In Vivo Correlate of Protection Against Plasmodium Vivax Duffy Binding Protein II in Sri Lanka

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Introduction

Plasmodium vivax is the most widely distributed human malaria parasite accounting for 80 to 300 million clinical cases per year globally andhalf the world's population is estimated to be at risk (1). In Sri Lanka though both *P. vivax* and *P. falciparum* were endemic, the majority of reported malaria cases (65–80%) were due to *P. vivax* (2). Over the past 10 years the numbers of malaria cases reported in the country have dropped significantly and Sri Lanka has entered malaria pre elimination stage from 2008 (3). From the year 2005, >90% of the reported malaria cases in Sri Lanka are due to *P. vivax* (4). With increasing global spread of mosquito strains resistance to insecticides and parasitestrains resistance to antimalarial drugs, traditional control measures used against malaria are increasingly becoming insufficient globally (5). Therefore, development of alternative control strategies such as effective vaccine is become primary requirement. Characterization of putative vaccine candidate antigen/s against malaria, in a specific geographical setting is vital for the development of vaccine constructs and for planning future vaccination strategies.

The *P. vivax*Duffy Binding Protein (PvDBP) stands out as the most promising vaccine candidate antigen against blood stage of *vivax* malaria. PvDBP is expressed on the merozoite surface and plays an essential role in RBC invasion by *Plasmodium* merozoite by forming the tight binding with its corresponding receptor on the RBC, the Duffy Antigen Receptor for Chemokines (DARC) (6). The exact receptor binding domain lies in the region II of the DBP (PvDBPII) (7).

Unlike *P. falciparum* which uses multiple invasion pathways, *P. vivax* invasion is solely dependent on interaction between PvDBPII and the DARC. Presence of naturally acquired IgM, IgG, IgG1-IgG4 antibodies and binding inhibitory antiboies directed against PvDBPII had been recorded previously from areas with moderate to high malaria transmission primarily in countries of South East Asia and South America (8,9,10,11,12,13). Of importance, children residing in hyperendemic areas for *P. vivax* develop anti-DBP binding inhibitory antibodies that confer protection against blood-stage infection (10). All these observations validated the candidacy of the PvDBPII as a prime vaccine against blood stage of *P. vivax* malaria.

The malaria conditions in Sri Lanka were described as unstable transmission with low malaria intensity, which is unique situation compared to the other areas of the world. To our knowledge, no large scale immune-epidemiological studies based on PvRII were conducted in the Indian subcontinent including Sri Lanka. Thus, a large scale research project was conducted to characterize the antibody mediated immune responses to PvDBPII of natural *P. vivax* infections, in Sri Lanka and the work reported in this paper is a component of this project. Here we exclusively reported on the existing associations between the PvDBPII specific antibody responses with the different host factors.

Materials and methods

Study population:

The study protocol approved from the Ethical Review Committee, Faculty of Medicine, University of Colombo (EC/04/103). After informed consent, blood samples were collected from age (>15 years) and gender matched patients with microscopy confirmed acute *P. vivax* infections from Anuradhapura (AP), Kataragama (KG) and Colombo (14). AP and KG are *P. vivax* endemic areas, and Colombo itself a malaria free area where patients were acquired the disease only after visiting malaria endemic regions of the country.

Assessment of the naturally acquired anti-PvRII antibody responses

Recombinant protein PvRII - representing the region II of the native PvDBPII with its functional activity, were used in different in house established Enzyme Linked Immunosorbent Assays (ELISAs) to assess the anti-PvRII IgM, IgG isotype, IgG1 to IgG4 subtype specific antibody responses and anti-PvDBPII specific binding inhibitory antibody (BIA) responses in the acute-phase sera, as described (15). The results of these assays were used to characterize the associations between naturally acquired antibody responses with different host factors.

Associations with the antibody responses and the host factors

At the time of screening for malaria parasites following information were collected from the patients using a previously validated questionnaire (16); the age of patients, number of past malaria infection they suffered and number of days each patient suffered from malaria clinical symptoms. Further, the parasite density of the patients blood at the time of the sample collection (% parasitaemia) were assessed using standard Giemsa stained thin blood smears. Where appropriate comparisons were drawn between patients with no previous exposure to malaria (PNE) with patients who have had previous exposure to the malaria (PE). Also patients were categorized to different groups according to the past malaria infections they suffered.

