Immunoepidemiology and Molecular Epidemiology of *Plasmodium vivax* Duffy Binding Protein II in Sri Lanka

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Abstract

The essential nature of the interaction between the region II of the Duffy binding protein of *Plasmodium vivax* (PvDBPII) and the Duffy Antigen (DA) of the human RBCs with lack of alternative invasion pathways make the PvDBPII as an important candidate vaccine for antibody mediated immunity against blood stage of vivax malaria. Exploration of the nature of naturally acquired immune responses, *in vivo* correlates of protection, genetic diversity and ensuing strain specific immunity of putative blood stage vaccine candidate antigen of malaria, such as the PvDBPII, in a specific geographical setting is vital for the development of vaccine constructs and for planning future vaccination strategies.

The present study, for the first time in the Indian sub continent, focused on PvDBPII, to characterize both the naturally acquired antibody mediated immune responses and the genetic diversity of *PvdbpII* gene in Sri Lanka, where unstable transmission with low malaria intensity prevails. Blood samples were collected from symptomatic *P. vivax* malaria patients from two malaria endemic areas, Anuradhapura and Kataragama, and from a malaria non-endemic area, Colombo.

In order to characterize the naturally acquired antibody responses, recombinant protein PvRII that structurally and functionally represent the native PvDBPII, was used in different types of in-house established Enzyme Linked Immunosorbent assays (ELISA). PvDBPII appeared to be immunogenic regardless of the endemicity of the three study areas. Total (IgG+IgM) antibody responses reflected that a higher proportion of non endemic patients responded to PvRII compared to their endemic counterparts, which was reiterated for IgM responses, indicative of primary immature antibody responses directed against PvDBPII. Conversely, functionally important IgG antibody responses were high in patients from the two malaria endemic areas. A mixed dominant cytophilic IgG1 and IgG3 isotype antibody responses were observed in all test areas. With increasing exposure to malaria, a marked isotype switch towards functionally active IgG responses with concurrent reduction of the IgM responses was collectively observed in the endemic areas, that was absent in the non endemic residents. Initial anti-PvDBPII antibody responses seem to be broadly directed against conformational epitopes of the molecule, while with increasing exposure, the responses were predominantly directed towards linear epitopes of PvDBPII. Relatively high level of local *P. vivax* patients (46%) contained PvDBPII specific binding inhibitory antibody responses that seem to be less effective in terms of complete inhibition of the binding of PvDBPII with DA. However, with increasing exposure, reduction of % parasitaemia in patients having binding inhibitory antibody responses suggested development of "immunity" against blood stage of *P. vivax*. Previous exposure of the patients to *P. vivax* malaria seem to be the only host factor that showed marked association with anti-PvDBPII total (IgG+IgM), IgG, IgG1 and IgG3 isotype specific "protective" antibody responses and the binding inhibitory antibody responses, implying that this host factor may be considered as an *in vivo* correlate of protection for asexual antibody mediated immunity in *P. vivax* malaria under unstable transmission and low malaria intensity in Sri Lanka.

Single clone *P. vivax* infections, identified by a combined polymerase chain reaction/ restriction fragment length polymorphism (PCR/RFLP) analysis of the *Pvmsp-3α* locus, were subsequently used to generate 100 nucleotide sequences of the *PvdbpII* gene (224 amino acid fragment including the critical binding motif) by nested PCR amplification followed by direct sequencing. The six amino acid residues predicted to be directly involved in binding with the

DA of RBC were totally conserved among the local isolates. A relatively high level of genetic diversity among the local *PvdbpII* gene was recorded with 21 amino acid polymorphisms that defined 33 amino acid haplotypes/alleles, in which more than 50% of the local isolates consisted of 3 dominant haplotypes. Most of the haplotypes were widely distributed throughout the country, while a few demonstrated area restricted distribution. However, lack of gene flow with strong geographical structure between the two endemic sites was evidenced by high *Fst* values. Mutations, recombination and balancing selection seem to maintain the observed local allelic diversity of *PvdbpII*. Some of the T-cell epitope polymorphisms on PvDBPII altered the binding capacity of those particular epitope/s to the HLA-DRB-1 alleles, may be indicative of disparity in host immune responses to this antigen. Of the 8 binding inhibitory linear B cell epitopes, 2 (H2 and M1) that lie in the vicinity of the exact binding region of PvDBPII are highly conserved among the local isolates, while these two epitopes showed relatively low level of polymorphism among global isolates also.

Among 271 world-wide isolates that included the 100 local isolates, 83 polymorphic amino acids and 117 amino acid haplotypes were recorded of which 3 polymorphisms and 26 haplotypes were unique to Sri Lanka. The *PvdbpII* phylogeny provided evidence for the presence of a few discrete PvDBPII alleles in different *P. vivax* endemic regions globally, but the Sri Lankan *P. vivax* parasites appeared to represents a sample of the global population.

Evidence was established for the existence of both strain specific and strain transcending naturally acquired antibody responses to PvDBPII among the local *P. vivax* patients. Among the naturally acquired binding inhibitory antibodies produced against 26 locally circulating PvDBPII amino acid haplotypes, 62% showed cross reactive inhibition against the reference Sal-1 strain, which may imply that a vaccine based on PvDBPII Sal-1 strain may be effective against most of the diverse PvDBPII haplotypes circulating among the natural *P. vivax* infections in Sri Lanka.

Thus, *in toto*, the results accrued from this study will contribute immensely to the development and deployment of a vaccine construct based on PvDBPII to be used against vivax malaria under Sri Lankan malaria endemic settings.