review article

Wien Klin Wochenschr (2010) 122 [Suppl 1]: 4–6
DOI 10.1007/s00508-010-1325-1

Printed in Austria

Wiener klinische Wochenschrift
The Middle European Journal of Medicine

Assessment of humoral immune responses in malaria vaccine trials

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Bestimmung der humoralen Immunität bei Malariaimpfstoffen

Zusammenfassung. In klinischen Studien zur Untersuchung neuer Impfstoffkandidaten wird zunächst Sicherheit, Verträglichkeit und teilweise auch Immunogenität des zukünftigen Impfstoffes erforscht. Immunologische Versuchsanordnungen müssen je nach Impfstoff etabliert und validiert werden und gegebenenfalls für die Durchführung in multizentrischen Studien weiterentwickelt werden. Die Etablierung von verschiedenen Versuchsanordnungen und die Anpassung an weltweit unterschiedliche Arbeitsbedingungen stellen Herausforderungen an die beteiligten Wissenschaftler. Im vorliegenden Artikel wird dieser Prozess am Beispiel einer kürzlich im Institut für Tropenmedizin in Tübingen, Deutschland durchgeführten Impfstudie zur Erprobung eines neuen Malaria Impfstoffkandidaten erläutert. Die Arbeitsgruppe für klinische Forschung und Immunologie ist hier die Schnittstelle für die Etablierung von immunologischen Untersuchungsmethoden und die Anwendung in klinischen Studien.

Summary. Clinical trials for new vaccine candidates evaluate safety and tolerability of future vaccines. In some studies immunogenicity is an additional exploratory aim. For this purpose immunological assays have to be established and further developed for future multicentre studies. The implementation of immunological assays and the different logistic conditions worldwide display new challenges for scientists working in this field. This article shows this process on a recently conducted phase Ia clinical trial at the Institute of Tropical Medicine in Tübingen, Germany, to evaluate the safety and immunogenicity of a new malaria vaccine candidate. The group for clinical trials and immunology is the interphase for the establishment of immunological assays and their implementation in clinical trials.

Key words: Humoral immunity, malaria vaccine trials, B-cell ELISPOT.

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Introduction

Vaccines against bacterial and viral antigens (Ags) are common and well implemented in public health systems of many countries, protecting millions of people from diseases. Vaccines against parasite Ags are currently not commercial available. Few parasite vaccine candidates are under investigation in the experimental stage but not yet commercially available [1–5]. A main focus is on malaria vaccine candidates [6] employing proteins from either the pre-erythrocytic or the erythrocytic stage of *Plasmodium falciparum* the causative agent of life threatening malaria tropica [7]. Most advanced is the RTS,S malaria vaccine candidate. Here the pre-erythrocytic vaccine Ag Circumsporozoite protein (CSP) is fused to hepatitis surface Ag [8–11]. A phase III trial with RTS,S started this year in several African countries.

Malaria affects all age groups and causes severe disease in non-immune individuals such as young children and travellers from non endemic areas. After a mosquito bite the parasite infects hepatocytes and undergoes asexual replication. The hepatic phase is asymptomatic and followed by the blood stage phase when parasite multiplication is amplified and symptoms like fever, malaise and weakness occur. In severe cases this may result in complications such as respiratory distress, coma or severe anaemia, sometimes with fatal outcome. Each stage of the parasite provides an opportunity for a malaria vaccine.

Methods, results and challenges

Recently, a phase Ia trial was conducted at the Institute of Tropical Medicine in Tübingen, in order to assess safety and immunogenicity of a new blood stage malaria vaccine candidate GMZ2, a fusion protein of a part of the Glutamate Rich Protein (GLURP) and the Merozoite Surface Protein 3 (MSP3) at different doses [12]. The results of this phase Ia trial provided the basis for further clinical trials in Lambaréné, Gabon. For the phase Ia trial 30 healthy non-immune volunteers were randomized into three groups to receive different dosages of the GMZ2 vaccine. Volunteers were vaccinated three times at intervals of four weeks. During this time safety data were assessed and recorded and blood samples were examined to determine immunogenicity of the GMZ2 vaccine candidate. Table 1 depicts timelines and assays for immunological investigations to assess humoral immunity effected by GMZ2.

Enzyme linked immunosorbent assay (ELISA), immunofluorescence (IFA) and vaccine Ag specific B-cell enzyme linked immuno-

Table 1. Immunological investigations to determine humoral immunity of the GMZ2 vaccine candidate

Day (d)	Vaccination	Vaccine specific Ag by ELISA	*P. falciparum* specific IFA	B-cell ELISPOT
d 0	1st	X	X	X
d 28	2nd	X		
d 56	3rd	X		
d 84	4 weeks post-vaccination 3	X	X	X
d 365	1 year follow-up	X		X

spot assay (ELISPOT) have been established for the implementation in these phase I trials.

All doses of GMZ2 were well tolerated and immunogenic [12] and a phase Ib trial in Lambaréné started in Lambaréné, Gabon. The aim was to determine the safety and the immunogenicity elicited by the vaccine in individuals exposed to *P. falciparum* infection. This is important because these adult individuals living in an area endemic for malaria have usually developed a certain degree of immunity (semi-immunity) against malaria which protects them from clinical complications or death but not from infection. If the vaccine is safe in this particular population another phase Ib trial in the target population of this vaccine will be initiated. The target population of a future malaria vaccine are children and infants and possibly pregnant women. These individuals are at high risk to develop severe malaria, in some cases with fatal outcome and harm of the unborn child *in utero*.