Statistical analysis

Statistical analyses of data were performed using SPSS 11 for windows (SPSS Inc., USA) and Epi Info 6 (version 6.04b to c upgrade; CDC, USA and WHO, Switzerland) computer programs. Associations between antibody responses and prevalence with host factors were derived using the Spearman and Pearson correlation coefficients, and the Chi-square for linear trend, respectively. The significance level was set at $P < 0.05$.

Results

Host factors and IgM antibody responses

Data of 57, 38 and 43 samples respectively from Colombo, Anuradhapura (AP) and Kataragama (KG) were used to this analysis. No associations were recorded between the antibody responses with the age, days of symptoms and % parasitaemia, in all test groups. However, samples from AP (Chi square for linear trend, $r = 2.619$, $P = 0.22$) and KG (Chi square for linear trend, $r = 2.309$, $P = 0.12$), a negative trend was observed between the prevalence of anti-PvRII IgM antibodies with previous

exposure to malaria, restricted only to the PE groups. In Colombo although no pattern was observed, patients with >6 past infections showed 100% prevalence for anti-PvRII IgM antibodies.

Host factors and IgG isotype and IgG1 and IgG3 subtype specific antibody responses

IgG isotype and IgG1 and IgG3 antibody responses did not show any specific pattern of association with age, % parasitaemia level and days of symptoms in all three test areas. In Colombo PE group, prevalence of anti-PvRII IgG antibodies showed a non-significant negative association with the number of past malaria infection groups (Chi square for linear trend, P>0.05). In AP prevalence of IgG was highest in patients with 1-3 past malaria infections, while in KG, the highest prevalence was recorded among the patients having > 6 past infections.

A non-significant positive trend was recorded between the IgG3 subtype response with exposure (Chi square for linear trend, P>0.05) in Colombo, while in two endemic areas the highest IgG3 prevalence was recorded in the patients who had >6 past malaria infections. For IgG1 response though no clear pattern recorded, the highest prevalence was found in the group of patients who had the most number of past malaria infections.

Isotype switch

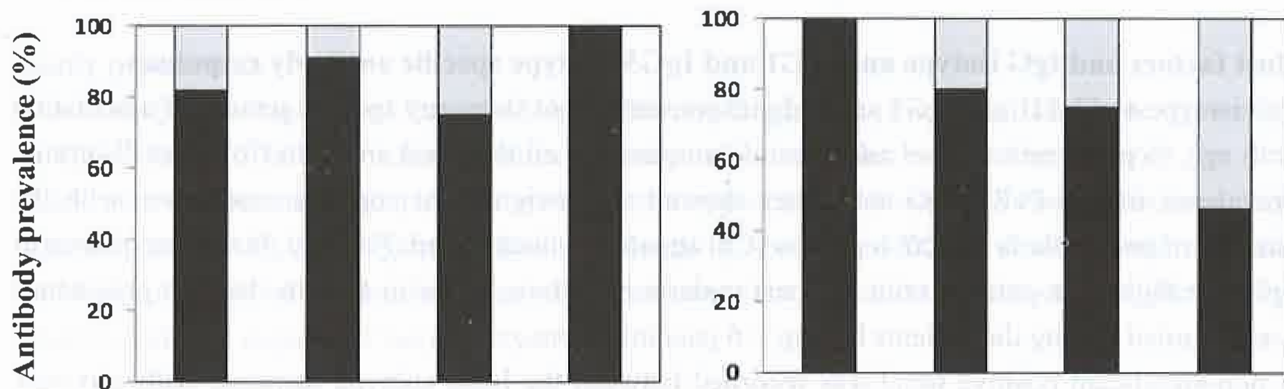
In order to explore the variation of anti-PvRII IgM and IgG isotype prevalence associated with previous exposure to malaria, antibody responses were classified into two types; (i) Type A response - IgM restricted and a combination of IgM and IgG response (ii) Type B response - IgG restricted response. Further, patients from the two endemic areas (AP and KG) were grouped together (N = 60) and were compared with non-endemic area Colombo (N = 41).

In endemic regions, PNE patients exhibited only Type A response, while in PE patients, with increasing exposure the prevalence of Type A response was reduced and minimal in those with >6 past malaria infections. In parallel, Type B response was increased with exposure and was maximized in patients with >6 past infections (Chi squared for linear trend 3.98, P = 0.044) (Figure 1). Compared to that, in non-endemic area, Type A response was increased with exposure (except those with 4-6 past infections) and in patients with > 6 past infections only Type A response was observed. In parallel, Type B response was decreased with exposure and none of the patients with > 6 past infections showed Type B response. Thus, there was a concurrent increase in the prevalence of functionally important IgG restricted response with increasing exposure to malaria in the endemic areas while this isotype switch was not obviously evident in the NEA group.

