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Conference report

The frontline of controlled human malaria infections: A report from the controlled human infection models Workshop in Leiden University Medical Centre 5 May 2016

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ABSTRACT

Controlled Human Malaria Infection (CHMI) is the most practiced controlled human infection model nowadays and there is an exponential increase in implementation of the model worldwide. During the Controlled Human Infection Models Workshop in Leiden, one day was dedicated to the discussion of the advances made and gaps in Controlled Human Malaria Infection (CHMI) trials. Factors contributing to this impressive expansion in the number of CHMI trials have been related to the ability to perform CHMI using injectable cryopreserved sporozoites (a product from Sanaria Inc. – PfSPZ Challenge), the development of a transmission blocking CHMI model and the need to test more vaccine candidates particularly in the field of whole-sporozoite vaccine development. However, with an increasing number of CHMI trials being undertaken, in an ever-growing number of trial sites, heterogeneity in trial design may compromise universal interpretation of results and require an ongoing dialogue on the need and feasibility of standardization. At the workshop, CHMI investigators convened to share their experiences in CHMI trials and discuss the possibilities for future trials.

1. Introduction

Since the first deliberate infection of volunteers with malaria as a treatment for neurosyphilis in the 1920s [1,2], controlled human malaria infections (CHMI) have been developed as a standardized model to investigate malaria pathophysiology, immunology and the efficacy of novel vaccines and drugs. With the exponential expansion of CHMI trials worldwide and an estimated >3000 volunteers being deliberately infected on five different continents to date, CHMI have taken a central position in the development of novel vaccines and medicine. Generally, CHMI testing novel vaccines and drugs will be performed after the phase 1 safety trial for the novel product has been completed. CHMI will then provide the first data on efficacy of the product. The general design of CHMI trials for evaluating novel vaccines or drugs is similar (Fig. 1). However, subtle differences in the way such trials are performed may influence trial results and interpretation.

In this report we present the different viewpoints on the use and interpretation of these models which were discussed at the Controlled Human Infection Models Workshop in Leiden.

2. Keys to CHMI trial design: Inoculum and endpoints

Traditionally, malaria drug and vaccine developers target different life cycle stages (pre-erythrocytic, blood- and sexual stage parasites). In analogy to vaccine development, CHMI models were developed to target specific life cycle stages. Exposure to mosquito bites is the most mature and frequently used model, which resembles the natural life cycle of malaria parasites in the human host. Alternatively, volunteers can be injected with blood stage parasites. The more recent availability of cryopreserved parasites (PfSPZ Challenge, Sanaria Inc.) for intravenous inoculation has changed the landscape for CHMI trials with sporozoites [3] (Fig. 2). With regards to the trial endpoints, the advance of molecular technology for fast and accurate parasite detection as an alternative to traditional microscopy of blood smears, has diversified the design of CHMI trials [4,5]. Benjamin Mordmüller (University of Tübingen, Germany), Jona Walk (representing the

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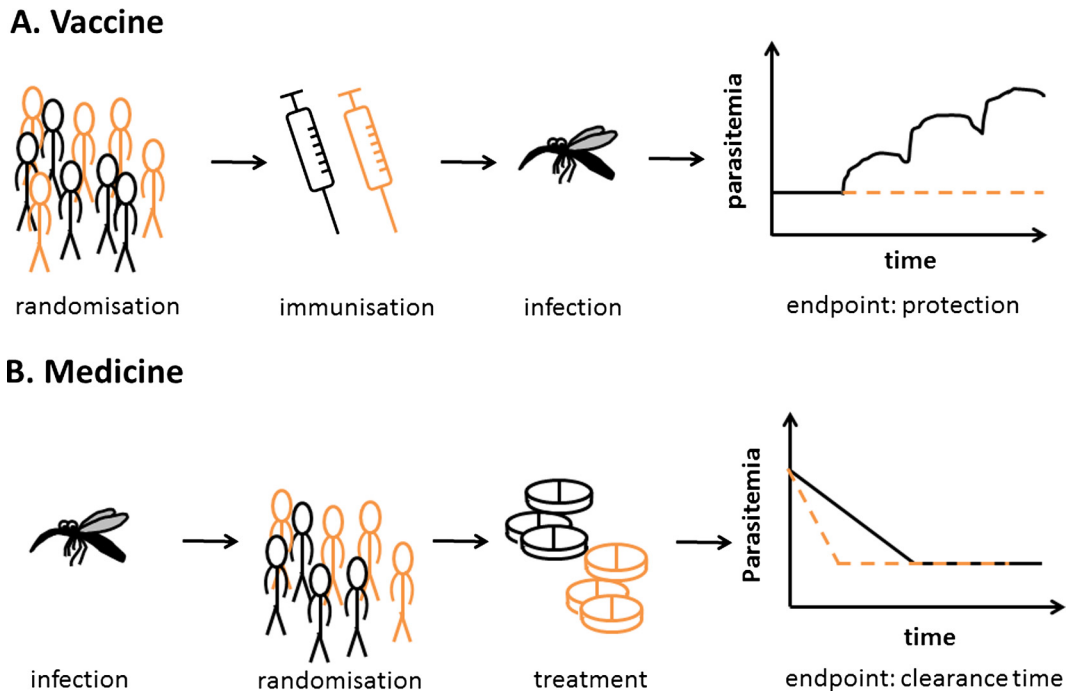

