

(B) Summary

Title: "Studies on the Antibody Response in Naturally Infected
Individuals to Purified Proteins of *Plasmodium vivax*"

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An understanding of the natural immune responses to potential vaccine candidates is important to unravel underlying immune mechanisms and to gain insights into vaccine development. The objective of this study was to identify the correlation between antibody levels and the specific

antibody isotypes in combination and the degree of protection *in vivo* in naturally exposed humans, with the aim of developing *in vivo* correlates of protection against the blood stages of vivax malaria.

Recombinant proteins, p42, p19 and PV66/AMA-1 representing native Merozoite Surface Protein1 and Apical Membrane Antigen-1 of *Plasmodium vivax*, were used in an indirect microplate ELISA to assess the total immunoglobulin (IgG and IgM) responses and titers of patients with acute *P. vivax* malaria infections (and at convalescence where possible) from two malaria endemic areas of the island, Anuradhapura and Kataragama and from a non-endemic area Colombo.

The proportion of responders for p42 was significantly higher ($P < 0.05$) than that for p19 and PV66/AMA-1 while there was no such difference between p19 and PV66/AMA-1 ($P > 0.05$), in each test area. No significant differences were apparent ($P > 0.05$) among responding proportions, magnitude of total antibody levels and antibody titers to p42, p19 and PV66/AMA-1, between the two endemic areas or between endemic and non-endemic areas. Thus, all three-vaccine candidates appear to be immunogenic in nature with p42 being more immunogenic than the other two proteins. The total antibody response to p42 and p19 in cohorts of acute and convalescent sera of *P. vivax* patients decline significantly during the 4th week after treatment in both Anuradhapura and Colombo. PV66/AMA-1 appears to be immunogenic during natural vivax malaria infections where antibody responses increase with exposure and are sustained during the first month of convalescence following treatment.

Sera from patients screened positive to anti-PV66/AMA1, anti-p19 and anti-p42 antibodies, were randomly selected from the three test areas and assessed for IgM, and IgG isotypes against the

three antigens using an antibody sandwich ELISA. Significant differences were apparent between endemic and non-endemic residents for IgM subclass specific antibody prevalence and levels for p19 and PV66/AMA1 ($P < 0.05$). Differences were apparent for anti-p42 IgM antibody prevalence between Anuradhapura and Colombo only ($P < 0.05$). The responses to different IgG isotypes for all three antigens indicated that among the four isotypes evaluated, marked cytophilic IgG1 responses compared to the other IgG isotypes were apparent in individuals of all three-test areas.

When data was analyzed using Best Subset Regression Analysis for the development of *in vivo* correlates of protection, R squared values obtained indicated that the associations between antibody parameters and host factors were not sufficiently strong to fit this data into a realistic model. Therefore, bivariate correlations were used to establish associations between antibody parameters and host factors. Antibody parameters to all three recombinant proteins were independent of age of patients in all test areas.

Total antibody levels and titers in residents of the two endemic areas for p42 and p19 confer positive correlation with parasitaemia ($P < 0.05$), which may reflect a current infection rather than protection.

In Anuradhapura, a positive correlation ($P < 0.05$) with both anti-p42 and anti-p19 antibody levels existed up to four past infections and a negative correlation was apparent thereafter for p19 only. However, for Kataragama a negative correlation ($P < 0.05$) for both p42 and p19 were established for total antibody levels and titers in patients with 2-10 past malaria infections. This was reiterated for their IgG1 and IgG3 isotype levels by showing a positive correlation up to six past infections and IgG1 levels declining thereafter. For PV66/AMA-1, considering total antibody levels a positive trend was evident between responding proportions and exposure up to five past

malaria infections in all three-test populations. However, both endemic areas showed a decrease of anti-PV66/AMA1 IgG1 levels with increasing exposure to malaria. Therefore, under unstable malaria conditions prevalent in the endemic areas of the island, it is apparent that with increasing exposure to malaria, the isotype switch from IgM towards the functionally important cytophilic IgG isotypes, IgG1 and IgG3, is more prominent in p19 and PV66/AMA1. Neither was this antibody switch apparent in non-endemic residents for these two antigens nor for p42 in the three test populations. Based on the results of this study, the only host factor that showed considerable association with either total or subclass specific antibody responses was the previous exposure to malaria, implying that this host factor may be considered as an *in vivo* correlate of protection for asexual immunity in *Plasmodium vivax* malaria under prevailing “unstable” malaria conditions in Sri Lanka.