Host factors with binding inhibitory antibody response

Anti-PvRII binding inhibition antibody titers did not exhibit significant associations with age and days of symptoms of the malaria patients from the three test areas. However, though significant (Chi square for linear trend, r = 7.35; P = 0.006) only in non-endemic Colombo, with increasing exposure, a parallel increase of the prevalence of anti-PvRII binding inhibitory antibodies (at 1:5 serum dilution) were evident in all there test areas (Figure 2).

Serum samples with PvDBPII BIAs showed a non-significant negative correlation between the number of past malaria infections with % parasitaemia (Pearson correlation coefficient; r = -0.208, P = 0.147) (Figure 3).



i) IgM restricted responses + combination of IgG and IgM responses (■) – Type A
ii) IgG restricted response (□) – Type B

Figure 1: Pattern of IgM and IgG antibody prevalence in patients categorized according to past exposure to malaria from malaria endemic and non-endemic areas.

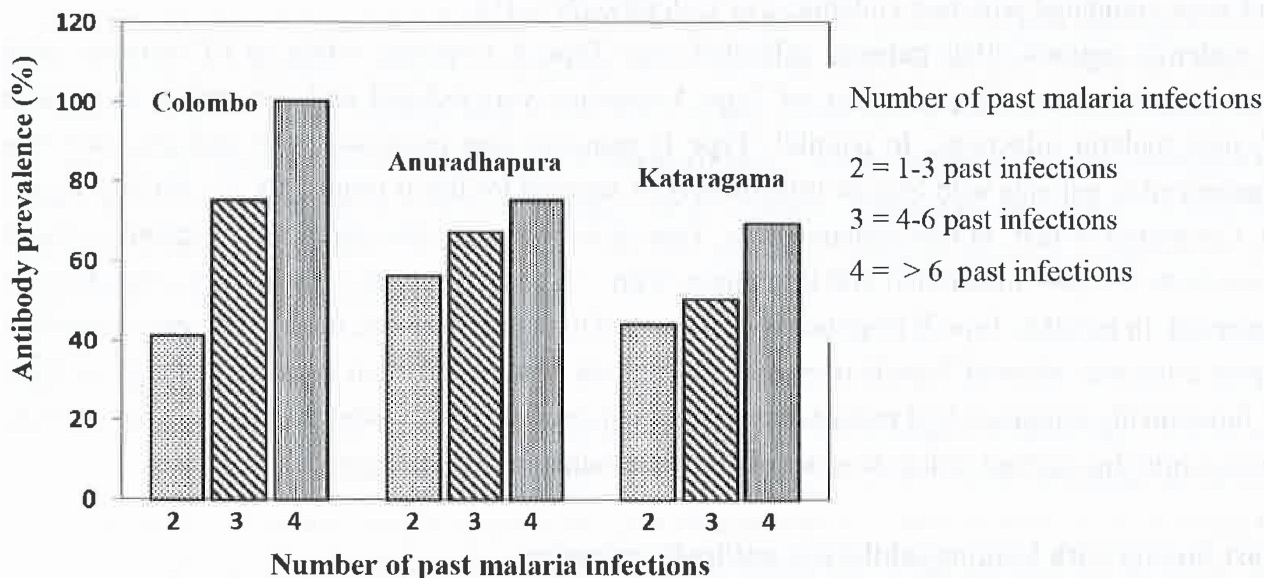


Figure 2. Prevalence of anti-PvRII binding inhibitory antibodies (at 1:5 serum dilution) in patients of different malaria exposure groups.

