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Functional antibody responses to the *Plasmodium falciparum* merozoite

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ABSTRACT

Plasmodium falciparum is a leading cause of death among children under the age of five and pregnant women in sub-Saharan Africa. More than one third of the world's population is at risk of contracting malaria, and 70 % of the cases are found in sub-Saharan Africa. Emerging drug resistance in parasites and limited effect of vector control calls for an effective vaccine. It is known that individuals living in malaria endemic countries develop naturally acquired immunity after repeated exposure. Antibodies are important components of acquired immunity, and it has been shown that passive transfer of antibodies from immune donors to individuals with *P. falciparum* infections reduced parasitemia and clinical symptoms. Antibodies against several *P. falciparum* merozoite antigens have been found to be associated with protective immunity. It is of great importance to understand the underlying functional role of antibodies in the development of protective immunity against severe malaria.

In a cross-sectional study in Uganda, the quantitative and qualitative differences in antibody responses to a panel of merozoite antigens in children with uncomplicated or severe malaria were evaluated using a set of assays including ELISA, Invasion Inhibition Assays (IIA), NH₄SCN-ELISA and Surface Plasmon Resonance (SPR). Children with uncomplicated malaria had higher antibody levels against PfEBA-181, MSP2-Fc27, MSP2-3D7 and PfAMA1 when compared to children with severe malaria. Acquired antibodies against PfRh2 and PfAMA1 in ELISA correlated with invasion inhibition of two clinical isolates in IIA, and anti-PfAMA1-antibodies in ELISA correlated with increased anti-PfAMA1-antibody affinity in SPR. Importantly, the only assay that correlated with initial parasitemia in the children was the IIA. Both MSP2-Fc27 and MSP2-3D7 allelic variants were present in both children groups, but there was a higher number of genotypes in uncomplicated malaria compared to in children with severe malaria.

P. falciparum clinical isolates collected from Ugandan children with uncomplicated malaria or severe malaria were further investigated for rosetting, parasite multiplication and RBC invasion. Optimal *in vitro* growth conditions were established, which allowed for phenotypic studies of clinical *P. falciparum* isolates. Presence of serum in growth cultures was found to be essential for optimal surface presentation of PfEMP1 and maintenance of rosettes. Higher peripheral parasitemia, higher rosetting levels and higher multiplication rates were observed in children with severe malaria and these correlated positively with one another. Rosetting might enhance successful merozoite invasion *in vivo*, hence could be the reason it is found to be associated with severe disease. Furthermore, parasite invasion into trypsin- and chymotrypsin-treated RBC differed between the uncomplicated and severe groups, and isolates from children with uncomplicated malaria showed higher sensitivity to enzyme treatment. The majority of clinical isolates used a sialic acid independent invasion pathway. Parasite invasion is central to parasite replication and virulence, and it is essential to know which invasion pathways are used for vaccine studies.

Naturally acquired antibody responses to *P. falciparum* merozoite antigens was further studied in a longitudinal study over almost one year in children and adults from Nigeria. The malaria protective effects of the hemoglobin S (HbAS) allele were also investigated. In both children and adults, the antibody response against PfEBA175 was more prominent than that against PfRh2, and cytophilic IgG1 and IgG3 against PfEBA175 were the predominant antibodies even though we could also see some response for IgG2 and IgG4. Individuals with higher total IgG responses against both PfEBA175 and PfRh2 had lower parasitemia over the course of the study period. Furthermore, children with HbAS had higher antibody responses against merozoite antigens compared to adults, and this might have protective effects against malaria.

In conclusion this thesis emphasizes the great importance of using a combination of functional assays, such as SPR and IIA, to study the functional role of acquired antibodies in the development of protective immunological responses against severe malaria. Furthermore, investigations of parasite invasion and rosetting in the context of pathogenesis of severe malaria are crucial as both are important for parasite replication and virulence. Taken together, future vaccine studies should include functional assays that would allow the investigation of differences in immunological responses against severe and uncomplicated malaria. Also, to consider the protective effects of red blood polymorphisms and their role in acquired immunity in the populations, would be of great value for future vaccine studies.