SENSITIVITY AND SPECIFICITY OF ISOLATED ANTIGEN FROM *PLASMODIUM FALCIPARUM* CULTURE SUPERNATANT

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ABSTRACT

Immunological sensitivity and specificity properties of isolated *Plasmodium falciparum* (GPL) antigen from culture supernatant have been measured and compared with malarial antigens and non malarial filtered paper blood sera for potency and efficacy. Latex bead coded GPL, Pf and RESA antigens immunoreaction properties of human filter paper blood samples (FPB) were studied by laser light scattering immunoassay (LIA) and Enzyme linked immunoabsorbent assay (ELISA) techniques. Results of GPL antigen sensitivity study by LIA method showed a very high malaria antibody binding response (MABR) i.e. 6% compared with 78% with RESA and 88% Pf antigens. Malaria detection by ELISA method also found similar results. Specificity study of GPL antigen for different non malarial filter paper blood sera (NMFS) showed no immunoreaction however Pf and RESA antigen showed few positive immunological responses. These results suggest that sensitivity and specificity properties of isolated GPL antigen is better than other antigens.

KEYWORDS:

GPL antigen, RESA antigen, Pf antigen, Non malarial sera, LIA, ELISA

INTRODUCTION

Plasmodium falciparum malaria is a common infectious disease of tropical countries and leads to heavy morbidity and mortalities (Butler, 1997) but it can be controlled if it is diagnosed at early stages of development (Makler *et al.*, 1998). There are several methods for detection of malaria and immunological detection techniques have been found better than other diagnostic methods and are commonly used for determination of malaria disease. (Kawamoto, 1991a; Peyron *et al.*, 1994; Dietz *et al.*, 1995; Clendennen *et al.*, 1995; Hang *et al.*, 1995; Lowe *et al.*, 1996; Warhurst and Williams 1996; Humar *et al.*, 1997; Antony *et al.*, 1998; Palmer *et al.*, 1998; Bhakat *et al.*, 1999; David *et al.*, 2000). Sensitivity and specificity of immunological detection methods mainly depend

upon immuno activity of antigen (McDougal and McDuffie, 1985; Nezlin, 2000). Also it have been seen that specific antigen immuno reaction help in precise response for diagnosis of disease and immunological properties are mainly depend upon specificity of antigen to the antibody (Frankenburg *et al.*, 1984; Roy *et al.*, 1995; Alaedini and Latov, 2000). Several Pf antigens have been developed for diagnosis of malaria but they failed to provide cent percent accurate diagnosis and some times gives erroneous results (Kabat and Mayer, 1961; Vial *et al.*, 1982; Van Oss, 1994; Kaslow *et al.*, 1994; Garraud *et al.*, 1999; George *et al.*, 1999; Nezlin, 2000). We have isolated an antigen (GPL) from Pf culture supernatant (Mya *et al.*, unpublished data) and our aim in this study to measure sensitivity and specificity properties of isolated antigen and compare with other antigens and non malarial sera for its efficacy in diagnosis of Pf malaria.

MATERIALS AND METHODS

50 controls and 50 Pf malaria positive human subjects were used for sensitivity study

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(Selected after microscopic examination of blood smear) and for specificity study 10 different types of non malarial antibodies containing subjects were selected and from each non malarial antibodies containing group (consisting of 10 subjects), blood were collected on filter paper for studying the properties of isolated GPL antigen. Blood samples were collected aseptically on filter paper disc by finger prick method. Also a drop of blood was put on a grease free clean slide for thick and thin smear for microscopic study of malaria parasite. Blood drop containing filter paper disc was dried at 25°C and stored at -20°C for sensitivity and specificity studies. Before the start of immunoassay's i.e. laser light scattering Assay (LIA) and Enzyme Linked Immunoabsorbent Assay (ELISA) blood soaked filter paper discs were punched twice by paper puncher and two punched discs were put in 500µl of PBS buffer (pH 7.2) and kept for 12 hours (over night) for dissociation of malarial antibody from discs. Malaria antibodies containing PBS has been studied with GPL, RESA and Pf antigens by ELISA and LIA method.

Isolation of antigens from Pf culture supernatant : Glycophospholipid of malaria (*P.falciparum*) has been developed by culturing the *P.falciparum* strain in RPMI 1640 LPLF medium supplemented with 25 mM Hepes, 25 mM Sodium bicarbonate and human O⁺ fresh serum (Trager and Jensen, 1976). And a better-modified preparation technique for malaria GPL has been developed (Mya *et al.* under publication).

Study of Chemical Chemistry of GPL antigen: Pf culture supernatant chemical chemistry has been studied by Gas Liquid Chromatography using Hewlett Packard capillary gas chromatogram technique, USA. The Pf culture supernatant was found to be composed of xylose, mannose, galactose and glucose based glycophospholipid (Fig. 1: Mya *et al.* unpublished data).

