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Antibody specificities of children living in a malaria endemic area to inhibitory and blocking epitopes on MSP-1_{19} of *Plasmodium falciparum*

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ABSTRACT

Merozoite surface protein-1_{19} (MSP-1_{19}) specific antibodies which include processing inhibitory, blocking and neutral antibodies have been identified in individuals exposed to *Plasmodium falciparum*. Here we intend to look at the effect of single and multiple amino acid substitutions of MSP-1_{19} on the recognition by polyclonal antibodies from children living in Igbo-Ora, Nigeria. This would provide us with information on the possibility of eliciting mainly processing inhibitory antibodies with a recombinant MSP-1_{19} vaccine. Blood was collected from children in the rainy season and binding of anti-MSP-1_{19} antibodies to modified mutants of MSP-1_{19} was analysed by ELISA. The MSP-1_{19} mutant proteins with single substitutions at positions 22 (Leu → Arg), 43 (Glu → Leu) and 53 (Asn → Arg) and the MSP-1_{19} mutant protein with multiple substitutions at positions 27 + 31 + 34 + 43 (Glu → Tyr, Leu → Arg, Tyr → Ser, Glu → Leu); which had inhibitory epitopes; had the highest recognition. Children recognised both sets of mutants with different age groups having different recognition levels. The percentage of malaria positive individuals (32–80%) with antibodies that bound to the mutants MSP-1_{19} containing epitopes that recognise only processing inhibitory and not blocking antibodies, were significantly different from those with antibodies that did not bind to these mutants (21–28%). The amino acid substitutions that abolished the binding of blocking antibodies without affecting the binding of inhibitory antibodies are of particular interest in the design of MSP-1_{19} based malaria vaccines. Although these MSP-1_{19} mutants have not been found in natural population, their recognition by polyclonal antibodies from humans naturally infected with malaria is very promising for the future use of MSP-1_{19} mutants in the design of a malaria vaccine.

1. Introduction

MSP-1 is probably the best characterized and most abundant merozoite surface protein of the malaria parasite. Most studies have focussed on MSP-1_{19} as a leading vaccine candidate against blood stage malaria (Chang et al., 1996; Daly and Long, 1993) and animals that are vaccinated with the protein develop high titres of anti MSP-1 antibodies with corresponding levels of protection (Kumar et al., 2000). There seems to be contrasting evidence for the role of MSP-1_{19} in providing protection in humans (Egan et al., 1996; Branch et al., 1998; Dodoo et al., 1999), thus the reasoning that the fine specificity of the antibodies other than the titres is more important (Corran et al., 2004; Okech et al., 2004). Monoclonal antibodies (mAbs) to MSP-1_{19} prevent the secondary processing of MSP-1_{42} and inhibit merozoite invasion in vitro (Guevara Patino et al., 1997).

However, there are some MSP-1 specific antibodies that do not inhibit processing, the first of these antibodies are defined as blocking antibodies because they block the binding of inhibitory antibodies and the other type of non-inhibitory antibody is neutral because it does not interfere with the binding of inhibitory antibodies, and has no biological effect when it binds to the antigen (Guevara Patino et al., 1997). Nwuba et al. (2002) reported the presence of processing inhibitory antibodies in about 12% of the population analysed in Igbo-Ora, Nigeria and they also showed that there was no significant correlation between MSP-1_{19} antibodies titre and processing inhibitory activity, thus it has been reported that the mere measurement of antibodies to malaria antigen might not be a useful indices of immune protection against malaria. In humans, it has been shown that antibodies against MSP-1_{19} comprise a large component of the total invasion inhibitory response in *Plasmodium falciparum* positive individuals (O'Donnell et al., 2001).

