Development and demise of Plasmodium liver stage parasites - The hunt for a Genetically Attenuated Malaria Vaccine

Ivo Ploemen1*

1 Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

* ivoploemen@gmail.com

Thesis details:
Year of publication: 2013
Subject category: Vaccinology
Keywords: Malaria, Plasmodium berghei, sporozoite, liver, vaccine
Supervisors: Professor Robert W. Sauerwein and Dr. Chris J. Janse

Thesis Summary

Abstract

A considerable effort is currently underway to develop a malaria vaccine based on live Plasmodium falciparum sporozoites. The first requisite of a sporozoite vaccine is the guarantee of parasite arrest prior to the onset of the pathogenic blood stage. Immunisation with genetically attenuated parasites (GAP) that arrest in the liver forms a promising approach. Work in this thesis describes the development and characterisation of a P. berghei Δb9Δslarp GAP that fully arrests in the liver. Immunisation of multiple mouse strains with low numbers of Δb9Δslarp GAP resulted in sterile protection. The Δb9Δslarp GAP is therefore the leading GAP vaccine candidate. Work in this thesis further describes the effect of varying the parameters of sporozoite inoculation on parasite liver load. These findings provide a rationale for the design of clinical trials aimed at the administration of live attenuated P. falciparum sporozoites.

Background

A vaccine against malaria and specifically against P. falciparum infection is pressingly needed. In human volunteers, live sporozoites are the only immunogens that, by immunisation through mosquito bites, have ever been shown to induce sustained and high levels of protection against a malaria infection. Immunisation with genetically attenuated parasites (GAP) is broadly considered the safest and best option for a live sporozoite vaccine. The critical first step in the clinical development of a GAP vaccine is the guarantee of complete parasite arrest in the liver. In mice, using the P. berghei and P. yoelii model, various GAP vaccine candidates have been described with clear gene orthologs in the P. falciparum genome. Unfortunately, none of these (murine) GAPs meet the requisites of safety and efficacy, essential for an ultimate P. falciparum GAP vaccine. In this thesis, we describe the development and characterisation of a safe and protective P. berghei GAP consisting of multiple gene deletions.

Aside from the safety and protective efficacy of a GAP candidate, the eventual route of sporozoite inoculation forms another obstacle in the development of a whole sporozoite vaccine. While mice can be immunised by intravenous sporozoite inoculation, most volunteers have been immunised through the bites of infected mosquitoes. It is evident that neither intravenous immunisation, nor immunisation through mosquito bites, are preferred vaccination routes for children in sub-Saharan Africa. We therefore explored alternative routes of sporozoite administration.

Objectives

The specific objectives of this thesis research were to: a) develop and characterise a GAP that fully arrests in the liver stage and induces long-lived protection in the murine P. berghei model; b) decipher the role of the route of sporozoite immunisation (e.g. intravenous or intradermal) on protection; c) optimise the administration of sporozoites in a P. berghei model and P. yoelii model in order to eventually improve the levels of conferred protection.

Methods

New GAP gene targets were selected based on the presence of transcripts in sporozoites and the absence of protein expression in sporozoites. A stringent and robust set of screening criteria was adopted to assess the adequacy of GAP sporozoite attenuation. Both BALB/c and C57BL/6 mice were inoculated with high numbers of P. berghei GAP. The development of these GAP candidates was monitored in live mice using in vivo imaging. The protective efficacy of GAP immunisation was addressed in multiple mouse strains.

A transgenic parasite, expressing the bioluminescent
reporter protein luciferase was used to describe the relation between protective efficacy and parasite development in the liver following sporozoite inoculation by various routes.

**Results**

In our search for a safe and protective GAP we first observed a lack of safety from two leading GAP vaccine candidates, *Ap*52+*p*36 and *Afabb/f*. Both GAPs are not adequately attenuated. Infection of mice with sporozoites of both *P. berghei* candidates resulted in blood stage infection. Moreover, we found that *Ap*52+*p*36 *P. falciparum* GAP can develop into replicating liver stages. A detailed analysis revealed that *P. berghei* *Ap*52+*p*36 GAP parasites can fully develop in hepatocytes in the absence of a parasitophorous vacuole membrane (PVM). Apparently, the formation of a PVM is not a necessity for the development of (*P. berghei*) liver stage parasites. These findings urged us to search for new GAP vaccine candidates that for their arrest do not rely on an impaired formation or maintenance of the PVM.

We developed and characterised a new GAP candidate; *Δ*b9, which has much the same characteristics as the *Ap*52+*p*36 GAP. While immunisation with low doses of *Δ*b9 sporozoites confers protection in mice, low numbers of mutant *Δ*b9 parasites fully mature into blood stage parasites. In the pursuit of a GAP candidate that is both safe and effective we generated and characterised a GAP consisting of multiple gene deletions; *Δ*b9 slarp. Immunisation with low numbers of *P. berghei* *Δ*b9 slarp GAP resulted in full protection in BALB/c and C57BL/6 mice and inoculation with high doses of sporozoites did not result in the development of late liver stage or blood stage parasites.

Immunisation of mice by intradermal injection is much less efficient compared to intravenous immunisation. We observed a relationship between the route of immunisation and conferred level of protection in mice on the one end and the subsequent parasite liver load following the various routes on the other. In C57BL/6 mice, the lack of protection following intradermal immunisation associated with a 30-fold lower liver load in vivo, compared to intravenous immunisation. Apparently, it is of high importance that upon immunisation, parasites reach the liver. We next explored alternative routes of sporozoite administration to increase the efficiency of liver infection. We found that, in a murine model, intramuscular injection of sporozoites resulted in a greater parasite liver load compared to intradermal and subcutaneous injection. The use of small inoculation volumes and multiple injections further increased the subsequent liver load. These findings provide a rationale for the design of clinical trials to optimise the administration of *P. falciparum* sporozoites by needle and syringe.

**Interpretation and conclusions**

Our results show that the *P. berghei* *Δ*b9 slarp GAP fully arrests in the liver stage. Immunisation of mice with low numbers of *Δ*b9 slarp resulted in long-lived sterile protection. At present *Δ*b9 slarp is therefore the leading GAP vaccine candidate. The orthologue mutant in *P. falciparum* has been generated and its development inside the hepatocyte will now need to be assessed prior to further clinical development. Future immunisation trials in human volunteers with *P. falciparum* (*Δ*b9 slarp) GAP will be key in determining the potential of this vaccine approach. Considering the precedent of efficacious whole sporozoite immunisations in human volunteers, the prospects for GAP immunisation are very promising. One major challenge will be to develop a non-intravenous immunisation route that is capable of delivering the sporozoites to the liver. Injection systems such as needle free jet-injectors and hollow microneedle arrays might be up for the task. Alternatively, a device might need to be newly designed. The work described in this thesis provides direction and guidance for the further clinical development of a *P. falciparum* GAP vaccine.

**Publications resulting from this thesis**

(As per 10 June 2013):


**Additional file**

Full copy of PhD thesis