous, reproducible conditions. The need for a dengue vaccine and/or products remains unmet, and thus a concerted effort among institutions and pharmaceutical companies to rapidly evaluate potential vaccine candidates using the dengue infection model could alleviate the morbidity suffered by the millions who contract dengue each year.

**Notes**

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