Therefore, in order to confirm that previous exposure to malaria is indeed an *in vivo* correlate of protection to *Plasmodium vivax* asexual immunity in Sri Lanka, it is imperative to conduct a longitudinal study on the precise dynamics of the antibody response based on the three vaccine candidates used in the present study.

Publications:

List of communications

a) Presented at International Conferences

- i) W T A Wicramarachchi, K L R L Perera, S Bandara, A Thomas, S Longacre, S M Handunetti and Prcethi V Udagama-Randeniya (2002). Antibody responses of naturally infected individuals to asexual erythrocytic stage vaccine candidates of *P. vivax* in Sri Lanka. Vivax Malaria Research: 2002 and beyond, February 3-8, Bangkok, Thailand, pp27, 28

ii) W T A Wickramarachchi, H P Premarathne, K L R L Perera, S Bandara, A. Thomas, S M Handunnetti and P V Udagama-Randeniya(2002).Profiles of IgG and IgM Antibodies to PV66/AMA-1 in individuals naturally infected with *Plasmodium vivax* from Sri Lanka. FIMSA Advanced Course and Conference, Molecular Mechanisms of Infection and Immunity, October 21-25, Ayutthaya, Thailand, p55.

b) Presented at National Conferences

i) Wickramarachchi W T A, Perera K L R L, Bandara S, Longacre S, Handunnetti S M and Randeniya P V (2000). Immunoepidemiology of p19: a potential vaccine candidate based on Plasmodium vivax MSP1. Proc.20th Ann.Sessions Institute of Biology, Sri Lanka, pp37.

ii)Wickramarachchi, W T A, Perera, K L R L, Bandara, S, Longacre, S, Handunnetti, S M and Randeniya, P V (2000). Seroepidemiology of potential vaccine candidate p42 representing a C-terminal fragment of Plasmodium vivax Merozoite Surface Protein -1. Proc.1st Ann.Sessions, University of Colombo, Sri Lanka, pp35.

iii)W. T. A. Wickramarachchi, R. J. Illeperuma, K L R L Perera, S Bandara, R S Lokupitiya, S. Longacre, S M Handunnetti and P V Udagama-Randeniya. Pattern of IgG1 and IgG3 isotype responses to C-terminal processing products of Plasmodium vivax Merozoite Surface Protein 1 in endemic populations of Sri Lanka. Proc.57th Ann.Sessions, Sri Lanka Association for the Advancement of Science, pp18.

iv) Wickramarachchi, W T A, Perera, K L R L, Bandara, S, Thomas, A, Handunnetti, S M and Udagama-Randeniya, P V (2001). Antibody responses to a leading vaccine candidate PV66/AMA-1 induced by natural infections of Plasmodium vivax in humans. Proc.21th

- v) W. T. A. Wickramarachchi, R. J. Illeperuma, K L R L Perera, S Bandara, S.Longacre, S M Handunnetti and P V Udagama-Randeniya (2002). Characterization of subclass specific antibody response to p19 representing *Plasmodium vivax* Merozoite Surface Protein 1 in endemic and non-endemic populations in Sri Lanka. Proc.22th Ann.Sessions, Institute of Biology, Sri Lanka, p55
- Manuscripts in preparation for submission to international journals
 1. Wickramarachchi, W T A *et al*, tentatively titled “Natural human antibody responses to PV66/AMA1 representing vaccine candidates for vivax malaria in Sri Lanka”.
 2. Wickramarachchi, W T A *et al*, tentatively titled “Antibody Responses of Naturally infected Individuals to Asexual Erythrocytic Stage Vaccine Candidates of *Plasmodium vivax* in Sri Lanka”.