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Inhibition of processing is one of a number of potential mechanisms that can neutralise merozoites. Understanding the immune mechanisms induced by vaccination will be essential for the evaluation of vaccine candidates and the validation of particular approaches. Results from experiments so far show the importance of the fine specificity of the antibody response. They also highlight the importance of measuring functional activity rather than antibody levels alone (Corran et al., 2004; Nwuba et al., 2002). It was reported that single and multiple amino acid substitutions were able to affect the binding of the different specific monoclonal antibodies, and some of the changes resulted in proteins that bound inhibitory antibodies and no longer bound blocking monoclonal antibodies (Uthaipibull et al., 2001). It is possible that the most important role of antibody against MSP-1 in suppressing blood stage parasitemia is by inhibiting MSP-1_{42} processing and erythrocyte invasion. A vaccine based on MSP-1 mutants that induce primarily inhibitory antibodies and not blocking antibodies may be an effective way to induce immunity to malaria. It will therefore be necessary to investigate if antibodies induced by natural malaria infection will recognise and bind to the mutagenically modified MSP-1. Thus the results generated here will be very helpful in deducing how humans could react to the mutant MSP1_{19} if they are used for future design of an effective malaria vaccine.

2. Materials and methods

2.1. MSP-1_{19} antigens

Wild type and mutant MSP-1_{19} recombinant proteins described by Uthaipibull et al. (2001) were used in this study.

2.2. Study area

The study sites Igbo-Ora and Idere towns, in Ibarapa local government area of Oyo state in south-western Nigeria, are located in the savannah region, which is heavily cultivated with numerous small streams. *Anopheles gambiae* and *A. funestus* are the mosquito species found in this area (Lawrence, 1965). The climate consists of a warm dry season (November–March) and a cooler rainy season (April–October). The main occupation of the men is farming and hunting while the women are peasant farmers and retail traders (Achidi et al., 1996).

2.3. Study design

This cross-sectional survey was carried out during the rainy seasons. The study protocol was reviewed and approved by the Joint Ethical Committee of the College of Medicine and the University College Hospital, Ibadan. Blood samples were collected randomly from 54 children, aged between 10 days and 15 years. The criteria for inclusion in this survey included the age, length of time spent in the study site and informed consent.

2.4. Blood collection

Blood, 0.5–2 ml; depending on the age of the children; was collected by venipuncture from the arm by qualified medical doctors into sample tubes containing 0.12 M trisodium citrate. The tubes containing the blood were carried on ice, and then transported to the Cellular Parasitology Laboratory in Ibadan within 3 h. The blood was then centrifuged at 8000 rpm for 2 min and the plasma obtained from it was then stored at −80 °C.

2.5. Parasitology

Blood was spotted on the slide, thick and thin smears were prepared, then *P. falciparum* positive and negative individual were determined.

2.6. Binding of serum samples to mutagenically modified variants of MSP-1_{19}

Binding of plasma samples obtained from 49 subjects in the rainy season to wild type and mutant MSP-1_{19} antigens produced by site directed mutagenesis were analysed by ELISA. The mutants used included 26 single amino acid and 7 multiple amino acid changes. Ninety-six well ELISA plates were coated with 100 μl mutants and wild type MSP-1_{19} (0.5 μg of MSP-1_{19} mutants/ml of sodium carbonate buffer) and incubated overnight at 4 °C. The plates were washed three times with 0.05% Tween 20 in phosphate buffered saline (PBS–Tween 20) and blocked with 1% bovine serum albumin in PBS/0.05 Tween 20 (PBS/BSA/Tween 20) for 1 h in an incubator at 37 °C. Washing was repeated (three times with PBS–Tween 20) and diluted plasma sample (1:50) was added per plate in PBS/BSA/Tween 20 at a working volume of 100 μl. The plates were incubated for 1 h at 37 °C and washed three times with PBS–Tween. Horplasmadish peroxidase-conjugated goat anti-human IgG at a dilution of 1:2000 in PBS/BSA/Tween 20 was added and the plates incubated in a moist chamber at 37 °C, for 2 h. The plates were washed again (three times with PBS–Tween 20) and 2-2′-azino-di (3-ethyl-benzthiazoline sulfonate (ABTS)) substrate/H_2O_2 (Kirkegaard and Perry) was diluted 1:1 and 100 μl of the substrate was added to the wells. The reaction was allowed to develop in the dark for 30 min at 37 °C and the absorbance read at 650 nm with a microplate reader. The absorbance of the wild type MSP-1_{19} was used as a cut-off to determine which individuals recognised the mutant MSP-1_{19}. The cut-off was determined based on the antibody titre of plasma that had equal or higher absorbance readings for the mutant MSP-1_{19} compared to the wild type MSP-1_{19}, these samples were then selected and categorised based on the fold increase over the absorbance of the wild type MSP-1_{19}.

