



AFRICAN MALARIA NETWORK TRUST

Workshop on Workshop on Molecular Biology and Immunology in Malaria Vaccine Development

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Workshop Report

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Introduction

This was a five-day workshop organized just before the fourth MIM Pan African Congress from 9 to 11 November 2005 at Prestige Palace Hotel in Yaoundé, Cameroon. The workshop aimed at forming a forum for African scientists working on malaria to gain update information from experts in the field as well as exchange views and experiences among themselves as part of strengthening of South-South collaboration, mentorship and networking among African molecular biologists and immunologists. By holding the workshop in Cameroon, AMANET provided an opportunity for young African scientists to participate at the MIM Pan African conference.

Overview of malaria immunity

The overview of malaria immunity set the pace for the subsequent lectures. The presentation touched on essential steps in anti-microbial defense, pathogen recognition, the TLR family, innate & acquired immunity (in the context of malaria), components of innate immunity, bridging innate and acquired immunity, Memory T cells and identifying TCM associated with malaria vaccine-induced protection, Factors affecting the outcome of Pf infection in African children and Pregnant Women and the Principles of Malaria vaccines. The lecture also touched on the issue that children with sickle cell (HbAS gene) had significantly higher levels of antibody with specificity for neo-antigens.

T-cell immunity

In this presentation, malaria immunity was said to be an essential step in malaria defense. In malaria, as in other diseases, cell mediated immunity appears to be crucial for prevention and control. T-cell immunity depends on both CD4+ and CD8+ cells recognizing presentation of exogenous and endogenous antigen epitopes. Cell mediated immunity (T-cell immunity) in malaria targets infected hepatocytes and asexual blood parasite multiplication. It was also established that the effector T- cell responses are driven by IFN-gamma release and that T-cells play a pivotal role in elimination of exoerythrocytic or liver parasite stages through the activity of CD8+ and CD4+ cells. The presentation gave results of a study in Gabonese children which showed that LSA-1 induced IFN-gamma responses reflecting immunity to malaria.

B-cell Immunity

B-cell immunity exists although it offers partial protection. The magnitude and nature of immunity depends on parasite and host genetic factors with the parasite manipulating the response. Antibodies released by B-Cells were said to be important acting in a stage-specific fashion (sporozoites, merozoites, red blood cell forms, gametocytes). Malaria pathogenesis was said to be largely an immunological response triggered by the release of schizont exoantigens and TNF inflammatory inducers linking TNF to the clinical presentations of the disease.

Malaria immunity in pregnancy and cellular immunological responses of new-borns

In this presentation malaria was said to be one of the main causes of high maternal mortality & morbidity as a result of severe anaemia in unknown numbers of pregnant women in endemic areas. The plight of malaria on the infancy was also pointed out and in this case malaria is associated with low birth weight and up to 200,000 deaths per annum. Malaria in pregnancy was said to be a result of molecular adhesive interactions between parasite ligands on infected erythrocytes and host receptors expressed on placental syncytiotrophoblasts. The interactions are mediated by molecules on syncytiotrophoblasts, including chondroitin sulphate A (CSA) and hyaluronic acid (HA). Primigravid mothers were said to have a greater risk of placental *P. falciparum* infection because they lack antibodies that interfere with binding of parasitized erythrocytes to syncytiotrophoblast receptors (CSA/HA). The impact of pregnancy associated malaria and the additional public health burden was also discussed

Role of AMANET in malaria vaccines development in Africa

The role of AMANET in malaria vaccines development was the final talk for the day highlighting the burden of malaria in Africa and pointing out methods used for malaria intervention which have so far been inadequate to contain the scourge therefore calling for new strategies such as vaccines. Vaccine development is a costly and time-consuming process which can take up to 20 years or more before the product becomes available. African participation in vaccine development process has been low because of malaria R&D weakness in most of African health research institutions. AMANET's role is that of building capacities of African R&D institutions and preparing them to be conducting malaria vaccine trials while at the same time promoting south-south cooperation. AMANET also supports the Afroimmuno Assay (AIA) network whose role is to develop validated and standardized assays for evaluating candidate malaria vaccines.

Malaria Vaccines

Day two kicked off with an overview on the history of vaccines and the advances achieved in this regard. Immunology was said to date started around 1000AD. Records from the Orient show evidence of variolation practises where small contents of smallpox pustules were inoculated intranasal. In 1796 Jenner demonstrated that, because of host specificity of virulence, an animal virus can (sometimes) provide an aborted infection in humans leading to reasonable protection against a human virus. Luis Pasteur introduced the term vaccine in honour of Jenner and his work. These efforts culminated into smallpox being the first and only communicable disease to be completely eradicated by vaccination by the end of 1977.

Development pipeline of candidate malaria vaccines: pre-clinical to cGMP production

This presentation discussed the research and development aspect for malaria vaccine from early stages of antigen isolation and definition, antigen validation, development of models and predictive markers, the limited GMP production of the candidate vaccine for clinical trial, the clinical phase Ia, b (safety) & IIa, b (efficacy), Phase III safety & efficacy trials in target population, to the late stages of scaling up the vaccine production, supply, access and utilisation. The role of the European Malaria Vaccine Initiative (EMVI) was also mentioned and includes providing a mechanism for accelerated development and clinical trials of malaria vaccines in Europe and Developing Countries and promoting affordability and accessibility of malaria vaccines in Developing Countries. It was further mentioned that Malaria vaccine development needs a clear definition of roles and responsibilities for the sponsor, investigator, inventor; insurance and indemnification, personal and institutional liability. Legal and institutional requirements for the sponsor were said to be governed by legislation in the sponsor's country.

