

## SHORT COMMUNICATION

### RELATIONSHIP BETWEEN ACUTE MALARIA AND ANTI-RESA ANTIBODIES IN SERA OF PATIENTS FROM TWO DIFFERENT ENDEMIC AREAS IN BRAZIL

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Several *P. falciparum* antigens which induce antibody responses have been studied (HOLDER et al., 1985)<sup>6</sup>, among them RESA (Ring-infected Erythrocyte Surface Antigen) or Pf155 described by PERLMANN et al. (1984)<sup>9</sup>. This antigen presents two regions of repetitive amino acid sequences: the carboxyl terminal end containing a repeating 11-amino acid sequence (DDEHVEEPTVA) and the amino terminal end containing an 8-amino acid sequence (EENVEHDA) followed by about 34 repeats of the 4-amino acid sequence (EENV) (FAVAROLO et al., 1986)<sup>5</sup>.

RESA/Pf155 may elicit an immune response interfering with reinvasion of noninfected erythrocytes (PERLMANN et al., 1984)<sup>9</sup>. These authors observed a higher frequency of negative anti-RESA antibodies reactions in sera from patients with acute malaria, specially in those who suffered their first infections. CHIZZOLINI et al. (1988)<sup>3</sup> reported an increased frequency of anti-RESA antibodies in patients with negative smears compared to those having acute malaria, suggesting that the presence of these antibodies may play a role in controlling parasitemia.

On the other hand, several authors (PETERSEN et al., 1989<sup>10</sup>; BJORKMAN et al., 1990<sup>2</sup>; CHUMPITAZI et al., 1991<sup>4</sup>) reported a lack of correlation between titers of anti-RESA antibodies detected by Modified Indirect Fluorescent Assay (MIFA) or ELISA against RESA peptides and parasite densities.

Levels of anti-RESA antibody detected by MIFA and ELISA against three peptides: (EENV)<sub>3</sub> (4x3); (EENVEHDA)<sub>2</sub>(8x2) and (DDEHVEEPTVA)<sub>2</sub> (11x2) in sera of patients with acute malaria from localities of two Brazilian States - Pará (KLOETZEL et al., 1990)<sup>7</sup> and Amapá - attending the local Hospital, are here reported.

Sera were from individuals, mainly miners, aged 13 years or above which came from several endemic areas. The exposure to the disease was different in these two groups: only 26.0% (6/23) of patients from Pará had been living more than one year in the area as compared with 66.6% (16/24) of patients from Amapá.

All sera had a positive Indirect Fluorescent Assay (IFA) with whole parasite *P. falciparum* an-

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tigen. They were tested by MIFA (PERLMANN et al., 1984)<sup>9</sup> and ELISA with the three peptides (BERZINS et al., 1986)<sup>1</sup>. Sera diluted at 1:100 were considered positive to peptides 4x3, 8x2 and 11x2 at absorbances > .053, .083 and .088 O. D., respectively as previously determined.

Both positivity and Geometric Mean Titers (GMT) of IgG anti-RESA antibodies, detected by MIFA, in sera from Amapá were higher than those observed in sera from Pará: frequency of positive MIFA was 51.1% (23/45) in sera from Pará and 80% (24/30) in those from Amapá, with GMT of 33.5 and 493, respectively.

There was no significant difference between the positivity and arithmetic mean of absorbances (A. M.) detected by ELISA, in sera from both States: the frequency of RESA antibodies in sera from Pará to 4x3, 8x2 and 11x2 peptides was respectively, 86.9% (20/23), 65.2% (15/23) and 73.9% (17/23) and in those from Amapá with the same peptides was 83.3% (20/24), 37.5% (9/24) and 70.8% (17/24). The A. M. for the 4x3, 8x2 and 11x2 peptides in sera from Pará was .537, .241 and .290, respectively and in those from Amapá .342, .258 and .215.

As expected, the lowest GMT (Table I) was observed in patients from Pará with a high parasitemia and suffering their first malaria attack.

These results were comparable to those reported by PERLMANN et al. (1984)<sup>9</sup>. However, we could not detect a significant difference between low and high parasitemia or level of anti-RESA antibodies (GMT) in sera obtained from patients with several malaria attacks, living in Pará.

A higher GMT of MIFA was obtained with sera from patients reporting several malaria attacks having low parasitemia, compared with those of high parasitemia levels. This was most conspicuous in sera from Pará, but differences were not statistically significant (Table I).

In patients from Amapá and Pará, as mentioned before, no significant difference was observed in levels of anti-RESA peptide antibodies, as measured by ELISA. These results were similar to those obtained from the individuals who were not infected at the moment of the collection, living in the same areas (MALAFRONTTE et al., 1994)<sup>8</sup>. Our findings are comparable to those reported by CHUMPITAZI et al. (1991)<sup>4</sup>, who did not observe, in patients from Burkina Faso, west Africa, any correlation between, the level of anti-RESA antibodies and the presence or not of parasitemia.