2.7. Statistical analysis

The levels of significance were estimated at $P < 0.05$ for ANOVA and Student's *t*-test. The software packages used were Microsoft EXCEL and SPSS.

3. Results

3.1. Parasitology

The percentage number of malaria positive individuals with antibodies that bound to the mutants MSP-1_{19} containing epitopes that recognise only processing inhibitory and not blocking antibodies, were significantly different from those with antibodies that did not bind to these mutants ($P < 0.05$) (Table 1).

3.2. The binding of plasma to single substituted mutations of MSP-1_{19}

The MSP-1_{19} mutants 14a (Gln → Gly), 14b (Gln → Arg), 20 (Arg → Glu), 22 (Leu → Arg), 43 (Glu → Leu), 48 (Thr → Lys) and 53 (Asn → Arg) elicited the best response with individual plasma (Fig. 1). Children aged 7–12 and 109–168 months elicited the highest response to these mutants (Fig. 3).

Table 1
The effect of presence of *Plasmodium falciparum* on the binding of antibodies to mutant MSP-1_{19} proteins, the epitopes shown here bind only processing inhibitory antibodies.

Mutants with processing inhibitory epitopes	Individuals sampled = 49			
	# Ab-positive	% Ab-positive/Mal+	# Ab-negative	% Ab-negative/Mal+
	N	*N* (%)	*N*	*N* (%)
34a	6	3 (50)	43	12 (28)
27 + 31 + 34	31	11 (35)	18	4 (22)
27 + 31 + 34 + 43	34	11 (32)	15	4 (26)
15 + 27 + 31 + 43	5	4 (80)	44	9 (21)

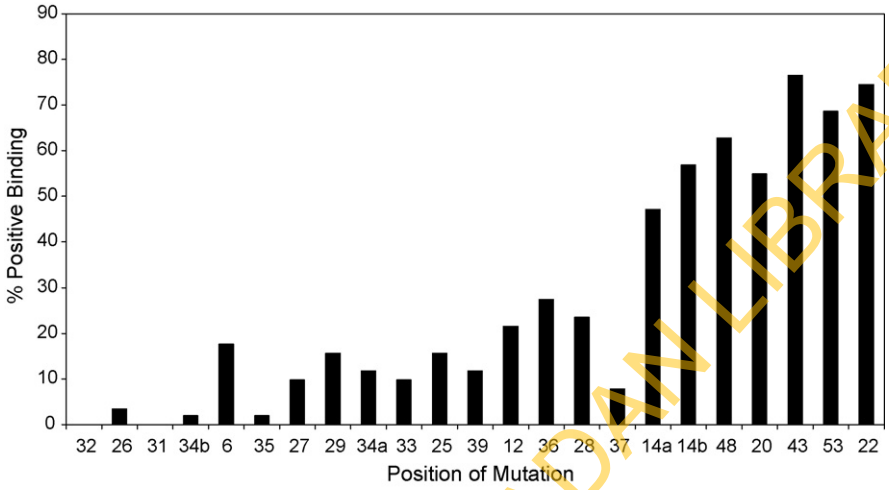

Fig. 1. The prevalence of plasma samples with positive binding to single substituted mutants of MSP-1_{19}. The number of individuals is expressed as a percentage.

3.3. The binding of plasma to multiple substituted mutations of MSP-1_{19}

MSP-1_{19} mutants proteins with amino acid substitutions; 43 + 48b (Glu → Leu, Thr → Asn), 27 + 31 + 43 (Glu → Tyr, Leu → Arg, Glu → Leu) and 27 + 31 + 34 + 43 (Glu → Tyr, Leu → Arg, Tyr → Ser, Glu → Leu) elicited the best response (Fig. 2). Children aged less than 6 and 7–12 months elicited the highest response (Fig. 4).

4. Discussion

Mutant MSP-1_{19} antigens, which have been modified to recognise inhibitory, blocking and neutral antibodies, respectively by Uthaipibull et al. (2001), were used to study the nature of recognition and antigenicity amongst children living in Igbo-Ora.

