RESEARCH NOTE

Prevalence of Antibodies to Potential Malaria Vaccine Antigens in an Endemic Area of the State of Rondônia (Brazil)

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Two of the most promising *Plasmodium falciparum* blood stage antigens to be included in a malaria vaccine are the gp190 and the Pf155/RESA, since these antigens are able to induce the production of antibodies with protective effects and their presence are related to the degree of immunity (LH Perrin, R Dayal 1982 *Immunol Rev 61:* 245-269, P Deloron et al. 1987 *Am J Trop Med Hyg 37:* 22-26).

Serocpidemiological studies using gp190 and Pf155/RESA have been conducted mainly in Africa (HM Muller et al. 1989 *Infect Immun 57:* 3765-3769, R Tolle et al. 1993 *Infect Immun 61:* 40-47, Deloron *loc.cit.*, C Chizzolini et al. 1989 *Trans R Soc Trop Med Hyg 83*: 147-151) and in Brazil there has so far been little research concerning the antibody response of *P. falciparum* malaria to these peptides (RS Malafronte et al. 1994 *Rev Inst Med Trop S Paulo 36*: 369-371).

We have recently conducted a survey in the municipality of Ariquemes (State of Rondônia, Brazil) with the main objective of establishing immunological criteria for the recognition of acute malaria infection. By studying the immune response against *P. falciparum* antigens we could identify a 40 kDa component associated to active disease (HC Balthazar-Guedes et al. *Parasitol Res* in press). This study enabled us also to analyze the pattern of antibody response to some of the previously described antigens in the migrant population of Ariquemes.

As the knowledge of the immune response to these polypeptides in different populations is pertinent to evaluate the potential use of the gp190 and Pf155/RESA polypeptides for subunit malaria vaccine, we report here the data concerning a sample of the Brazilian population.

The population studied, comprised mainly young migrants (x=30 years) living for nearly five years in the endemic region. The 2486 Brazilian isolate of *P. falciparum*, maintained asynchronously *in vitro*, was used as source of antigen (W Trager, J Jensen 1976 *Science 193*: 673-675). The components of the parasite were fractionated by SDS-PAGE on a discontinuous SDS buffer system 7% to 10% (VK Laemmli 1970 *Nature 227:* 680-685) adapted for slabs (FW Studier 1973 *J Mol Biol 79*: 237-248), associated to the immunoblotting technique (HU Towbin et al. 1979 *Proc Natl Acad Sci USA 76:* 4350-4354). In order to perform all the immunoblots in strictly comparable conditions, an improved immunoblotting system was utilized (J Thelu et al. 1991 *J Clin Microbiol 29*: 510-518). Using this technique, we analyzed a total of 76 individuals. Twenty five of them were primeinfected (14 with positive and 11 with negative thick blood smear [TBS]) and 51 were polyinfected (median=10.2 past attacks of malaria) subjects (26 with positive and 25 with negative TBS).

The mean time elapsed after the last malaria attack referred by individuals with negative TBS was 48 months in primeinfected and 12 months among polyinfected subjects.

Several polypeptides distributed between 190 and 10 kDa were recognized by most of the IgG antibodies present in the serum of prime and polyinfected individuals. Among them, the polypeptides of 190 and 155 kDa, were the most frequently recognized (66% and 24%, respectively).

As expected, polyinfected individuals showed the highest percentage of reactivity against both polypeptides. The polypeptide of 190 kDa was recognized by all the 26 parasitized polyinfected individuals regardless the number of past attacks of malaria and by 7 out of 14 (50%) of primeinfected with positive thick blood smear. Similar percentages were observed in 5 out of 11 prime and 12 out of 25 polyinfected subjects with negative TBS (45% and 48%, respectively) (Table I). These differences were also apparent when we compared only prime and polyinfected subjects having had the last malaria attack less than one year before examination (Table II).

Only polyinfected individuals were able to recognize the component of 155 kDa. The prevalence of IgG antibodies against this polypeptide was

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TABLE I

Percentage of recognition of gp190 and Pf155/RESA *Plasmodium falciparum* polypeptides by the IgG present in sera from all prime and polyinfected individuals studied

Polypeptide	Primeinfected		Polyinfected		
	TBS		TBS		
	Pos (np/n)	Neg (np/n)	Pos (np/n)	Neg (np/n)	Total
gp190 (%)	7/14 (50%)	5/11 (45%)	26/26 (100%)	12/25 (48%)	50/76 (66%)
Pf155/RESA (%)	0/14 (0%)	0/11 (0%)	12/26 (46%)	6/25 (24%)	18/76 (24%)

TBS: thick blood smear; Pos: positive; Neg: negative; np: number of positive; n: number of individuals

TABLE II

Percentage of recognition of gp190 and Pf155/RESA *Plasmodium falciparum* polypeptides by the IgG present in sera from prime and polyinfected individuals having had the last malaria attack in a period ≤12 months

Polypeptide	Primeinfected		Polyinfected	
	TBS		TBS	
	Pos (np/n)	Neg (np/n)	Pos (np/n)	Neg (np/n)
gp190 (%)	7/14 (50%)	1/4 (7%)	26/26 (100%)	10/19 (53%)
Pf155/RESA (%)	0/14 (0%)	0/4 (0%)	12/26 (46%)	5/19 (26%)

TBS: thick blood smear; Pos: positive; Neg: negative; np: number of positive; n: number of individuals

higher among the 26 parasitized polyinfected individuals (46%) than in non parasitized ones (24%) (Table I), stressing the importance of boosting to the appearance of the immune response directed to the 155 kDa component.

Previous studies suggest that the anti-Pf155 and the anti-gp190 antibodies are involved in controlling parasitemia or providing protection against clinical malaria (Chizzolini *loc. cit.*, Tolle *loc.cit.*). In our study the parasite rates in infected subjects with no antibodies to these antigens were similar to those recorded in subjects having such antibodies. These data could reflect the short time of exposure and the low grade of immunity of these individuals. In fact, this population comprises migrant individuals and it is well known that high levels of antibody to the polypeptides in human primed by natural infection require repeated infections for full expression (H Perlmann et al. 1984 *J Exptl Med 159*: 1686-1704, Deloron *loc.cit.*, Tolle *loc.cit.*). Together with previously reported results, the present data suggest that the population studied here must probably be considered a priority target for immunoprophylactic campaigns in the future. The existence of baseline data concerning the immune status of different populations in the Amazon basin could help in the evaluation of the impact of implementation of malaria control measures.

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