Strain-specific immunity to *Plasmodium vivax* asexual erythrocytic stage antigens in Sri Lanka

A thesis submitted for the Degree of Doctor of Philosophy

Kehelkaduwa Vithanage Geethika Sajani Dias

Faculty of Science

University of Colombo

June, 2010

Abstract

The efficacy of a vaccine is hindered by the antigenic polymorphism elicited by the parasite to escape the host immune system. Thus, the present study for the first time characterized the prevailing genetic diversity of two major asexual stage putative vaccine antigens of *Plasmodium vivax*, the Apical Membrane Antigen-1 domain II (*ama-1DII*) and the Merozoite Surface Protein-1_{42} (*msp-1_{42}*) in Sri Lanka, where unstable malaria prevails with low transmission. Parasite isolates (N=217) were collected from patients infected with *P. vivax* malaria from two malaria endemic areas, Anuradhapura and Kataragama, and from non-endemic Colombo. The selected single clone isolates (N=169) identified by a combined polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) of the *Pvmsp-3α* locus were amplified at the *Pvama-1DII* and *Pvmsp-1_{42}* locus by the standard PCR technique. Both the local and the worldwide genetic diversity of these two antigens were analysed.

The *ama-1DII* gene in Sri Lanka showed meagre meiotic recombination with the enclosure of single nucleotide polymorphisms (SNPs), where eleven amino acid (a.a) variant positions defined 21 a.a. haplotypes with 10 unique to Sri Lanka. The H1 a.a. haplotype predominant in Sri Lanka was identical to the reference Salvador I strain, while haplotypes H14 and H16 were observed in Sri Lanka, India and Venezuela. Further, 145 globally dispersed isolates defined 12 a.a. haplotypes (H22-H33), where 7 and 1 haplotypes were unique to India and Venezuela, respectively. A novel observation where all a.a. sequences locally and globally were identical at the domain II loop of PvAMA-1 was recorded. The evolutionary relationship in the phylogenetic tree revealed fewer clustering where most isolates had a very recent common origin. Evidence of reduced genetic diversity in Venezuela reflected the effects of recent spread of the parasite to the new world, whereas those from the old world appeared to reflect a very ancient selectively maintained polymorphism.

At the PvMSP-1_{19} locus, all a.a. sequences were identical. The analysis of 39 variant a.a. positions upstream of PvMSP-1_{19}, at the PvMSP-1_{33} region, documented several novel observations: i) this region defined 27 a.a. haplotypes with 19 unique to Sri Lanka, ii) 24 of the 27 PvMSP-1_{42} haplotypes represented 7 basic a.a. sequence types at the hypervariable region (HVR) while the remaining 3 were generated by interallelic double recombination, iii) sequences from widely dispersed isolates in the database defined 62 more a.a. haplotypes, 43 of which corresponded to 9 of the 10 HVR types (excluding HVR-T7, unique to Sri Lanka), iv) two novel HVR types, HVR T11 and T12, with a double recombination were derived from South America and Thailand, respectively, and v) intragenic recombination accounted for a.a. haplotypes HVR-T3 to T7, and for the generation of H71-H89. T cell epitope polymorphism arising due to non-synonymous substitutions at PvMSP-1_{33} may result in differential binding of the polymorphic peptides to class II MHC alleles, and may evoke different host immune responses. Hence, the extensive allelic polymorphism evident at *Pvmsp-1_{33}* was due to recombination, mutation and positive selection.

This study for the first time reported 33 combined amino acid haplotypes that were generated by the PvAMA-1DII and the PvMSP-1_{33} amino acid sequences that demonstrated the extensive allelic polymorphism present in Sri Lanka.

Subsequently, to evaluate the ensuing strain-specific immunity arising due to extensive antigenic polymorphism, amino acid sequences of PvAMA-1DII were aligned with the homologous total (IgM+IgG) antibody responses assayed by an indirect enzyme linked immunosorbant assay (ELISA) established in-house against seven PvAMA-1DII synthetic peptides (P01-P07). This analysis was repeated for the a.a. sequences of PvMSP-1_{42}, and PvMSP-1_{19} with the previously published antibody responses assayed against PvMSP-1_{42}, and PvMSP-1_{19} recombinant proteins. A strain-transcending (cross reactive) immune response was clearly prevalent against peptide P07 of PvAMA-1DII and the PvMSP-1_{42} protein. Conversely, anti-P04peptide and anti-PvMSP-1_{19} antibody prevalence precluded strain-specific immune response against the domain II loop of PvAMA-1 and PvMSP-1_{19}, respectively. More importantly, both these regions clearly elicited a protective antibody response where an isotype switch was observed from a primary IgM response to a functionally important cytophilic IgG with increasing exposure to malaria in endemic residents.

A multi-component vaccine containing a cocktail of parasite antigens would be the final goal of vaccine development against the asexual stages of *Plasmodium*. Thus, it is crucial to identify multiple protective domains or epitopes from different antigens that are protective against the natural malaria infections. Hence, this study signifies that the highly conserved domain II loop of PvAMA-1 and the 19 kDa fragment of PvMSP-1_{42} may be suitable as veritable vaccine components to develop "protective" immunity against *P. vivax*.