Because the target population represents subjects from vulnerable groups (meaning that they are under physical and/or mental development) it is ethically not arguable to take the same amount of blood for experimental clinical and immunological examinations as from healthy adults. Here, a balance of strictly required examinations and scientific interest has to be found.

Furthermore, it is important for future vaccines and vaccine candidates to gain more information about the impact of the adjuvant, dose of the vaccine Ag, route of injection and time schedule for vaccination to maximize vaccine efficacy. The success in terms of safety and also of immunogenicity of these trails is the basis of subsequent phase II clinical trials. Here, a larger number of individuals will be included and these trials are generally performed as multicentre studies. Besides assessment of safety and immunogenicity phase II clinical trials allow to evaluate at least partially the efficacy of the malaria vaccine candidate.

The start of GMZ2 phase II trials is envisaged for end of 2010. Therefore, it is crucial to evaluate physical examination methods, case report forms (CRFs) and experimental methods for the use in multicentre studies and to adapt them to different circumstances in the participating countries like the availability of materials, the climate and the equipment of laboratories. Study specific standard operation procedures (SOPs) have to be generated and adapted for their practicability in different centres as well as centres have to be prepared and evaluated for their participation. A suitable set of assays has to be developed and established comprising detection of vaccine specific humoral immune response, determination of vaccine induced cytokines and cell subsets alteration during immunization with the vaccine Ag. Currently, members of my group and of the group of Mordmüller adapt immunological methods applied in the phase Ia and phase Ib trials for the use in different centres and regions for the future phase II trial. Malaria vaccine trials in endemic areas often take place under constrained logistic conditions. For example, lack of electricity under field conditions may force investigators to find solutions independent from public power supply [13]. As transport and storage of frozen serum samples might be difficult, we modified a method to detect Abs from dried bloodspots on filter paper [14] in the Venezuelan Amazon using the GMZ2-, MSP3- and GLURP-Ags (unpublished data).

Detection of vaccine specific antibodies (Abs)

The main surrogate marker of vaccine protection are vaccine specific Abs.

These are usually detected by ELISA. The ELISA technology is widely used because of its simplicity and the objective interpretation by optic density (OD) values. The major problem of this assay is the assessment of Ab concentration by different protocols. Different results are obtained using commercial available standards or positive and negative reference sera. Thus, results obtained of one sample may differ from one method to another making the reproduction and the comparison of results from different laboratories difficult. Therefore we investigated different ELISA methods to obtain the most stable results under varying environmental conditions. Additionally we compared the results from ELISA with those obtained by Westernblot.

Another possibility to determine Abs against *P. falciparum* proteins is the IFA. Here parasite coated slides are incubated with human serum and later with a fluorescent detection Ab to detect parasite directed Abs under a fluorescence microscope. This is important because with this assay it can be shown that Abs generated by the vaccine are able to bind to native parasite surface molecules. Because GMZ2 is expressed in *P. falciparum* merozoites we established the IFA based on the protocol of Hermsen et al. [15] with synchronized merozoites.

Assessment of vaccine specific Ab secreting cells (ASCs)

Ag specific memory is characterized by fast and efficient immune reaction upon repeated contact with the Ag. This is conducted by Abs which are secreted from Ab secreting cells (ASCs) or plasmablasts. These are terminally differentiated cells and homed in the spleen or the bone marrow and not circulating in the periphery. Therefore we used a cell culture system to obtain ASCs indirectly from peripheral blood mononuclear cells (PBMCs). In 2002, Bernasconi et al. showed that memory B-cells switch to IgG secreting plasmablasts in the presence of CpG and interleukin-15 [16].

Plasmacells are thought to provide immunity even when the amount of circulating Abs decreased. Because B-cell memory plays a central role in the persistent maintenance of Ab levels after infection or following vaccine immunization it would be beneficial to establish a reproducible test to detect ASCs of defined specificities.

Therefore the development of a reliable B-cell ELISPOT was a major interest before the phase Ia study started in Tübingen. This was done with PBMCs from individuals from malaria endemic areas and from non-immune individuals. In brief microtiterplates were coated with vaccine Ag and PBMCs were seeded to allow them secreting Abs. Different development buffer systems were investigated to identify the most stable and reproducible method. To establish the method, ASCs secreting anti-tetanus toxoid (TT) Abs were examined and it was found that individuals with serum Ab titres detected by ELISA in some cases displayed only very few spots representing Ab secreting cells in the assay. The history of these "low responders" showed that they received their last vaccination against TT around ten years ago suggesting that protection against TT is very low and booster vaccination is recommended. A few weeks after a new vaccination the

number of Ab secreting cells detected by ELISPOT increased (unpublished observation by Esen).

Because most malaria endemic regions usually face problems like poverty, malnutrition, poor hygienic conditions and other endemic diseases like AIDS, tuberculosis and intestinal parasite infections, these circumstances have to be considered during the conduct of clinical vaccine trials.

For example, impaired immunity sometimes induced by helminth infection results in reduced immunogenicity of vaccines [17–19]. Therefore new strategies for vaccine trials in malaria endemic regions have to be developed to maximize vaccine efficacy.

Conclusion

In conclusion the members of my group investigate safety and immunogenicity of vaccines in clinical trials phase I to III. For that purpose immunological assays are and will be investigated and established. Furthermore quality controlled and standardized well established protocols for clinical multicentre trials conducted in malaria endemic areas are implemented. This includes training activities for physicians and scientists as well as development of suitable databases for secure and useful data management.

Conflict of interest

The author declares no conflict of interest.

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