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Naturally occurring plasmodium-specific IgA antibody in humans from a malaria endemic area

SUKLA BISWAS*, QUEEN B SAXENA, ARATI ROY and LALITHA KABILAN
Malaria Research Centre, 22 Sham Nath Marg, Delhi 110 054, India

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Abstract. Blood samples collected from individuals belonging to an endemic area in Uttar Pradesh, were tested for plasmodial antigen specific immunoglobulin A (IgA) by enzyme immuno assay using soluble extract of *Plasmodium falciparum* from culture. Among 773 (20·18%, $P < 0·0001$) samples 156 sera demonstrated a detectable seropositivity for antigen specific IgA. IgA levels were higher among individuals who experienced repeated attacks of malaria compared to acute infected patients. Among seropositive individuals the IgA titers were found increased with the age. Immunoglobulin isolated from sera having high level of IgA showed growth inhibitory effect in *Plasmodium falciparum in vitro.* A group of sera with high IgA antibody against *Plasmodium falciparum* crude antigen showed seronegativity with specific peptides. Statistically, no positive or negative correlations were observed between antigen specific IgG and IgA. However, there was a tendency towards negative correlation between IgA and IgM. Mechanisms for the parasite specific IgA production remain to be established.

Keywords. Malaria; IgA; enzyme immuno-assay; *Plasmodium falciparum* peptide antigens.

1. Introduction

During malarial infections there is a marked increase in serum immunoglobulin levels, part of which is specific to malarial parasites (Biswas *et al* 1990). However, there is a poor or no correlation between immune status of the host and the levels of antimalarial antibody. Much of this antimalarial antibody is non-protective (McGregor 1981). It was already established in earlier studies that, malaria specific antibodies belong to IgM for primary response, isotype IgG for secondary response. So far nothing is known about the production of parasite specific IgA and the relevance of this antibody in protection and the mechanism of production of this isotype in malarial infection.

Serum IgA is primarily produced in the bone-marrow. The daily production rate of IgA in human exceeds the production of immunoglobulins of all other isotypes. In accordance with the high general production rate, the IgA level in external secretions is not significantly high. The isotype IgA not only provides localized specific humoral protection of mucosal surfaces but also acts as an integral component of the entire immune system.

In our present study the profile of malaria specific IgA in the sera of individuals of various age groups belonging to endemic malarious areas of India was investigated

*Corresponding author.

and the relation to disease and age dependent relationship to this antibody was carried out. Effect of antimalarial IgA in *Plasmodium falciparum* growth has been demonstrated in a group of sera and these sera were also tested with *P. falciparum* stage specific peptides, as circumsporozoite antigen (Zavala *et al* 1986), ring infected erythrocyte surface antigen (Perlmann *et al* 1989) and merozoite surface antigen (Fruh *et al* 1991).

2. Materials and methods

2.1 *Study area*

The study includes the inhabitants of village Piyawli in Ghaziabad, Uttar Pradesh (India), where malaria is seasonal. Early and prolonged monsoons are responsible for intensive transmissions of both *P. vivax* and *P. falciparum* in these areas.

2.2 *Collection of blood samples*

Blood samples were collected from 773 individuals of Piyawli village of various age groups during a cross sectional survey in the study area from July to December, 1990. Thirty five samples were collected from healthy normals from Delhi and 20 blood samples were collected from tourists belonging to non-endemic, non-malarious areas. Sera, after separation, were stored at –70°C until processed. Thick and thin blood films were prepared for microscopic examination.

2.3 *Parasites for antigen preparation*

The Indian *P. falciparum* isolates (FSJ-from Shahjahanpur, P-30 from Piyawli, Ghaziabad) were used for antigen preparation. Parasites were maintained in routine *in vitro* culture using O + RBCs and AB + serum by candle jar technique (Trager and Jensen 1976). Antigen was prepared from *in vitro* parasite cultures enriched with late trophozoites and schizonts. Parasites were freed by saponin lysis and soluble extract was obtained by sonication in MSE Soniprep at 14 μA for 90s. The antigen was purified after adsorption with rabbit anti human erythrocytes by the method described elsewhere (Avrameas and Ternynck 1969).