1. Laser light scattering assay (LIA):

(a) Sensitivity study of GPL, RESA and Pf antigens:

Preparation of antigen coated latex polystyrene beads: 25 µl of 1mg/ml of antigen and 1ml of 1% latex bead aqueous solution (900 µl of coating buffer (carbonate bicarbonate buffer pH 9.2) in 100 µl of 10% latex polystyrene beads) was mixed and kept at 4°C for over night. Next day the latex bead suspension was incubated at 37°C for

one hour in incubator. The particles were washed three times with washing buffer by centrifugation at 20,000 rpm at 25°C for 30 min. The washed beads were re-suspended in 100 µl of 1% BSA. Suspended beads were re-incubated for 1hr at 37°C. After incubation the beads were washed again three times with washing buffer as above. After final washing, the volume was made to 1ml by adding of coating buffer and kept at 4°C for the testing of immunosensitivity by Laser light scattering technique.

Detection by LIA : 20 µl of antigen coated latex bead and 2 µl of 1:100 diluted filter paper blood serum was thoroughly mixed. The mixture was immediately drawn into a 3mm diameter glass capillary tube and filled up to one third of capillary tube. Both the ends of the tube were closed with paraffin wax and kept for 1 hour at room temperature. Then the tube was placed inside the goniometer of Laser light scattering apparatus. He-Ne Laser (Aerotech, USA) system was operated at 632.8 nm and the scattered light generated after passing through latex beads goes to photo multiplier tube which change the scattered light as photocurrent. Photocurrent was then calculated by Brookhaven Instruments, USA Model 9000 AT digital Correlator.

(b) Specificity Study of GPL, RESA and Pf antigens: GPL, RESA and Pf antigens were studied individually with each of the 10 different types of non malarial antibody producing human blood by LIA method.

2. Enzyme linked immunoabsorbent assay (ELISA)

(a) Sensitivity study of GPL, RESA and Pf antigen:

ELISA immunoassay has been performed on developed GPL and RESA and Pf malaria antigens coated plates. Sensitivity and efficacy of GPL, RESA and Pf antigens have been studied with endemic malaria human antibodies content, PBS extracts and ELISA reader (Pasteur LP300) has been used for the measurement of immuno-reaction.

Detection by ELISA: 1:1000 dilution of glycolipid (GPL), 1:1000 dilution of RESA and Pf antigen were freshly prepared and 50 µl of 50 ng/well RESA and GPL diluted antigen were added in each well of the costar plates (one for GPL, one for RESA and

one for Pf) and kept over night at 4°C. Next day plate were taken out and put in the incubator at 37°C for 1 hour. After incubation the plates were washed three times with PBS-Tween 20 (phosphate buffer saline with Tween 20) for 5 min. Wells were blocked with 100 µl of 1% BSA in each well of the plates. Plates were incubated again at 37°C for 1 hour. Then the plates were again washed three times with PBS-Tween 20. 50 µl of diluted serum was then added in each well of the plates as duplicate and incubated at 37°C for one hour. After incubation plates were washed as above. 50 µl of freshly prepared Rabbit antihuman IgG R1- HRPO conjugate (purchased from Bangalore Genei) (1:1000 dilution) was added each well of the plates and incubated at 37°C for one hour. After incubation plates were washed as above. 50 µl of freshly prepared orthophenylenediamine (OPD) was added in each well of the plates and incubated at room temperature for 10 min in dark condition for colour reaction. After 10 min the reaction stopped by adding 25 µl of 8N sulphuric Acid in each well of the plates. The absorbency of each well of the plates was measured with ELISA reader (Diagnostic Pasteur LP300) at 492nm.

(b) Specificity Study of GPL, RESA and Pf antigens: GPL, RESA and Pf antigens were studied individually with each 10 different types of non malarial antibody producing human blood by ELISA method.

The results of the sensitivity and specificity studies of GPL, RESA and Pf antigens were analyzed for comparison by using statistical cut of value test.

RESULTS AND DISCUSSION

The results of this study show that isolated GPL antigen purified from Pf culture supernatant has a very high immuno activity compared to other antigens. Also specificity characteristics showed

better specific efficacy than other antigens. Results of GPL antigen in LIA study showed a significant increase in antigen antibodies reaction in LIA (96% +ve) and in ELISA method it was (88% +ve) after testing. However, RESA antigen sensitivity studies showed 78% positive immuno reaction in LIA and in ELISA it was 66%. Pf antigen in LIA measurement showed 88% positivity for malaria antibodies in patients filter paper blood sera however ELISA studies showed no change in sensitivity activities and found to be similar to RESA. RESA and Pf antigens in LIA and ELISA methods showed 2 to 8 % false malaria positivity when compared with control (No. 50) filter paper blood sera (Table 1) but GPL antigen showed no false malaria positive result when studied with control filter paper blood serum. Table 2 shows specificity results of GPL, Pf and RESA antigens with different non-malarial antibodies filter paper blood sera studied by LIA and ELISA methods. It is found that different non malarial sera produced no immuno-reaction with GPL antigen (100% specificity) however few false malaria diagnosis (90% specificity) has been observed with RESA and Pf antigens. Study of Quarles and Dalakas (1996) found that glycosylphosphatidylinositols (GPLs) can provide valuable prognostic information for blood antibodies diseases and the result of our study of isolated Pf antigen (glycophospholipid) showed a very high sensitivity and specificity for detection of serum Pf malaria antibody.

