Naturally Acquired Antibodies Against the Major Merozoite Surface Coat Protein (MSP-1) of *Plasmodium falciparum* Acquired by Residents in an Endemic Area of Colombia

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A preliminary baseline epidemiological malaria survey was conducted in the village of Punta Soldado, Colombia. Parasite prevalence and density as well as serological data were obtained from 151 asymptomatic children and adults. Fifty individuals were infected with Plasmodium falciparum. The mean parasite density was 184 parasites/mm³. Greater than 90% of the sample population were *P. falciparum* antibody positive as detected by the indirect immunofluorescent antibody test (IFAT). The enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies against the major merozoite surface protein (MSP-1) of *P. falciparum*. In this population, anti-MSP-1 antibody concentration is acquired in an age dependent manner with equal immunogenicity to both the N- and C-terminal regions of the molecule. Infection at the time of sampling was associated with a higher anti-MSP-1 antibody concentration than that found in non-infected individuals. Further studies are planned to assess the role of immune and non-immune factors in limiting the number of cases of severe malaria seen in this population.

Key words: *Plasmodium* - immunity - antibody - asexual stages

Malaria is a significant cause of morbidity and mortality in tropical and subtropical regions of the world. In endemic areas, malaria mortality is restricted mainly to young children. Immunity to the disease, but not to infection, develops and is maintained through repeated infections. Human malaria studies have demonstrated that the development of immunity requires repeated infections with prolonged exposure of susceptible hosts to many antigenically-distinct parasite strains, or repeated exposure to poorly immunogenic epitopes involved in protection (McGregor 1988). Taylor (1989) hypothesized that repeated exposure to the parasite would result in (1) increased antibody titers, (2) recognition of antigens not previously recognized (i.e. minor antigenic determinants or antigenic variation) and/or increased antibody quality (i.e., affinity, avidity, isotype, etc.). Numerous seroepidemiological studies have been conducted aimed at explaining the role of the humoral immune response in naturally acquired immunity to malaria. Although there is an association between the number of malaria infections and antibody titer, it has been difficult to consistently demonstrate a relationship between anti-malarial antibody titers and protection from infection or disease. This paradox maybe due to the fact that investigators have been examining different malarial antigens under different endemic conditions.

Due to the increasing complexity and difficulty of malaria control, vaccination against malaria has taken on increased importance. One of the primary candidate antigens for a vaccine against *P. falciparum* is MSP-1, also known as MSA-1, gp195, p190 and PMMSA (Hall et al. 1984, Perrin et al. 1984, Cheung et al. 1986, Siddiqui et al. 1987, Patarroyo et al. 1987, Herrera et al. 1992). MSP-1 is synthesized by the parasite as a high molecular weight precursor which is cleaved into smaller polypeptide fragments found on the surface of the merozoite (Holder & Freeman 1984). The gene for MSP-1 occurs as a dimorphic allele with the amino acids arranged in blocks of conserved and allele-specific regions (Tanabe et al. 1987). MSP-1 may
play a role in the merozoite invasion of the red blood cell (Perkins & Rocco 1988) and antibodies against MSP-1 have been shown to interfere with parasite invasion in vitro (Schmidt-Ulrich et al. 1986, Hui & Siddiqui 1987, Blackman et al. 1990). B-cell epitopes have been identified in both the N- and C-terminal regions of the MSP-1 molecule (Sinigalia et al. 1988, Burns et al. 1988, Cooper et al. 1992). Naturally acquired anti-MSP-1 antibodies have been detected in populations living in malaria endemic regions of the world (Holder & Freeman 1984, Gabra et al. 1986, Chizzolini et al. 1989, Kramer & Oberst 1992). Cellular and humoral responses to MSP-1 are acquired in an age-dependent manner and antibodies against specific regions of the MSP-1 molecule have been associated with a decreased frequency of disease (Riley et al. 1992).

In this preliminary cross-sectional study, we examined the malaria parasite prevalence and the humoral immune response to blood stage \textit{falciparum} malaria antigens in children and adults living in Punta Soldado, Colombia, South America. Specific attention was paid to the detection of naturally acquired antibodies against parasite-derived, recombinant and synthetic MSP-1 antigens of \textit{P. falciparum}.

**MATERIALS AND METHODS**

**STUDY AREA**

The study site is located in the village of Punta Soldado, a few miles from the pacific coast seaport of Buenaventura in the Department of Valle del Cauca. Malaria in this region is considered unstable. One-hundred and fifty-one individuals, out of a population of approximately 250, agreed to participate in this study. The villagers are of African origin and live in wooden houses along the beach. The main activities of the villagers are farming and fishing. \textit{P. falciparum} is the predominant species followed by \textit{P. vivax}. Severe falciparum malaria is rarely seen in this population (unpublished observation).