Fig. 1. Overall design of CHMI trials testing novel vaccines (A) or medicine (B). When testing vaccines, two groups of volunteers will be randomized, immunized with the vaccine or (placebo) comparator and subsequently infected with malaria. Endpoint in such trials is blood stage parasitemia which may not occur (full protection) or be reduced (partial protection). When testing novel antimalarial medicines, volunteers will be infected and subsequently randomized to receive novel medication or the comparator drug. Endpoint in such trials is generally parasite clearance time in the blood.

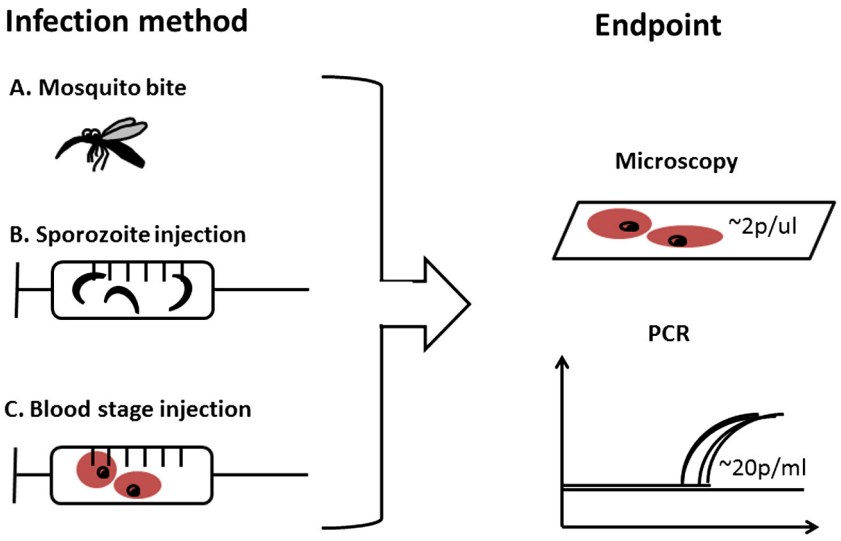

Fig. 2. Illustration of the heterogeneity in CHMI trial design. Volunteers can be infected by mosquito bite (A), injection of sporozoites (B) or injection of blood stage parasites (C). Subsequently, parasitemia in the blood can be measured by microscopy (thick smears) or by PCR. The detection threshold for these two techniques are 2 parasites per ul and 20 parasites per ml respectively. p = parasites, ml = millilitre, ul = microliter.

Robert Sauerwein group, Radboud University Medical Center, The Netherlands), Jim Kublin (Seattle Malaria Clinical Trials Center, USA) and Matt Laurens (University of Maryland, USA) discussed the optimal design of CHMI including their infection procedures and trial endpoints.

The panel discussion focussed around different infection techniques using sporozoites: more natural mosquito bites versus the more standardized direct venous inoculation. Inoculation of parasites by needle is clearly easier to standardize and can be used across the world also in centres lacking an insectary. The direct venous inoculation has shown to be the most efficient method of infection [5]. Other methods of administration (intradermal, subcutaneous, intramuscular) have been tried but were less efficient in inducing parasitemia and clinical malaria [6–10]. The use of the natural mosquito vector to deliver the controlled malaria infection is thought to more closely resemble the field situation by some researchers, as it does not circumvent the skin inoculation site. The skin may be an important immunological site for both the induction and the effector mechanism of immunity, but its relevance in malaria is still under debate [11]. In any case, even in mosquito bite infections a portion of parasites are inoculated directly in the blood stream. There are discussions on the requirement of five

mosquito bites to achieve infection in all trial volunteers. This might be "supra-natural" as five infectious bites almost never occur simultaneously and without interruption during feeding in nature.

Because valid arguments for both techniques can be made, the trial design should clearly be hypothesis-driven and tailored to the specific trial objectives. A head-to-head comparison of mosquito bite versus needle infection may be needed to allow researcher to make an informed choice when possible.