The results from this study show that the presence of parasites has a role to play in the recognition of these mutants. This is in line with the results which showed the presence of anti-MSP-1_{19} antibodies in children with asymptomatic infection (Egan et al., 1996; Omosun et al., 2005). The mutants that have been shown to bind processing inhibitory antibodies but not blocking antibodies were investigated to see if the presence of *P. falciparum* in the blood sample of the children has any relationship with binding properties of the plasma antibodies. The relationship between these mutants and the presence of parasites was apparent as there was a significant difference in the percentage of malaria positive individuals that had antibodies that recognised these mutants compared with

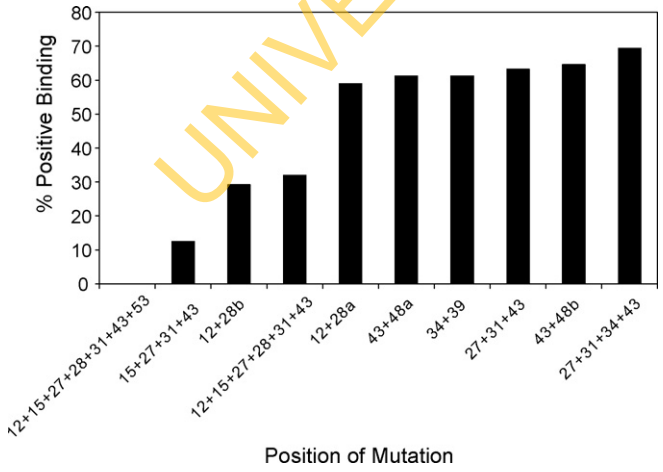

Fig. 2. The prevalence of plasma samples with positive binding to multiple substituted mutants of MSP-1_{19}. The number of individuals is expressed as a percentage.

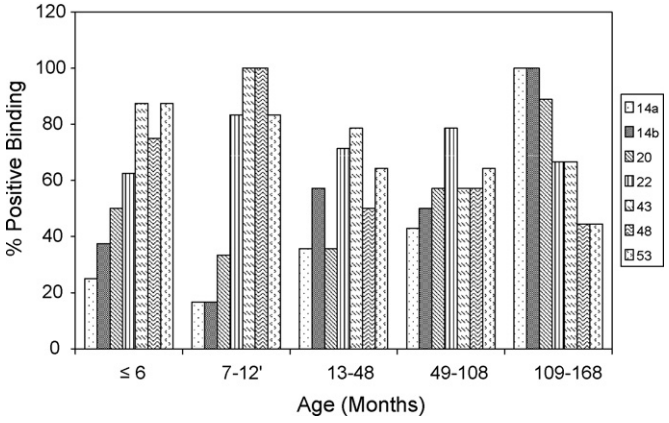

Fig. 3. The relationship between binding of plasma samples with single substituted mutants of MSP-1_{19} protein and age. The number of individuals is expressed as a percentage of the individuals within that age group.

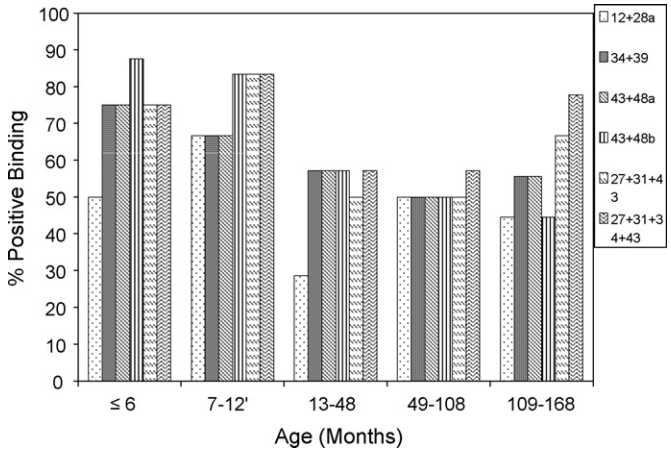

Fig. 4. The relationship between binding of plasma samples with multiple substituted mutants of MSP-1_{19} protein and age. The number of individuals is expressed as a percentage of the individuals within that age group.

those individuals who did not have antibodies to these mutants (Table 1). This result is different from that obtained by Corran et al. (2004) this could be due to differences in study population or seasonal variability. The result implies that the high prevalence of parasites in the rainy season may have influenced the production of antibodies that recognise these modified MSP-1_{19} molecule.

