

Original articles

Total immunoglobulin G and IgG1 subclass levels specific for the MSP-1₁₉ of *Plasmodium falciparum* are different in individuals with either processing-inhibitory, blocking or neutral antibodies

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Abstract

Background: Some MSP-1₁₉ specific antibodies that inhibit merozoite invasion also inhibit the secondary processing of MSP-1. However the binding of these inhibitory antibodies can be blocked by another group of antibodies, called blocking antibodies, which recognize adjacent or overlapping epitopes, but themselves have no effect on either MSP-1 processing or merozoite invasion. These antibodies have been reported to be present in individuals living in a malaria endemic area.

Methods: Blood samples were obtained from children shown to have processing inhibitory, blocking, and neutral antibodies in a previous study. Enzyme linked immunosorbent assay (ELISA), was used to determine the total IgG, IgM and IgG subtypes.

Results: There was a significant difference in anti-MSP-1₁₉ IgG, while there was no significant difference in the anti-MSP-1₁₉ IgM. Only anti MSP-1₁₉ IgG1, amongst the IgG subtypes was significantly different between the groups.

Conclusion: This study shows that antibodies against MSP-1 are different not only in specificity and function but also in the amount of total IgG and IgG subtype produced.

Key Words: IgG subtypes; MSP-1; malaria

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Introduction

There are 300-500 million cases of malaria each year, resulting in over 1 million deaths, mainly of children under 5 years of age in Africa¹. Deployment of an effective malaria vaccine would have a significant public health impact. There are several antigens that are currently being evaluated from the various stages of the malaria parasite life cycle. One of these is merozoite surface protein-1 (MSP-1), which is found on the surface of the merozoites of the parasite (asexual stage). Immunization with purified *Plasmodium falciparum* MSP-1 has protected monkeys from malaria². Protective immunity induced by immunization with MSP-1 derived polypeptides is thought to be primarily antibody dependent³.

Monoclonal antibodies to MSP-1 have been shown to inhibit parasite growth in vitro^{4,5}. MSP-1 is synthesized during schizogony as a 190–200-kDa glycoprotein⁶. It is subsequently proteolytically processed into a range of defined fragments⁶. MSP-1 is cleaved by two processing events. The primary processing products include a 42 kDa C-terminal fragment (MSP-1₄₂) while the secondary processing cleaves MSP-1₄₂ into two fragments, one of which is the 19 kDa C-terminal fragment MSP-1₁₉.

Antibodies specific for the C-terminus of MSP-1 can inhibit erythrocyte invasion by a mechanism that involves inhibition of protease activity⁷. Some MSP-1₁₉ specific antibodies that inhibit merozoite invasion also inhibit the secondary processing of MSP-1; these are called processing inhibitory antibodies⁸. Some MSP-1₁₉ specific antibodies that do not inhibit processing are defined as blocking antibodies because they block the binding and functioning of these processing inhibitory antibodies and thereby facilitate processing⁹. The last group of MSP-1₁₉ specific antibodies are termed

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neutral antibodies because they do not interfere with the binding of any of the inhibitory antibodies, and when they bind to the antigen they have no known biological effect. In one study processing inhibitory antibodies were found in children but there was no correlation between MSP-1₁₉-specific total antibody titre and processing inhibitory activity^{10,11}.

The ratio of inhibitory to blocking antibody (and their respective avidities) in a polyclonal response will determine whether or not the outcome is inhibition of invasion⁷. Therefore a vaccine based on MSP-1 variants to induce primarily inhibitory antibodies and not blocking antibodies may be an effective way to induce immunity to malaria¹². In addition it has been shown that protection against the asexual stage of malaria seems to rely largely on specific IgG1 and IgG3 antibodies¹³, but whether or not such antibodies are directed against MSP-1₁₉ is unknown. The objective of the study was to determine whether or not processing-inhibitory activity and/or the presence of blocking antibodies are associated with levels of IgG, IgM or a specific IgG subclass.

Methods

Study area

Igbo-Ora and Idere towns in Ibarapa local government area of Oyo state in southwestern Nigeria were the study sites. *Anopheles gambiae* and *A. funestus* are the mosquito species found in this area¹⁴. 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 traders¹⁵.

Study design

The blood samples used in this study were obtained from a cross-sectional survey carried out during the dry season (January–March) of 1999. The samples had previously been reported to show the presence of processing inhibitory, blocking and neutral antibodies^{10,11}. The study protocol was reviewed and approved by the Joint Ethical Committee of the College of Medicine and the University College Hospital, Ibadan. The subjects of the study included infants and children from 10 days to 15 years.

