



**Karolinska  
Institutet**

**Institutionen för mikrobiologi, tumör-och cellbiologi**

**Virulence in *Plasmodium falciparum* malaria:  
mechanisms of PfEMP1-mediated rosetting**

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska  
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av

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## ABSTRACT

Malaria is one of the most important infectious diseases in the world and the *Plasmodium falciparum* parasite is the causative agent of most of the severe cases. The pathogenesis of the disease is complex but sequestration and hence microvascular obstruction is associated with virulence of the parasite. Rosetting, the adhesion of a parasitized red cell (pRBC) to two or more non-parasitized RBC is central in the adhesion phenomena. The adhesin *Plasmodium falciparum* Erythrocyte Membrane Protein-1 (PfEMP1) mediates rosetting through its adhesive head structure composed of the NTS-Duffy Binding Like (DBL) 1 $\alpha$  domain. Specific PfEMP1 antibodies (Abs) acquired after repeated exposures to parasites are associated with immunity to severe disease. In order to design effective therapies against severe malaria a deeper knowledge of the rosetting phenomenon is required.

A panel of monoclonal antibodies (mAbs) to NTS-DBL1 $\alpha$  was generated by vaccination with recombinant protein. Epitopes recognized by the antibodies were mapped using a peptide array revealing that the reactivity of rosette disruptive monoclonal antibodies is localized in a specific region of subdomain 3 of DBL1 $\alpha$ , independently of the parasite strain tested. In addition, the majority of anti-rosetting antibodies in a polyclonal IgG preparation towards NTS-DBL1 $\alpha$  targeted the same area. This suggests subdomain 3 of NTS-DBL1 $\alpha$  to be one of the major targets for rosette-disruptive antibodies. Further, generation of biologically active antibodies was consistent in different animal species and cross-recognition of heterologous rosetting domains was common in ELISA but not on live pRBC.

In parallel, to overcome the strain-specificity of the antibodies, a sequence motif present in subdomain 2 of the DBL1 $\alpha$  sequence and previously associated with severe malaria was used for immunization. The peptide elicited a strain-transcending antibody response, with immune IgG recognizing a number of genetically distinct parasites, including both laboratory strains and patient isolates. Our results demonstrate the possibility to generate cross-reactive antibodies that recognize the pRBCs surface.

In addition, investigations were carried out on the naturally acquired human antibody repertoire as found in individuals living in an area of high malaria endemicity. Patients plasma samples were analysed for their biological activity towards a laboratory parasite strain. Findings were correlated with clinical symptoms and the epitopes recognized by the Abs on a peptide array. Reactivity of the plasma samples towards six of the peptides was correlated with the sample capacity to disrupt rosettes. The identified peptides were distributed along the NTS and DBL1 $\alpha$  sequence, but mainly localized in subdomain 2.

Finally, by combining site directed mutagenesis with RBC binding and rosette inhibition studies, the localization of the binding site of one rosetting NTS-DBL1 $\alpha$  domain was mapped to subdomain 2. Our results also demonstrate that rosetting inhibition by mAbs is not mediated by direct blockage of receptor binding but rather by modifications distal from the paratope.

In conclusion this thesis provides new insights into targets for vaccination-induced and naturally acquired antibodies towards PfEMP1-NTSDBL1 $\alpha$  and it describes a receptor-binding site important for rosetting. Overall this thesis increases the knowledge on the molecular mechanisms underlying rosetting and could be helpful for the future rational development of therapeutic means against severe malaria.

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