Our findings do not agree with those reported by PETERSEN et al. (1989)<sup>10</sup>, who observed low rates of seropositivity to the three RESA peptides

TABLE I

Relationship between Geometric Mean Titers (GMT)<sup>+</sup> of anti-RESA antibodies by Modified Indirect Fluorescent Assay (MIFA), parasitemia levels and malaria episodes of patients from Pará and Amapá

Localities	Parasitemia First Attack				Parasitemia Several Attacks			
	Low*		High		Low*		High	
	%	GMT	%	GMT	%	GMT	%	GMT
Pará	27.0	100	73.0	18.3	58.0	53.8	42.0	22.9
(23)	(3/11)		(8/11)		(7/12)		(5/12)	
Amapá	75.0	126	25.0	1280	70.0	819.7	30.0	253.9
(24)	(3/4)		(1/4)		(14/20)		(6/20)	

\* - up to 1 parasite/microscopic field.

MIFA cut-off value  $\geq 1/5$

+ - GMT of MIFA positive sera.

in acute patients. A low seropositivity rate (37.5%) was observed only with the 8x2 peptide in sera from Amapá. This does not necessarily mean that high A.M. to the three peptides confers some protection against malaria infections.

It is unknown if anti-RESA antibodies are relevant, by themselves, for protection against malaria. Continuous exposure to the disease for several years, elicits the response of other antibodies that may also contribute to resistance against the infection.

We believe that there is a need to study the profile of anti-RESA antibodies in individuals from Brazil in other regions, with different epidemiological conditions, in order to know the real role played by this antibody.

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#### REFERENCES

1. BERZINS, K; PERLMANN, H; WAHLIN, B. et al. - Rabbit and human antibodies to a repeated amino acid sequence of a *Plasmodium falciparum* antigen, Pf155, react with the native protein and inhibit merozoite invasion. *Proc. nat. Acad. Sci. (Wash.)*, **83**: 1065-1069, 1986.
2. BJORKMAN, A.; PERLMANN, H.; PETERSEN, E. et al. - Consecutive determinations of seroreactivities to

Pf155/RESA antigen and to its different repetitive sequences in adult men from a holoendemic area of Liberia. *Paras. Immunol.*, **12**: 115-123, 1990.

3. CHIZZOLINI, C.; DUPONT, A.; AKUE, J.P. et al. - Natural antibodies against three distinct and defined antigens of *Plasmodium falciparum* in residents of a mesoendemic area in Gabon. *Amer. J. trop. Med. Hyg.*, **39**: 150-156, 1988.
4. CHUMPITAZI, B.F.F.; DELORON, P.; PEYRON, F. et al. - Relationships between clinical protection and antibodies to *Plasmodium falciparum* RESA (ring-infected erythrocyte surface antigen) peptides. *Int. J. Parasit.*, **21**: 271-274, 1991.
5. FAVAROLO, J.M.; COPPEL, R.L.; CORCORAN, L.M. et al. - Structure of the RESA gene of *Plasmodium falciparum*. *Nucleic Acids Res.*, **14**: 8265-8277, 1986.
6. HOLDER, A.A.; LOCKYER, M.J.; ODINK, K.G. et al. - Primary structure of the precursor to the three major surface antigens of *Plasmodium falciparum* merozoites. *Nature*, **317**: 270-273, 1985.
7. KLOETZEL, J.K.; MALAFRONTA, R.S.; GONDO, S. & NOGUEIRA, M.C.L. - Anti-RESA (Pf155) antibodies in malaria patients from Pará and Rondônia, Brazil. *Ann. trop. Med. Parasit.*, **84**: 531-533, 1990.
8. MALAFRONTA, R.S.; VALDIVIA, J.L.; NAKAIE, C. & KLOETZEL, J.K. - Seasonal variation of anti-RESA (Pf155) *Plasmodium falciparum* antibodies in three localities from the State of Amapá, Brazil. *Rev. Inst. Med. trop. S.Paulo*, **36**: 237-243, 1994.
9. PERLMANN, H.; BERZINS, K; WAHLGREN, M. et al. - Antibodies in malarial sera to parasite antigens in the membrane of erythrocytes infected with early asexual stages of *Plasmodium falciparum*. *J. exp. Med.*, **159**: 1686-1704, 1984.
10. PETERSEN, E.; HOGH, B.; PERLMANN, H. et al. - An epidemiological study of humoral and cell-mediated immune responses to the *Plasmodium falciparum* antigen Pf155/RESA in adult Liberians. *Amer. J. trop. Med. Hyg.*, **41**: 386-394, 1989.

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