Both the single substituted MSP-1_{19} and combined mutants were well recognised (Fig. 1), this could be because of the prevalence of circulating antibodies to *P. falciparum*, due to the increase in infection rate and endemicity of malaria in the rainy season when these samples were collected. The implication is that these modified MSP-1_{19} mutants could be used in the eventual MSP-1_{19} based vaccine. 76% of the individuals had plasma antibodies that recognised Glu 43-Leu mutant MSP-1_{19} protein (Fig. 1) implying that there were a lot of samples with specificity for this epitope which is meant to reduce the effect of blocking antibodies. 65% of the individuals had plasma antibodies that bound to Arg 20-Glu mutant MSP-1_{19} protein, which is supposed to reduce the binding of two processing inhibitory antibodies. Thr 48-Lys and Asn 53-Arg mutant MSP-1_{19} proteins which abolished the binding of a neutral antibody were also well recognised within the population.

Mutant MSP-1_{19} (27 + 31 + 43) single protein with 3 amino acids substitutions; was shown by Uthaipibull et al. (2001), to bind the processing inhibitory antibodies but not blocking antibodies 1E1, 2.2 and 111.4, although it bound mAb 7.5 (a blocking monoclonal antibody); however the additional substitution of Asn 15 → Arg or Tyr 34 → Ser to form 15 + 27 + 31 + 34 + 43 mutant MSP-1_{19} protein resulted in polypeptides that no longer bound to any of the known monoclonal blocking antibodies but still bound to processing inhibitory antibodies. The number of individuals in the population that recognised the 15 + 27 + 31 + 43 mutant MSP-1_{19} protein was low (13%, Fig. 2), which is in support of the low percentage of children within the same population that had processing inhibitory antibodies (Nwuba et al., 2002). These plasma samples thus had antibodies that recognised the substituted epitopes of MSP-1_{19} that is not found in the natural population indicating the possibility of using these mutants as possible vaccine candidates. It has been shown that the percentage of plasma from individuals that bound to recombinant MSP-1_{19} antigens which no longer bound to blocking antibodies were low (Okech et al., 2004). This is similar to the results obtained in this study for the mutant MSP-1_{19} proteins Tyr 34 → Ser and 15 + 27 + 31 + 43 (Figs. 1 and 2). However, this was not the case for the mutant proteins with substitutions at positions 27 + 31 + 43 and 27 + 31 + 34 + 43. Recognition of these two mutant proteins was high, 63% (27 + 31 + 43) and 69% (27 + 31 + 34 + 43) (Fig. 2). Here we associate the presence of parasitemia with binding to processing inhibitory epitopes. We state that the fine specificity of the plasma is important but varies widely within the population and that some mutations could be more important than the others, thus the least recognized mutant proteins might putatively be the most protective. This is all the more revealing when compared with results from Nwuba et al. (2002) who showed through processing inhibitory assays that 16% of individuals in the population studied could inhibit processing. The percentage of individuals who recognize the mutant proteins Tyr 34-Ser and 15 + 27 + 31 + 43 come closest to this finding, thus plasma which did not bind to these MSP-1_{19} mutant proteins might contain predominantly blocking antibodies.

Results in this study showed that children aged 7–12 and 109–168 months had more response to the single MSP-1_{19} mutant proteins (Fig. 3), while children aged less than 6 and 7–12 months had the highest response to multiple substituted mutant proteins (Fig. 4). These finding show that children of all age groups recognise the mutants and this is good for the production of vaccines. A malaria vaccine that would produce such an effective immune response in this age group will surely be of great benefit for many African countries. The fact that the children recognised the modified protein is favourable for the eventual design of the MSP-1_{19} vaccine. MSP-1_{19} can be so modified that only processing inhibitory antibodies, would bind to it totally abrogating the binding of blocking antibodies which are induced by natural infection and might be a successful immune evasion strategy for the malarial parasites.

The fine specificity of the antibodies to MSP-1_{19} rather than their prevalence might be necessary for their protective efficacy. We report that children living in Igbo-Ora produce antibodies against MSP-1_{19} mutants providing more information that will assist in the eventual production of an effective genetically modified MSP-1 based vaccine.

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