Original articles

Total immunoglobulin G and IgG1 subclass levels specific for the MSP-1_{19} of *Plasmodium falciparum* are different in individuals with either processing-inhibitory, blocking or neutral antibodies

*Omosun YO[1,2], Adoro S[1§], Anumudu CI[2], Odaibo A[2], Holder AA[3], Nwagwu M[2] and Nwuba RI[2]

1 Department of Biotechnology, College of Food Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.
2 Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Ibadan, Nigeria.
3 National Institute for Medical Research, Mill Hill, London, England.
§ Present address: Immunology Graduate Group, University of Pennsylvania

Abstract

Background: Some MSP-1_{19} 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-1_{19} IgG, while there was no significant difference in the anti-MSP-1_{19} IgM. Only anti MSP-1_{19} 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
African Health Sciences 2010; 10(2): 106 - 110

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 Africa[1]. 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 malaria[2]. Protective immunity induced by immunization with MSP-1 derived polypeptides is thought to be primarily antibody dependent[3].

***Correspondence author:**
Dr. Yusuf Omosun
Department of Biotechnology
College of Food Sciences
Bells University of Technology
Ota, Ogun State, Nigeria
Phone: (234) 8033309937
E-mail: yusufomosun@yahoo.com

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 glycoprotein[6]. It is subsequently proteolytically processed into a range of defined fragments[6]. MSP-1 is cleaved by two processing events. The primary processing products include a 42 kDa C-terminal fragment (MSP-1_{42}) while the secondary processing cleaves MSP-1_{42} into two fragments, one of which is the 19 kDa C-terminal fragment MSP-1_{19}.

Antibodies specific for the C-terminus of MSP-1 can inhibit erythrocyte invasion by a mechanism that involves inhibition of protease activity[7]. Some MSP-1_{19} specific antibodies that inhibit merozoite invasion also inhibit the secondary processing of MSP-1; these are called processing inhibitory antibodies[8]. Some MSP-1_{19} 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 processing[9]. The last group of MSP-1_{19} specific antibodies are termed

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-1_{19}-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 invasion[7]. 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 malaria[12]. In addition it has been shown that protection against the asexual stage of malaria seems to rely largely on specific IgG1 and IgG3 antibodies[13], but whether or not such antibodies are directed against MSP-1_{19} 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 govern-ment area of Oyo state in southwestern Nigeria were the study sites. *Anopheles gam-biae* and *A. funestus* are the mosquito species found in this area[14]. 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 farm-ers and retail traders[15].

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 proto-col was reviewed and approved by the Joint Ethical Committee of the College of Medicine and the Uni-versity 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 qualiûed 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 ^{o}C (Forma Scientiûc, Marietta, OH, USA).

Determination of anti-MSP-1_{19} Antibodies

Total IgG antibodies and specific IgG subclass antibodies were detected by ELISA using recombinant MSP-1_{19} antigen and a method that has previously been described[16].

MSP-1_{19} 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-1_{19} (0.5 µg of MSP-1_{19}/ml of sodium carbonate buffer, pH 9.6) and incubated overnight at 4^{0} 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^{0}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^{0}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 (?-chain specific) peroxidase conjugate in boiled casein was added to the plates which were then incubated for 1 hour at 37^{0}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/H_2O_2 was added to the plates and the colour allowed to develop at 37^{0}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-1_{19} 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-1_{19} 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-1_{19}-specific total IgG (p=0.0122) while there was no significant difference in anti-MSP-1_{19} total IgM for individuals having processing inhibitory antibodies, blocking antibodies or neutral antibodies (Table 1).

The mean and median anti-MSP-1_{19} 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-1_{19} antibodies).

Table 1: Mean and Median anti-MSP-1_{19} IgG and IgM of individuals with processing inhibitory, blocking and neutral antibodies

	Processing inhibitory antibodies N=6	Blocking antibodies N=9	Neutral AntibodiesN=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-1_{19} 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-1_{19} 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-1_{19} antibodies).

Table 2: Mean anti-MSP-1_{19} 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-1_{19} 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-1_{19} 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 important[10]. 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-1_{19} antibody titres respectively[16].

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-1_{19} 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-1_{19} IgM titres in these individuals. The results from this study not only validates the previous study[10], 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-1_{19} IgG subtypes showed that only the level of anti MSP-1_{19} 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-1_{19} 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 cytophillic and has been reported to be protective[17], but it is unclear whether or not IgG1 antibodies specific for MSP-1_{19} are important for Fc mediated mechanisms[18]. Some individuals who had high anti-MSP-1_{19} IgG titre still had clinical malaria[19], 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.