Traditionally, microscopy of thick blood smears is used as endpoint for CHMI trials. In exceptional circumstances, parasitemia can be allowed to increase beyond the thick smear threshold based on clinical criteria. The turnaround time of sensitive PCRs for the diagnosis of *Plasmodium falciparum (Pf)* malaria has decreased to six hours and is competing with traditional microscopy of thick smears as the endpoint of CHMI. The sensitivity of PCR can be as low as 20 parasites per mL of blood, compared to microscopy (~2000 parasites per mL) [4,12–14]. Therefore, PCR can diagnose malaria 2–4 days (1–2 growth cycles) earlier than thick blood smear. This may decrease the number of adverse events and improve the tolerability of CHMI trials. Whereas the consumables for PCR are more expensive, it is less labour intensive when multiple samples are tested in one run. In low-income settings, traditional microscopy is still often the preferred option as it is cheaper, comparatively easy to implement and does not require complicated technical infrastructure.

3. Increasing diversity of CHMI: more species and more strains

Malaria parasites are known for their antigenic variation [15]. Most CHMI trials have been performed using either the NF54/3D7 strain, derived from the Schiphol area. The 3D7 strain has been obtained from NF54 by cloning. Because this strain has been cultured for years, it may have diverged from field strains. Particularly for vaccine testing, the strain used in CHMI trials may be critical to the trial outcome because homology between the vaccine antigen and the CHMI strain may lead to increased vaccine efficacy, which can be an overestimation as compared to the field. This holds true for subunit vaccines as well as whole sporozoite vaccines. To bridge the gap between CHMI and endemic settings, strain specific CHMI have been developed for *Pf*.

Isaie Reuling (Radboud University Medical Center, The Netherlands), Ilin Chuang (Armed Forces Research Institute of Medical Sciences, Thailand), Steve Hoffman (Sanaria Inc.) discussed the need for an increasing number of *Pf* strains for CHMI. Three additional strains are currently being characterised in CHMI: 7G8 from Brazil, NF135.C10 from Cambodia and NF166.C8 from Guinea [16,17]. Possibilities to mix strains or infect with several small doses of different strains at short intervals was debated with some reservations as interpretation of the trial particularly when infectivity of strains differ becomes highly complicated.

Considerable efforts have also been undertaken to develop a *Plasmodium vivax* (*Pv*) challenge model, facing major logistical challenges as the *Pv* parasite cannot be continuously cultured *in vitro* [18]. Socrates Herrera (Caucaseco Scientific Research Center, Colombia) showed the advances which have been made to establish a *Pv* controlled infection model. Although *Pv* strains are always directly obtained from the field, natural *Pv* isolate heterogeneity and mosquito species diversity does not affect endpoint readout, so that *Pv* CHMI does not need further standardisation [19,20]. Because *Pv* produces gametocytes very early in infection, the spread of the challenge strain through the bites of naturally occuring *Anopheles* mosquitoes has been a concern. However, in the initial phase these gametocytes do not seem to be able to infect mosquitoes. The *Pv* model has been taken further to, very successfully, test efficacy of radiation-attenuated *Pv* sporozoites administered by mosquito bite [21].

4. CHMI in endemic areas: mapping pre-exposure and building the capacity

The technique of direct intravenous inoculation of cryopreserved sporozoites has expanded the field of CHMI into endemic areas [7,8]. CHMI are now being performed in endemic areas in Africa to test the efficacy of novel vaccines (NCT02132299, NCT02627456). In these trials, a small group of volunteers which may be selected based on their geographical location or pre-exposure to malaria, is randomized, immunized and subsequently infected by intravenous inoculation of *Pf* sporozoites. African CHMI require modification of the trial endpoints because Africans, unlike non-endemic populations, can harbour malaria parasites without clinical symptoms and are able to (partially) clear parasites without treatment [7,8]. As such, the endpoint of African CHMI can be based on microscopy and/or clinical malaria rather than detection of any parasite by PCR.