Blood collection

Blood (1–2 ml) was collected by qualified medical doctors. The blood was then stored in sample tubes containing 0.12 M trisodium citrate and labeled.

Plasma obtained from the blood was stored at “80 °C (Forma Scientific, Marietta, OH, USA).

Determination of anti-MSP-1₁₉ Antibodies

Total IgG antibodies and specific IgG subclass antibodies were detected by ELISA using recombinant MSP-1₁₉ antigen and a method that has previously been described¹⁶.

MSP-1₁₉ specific IgM was also determined by ELISA. Flat bottom polyvinyl chloride plates (Corning Incorporated-Life Sciences, MA, USA) were coated with 50 µl of MSP-1₁₉ (0.5 µg of MSP-1₁₉/ml of sodium carbonate buffer, pH 9.6) and incubated overnight at 4 °C. The antigen solution was then poured off and the plates were blocked with 150 µl of 0.5% Boiled Casein for 1 hour at 37 °C. The plates were washed three times with PBS-Tween 20. Plasma samples serially diluted from 1:20–1:2560 in boiled casein were added to the plates that were then incubated for 1 hour at 37 °C in an incubator (Forma Scientific, Marietta, Ohio, USA). The plates were washed three times with PBS-Tween 20 and 50 µl of 1:1000 dilution of Goat anti Human IgM (γ-chain specific) peroxidase conjugate in boiled casein was added to the plates which were then incubated for 1 hour at 37 °C in the incubator (Forma Scientific, Marietta, Ohio, USA). The plates were then finally washed three times with PBS-Tween 20. 50 µl of ABTS/H₂O₂ was added to the plates and the colour allowed to develop at 37 °C for 30 minutes in the incubator (Forma Scientific, Marietta, Ohio, USA), the absorbance was then read at 650nm with a microplate reader (Molecular Devices, Menlo Park, CA, USA) without stopping the reaction. The end point titre was defined as the highest dilution that gave an absorbance value above the highest absorbance of the negative control. The reciprocal end titres were log transformed and expressed as log reciprocal titres.

Statistical analysis

The results were analyzed using non parametric ANOVA. The level of significance was estimated at $p < 0.05$ using Graph pad prism.

Results

Plasma from fifty individuals who had been selected from a previous study and analysed for the type of anti-MSP-1₁₉ antibodies they contained were found to possess processing inhibitory, blocking and neutral antibodies^{10,11}. However in this study these samples were examined to determine whether the kind of

anti-MSP-1₁₉ antibodies was an effect of the total IgG, IgM and IgG subtypes produced by these children. There was a significant difference in the level of anti MSP-1₁₉-specific total IgG (p=0.0122) while there was no significant difference in anti-MSP-1₁₉ total IgM for individuals having processing inhibitory antibodies, blocking antibodies or neutral antibodies (Table 1).

The mean and median anti-MSP-1₁₉ IgG and IgM titres were determined for each group of individuals with processing inhibitory, blocking and neutral antibodies. The antibody titre was expressed as log reciprocal titres. (N: number of individuals with anti-MSP-1₁₉ antibodies).

Table 1: Mean and Median anti-MSP-1₁₉ IgG and IgM of individuals with processing inhibitory, blocking and neutral antibodies

	Processing inhibitory antibodies N=6	Blocking antibodies N=9	Neutral Antibodies N=32 ^a	P Value
Anti-MSP-1 ₁₉ IgG				
Mean±SD	2.800±0.675	2.930±0.380*	2.440±0.456	0.012
Median	2.750	2.900	2.300	
Anti-MSP-1 ₁₉ IgM				
Mean	1.550±0.226	1.400±0.212	1.400±0.178	NS
Median	1.600	1.300	1.300	

* Significant difference between individuals with blocking antibodies and neutral antibodies, using non parametric ANOVA. ^a Anti-MSP-1 IgG was determined in 35 individuals.

When the levels of the individual IgG subclasses was examined there were also no significant differences between the titres of anti MSP-1₁₉ specific IgG2, IgG3 and IgG4 for individuals with processing inhibitory antibodies, blocking antibodies or neutral antibodies (Table 2). However, in contrast, samples from individuals defined as having blocking

antibodies had significantly higher levels of IgG1 relative to the other two groups (p=0.0033). In table 2, the mean anti-MSP-1₁₉ IgG subtypes (IgG1, IgG2, IgG3 and IgG4) were determined for each group of individuals. The antibody titre was expressed as optical density (OD). (N; number of individuals with anti-MSP-1₁₉ antibodies).