2.4 *Detection of malaria specific immunoglobulins by ELISA*

Assay was performed in 96 well round bottom microtitre plates (Coster, USA). *P. falciparum* antigen was absorbed on solid phase at protein concentration of 20 μg/ml incubated 1 h at 37°C then overnight at 4°C. Sera tested were used at dilution of 1 : 1024 for IgG and 1 : 128 for IgM and IgA. Three types of antibodies were trapped by using peroxidase labelled specific antihuman globulin conjugates. The assays were visualized by addition of *o*-phenylenediamine/H_2O_2substrate and results were recorded in ELISA reader (Biotek, USA) at 490nm.

2.5 *Peptide ELISA*

ELISA plates were coated with three peptide antigens (CSP, RESA and MSA 1)

at protein concentration of 20 μg/ml like *P. falciparum* crude antigen. Ten sera with antimalarial IgA positivity against crude antigen were tested with peptides at 1 : 128 dilution. Method remained same as above.

2.6 *Parasite growth inhibition assay*

The assay was done by the method described earlier (Biswas and Sharma 1991). Briefly, parasites synchronized at ring stage having 1% parasitaemia were cultured in 96 well tissue culture plate in the presence of 1 μg immunoglobulin isolated from a batch of 46 sera, 26 sera having high titre of IgA and 20 sera with IgA negative. They were allowed to grow for 48 h and then the parasitaemia was determined by light microscopy after staining. The percentage of total parasite growth inhibition was calculated.

2.7 *Statistical analysis*

Probability value for antigen specific IgA reactivity was calculated using the normal

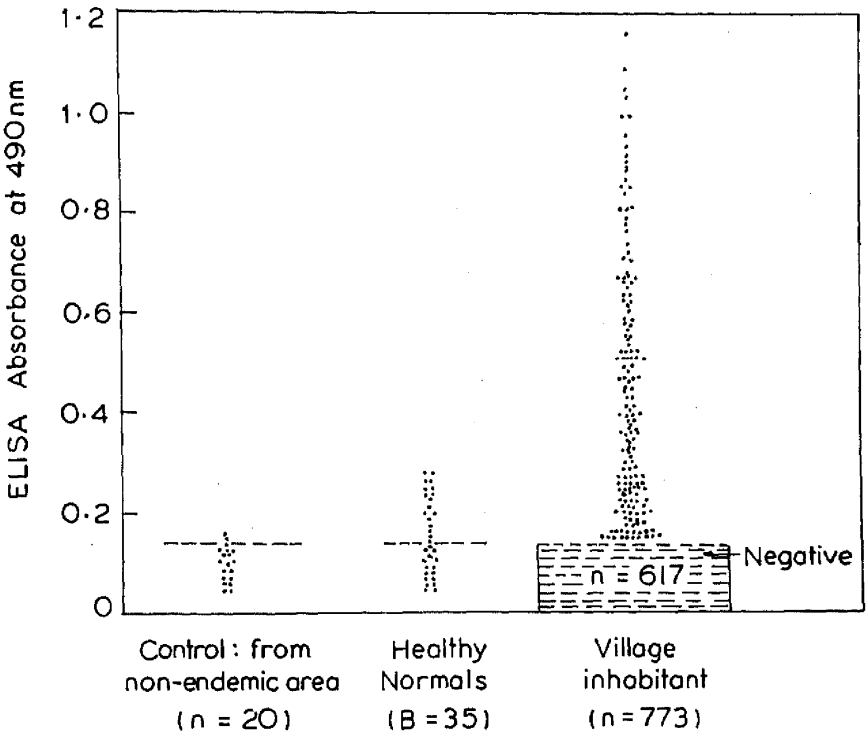

Figure 1. Scatter diagram of *P. falciparum* antigen-specific IgA level in sera from individuals belong to malaria endemic and non-endemic areas. ELISA mean OD ± SD values for control: 0·09 ± 0·03; Healthy normals. 0·14 ± 0·07. OD values > 0·15 for study group has taken as positive.

test for proportions, and a regression analysis was used to establish the correlations between immunoglobulin isotypes and parasite growth inhibition *in vitro.*