Acknowledgements

We acknowledge the cooperation Staff and children of the schools used in this study. This investigation received financial support under the Multilateral Initiative on Malaria (MIM) projects A980050 and A10581 through the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

References

1. TDR Progress 1997-98 Fourteenth Programme Report UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases 1999.
2. Kumar S, Collins W, Egan A, et al. Immunogenicity and efficacy in *Aotus* monkeys of four recombinant *Plasmodium falciparum* vaccines in multiple adjuvant formulations based on the 19- kilodalton C Terminus of merozoite surface protein – 1. *Infection* & *Immunity* 2000; 68(4): 2215- 2223.
3. Hui GS, Siddiqui WA. Serum from Pf195 protected Aotus monkeys inhibit *Plasmodium falciparum* growth in vitro. *Experimental Parasitology* 1987; 64(3): 519-22.
4. Blackman MJ, Heidrich HG, Donachie S, McBride JS, Holder AA. A single fragment of a malaria merozoite surface protein remains on the parasite during red cell invasion and is the target of invasion-inhibiting antibodies. *Journal of Experimental Medicine* 1990; 172: 379-382.
5. Locher CP, Tam LQ, Chang SP, Mcbride JS, Siddiqui WA. *Plasmodium falciparum:* gp195 tripeptide repeat-specific monoclonal antibody

inhibits parasite growth in vitro. *Experimental Parasitology* 1996; 84(1): 74-83.
6. Holder AA, Freeman RR. The three major antigens on the surface of *Plasmodium falciparum* merozoites are derived from a single high molecular weight precursor. *Journal of Experimental Medicine* 1984; 160: 624-629.
7. Holder AA, Guevera Patino JA, Uthaipibull C, et al. Merozoite surface protein –1, immune invasion, and vaccines against asexual blood stage malaria. *Parasitologia* 1999; 41: 409- 414.
8. Blackman MJ, Scott-Finnigan TJ, Shai S, Holder AA. Antibodies inhibit the protease- mediated processing of a malaria merozoite surface protein. *Journal of Experimental Medicine,* 1994; 180: 389-393.
9. Guevara Patino JA, Holder AA, McBride JS, Blackman MJ. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. *Journal of Experimental Medicine* 1997; 186(10): 1689-99.
10. Nwuba RI, Sodeinde O, Anumudu CI, et al. The human immune response to *Plasmodium falciparum* includes production of antibodies that inhibit MSP-1 secondary processing. *Infection* & *Immunity* 2002a; 70(9): 5328-5331.
11. Nwuba RI, Adoro SA, Anumudu CI, et al. Specificities of Antibodies to *Plasmodium falciparum* Merozoite Surface Protein (MSP)-1_{19}. Proceedings of the 10th International Congress of Parasitology, Vancouver, Canada, 2002b; 477-486.
12. Uthaipibull C, Aufiero B, Syed SEH, et al. Inhibitory and blocking monoclonal antibody epitopes on merozoite surface protein 1 of the malaria parasite *Plasmodium falciparum*. *Journal of Molecular Biology* 2001; 307: 1381-1394.
13. Diallo TO, Spiegel A, Diouf A, Perraut R, Kaslow DC, Garraud O. Short Report: IgG1/ IgG3 Antibody Responses to Various Analogs of Recombinant yPfMSP119- A Study in Immune Adults Living in Areas of *Plasmodium falciparum* Transmission. *American Journal of Tropical Medicine and Hygeine* 2001; 64 (3, 4): 204-206.
14. Lawrence BR. Medical entomology at Igbo-Ora, western Nigeria. *Journal of Nigerian Medical Association* 1965; 2: 198-205.
15. Achidi EA, Salimonu LS, Asuzu MC, Berzins K, Walker O. Studies on *Plasmodium falciparum* parasitemia and development of anemia in Nigerian infants during their first year of life. *American Journal of Tropical Medicine and Hygiene* 1996; 55(2): 138-43.
16. Omosun YO, Anumudu CI, Adoro S, et al. Variation in the relationship between anti-MSP-1_{19} antibody response and age in children infected with *P. falciparum* during the dry and rainy season. *Acta Tropica;* 2005; 95 (3): 233-247.
17. Druilhe P, Bouharoun-Tayoun H. Natural Immunities. *Research Immunology* 1991; 142(8): 637-643.
18. McIntosh RS, Shi J, Jennings RM, et al. The importance of human FcgammaRI in mediating protection to malaria. *PLOS Pathogens* 2007; 3(5): e72, 647-658 doi:10.1371/journal.ppat.0030072
19. Dodoo D, Theander TG, Kurtzhals JAL, Koram K, Riley E, Akanmori BD, Nkrumah FK, Hviid L. Levels of Antibody to Conserved Parts of *Plasmodium falciparum* Merozoite Surface Protein-1 in Ghanaian Children Are Not Associated with Protection from Clinical Malaria. *Infection* & *Immunity* 1999; 67(5): 2131-2137.