# IMMUNO AND MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM VIVAX* BLOOD STAGE ANTIGENS UNDER LOW TRANSMISSION AND UNSTABLE MALARIA CONDITIONS IN SRI LANKA

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## ABSTRACT

Data generated through Immuno and molecular–epidemiology studies are imperative in vaccine development studies. In this context, exploration of the nature of naturally acquired immune responses, *in vivo* correlates of protection and the genetic polymorphism among *Plasmodium* parasite populations, are of significant relevance for anti-malarial vaccine development.

The 42-kDa and 19-kDa C-terminal processing products of *Plasmodium vivax* Merozoite Surface Protein-1 (PvMSP-1p42 and PvMSP-1p19) and the 66-kDa surface accessible region of the *P. vivax* Apical Membrane Protein-1 (PvAMA-1) are leading vaccine candidates against vivax malaria. Naturally acquired total and isoytpe antibody responses to PvMSP-1p42, PvMSP-1p19 and PvAMA-1 were assayed by ELISA against p42, p19 and PV66/AMA-1 recombinant proteins, representing the native antigens, respectively. Test populations included two *P. vivax* malaria endemic regions in Sri Lanka with low transmission and unstable malaria conditions, and a non-endemic urban area.

All three-vaccine candidates appeared to be immunogenic in nature with p42 being more immunogenic than the other two proteins. Analysis of data of individuals with a history of malaria indicated that a significantly higher proportion of individuals from non-endemic Colombo responded to all three antigens, compared to individuals from both malaria endemic areas. Further, a significantly higher number of individuals from Colombo with a history of malaria responded to at least one of the antigens tested compared to individuals from both endemic areas. Significant differences were apparent between endemic and non-endemic

residents for IgM subclass specific antibody prevalence and levels for p19 and PV66/AMA1. A marked cytophilic IgG1 response among the four IgG isotypes evaluated was apparent against the three antigens tested in individuals of all three-test areas. The high proportion of IgM antibodies directed against the polymorphic region of p42 may interfere with a p19 "protective" isotype switch, which may insinuate that immunological "cross-talk" between epitopes of p42 and p19 may complicate MSP-1 based vaccine development.

The only host factor in all test populations that was significantly associated with total and isotype specific antibody responses to all three antigens, was the previous exposure of the patient to malaria. Furthermore, it was apparent that with increasing exposure to malaria, in both endemic areas, anti-p19 and anti-AMA-1 antibody responses were dominated by the functionally important IgG1 and IgG3 isotypes, with a concurrent reduction in IgM, that was absent in the non-endemic residents. In contrast, this antibody switch for p42 was restricted to endemic residents with more extensive exposure, implying that this host factor may be considered as an *in vivo* correlate of protection for asexual humoral immunity in *P. vivax* malaria under low and unstable malaria conditions in Sri Lanka.

The genetic variation of PvAMA-1 among field isolates of *P. vivax* in Sri Lanka was assessed using standard methodology. The sequencing of PvAMA-1 of 15 parasite isolates resulted in a lack of size polymorphism in the coding region. There were 11 haplotypes with 29 single nucleotide polymorphisms (SNPs) encoding 9 different alleles

among the 15 isolates examined. All mutations were restricted to the coding reagion and 19 polymorphic sites were displayed in Domain I, while Domains II and III had only 7 and 3 polymorphic sites, respectively. From the total number of mutations observed, five mutations showed synonymous polymorphisms, where as the rest of the mutations were non-synonymous. Significant deviation from neutrality was found in the entire gene as well as in ectodomains I and II. Ectodomains I and II probably are the most suitable regions to be developed as vaccine candidates against vivax malaria, as these domains probably carry protective epitopes suitable to be incorporated as part of a subunit vaccine. The similarity in observed SNPs between the present and previously published work, imply that a vaccine composed of *P. vivax* AMA-1 could be expected to be equally effective in different populations residing in different geographical settings worldwide.

In order to utilize data from a future clinical trial based on *P. vivax* asexual erythrocytic stage malaria vaccine(s) in Sri Lanka, this study amply demonstrated the significance of a strong interlink between the molecular immunology of the vaccine construct, host and parasite biology, and the prevailing epidemiological conditions of the field. Thus, *in toto*, the data accrued from this study will contribute immensely to the improvement of vaccine constructs based on PvMSP-1 and PvAMA-1, and also provide vital information on the development of a multi-component vaccine representing PvMSP-1 and PvAMA-1.