Isolated GPL antigen is showing better sensitivity than RESA and Pf antigens. Also it has been seen that GPL can detect fresh Pf malaria infection (IgM antibody) and may be very useful for large scale Pf antibody survey (IgM study: unpublished data of Mya *et al.*). Results indicate that isolated GPL antigen is very sensitive for detection of early Pf malaria antibodies in human serum and it is highly specific for malaria disease.

Table 1

Sensitivity study of GPL, RESA, and Pf antigens by LIA and ELISA methods

S. No.	Particulars	GPL antigen		RESA antigen		Pf antigen	
		LIA (nm)	ELISA (OD)	LIA (nm)	ELISA (OD)	LIA (nm)	ELISA (OD)
1	P.falciparum(n=50)						
	Mean	804.96	0.7868	551.51	0.5630	492.54	0.5428
	±SD	±182.23	±0.1314	±272.64	±0.2127	±223.29	±0.2069
	No. of +ve subjects	48	44	39	33	44	35
2	(% +vity)	96%	88%	78%	66%	88%	70%
	Control(n=50)						
	Mean	98.36	0.1954	98.72	0.1524	97.1264	0.1562
	±SD	±3.8532	±0.0231	±4.89	±0.0559	±3.4464	±0.0494
	No. of +ve subjects	0	0	1	4	1	3
	(% +ve)	0%	0%	2%	8%	2%	6%
	Cut of value (mean ± 2SD)	106.07	0.2416	108.5	0.2642	104.02	0.255

Table 2

Specificity study of GPL, RESA and Pf antigens with different non malarial antibody sera

S. No.	Non malarial antibody blood	% specificity					
		GPL antigen		RESA antigen		Pf antigen	
		LIA	ELISA	LIA	ELISA	LIA	ELISA
1	Healthy subject (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%
2	<i>P.falciparum</i> (n=10)	+ve 100%	+ve 100%	+ve 100%	+ve 90%	+ve 100%	+ve 90%
3	Polio (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 90%
4	HIV (n=10)	-ve 100%	-ve 100%	-ve 90%	-ve 90%	-ve 100%	-ve 90%
5	Tuberculosis (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%
6	Pregnancy (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%
7	Asthma (n=10)	-ve 100%	-ve 100%	-ve 100%	=ve 100%	=ve 100%	-ve 100%
8	Hepatitis- B (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%
9	Leprosy (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%
10	Filariasis (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%
11	Leishminiasis (n=10)	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%	-ve 100%

Fig. 1

Chemical chemistry of isolated GPL antigen separated from Pf culture supernatant studied by Hewlett Packard Capillary Gas Liquid Chromatography, USA