Past exposure to malaria parasites, but also parasitic helminths or other co-infections are known to affect immunological responses to infection and vaccination [22]. Seif Shekalage (Ifakara Health Institute, Tanzania), Issaka Sagara (Malaria Research and Training Center, University of Bamako, Mali), Bernhards Ogutu (Kenya Medical Research Institute, Kenya), Ally Olotu (Equatorial Guinea Malaria Vaccine Initiative, Equatorial Guinea) and Ulysse Ateba Ngoa (Centre de Recherches Médicales de Lambaréne, Gabon) represented the African CHMI community and shared their experiences of their trials. They emphasized hurdles faced when performing CHMI in the field. First of all, they are facing the need to map previous exposure to malaria parasites and other pathogens including the presence of co-infections as parameters in the CHMI equation [8,23]. Whereas they agree to provide antimalarial treatment for parasitemic subjects prior to immunisation or infection, this should potentially be expanded to also include treatment for concurrent helminth infections [24]. As yet, there is no consensus on the type and number of parasites that should be tested and treated before the trial. The preferred timing and treatment regimen for malaria was discussed, but head-to-head comparisons are lacking and investigators are often bound by local malaria treatment customs. Drugs most commonly provided to clear infections before CHMI were artesunate monotherapy, artemisinin combination therapy or clindamycin. Paramount to the choice of drug is the mechanism of action and the drug's half-life. In addition, it is yet unclear how to best characterise individual pre-exposure to *Pf*. In order to shed light on co-infections and their role in modulating immune responses to subsequent infections or vaccinations, full characterisation of the co-infection status and an assessment of the serological malaria status is needed before inclusion. As a consequence of the heterogeneity in trial volunteers, African CHMI may require increased sample size particularly in sites where pre-exposure and co-infection induces variation in the clinical trial endpoints. For example, pre-exposure to malaria may decrease susceptibility to CHMI for individual volunteers regardless of the trial intervention and co-infection may influence immune status and subsequent vaccine responses.

Most importantly, the African researchers emphasized the importance of training of personnel at endemic sites, including capacity building regarding ethics committees to review such trials as well as clinical and technological capabilities.

5. CHMI for different life cycle stages

In addition to the most frequently used CHMI with the use of sporozoites, the model has also been adapted for evaluation of blood stage and gametocytocidal drugs or vaccines [25,26]. Benjamin Mordmüller (University of Tübingen, Germany), Jim Kublin (Seattle Malaria Clinical Trials Center, USA), Simon Draper (Univer-

sity of Oxford, UK), Jörg Möhrle (Medicines for Malaria Venture, Switzerland) and James McCarthy (University of Queensland, Australia) shared their experiences with CHMI for drug and vaccine development as well as its potential to assess transmission blocking interventions. The use of blood stage challenge to assess the parasite multiplication rates was discussed by Simon Draper [26].

Medicines for Malaria Venture, in collaboration with the Universities of Queensland (blood stage activity) and Tübingen (liver stage activity) have very successfully set up a pipeline whereby drugs are selected based on data from PK/PD as well as the CHMI data [27]. In drug CHMI, volunteers are treated with the experimental drugs until they are cured [28]. In addition, a clinical symptom score was developed which allowed for extended observation of participants. It was found that the obtained parasite reduction rates were comparable between CHMI and field studies.

James McCarthy also alluded to a newly developed model to assess interventions targeting gametocyte by follow up of CHMI participants after treatment with piperaquine to clear the asexual parasites [25]. Considerable efforts to improve this model are currently ongoing. The use of *Pv* asexual blood stage parasites for CHMI is another interesting approach, since it eliminates the risk of relapse.

6. CHMI for advanced product testing/development

Now that CHMI can be performed with heterologous strains in endemic settings, CHMI trials may cover a large part of phase 2 clinical development. The question arises whether CHMI should be used to also provide phase 3 clinical data and eventually replace field trials, as was recently done for the FDA approved Vaxchora, a cholera travellers vaccine [29]. Steve Hoffman (Sanaria Inc.), Adrian Hill (Oxford University, UK), Leo Visser (Leiden University Medical Center, The Netherlands) weighed the advantages of fast and possibly cheaper testing with the use of CHMI against the benefits of natural infections. Because speed and costs are paramount in clinical product development, the availability of well-equipped, efficient, highly endemic sites is essential. Unfortunately, these sites may be limited in their capacity, restricting the trial design and timelines. In addition, there are limitations with regards to phase 3 trials for travellers. For example, a proper phase 3 evaluation in which malaria prophylaxis is compared with a novel intervention under a non-inferiority hypothesis is logistically not possible. In such cases, data from endemic areas, safety data from travellers and non-endemic heterologous CHMI will need to be compiled to provide sufficient evidence.