3. Results

Sera tested in this study were collected form a group of individuals who were suffering from both *P. vivax* and *P. falciparum* malaria and also had multiple attacks. Among these 773 samples, 407 sera (52·65%) were positive for antigen specific IgG, 288 (37·26%) were positive for IgM and 156 sera (20·18%) showed a varied degree of positivity for antigen specific IgA (figure 1). Sera collected from healthy normals from Delhi city had an average absorbance value of 0·15; whereas non-endemic control sera had average absorbance value of 0·09. For determination of serological positivity, mean + 2 SD value (0·15) of non-endemic control sera was considered. In individuals with repeated attacks of malaria, higher IgA antibody level were detected compared to acute (figure 2), and age dependent

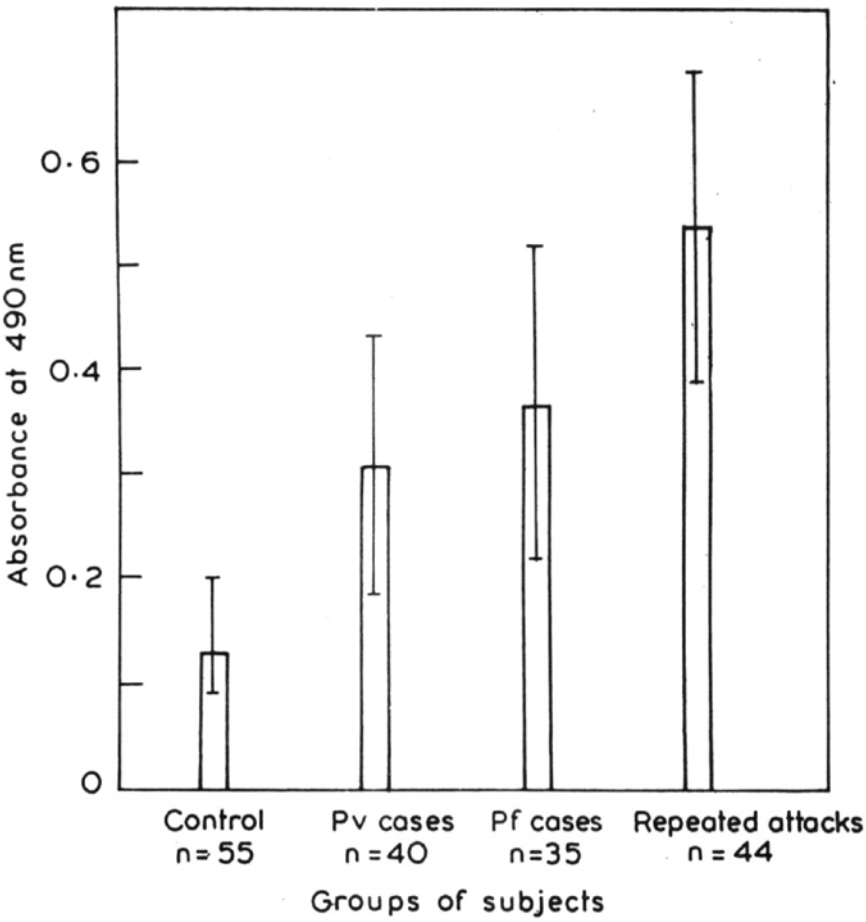

Figure 2. Malaria antigen-specific IgA profile in human subjects with acute and repeated infections. Graph plotted with mean ± SD values.

increase in IgA level was noticed (figure 3). Twentysix sera from a group of individuals with high malarial antigen specific IgA showed significant growth inhibition in *P. falciparum* culture (r = 0·8) (table 1). These sera were also tested against parasite stage specific peptides as RESA, MSA 1 and CSP to check the antipeptide IgA antibodies. No significant seroreactivity has been observed against these peptides (data not shown), which may reflect that IgA epitope might be associated with some antigens other than these three peptides. No positive or negative correlations was observed between antigen-specific IgG and IgA. However, a negative correlation could be seen between low IgA positivity and high IgM positivity and vice versa (figure 4).