Table 2: Mean anti-MSP-1₁₉ IgG subtypes of individuals with processing inhibitory, blocking and neutral antibodies

Anti-MSP-1 ₁₉ antibodies	Processing inhibitory antibodies(N=6)	Blocking antibodies (N=8)	Neutral antibodies(N=25)	P value
IgG1*	0.095±0.15	0.227±0.16	0.067±0.07	P=0.0033
IgG2	0.029±0.04	0.031±0.05	0.037±0.04	NS
IgG3	0.049±0.03	0.112±0.16	0.063±0.07	NS
IgG4	0.001±0.001	0.007±0.02	0.002±0.004	NS

* Significant at P<0.05, NS is not significant

Discussion

The fine specificity of the antibody response to MSP-1₁₉ is crucial for the antibody's function. Some monoclonal antibodies bind to the C-terminal part of the protein and inhibit the proteolytic processing of MSP-1 that occurs at or just before erythrocyte invasion. Antibodies that inhibit this processing inhibit erythrocyte invasion suggesting that the mechanism by which they prevent erythrocyte invasion is by

inhibiting processing. Some other antibodies that bind to MSP-1 are blocking antibodies; when these bind they block the binding of the inhibitory antibodies, allowing MSP-1 processing and erythrocyte invasion to proceed even in the presence of inhibitory antibodies^{7,9,12}.

It was reported previously that there was no correlation between the anti-MSP-1₁₉ total IgG titre and its processing inhibitory activity. Only 12% of the 50 plasma samples analysed had significant processing inhibitory activity, suggesting that the specificity rather than the total level of specific antibody is important¹⁰. This observation might have implications for the design of MSP-1 based vaccines. A cross sectional survey of which this subset of children belongs had reported that children positive for *P. falciparum* had a negative correlation between parasitemia and IgG 1 and IgG3 anti-MSP-1₁₉ antibody titres respectively¹⁶.

The same samples, which had been analysed previously for processing inhibitory antibodies, blocking antibodies and neutral antibodies^{10,11}, were examined in more detail. There was a significant difference in the anti MSP-1₁₉ specific total IgG ($p=0.0122$) for individuals with processing inhibitory antibodies, blocking and neutral antibodies (Table 1) while there was no difference in anti MSP-1₁₉ IgM titres in these individuals. The results from this study not only validates the previous study¹⁰, but goes a little further to suggest that in individuals with blocking antibodies there might be a relationship between the total IgG and blocking activity. This might be explained in terms that the specificities for the blocking epitopes on MSP-1 increases with increasing IgG levels.

The analysis of the anti MSP-1₁₉ IgG subtypes showed that only the level of anti MSP-1₁₉ IgG1 was significantly different ($p=0.0033$) between the groups. The samples from the individuals with blocking antibodies have the highest absorbance values (Table 2). This suggests that the anti MSP-1₁₉ IgG1 in these individuals are the blocking antibodies. This suggestion could in principle be tested experimentally by examining the epitope specificity and avidity and ability to compete with processing inhibitory monoclonal antibodies of affinity purified IgG1 antibodies from the plasma samples. This increase in blocking IgG1 might lead to the masking of the effect of processing inhibitory antibodies, which is why the percentage of individuals with this antibody phenotype is small in the population and thus the need for a vaccine that can lead to increased production of processing inhibitory antibodies. The contribution of processing inhibitory, blocking and neutral antibodies to protection against malaria still needs to be established and the importance in other protective mechanisms needs to be considered. For example, IgG1 is cytophilic and has been reported

to be protective¹⁷, but it is unclear whether or not IgG1 antibodies specific for MSP-1₁₉ are important for Fc mediated mechanisms¹⁸. Some individuals who had high anti-MSP-1₁₉ IgG titre still had clinical malaria¹⁹, the reasons for this is not known, but it could be presumed that not all the IgG1 antibodies produced were beneficial.

Conclusion

This study shows that antibodies against MSP-1 are different not only in specificity and function but also in the amount of total IgG and IgG1 subtype produced. Differences in functionality may be due to these subtype variations. More studies need to be done to ascertain the role of subtypes in different individuals.

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