7. Immunology in CHMI

CHMI trials can open a gateway to the basic mechanism behind antimalarial immune responses and the immune correlates of protection against malaria. Antibodies obtained from immunized volunteers can be tested for their invasion inhibiting capacity *in vitro*, but also in humanized mouse models [30,31]. Increasing numbers of cells are obtained from volunteers during and after CHMI to identify possible crucial effector cells. Phil Felgner (University of California, USA), Bob Seder (National Institute of Allergy and Infectious Diseases NIH, USA), Carlota Dobaño (Barcelona Center for International Health Research, Spain), Claudia Daubenberger (Swiss Tropical and Public Health Institute, Switzerland), Sanne de Jong (Leiden University Medical Center, The Netherlands), Chris Ockenhouse (PATH Malaria Vaccine Initiative, USA) and Ken Stuart (Center for Infectious Disease Research Seattle, USA) presented their recent advances in dissecting the immunological responses following CHMI. Phil Felgner highlighted the developments in protein array technologies which have provided unprecedented information on the antibody profile [32–34].

Bob Seder stated that the Achilles' heel of malaria immunity is probably in the liver and questioned whether PBMCs accurately reflect liver-specific immune responses [35]. He made a strong plea for investment into a peptide library of the *Pf* genome to identify dominant antigens. Chris Ockenhouse supported a comprehensive approach, but highlighted that data from antibody assays, PBMCs, RNA seq and stimulation assays should be combined in an attempt to tackle antigenic heterogeneity. Claudia Daubenberger stressed the need to investigate innate immune responses at baseline to dissect the heterogeneity of responses in African populations but also cutting-edge B-cell cloning technology that might help the malaria field in the same way that it has helped to identify broadly neutralizing antibodies for viral infections [36]. Carlota Dobaño shared her work on the cellular immune response in PBMCs of several CHMI and vaccine trials showing that antigen-specific Th2 responses can be correlated to the risk of malaria, emphasizing the need to expand the breadth of immune-profiling in African populations exposed to other environmental factors and thus include Th2 and Treg biomarkers (unpublished data). These arguments were further supported by data from Sanne de Jong (on behalf of Maria Yazdanbakhsh), who showed the strikingly different immune fingerprint of Africans and Europeans before and after CHMI with the use of mass cytometry technology (unpublished data). Ken Stuart presented work on CHMI studies where responses to radiation-attenuated parasites were tested. The flow cytometry, cytokine and RNA-Seq data have been generated and are being integrated and analysed. The studies showed different transcriptional patterns when one considered protected and non-protected subjects. In addition, a possible role for Mucosal Associated Invariant T-cells in response to malaria infection was put forward. Stuart emphasized that a network modelling the immune responses will be necessary for fully understanding responses to infections and successful vaccines where controlled human infections can play an important role.

8. Challenges and perspectives

Controlled human malaria infection, at the forefront of controlled human infection models, is a multifaceted dynamic model which is adapted to suit specific research questions related to vaccine or drug development. As the field of malaria research is still struggling with many essential questions, such as the correlates of protection, mechanisms of protection, cross-strain protection and heterogeneity of responses in African populations, the use of the CHMI model has expanded to endemic areas and a portfolio of different strains, species and lifecycle stages are being built. Omics approaches will facilitate the further dissection of these important parameters if the right questions are asked. Integration of immunological parameters such as antibody, RNA seq, mass cytometry and other novel tools to obtain an integrated signature of immunity, will remain a challenge. Collaboration between research centres performing these demanding trials will prove to be essential to draw meaningful conclusions from big-data approaches. In addition, an increasing heterogeneity in trial design complicates data interpretation and pooling but the efforts of putting data together are essential to maximize the scientific benefit.

9. Recommendations

The expansion of CHMI trials into endemic areas was perceived as one of the major advances for the field of malaria immunology, drug and vaccine development. Although CHMI in endemic areas adds to the complexity of the model, it is essential for product development and provides unprecedented opportunities for understanding malaria immunology. CHMI shifts the risk curve of clinical development, decreasing the chances of failure at later

phase 2 and 3 stages. As such it decreases the need for large phase 2 field trials and acts as a gatekeeper before moving into field efficacy trials. Although field efficacy should always be targeted, whenever possible the need and size of phase 2 trials may decrease if they add to previously acquired endemic CHMI data. In order to keep up with the high standard of care to safeguard volunteer's safety there is a clear need to establish an international network of CHMI researchers to allow for real-time sharing of adverse events and facilitate capacity building at trial sites including ethical boards. Taking into account the already existing heterogeneity in trial design, investigators recommended not to stretch the model initially into mixing strains or repeated dosing as this will lead to an unduly increase of trial complexity, but rather test protection against each strain separately first before further combined strains CHMI can be considered. Nonetheless, the already existing diversity of models such as the blood stage CHMI or the Pv CHMI, were considered valuable tools for the drug and vaccine development pipeline.

Continuing the discussion on CHMI safety, design and harmonisation is paramount to provide a framework for the ethical and scientific acceptance of the CHMI trials and secure the ongoing use of this highly valuable tool.

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