References

1. Antony, T., Saxena, A., Roy, K.B. and Bohidar, HB. 1998 Laser light scattering immunoassay: an improved data analysis by CONTIN method. *J. Biochem Biophys Methods* 36(2-3), 75-85.
2. Alaedini, A. and Latov, N. 2000 Detection of antiGMI Ganglioside antibodies in patients with Neuropathy by Noval Latex agglutination assay. *J. Immuno.* 21(4), 377-386.
3. Bhakat, P., Arati Roy. and Kunal B. Roy 1999 Laser Light Scattering Immunoassay for Malaria *J. Immunol.* 20(3), 103-114.
4. Butler, D. 1997. Time to put malaria control on the global agenda. *Nature* 386 535-541.
5. Clendennen, T. E., Long, G. W. and Baird, J. K. 1995 QBC and Giemsa stained thick blood films diagnostic performance of laboratory technologists. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 89, 183-184.
6. David, J., Fryaufe Purnomo., Mochammad, A., Sutamihardja., Iqbal, R. S., Elyazar., Ika Susanti., Krisin, Budi Subianto. and Harijani Marwoto. 2000 Performance of the Opo:MAL assay for detection and identification of malaria infections in asymptomatic residents of Irian Jaya, Indonesia. *Am. J. Trop. Med. Hyg.* 63(3-4), 139-145.
7. Dietz, R., Perkins, M., Boulos, M., Luz, F., Reller, B. and Corey, C.R. 1995 The diagnosis of *Plasmodium falciparum* using a new antigen detection system. *Am. J. Trop. Med. Hyg.* 88, 187-188.
8. Frankenburg, S., Sllutzky, G. M., Gitler, C. and Lonhner, M.V. 1984 Lipid antigens derived from erythrocytes infected with *Plasmodium falciparum*. *Zeitschrift Fir Parasitenkunde* 70, 331-335.
9. Garraud, O., Diouf, A., Nguer CM., Dieye, A, Longacre S., Kaslow DC., Holder A., Tall A., Molez JF., Perraut, R. and Mercereau Puijalon, O. 1999 Differential in vitro stimulation of T lymphocytes using *P.falciparum* MSP119 antigens in individuals from different endemic areas, *Scand. J. Immunol.* 97, 204-210.
10. George, J., Gilburd, B., Langevitz, P., Levy, Y., Nezlin, R., Harats, D. and Shoenfeld, Y. 1999 $\beta 2$ glycoprotein-1 containing immune-complexes in lupus patients: associated with thrombocytopenia and lipoprotein (a) levels. *Lapus* 8, 116-120.
11. Hang, V.T., Be, T.V., Tran, P.N., Thanh, L.T., Hien, L. V., O'Brine, E. and Morris, G. E. 1995 Screening donor blood for malaria by polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 89, 44-47.
12. Humar, A., Ohrt, C., Harrington, M. A., Pillai, D. and Kain, K.C. 1997 ParaSight F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travelers. *American Journal of Tropical Medicine and Hygiene* 56, 44 - 48.
13. Kabat, E.A. and Mayer, M. .M. 1961 *Experimental immunochemistry*, C.Thomas, Springfield, Illinois.
14. Kaslow, DC., Hui, G. and Kumar, S. 1994 Expression and antigenicity of *Plasmodium falciparum* major merozoite surface protein-1 (MSP119) variants secreted from *Saccharomyces cerevisiae*, *Mol. Biochem. Parasitol.* 63, 283-289.
15. Kawamoto, F. 1991a Rapid diagnosis of malaria by fluorescence microscopy with light microscopy and interference filters. *Lancet* I, 200-202.
16. Lowe, B. S., Jeffa, N. F., New, L., Pedersen, C., Engbaek, K. and Marsh, K. 1996 Acridine orange fluorescence techniques as alternatives to traditional Giemsa staining for the diagnosis of malaria in developing countries. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 90, 34 - 36.
17. Makler, M.T., Palmer, C.J. and Ager, A. L. 1998 A review of practical techniques for the diagnosis of malaria. *Annals of Tropical Medicine and Parasitology* 92 (4), 419-433.
18. Nezlin, R., 2000 A quantitative approach to the determination of antigen in immune complexes. *J. Immunol. Methods* 237, 1-17.
19. Mya, M. M., Roy, A. and Saxena, R.K. Isolation, part characterization and antigenecity of a glycopospholipid from *Plasmodium falciparum* culture supernatant. (Unpublished data: Sent for

publication in Current Science).

20. McDougal, J.B. and McDuffie, D.C. 1985 Immune complexes in man detection and clinical significance, *Adv.Clin.Chem.* 24, 1-60.
21. Palmer, C.J., Lindo, J. F., Klakala, W., Quesada, J., Kaminsky, R. and Ager A.L. 1998. Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J. Clin. Microbiol.* 36, 203 - 206.
22. Peyron, F. G., Martet, J. P. and Vigler, A. 1994 Dipstick antigen-capture assay for malaria detection. *Lancet* I, 1502-1503.
23. Quarles, R.H. and Dalakas. M.C. 1996 Do anti-Glycolipid antibodies cause human peripheral neuropathies? *J. Clin. Invest.* 97, 1136-1137.
24. Roy, A., Biswas, S., Kabilan, L. and Sharma, V. P. 1995 Application of simple peptede ELISA for stratification of malaria endemicity . *Indian J. Malariology* 32, 164-173.
25. Trager, W. and Jenson, J. B. 1976 Human malaria parasites in continuous culture. *Science* 193, 673-675.
26. Van Oss, C. J. 1994 Nature of specific ligand- receptor bonds, in particular the antigen- antibody bond. In: Van Oss C.J. and van Regenmortel, M.H.V. (Eds), *Immunochemistry*, Marcel Dekker, New York , pp 581-614.
27. Vial, H.J., Thuet, M,J. and Philippot, J. R. 1982 Phospholipid biosynthesis in synchronous *Plasmodium falciparum* antigen efficiently inhibit merozoit invasion *Proceedings of the National Academy of Sciences, USA.* 81, 7912-7916.
28. Warhurst, D.C. and Williams, J.E. 1996. Laboratory diagnosis of malaria. *Journal of Clinical Pathology* 49, 533 - 538.