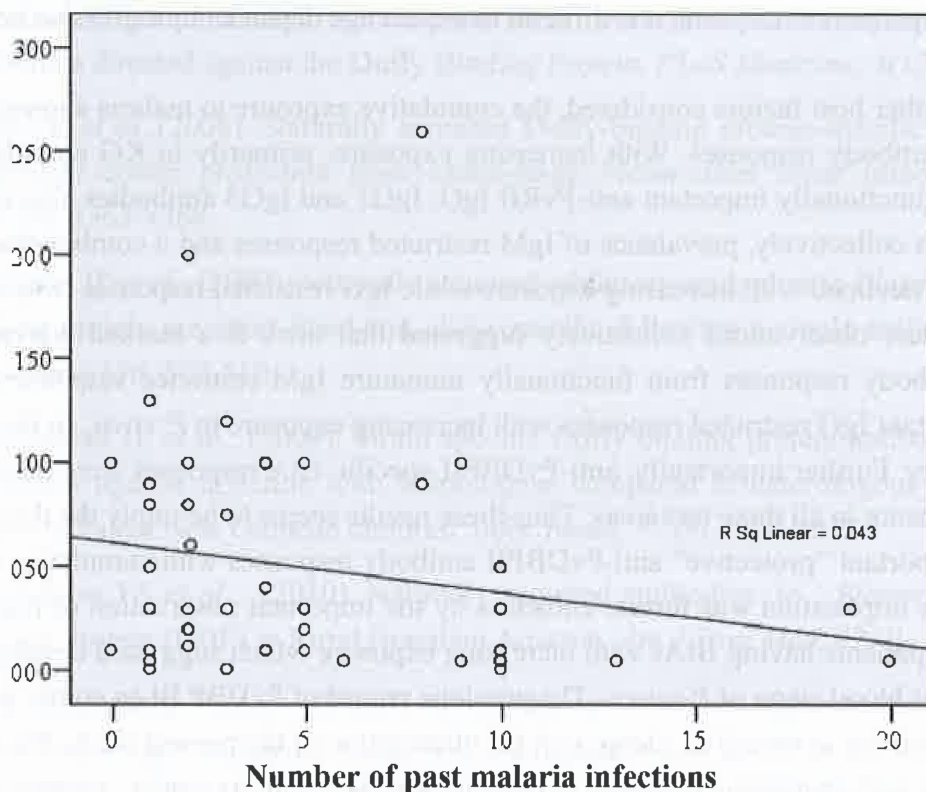


Figure 3. Correlation of % parasitaemia and the number of past malaria infections of the individuals with anti-PvRII binding inhibitory antibody responses.

Discussion:

The unavailability of reliable *in vitro* and *in vivo* correlates of protective anti-parasite immunity against the blood stages of the malaria parasite has been one of the greatest obstructions to the development of a malaria vaccine. Here, based on previously available data on naturally acquired antibody responses to PvDBPII and different host factors, attempts were made to develop *in vivo* correlates of protective immunity against the blood stage of vivax malaria parasite in relation to prevailing unstable transmission with low malaria intensity in Sri Lanka.

According to the available records, PvRII appeared to be immunogenic regardless of the endemicity of the three study areas (15). Prevalence of anti-PvRII IgM antibody responses were high in non endemic patients compared to their endemic counterparts, which indicates the presence 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. Further importantly, in all three relatively high level of local *P. vivax* patients recorded to contain PvDBPII specific binding inhibitory antibody responses.

Of the host factors considered age of the patient, days of symptoms and % parasitaemia did not show any type of association with antibody responses. The age and/or cumulative exposure dependent progressive acquisition of antibodies against PvDBP was previously demonstrated in areas with high malaria transmission, suggested development of boosting effect due to recurrent exposure through repeated infection (8, 11, 12). Primarily due to the presence of comparatively low malaria transmission intensity in Sri Lanka, heterogeneous exposure to inoculation amongst different age

groups and lack of patients < 15 years, it is difficult to expect age dependent progressive acquisition of antibodies.

In contrast to the other host factors considered, the cumulative exposure to malaria showed marked association with antibody responses. With increasing exposure, primarily in KG and also in AP, prevalence of the functionally important anti-PvRII IgG, IgG1 and IgG3 antibodies also increased. Further in two EAs collectively, prevalence of IgM restricted responses and a combination of IgM and IgG responses declined with increasing exposure while IgG restricted responses was increased with exposure. These observations collectively suggested that there is a marked isotype switch of anti-PvRII antibody responses from functionally immature IgM restricted responses towards functionally important IgG restricted responses with increasing exposure to *P. vivax*, in the endemic areas of the country. Further importantly, anti-PvDBPII specific BIA responses were also increase parallel to the exposure in all three test areas. Thus these results seems to be imply the development of functionally important "protective" anti-PvDBPII antibody responses with cumulative exposure in Sri Lanka. This implication was further enhances by the important observation of reduction of % parasitaemia in patients having BIAs with increasing exposure which suggested development of "immunity" against blood stage of *P. vivax*. The previous record of PvDBP BIAs confer protection against *P. vivax* infection *in vivo* (10), along with the observation of the present study, the reduction of the parasite load with increasing exposure in patients with BIAs in Sri Lanka, provide plausible evidence for an important immune correlate for protective immunity against *P. vivax* in endemic populations. Thus, all these observation provie evidences that previous exposure of the patients to *P. vivax* malaria seem to be the only host factor that 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.

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