Blood-stage malaria vaccines

In a presentation on blood-stage vaccines, it was mentioned that protective antibodies against variant surface antigens disrupt cytoadhesion between infected erythrocytes and human receptors on endothelial cells. The protective antibodies may act directly on parasite growth by neutralizing receptors / ligands expressed on the surface of the free merozoites or merozoites agglutination. An ideal vaccine against blood stages should contain epitopes that are recognized by human antibodies which act through biological mechanisms that are effective only in clinically immune individual. Evidence was presented from studies which have identified targets of merozoites neutralizing antibodies; the targets include the merozoites surface antigen-1 (MSP1) and whose protection was said to depend on the relative amount of cytophilic subclasses rather than on the total quantity of immunoglobulins.

Pregnant associated malaria

This presentation discussed the consequences of pregnancy associated malaria to both the mother and the foetus. It was further said that primigravid women are more susceptible to malaria compared to secundigravid or multigravid women and that pregnancy associated malaria (PAM) is sex specific. Sera from secundigravid and multigravid women significantly inhibit binding of parasites to clonal specific antigens (CSA) *in vitro*. On CSA, the presentation concluded that CSA-selected parasites are not recognised by males; they very well recognised by 3rd-trimester pregnant women from a malaria-endemic area; levels of PfEMP1_{CSA}-specific antibodies depend on parity and levels of PfEMP1_{CSA} -specific antibodies are positively associated with birth weight and maternal haemoglobin levels.

Regulatory Issues in Malaria Vaccine Research & Development

The last day began with a presentation on Regulatory Issues in Malaria Vaccine Research & Development. Regulatory Authorities (RA) are empowered by governments or international organisations, to decide on and promulgate laws to evaluate product data on

its quality, safety and efficacy. The talk pointed out the weakness in most of RA in sub-Saharan Africa and the efforts being undertaken to strengthen them. Since product development is undertaken with a view to eventually register and market a product, product developers need to be informed of regulatory requirements. Early consultations with the corresponding RA is a pre-requisite for proper conduct of the development process

Clinical evaluation of candidate malaria vaccines

Vaccine development takes long involving pre-clinical and clinical studies that cost a lot of money and requires multi-disciplinary expertise and production under current Good Manufacturing Practices. The pre-clinical period that includes basic research, pre-requisite for clinical development, animal data and the early clinical period (profile for), phase Ia and IIa trials

Immunological correlates in the evaluation of malaria vaccines

Clinical phase II trials will offer the opportunity to assess the predictive value of the various *in vitro* assays, validating immunology and immunogenicity of the T-cell controlled antibody productivity. It has been argued that antimalarial immunity against blood stages is age dependent. Passive transfer of IgG was related to clinical protection by reducing parasite multiplication and hence morbidity. Moreover, IgG3 was said to be short-lived and could explain why antimalarial immunity is not long lasting. In addition, IgG1, IgG3 and sometimes IgG2 levels correlate with protection, IgG4 often presents in low quantities. In terms of cellular immunity, it was reported that CD8+ cells protect against pre-erythrocyte parasite stages.

Role of the Afro-immunoassay initiative in evaluation of malaria vaccines in Africa

A presentation was made on the role of Afro-immunoassay initiative in evaluation of malaria vaccines in Africa. Afro-Immuno Assay (AIA) network project standardizes immunological assays to enable comparison of data emanating from studies and clinical trials conducted in different areas of Africa. AIA aims to develop standardized assays using the same reagents and statistical tools to assess the relationship between acquisition of malaria specific antibody responses to potential malaria vaccine candidate antigens and protection from clinical malaria.

Standardized assays developed by AIA can be used to validate malaria candidate antigens, provide essential baseline information for clinical trials and enhance quality assured laboratory capacity and capability. The network (currently involving six institutions in six countries) aims at expanding to include more African partners, and involve more additional antigens as well as establish standardized T-Cell and parasite growth inhibition assays (ADCI, ADCC).

Transmission blocking vaccines

Regarding transmission blocking vaccines, immunity against sexual stage antigens has been demonstrated to be capable of blocking malaria parasite transmission from human to mosquitoes through antibody mediated mechanism including monoclonal antibodies and naturally acquired antibodies. It was said that such a vaccine would prevent malaria epidemics, malaria (re)-introduction, escape and spread of resistant mutants. It was further argued that interventions with transmission blocking vaccines in low to medium

endemic conditions could reduce or even eradicate malaria in proportion to effective coverage while in high endemic areas, coupled with other interventions, could lead to an overall reduction in morbidity and mortality. An association was said to be present between sexual stage specific antibodies against TBV candidate antigens (pfs48/45, pfs230, and pfs25) and transmission blocking activity.

Clinical trials in the evaluation of candidate malaria vaccines

In the background the presentation acknowledged that vaccine development costs a lot of money, requires multi-disciplinary expertise, takes a long time, needs international acceptability (basis for ICH) and an “enabling environment”-stake holder’s role

Pre-clinical evaluation is a prerequisite to the initiation of clinical trials. Together with the laboratory studies, preclinical studies aim at defining the characteristics of the product’s physical, chemical, biological profile, safety indicators and immunogenicity in appropriate animal models. Close collaboration between the preclinical and the clinical team is essential before the product could be used in humans. Throughout the product development process, cGMP issues should be adhered to in order to ensure quality of medical product and some countries might require samples and test results for control tests at RA laboratories. After meeting profile for in human use, conduct of trial should be in compliance with GCP requirements and measures of its efficacy including safety etc should still continue during the clinical trial. The presentation noted that there is not yet a known and validated immunological parameter that correlates or is predictive of clinical protection. Other important issues to consider may include adjuvant, combination vaccines, additives –*preservative & excipients* in product use and delivery.