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学 位 論 文 要 旨
Dissertation Summary

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論 文 名(Dissertation Title): **NATURALLY ACQUIRED IMMUNITY TO PLASMODIUM FALCIPARUM MALARIA: PROFILING ANTIBODY RESPONSES FOR NOVEL VACCINE ANTIGEN DISCOVERY**

Malaria, a mosquito-borne disease, remains an important global health issue, with around 40% of the world's population at risk, and an estimated 212 million illness episodes and 438 000 deaths being reported in 2015; *Plasmodium falciparum* is the major cause of this disease burden. The disease is a major cause of morbidity and mortality among young children in sub-Saharan Africa. Emerging drug resistance to both the parasites and the mosquito vectors control calls for development of improved and novel approaches towards malaria elimination. Malaria vaccines present a feasible option. There is strong evidence that effective and protective immunity develops in individuals exposed to *P. falciparum* infections after repeated infection. Although the mechanism of the observed immunity remains largely unknown, passive transfer of purified immunoglobulins from malaria immune donors to malaria patients confers protection from hyper parasitemia and symptomatic malaria, indicating that, antibodies play an essential role in protective immunity.

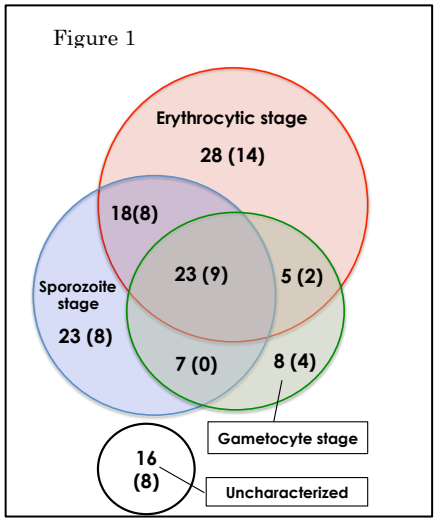
Several immuno-epidemiological studies have explored antibody profiles in malaria-exposed individuals to identify the key targets of the protective immunity. In such studies, recombinant parasite proteins expressed in bacteria and printed on microarray chip were probed with malaria-exposed human sera. These strategies proved tremendously valuable to leverage several antigens as candidate vaccines. However, the major drawback is the possibility of proteins expressed by the bacterial system not attaining optimal native conformations, which appears to be important for the induction of protective antibodies. In contrast, only a limited

number of proteins expressed by eukaryotic system have been analyzed. Furthermore, extensive malaria vaccine discovery efforts have yielded only a handful of vaccine candidates, with few that progressed to clinical trials having unsatisfactory low or moderate efficacies. There is therefore, an urgent need to carefully profile antibody responses against *P. falciparum* antigens to gain deeper insights into the mechanism of acquired immunity as well as identify the targets of malaria protective immunity to form the bases of second-generation vaccine targets.

In this study, immunoreactivity to a large library of recombinant proteins (n=1827) derived from ~30% (1565 genes) of the entire *P. falciparum* genome, was determined for identification of novel malaria vaccine candidates. The recombinant proteins were expressed by wheat germ cell-free system; a eukaryotic platform that can synthesize quality plasmodial proteins that induce biologically functional antibodies in immunized animals. Human sera were obtained from indigenous residents of a malaria endemic region in Northern Uganda who were naturally exposed to *P. falciparum* infections. The volunteers were enrolled at the start of a rainy season and prospectively monitored for symptomatic malaria episodes for a year. Protein immunoreactivity to the sera was determined by AlphaScreen; a homogeneous high-throughput system that detects protein interactions.

Analysis revealed broader protein immunoreactivity than previously observed in similar immuno-epidemiological studies. Fifty-one percent of the proteins (938/1827) in the library were immunoreactive against the Ugandan sera. This affirmed that the pool of target antigens is much larger than previously described, further underscoring the complexity of identifying key targets of protective antibodies and novel vaccine candidates. Antibody responses to 128 single proteins, expressed in different stages of parasite lifecycle, significantly associated with protection from symptomatic malaria; defined as fever ≥37.5°C and asexual parasitemia of ≥ 2500/μl of blood (Figure 1). Fifty-three antigens (in parenthesis, Figure 1) were down-selected as the most plausible targets of host protective immunity by virtue of having a predicted signal peptide and/or transmembrane domain(s), or confirmed localization on the parasite surface. The 53 proteins comprised of not only previously characterized vaccine candidates with known parasite expression, localization and function but also novel uncharacterized proteins. Specifically, three well-known rhoptry proteins (rhoptry neck protein 4 (RON4), RON2, cytoadherence linked asexual protein 3.1 (CLAG3.1)) and two merozoite surface proteins (MSPs; MSP8 and MSP7-like protein 5), remained significantly associated with protection even after adjusting for potential confounders, with RON4 having the highest adjusted potential protective efficacy. Eight proteins exclusively expressed in the sporozoites were also selected; three of them including one promising vaccine candidate, sporozoite surface protein 2 (TRAP), remained significant after the adjustment. Altogether, responses to 15 antigens remained significantly associated with protection from symptomatic malaria irrespective of confounders. Considering the increasing body of evidence on important functions and potential roles of secreted proteins; RON2, RON4 and CLAG3.1, in host-parasite interaction and invasion and potential roles of sporozoite invasion-associated protein 2 (SIAP-2), TRAP and cell traversal protein for ookinetes and sporozoites (CelTOS) in pre-erythrocytic stage parasite development, the antigens were recommended for prioritization

Figure 1



for further evaluation as vaccine candidates.

Analysis with combinations of antibodies to multiple antigens lead to small not significant increases in potential protective efficacy which, although needs to be confirmed in a study with a larger sample size, clearly suggest that the breadth of antibody response is important to confer adequate protection rather than a strong response to a single antigen.

In conclusion this thesis demonstrates and emphasizes the great importance of using a combination of (i) an unbiased genome-wide recombinant protein library to comprehensively identify potential targets of immunity in malaria, (ii) use of WGCFS generated proteins, that are comparatively more soluble, intact, biologically active and immunoreactive to human sera; and (iii) application of high-throughput immunoscreening system (AlphaScreen) for unbiased antibody quantification and profiling. The study offers new options for rational discovery and selection of potential malaria vaccine candidates. Future vaccine studies are now needed to investigate acquisition, maintenance quality and mode of action of immunological responses against malaria.