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Title of the PhD Thesis : Receptor-Ligand Interactions in Erythrocyte Invasion by Malaria Parasites: Targets for Intervention

Abstract

Malaria is an important public health problem worldwide with a WHO estimate of ~250 million cases annually. Five species of *Plasmodium* can cause malaria in humans but two species, *P. falciparum* and *P. vivax,* account for bulk of the disease burden. *P. vivax* is the leading cause of the disease in South America, South and South East Asia responsible for 100 million cases or more each year. Recently, it has been found that *P. vivax* can cause severe illness similar to that caused by *P. falciparum*, and that *P. vivax* has a far wider geographical reach than estimated earlier. Clinical symptoms of malaria in humans are associated with invasion of erythrocytes by malaria parasites, that is mediated by interaction of specific receptors and ligands located on the surface of erythrocytes and the parasite respectively.

P. vivax uses the Region II of its Duffy binding protein (PvDBP) for binding to the Duffy blood group antigen (also known as Duffy Antigen Receptor for Chemokines – DARC) as a key receptor-ligand interaction necessary for erythrocyte invasion. A preventive vaccination strategy based on targeting the PvDBP-DARC interaction can block parasite entry into the erythrocytes, and thus can prevent or reduce malaria clinical disease.

In order to design and formulate a preventive vaccine for *P. vivax* malaria, first we validated the Region II of Duffy binding protein as a potential vaccine candidate. We successfully produced recombinant Region II of Duffy binding protein (PvRII, based on SalI strain of *P. vivax*) in *E. coli,* refolded it to native conformation, and evaluated its immunogenicity in BALB/c mice upon formulation with five human-compatible adjuvants namely, Alhydrogel, Montanide ISA720, AS02A, QS21 and MF59. Montanide ISA720, AS02A and Alhydrogel formulations yielded high IgG end point titres by ELISA and inhibited binding of Duffy positive erythrocytes to PvRII in a PvRII-expressing COS cell binding assay. Thus we selected the adjuvants Montanide ISA720, AS02A and Alhydrogel as promising adjuvants for further evaluation in rhesus macaques.

As an improved method to study immune responses against PvRII, we developed a new *in vitro* 96-well plate receptor-ligand binding assay based on recombinant PvRII and recombinant N-terminal region of DARC. This assay design improved throughput and quantitation, and proved to be a useful method for measuring the neutralizing potential of antibodies against PvRII that may serve as an indicator of protective immunity.

The three vaccine formulations down selected form the mice study were re-tested in rhesus macaques. These formulations yielded high IgG antibody titres by ELISA that correlated to the 50% binding inhibitory titres (Responses of Montanide ISA 720 and AS02A formulations were significantly higher than that of Alhydrogel). Thus, we concluded that PvRII-based vaccine formulations can drive functional antibody production to sufficiently high levels that can also potentially block *P. vivax* invasion of human erythrocytes.

With an aim of finding mechanisms of protective immunity against *P. vivax* malaria, we analysed sera from children residing in Mugil, Papua New Guinea (a *P. vivax*-endemic region). Nine percent of the 206 children had acquired >90% inhibition of binding inhibitory antibodies. This level was associated with a species-specific protection against blood-stage *P. vivax* infection. Various parametres compared between children with high blocking antibodies (>90%) vs low blocking antibodies (<50%) were as follows : Delay in time to re-infection after clearance of blood-stage infection - Median time to re-infection of 96 days vs 62 days; Frequency of infection 5.1% vs 13.7%, and parasite density ~40% less when compared to low blockers. Further, children with high blocking antibodies had strain-transcending antibodies that blocked binding of diverse PvRII domains to DARC. Thus a vaccine based on SalI sequence is expected to be effective against a range of vivax strains. These data proved the hypothesis that antibodies directed against region II of Duffy binding protein are an important component of naturally acquired immunity that can protect against vivax infection.

Further, antibodies elicited upon immunization of rhesus with PvRII were found to be at least 25 fold higher and more potent in terms of inhibiting PvRII-DARC binding when compared to naturally acquired antibodies. Rhesus sera also inhibited binding of diverse PvRII variants with high efficiency. Thus a high-titre inhibitory antibody level induced upon immunization of humans with PvRII could provide strain-transcending action against *P. vivax* infection. In this study we have made promising advances in pre-clinical development of PvRII as a vaccine candidate. These data validate the rationale for development of a vaccine based on PvRII for *P. vivax* malaria.