**SAMPLE COLLECTION**

Demographic data, thick blood smears and peripheral blood for sera were collected from April 5-7, 1993; this time frame corresponds to approximately the beginning of the transmission season (Rojas et al. 1992). The serum samples were stored at -20°C until used. Thick blood smears were stained with Giemsa stain and examined under oil immersion for the presence of parasites. Parasite density, expressed in parasites/mm3, was determined by counting the number of parasites per 100 white blood cells and multiplying the resulting proportion by an average leukocyte count of 8000/mm³. The parasite density was grouped into four classes: class 1: 1-100, class 2: 101-200, class 3: 201-400, class 4: 401-600 parasites/mm³.

**PARASITE STRAINS AND ANTIGENS USED IN THE STUDY**

The FUP (\textit{Falciparum}-Uganda-Palo Alto) strain of \textit{P. falciparum} was used in this study (Chang et al. 1988). Parasite-derived MSP-1 was isolated by monoclonal antibody affinity chromatography as described previously with the exception that the elution buffer was 0.1 M glycine-HCL, pH 2.5 (Siddiqui et al. 1987). The N-terminal region of MSP-1 (195A) was expressed using a yeast expression system and purified by FPLC as described elsewhere (Hui et al. 1991). 195A is equivalent to the first 56% of the 83 kDa N-terminal processing fragment. The C-terminal region of MSP-1 (BVp42) was expressed using a baculovirus expression system and purified as described previously (Chang et al. 1992). BVp42 is equivalent to the entire 42 kDa C-terminal processing fragment minus the anchor sequence. The synthetic peptides were derived from predicted B cell epitopes on the N-terminal region of MSP-1. The sequences of the \textit{P. falciparum} MSP-1 based synthetic peptides were: peptide 3 (a.a. 203-220; ELYYKLNFYFVDLARRKNDV-NH2) and peptide 16 (a.a. 333-352; IDTLKKNENIKLLDKINEI-NH2) (amino acid numbering according to Miller et al. 1993). These sequences are found within the FUP sequence. Peptide 3 is within a conserved region (block 3) of the molecule while peptide 16 is found within a semi-conserved region (block 4).

**ANTIBODY DETECTION ASSAYS**

Antibodies against MSP-1 were detected by ELISA. Briefly, antigen (1 μg/ml, 50 μl/well) diluted in BBS (borate buffered saline; 167 mM boric acid, 134 mM NaCl, 27.5 mM NaOH, pH 8.0) was coated to polystyrene plates for 2 hours in a humid box. Unbound antigen was removed by washing the plate three times with HSBBS (BBS with NaCl at 0.5 M). The wells were blocked with 150 μl of 5% milk in BBS for 2 hours in a humid box. The plate
was washed three times with HSBBS and frozen at -70°C until needed. The serum samples were diluted 1/200 in BBS with 1.5% powdered low fat milk. Diluted sera were tested in duplicate wells in which 50 µl/well were reacted for 2 hours. The unbound antibodies were removed by washing. Bound antibodies were detected using a goat-anti human IgG peroxidase conjugated antibody (ZYMED, gamma chain specific) diluted in BBS with 1.5% milk. After two hours the plates were washed and 50 µl/well of substrate was added. The OD410 was read after 30 minutes using a Dynatech MR650 Microplate Reader. Antibody units were determined using a standard curve derived from a pool of adult sera from Punta Soldado. Wells were similarly reacted with sera from unexposed persons served as a negative control. An individual was considered negative if the mean OD value of 2 wells was less than the mean plus 2 standard deviations of the negative control wells on each plate.

The IFAT was performed as described elsewhere (Voller 1971). Titers greater than 1/16 were considered positive.

Categorical data were analyzed using the Chi square test. Serological and parasitological data were analyzed using the Mann-Whitney Rank Sum Test for comparison of two groups and the Kruskal-Wallis One Way ANOVA on Ranks to compare multiple groups with Dunn's method to isolate which group(s) were different. Spearman rank order correlation was used to assess the linear relationship between variables. Differences of p < 0.05 were considered significant.

**RESULTS**

Fifty (33.1%) villagers were positive for *P. falciparum* by Giemsa stained thick blood smears (Table I). During the survey, no severe or complicated cases of *falciparum* malaria were noted. *P. vivax* parasites were not found. Parasites were not detected in the youngest age group (1-4 years). Infected individuals had parasite densities between 80 and 560 parasites/mm³. Twenty individuals (40%) had a class 1 infection, 12 (24%) had a class 2, 16 (32%) had a class 3 and 2 (4%) had a class 4 infection. The frequency of parasite positive blood smears increased with age, reaching a maximum of 48% in the 15-29 age group. There was no significant difference in the parasite densities between the three age groups (Table I; Kruskal-Wallis One Way ANOVA, p = 0.09).