4. Discussion

It is well documented by several studies in human that malaria infection trigger

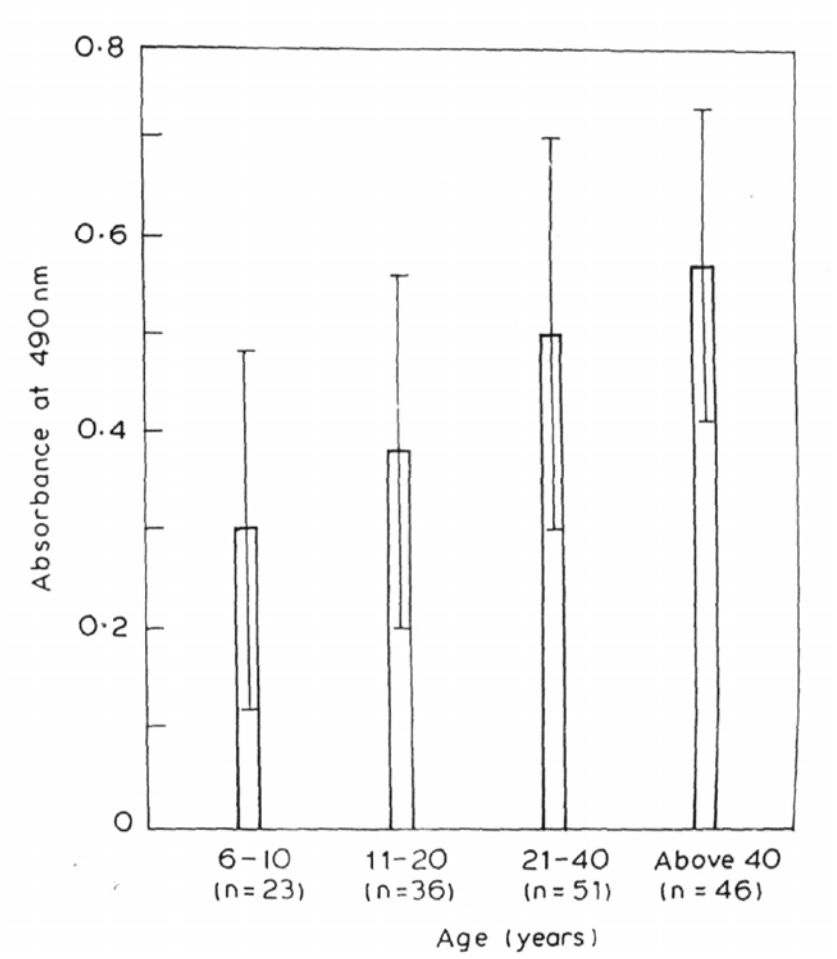

Figure 3. Age relatd increase in antigen-specific IgA level. Graph plotted with mean ± SD values.

Table 1. Comparative profile of sera (26) from individuals with high IgA level (group 1), and sera (20) from individuals with negative IgA level (group II) for their IgG and IgM level and per cent growth inhibition (GI) of *P. falciparum* in culture.

ELISA OD values at 490 nm							
IgG		IgM		IgA		Parasite growth inhibition (%)	
Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0·56	0·39	0·28	0·49	0·73	0·08	68	8
0·72	0·49	0·30	0·32	0·51	0·10	77·8	22
0·77	0·46	0·32	0·21	0·25	0·12	52	20
0·87	0·74	0·34	0·51	0·67	0·04	92·6	29
0·88	0·77	0·44	0·52	0·58	0·06	73·4	44·4
0·64	0·69	0·22	0·46	0·44	0·11	49·5	10·5
0·74	0·85	0·51	0·53	0·48	0·06	34·4	13·2
0·86	0·88	0·26	0·49	1·04	0·12	96	35
0·70	0·25	0·31	0·44	0·68	0·08	66	29
0·68	0·22	0·27	0·64	0·62	0·12	49·5	12
0·84	0·42	0·34	0·29	0·99	0·08	64	12
0·59	0·45	0·52	0·24	0·28	0·12	50	26
0·57	0·58	0·30	0·30	0·52	0·09	54	19
0·65	0·43	0·59	0·27	0·41	0·07	58	11
0·46	0·58	0·61	0·28	0·48	0·04	62	22
0·50	0·62	0·30	0·33	0·93	0·10	85	34
0·39	0·54	0·10	0·31	0·65	0·05	76	37
0·37	0·60	0·23	0·22	0·56	0·10	45	45
0·53	0·70	0·36	0·15	0·68	0·08	77	36
0·62	0·46	0·29	0·08	1·02	0·04	80	27
0·70		0·47		1·07		92	
0·64		0·36		0·43		63	
0·41		0·26		0·74		66	
0·55		0·25		0·63		75	
0·69		0·33		0·84		84	
0·67		0·31		0·80		79	