The overall seroprevalence of anti-*P. falciparum* antibodies, as measured by IFAT, was 92%. Seventy percent of the youngest age group (1-4 years) were IFAT positive (Table I). The IFAT titers of the two older age groups (15-29 and 30+ years) were significantly higher than the IFAT titers of the younger age groups (Kruskal-Wallis One Way ANOVA, p > 0.05). There was no difference in IFAT titers between infected and uninfected individuals (infected individuals: mean = 38, median = 32; uninfected individuals: mean = 33, median = 32; Mann-Whitney Rank Sum Test, p (0.05).

We evaluated the antibody response to the blood stage antigen MSP-1 by ELISA using purified parasite-derived MSP-1 and the recombinant

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Parasite and antibody prevalence for <em>Plasmodium falciparum</em> by age group in villagers of Punta Soldado, Colombia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group (Years)</td>
<td>N</td>
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<td>1 - 4</td>
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<td>5 - 14</td>
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<td>30</td>
<td>46</td>
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<td>TOTAL</td>
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*a* Kruskal-Wallis One Way ANOVA, p=0.09

*b* Kruskal-Wallis One Way ANOVA, p<0.05
polypeptides 195A and BVp42 (Table II). The correlation coefficients (r) between the antibody concentrations against parasite-derived MSP-1 versus the recombinant antigens were 0.725 and 0.713 for 195A and BVp42 respectively (p < 0.001 for both comparisons). The overall seroprevalence for anti-MSP-1, anti-195A and anti-BVp42 antibodies was 74%, 68% and 68% respectively. Both the mean and median antibody units against the three antigens increased with age. In general, the older age groups had higher concentrations of anti-MSP-1 antibodies than did the younger age groups. There was no significant difference between the number of females and males with detectable antibodies to parasite-derived MSP-1 and BVp42 (X² = 0.962 and 2.793 respectively, p > 0.05). However, a larger number of males than females had antibodies which recognized 195A (X² = 5.99, p < 0.05).

Infection appeared to boost the antibody concentration to MSP-1. Infected individuals had significantly higher anti-MSP-1 antibody concentrations than non-infected individuals (parasite-derived MSP-1: infected individuals: mean = 224, median = 26, non-infected individuals: mean = 125, median = 7; 195A: infected individuals: mean = 310, median = 80, non-infected individuals: mean = 207, median = 69; BVp42: infected individuals: mean = 139, median = 43, non-infected individuals: mean = 139, median = 36; Mann-Whitney Rank Sum Test, p = 0.02, 0.04 & 0.03 respectively). Among the infected individuals, boosting of the antibody response was also reflected in a very strong positive correlation between antibody con-

<table>
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<tr>
<th>Antigen</th>
<th>Age Group (Years)</th>
<th>Percent Antibody Positive</th>
<th>Reciprocal Median Antibody Units</th>
<th>ELISA results</th>
<th>Difference in Rank</th>
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<td>Reciprocal Median Antibody Units</td>
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<td>136</td>
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<td>46.6* 27.0* -</td>
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<td>59</td>
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<td>87</td>
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<td>54</td>
<td>65.5* 42.7* 22.1</td>
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<tr>
<td></td>
<td>Total</td>
<td>68</td>
<td>101</td>
<td>39</td>
<td>- - - -</td>
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</table>

* Kruskal-Wallis One Way ANOVA, p < 0.001
b Dunn's Comparison, p < 0.05

TABLE II
Anti-MSP-1 antibodies by age group in villagers of Punta Soldado, Colombia
centrations of the three antigens tested (r = 0.998, 0.994 & 0.994 for MSP-1 vs 195A, MSP-1 vs BVp42 & 195A vs BVp42 respectively, p < 0.005 for all comparisons).

The specificity of the detectable anti-MSP-1 antibodies were analyzed using immunoblotting (data not shown). In general, individuals who recognized the respective antigens by ELISA also recognized appropriate molecular size bands on the immunoblots. Thirty-six sera, which reacted with 83 kDa N-terminal processing fragment by immunoblotting against parasite-derived MSP-1, were tested by ELISA against two N-terminal synthetic peptides (peptide 3 and peptide 16) derived from conserved 190L region P. falciparum MSP-1. P16 has been identified as a MSP-1 / Spectrin binding region (Herrera et al. 1993). Five (13.9%) of these sera reacted with peptide 3, eighteen (50%) reacted with synthetic peptide 16. Thirteen sera (36%) reacted only with peptide 16. The five individuals who recognized peptide 3 were adults whereas individuals of all age groups, including children, recognized peptide 16. There was no association between a positive ELISA value for either synthetic peptide and parasite density or MSP-1 (parasite-derived MSP-1, 195A and BVp42) antibody concentrations.

**DISCUSSION**

In this study, we evaluated the anti-*P. falciparum* antibody response and parasite prevalence and density of 151 asymptomatic individuals from the malaria endemic village of Punta Soldado, Colombia, South America. The overall *P. falciparum* infection rate was 33.1%. The highest parasite prevalence was found in the 15-29 age group. The maximum prevalence of IFAT anti-falciparum antibodies was not reached until the 30+ age group. In highly endemic areas of Africa, maximum parasite prevalence is reached in childhood; ≤ 2 years in holoendemic, 2-4 years in hyperendemic and 5-9 years in mesoendemic regions (Boyd 1949, Schwetz 1949). Under intense malaria transmission, 100% of the children between 1-4 years would be predicted to be IFAT antibody positive (Voller et al. 1980). In the absence of detailed epidemiological data from Punta Soldado, these two criteria lead us to conclude that transmission in this region is probably low and seasonal. However we can not rule out that the time frame of the study, which was just before the peak transmission season, could have accounted for the low parasite prevalence and densities observed in the study population. On the other hand, the *P. falciparum* infection rates by age group in a placebo group from another Pacific coast village in Colombia (Valero et al. 1993) were very similar to the parasite prevalence data reported in this study. Future studies are planned to accurately define the epidemiology of malaria in Punta Soldado.

Despite significant differences in the antibody response to the blood stage antigens tested, the similar low parasite densities noted in the three age groups suggests that this population's resistance to severe *falciparum* infections may be the result of several mechanisms rather than solely due to acquired immunity. Other reasons for this observation may be genetic factors such as the presence in this population of haemoglobin S, thalassemias and glucose-6-phosphate dehydrogenase deficiency or the unreported use of antimalarial drugs or folk medicines (Bruce-Chwatt 1990).

When parasite-derived MSP-1 was used in an ELISA assay, 74% of the people of Punta Soldado had detectable antibodies to this prominent merozoite surface polypeptide. Both prevalence and concentration of anti-MSP-1 antibodies increased with age; indicating a positive relationship between exposure to the parasite and the development of anti-MSP-1 antibodies. Furthermore, infected individuals had higher concentrations of anti-MSP-1 antibodies than non-infected individuals. The prevalence of anti-MSP-1 antibodies in a population would be expected to indicate the intensity of transmission for a given endemic area. In the Gambia, Gabra et al. (1986), using an *E. coli* recombinant polypeptide (31-1) representing a portion of the 83 kDa N-terminal processing fragment, reported a high proportion (>90%) of individuals, 5 years and older, with positive ELISA values. Kramer and Oberst (1992), also, found a seroprevalence of 90% of anti-MSP-1 antibodies in a population living in a hyperendemic area of the Philippines. Conversely, using the 31-1 antigen, Chizzolini et al. (1989) reported a much lower prevalence (~15%) of anti-MSP-1 antibodies in a population living in a low malaria transmission region of Gabon. The similar seroprevalence rates and high correlation of antibody concentrations for the three antigens tested in this study, which included a purified parasite-derived polypeptide (MSP-
1) and two recombinant polypeptides (195A and BVP42), suggests a large overlap of epitopes present on the parasite and recombinant polypeptides.

The humoral responses to the two recombinant antigens, 195A and BVP42, were very similar to the parasite-derived MSP-1 antigen. According to our data, the prevalence and concentration of antibodies to MSP-1 increased with age with equal immunogenicity to both N-terminal and C-terminal regions of the molecule. In fact, the antibody prevalence and concentration for the three MSP-1 antigens continued to increase with age. The recombinant antigens used in this study were based on the reported sequence of the PUP strain of *Plasmodium falciparum*. The representation of this dimorphic allele within the parasite population of Punta Soldado needs to be investigated. The synthetic peptides used in this study were derived from predicted B cell epitopes within the 83 kDa N-terminal processing fragment. Fifty percent of the tested sera reacted with one of the two synthetic peptides, which included a semi-conserved site, indicates that these B cell epitopes are present in the parasite population of Punta Soldado. However we were unable to find an association between individuals which reacted with the peptides and parasite density or antibody concentrations to MSP-1. This may be due to the small number of serum samples tested and an expanded study using these peptides is planned.

Studies are presently underway to determine the IgG subclass of anti-MSP-1 antibodies and to examine the *in vitro* growth inhibition of the serum samples collected in this study. Future studies are planned in Punta Soldado to define the epidemiology of *P. falciparum* as well as to examine the relative contributions of immune and nonimmune factors in resistance to severe falciparum malaria in this population.

**ACKNOWLEDGEMENT**

To the residents of Punta Soldado for their participation in these studies, to Drs Maria Fernanda Rizzo and Nancy Torres for field and technical assistance.

**REFERENCES**


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