Correlations between ELISA OD values for 3 immunoglobulin isotypes and parasite growth inhibition: Group I, r = 0·22 for IgG vs GI; r = 0·24 for IgM vs GI; r = 0·8 for IgA vs GI. Group II, r = 0·39 for IgG vs GI; r = 0·21 for IgM vs GI; r = 0·35 for IgA vs GI.

polyclonal activation producing IgG, IgM isotypes of immunoglobulins and part of which are specific to malaria (Collins *et al* 1971). The results of the present study in a small holoendemic area reveal that about 20% of the total sample size have only *Plasmodium-specific* IgA antibody, but all those sera were not significantly positive for IgG or IgM. Among these 156 antigen specific IgA positive sera only 30 showed significant level for IgG and 18 for IgM. These findings may also suggest the specificity of the assay and the immuno-reagents used in detecting parasite-specific IgA antibody.

The present findings show that human individuals residing in endemic area with a history of repeated malaria attacks have substantial amount of antigen-specific IgA in circulation. Moreover, there is an age-wise increasing pattern of this isotype. This age-related increase pattern has been observed in IgG isotype in earlier studies

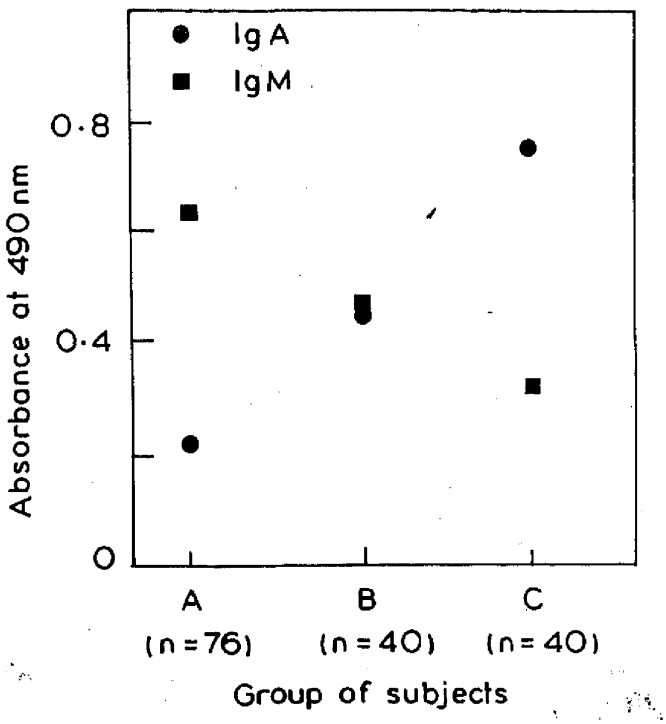

Figure 4. Individuals with low **(A),** medium **(B)** und high **(C)** level of parasite specific IgA antibody and their IgM profile. ELISA OD values for **(A)** 0·15-0·35; **(B)** 0·36-0·55; (C) ≥ 0·56.

(Deloron *et al* 1987). Malarial parasites may have some components, which can trigger the IgA response and this along with IgG can help in the development of protective immune response by providing defense mechanism to the invading organisms. Negative correlation between high IgM and low IgA profile or vice versa may indicate the switching over of individual IgM producing cells to IgA as it is evidenced in switch over from IgM to IgG production.

The present study has been conducted in a small area having two bouts of seasonal *Plasmodial* infections; children are most vulnerable to this disease, a portion of adults have developed immunity because of repeated attacks. So far, no such studies have been reported in any malaria endemic population; therefore, it would be a subject of interest to conduct studies in some other endemic areas to confirm the presence of naturally occurring antimalarial IgA antibody.

Though it has been observed that persons with acute infections of *P. vivax* and *P. falciparum* and also the immune adults are having this immunoglobulin A, yet identification of species contributing more for the production of IgA needs to be explored. Only 10% of the sample size has shown a medium to high level of antigen-specific IgA antibody, it is necessary to identify the IgA specific *Plasmodial* epitope and also to screen those individuals with elevated malaria specific IgA in circulation. This type of study may help to obtain some baseline informations regarding the inclusion of a group of individuals prior to any vaccine action programme or before launching new drugs or drug combination trial.

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