SHORT COMMUNICATION

Genetic polymorphism of the serine rich antigen N-terminal region in *Plasmodium falciparum* field isolates from Brazil

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In this work we investigated the frequency of polymorphism in exon II of the gene encoding most of the amino-terminal region of the serine rich antigen (SERA) in Plasmodium falciparum *field samples. The blood samples were colleted from* P. falciparum *infected individuals in three areas of the Brazilian Amazon. Two fragments have been characterized by polymerase chain reaction: one of 175 bp corresponding to the repeat region with 5 octamer units and one other of 199 bp related to the 6 repeat octamer units of SERA protein. The 199 bp fragment was the predominant one in all the studied areas. The higher frequency of this fragment has not been described before and could be explained by an immunological selection of the plasmodial population in the infected individuals under study. Since repeat motifs in the amino-terminal region of SERA contain epitopes recognized by parasite-inhibitor antibodies, data reported here suggest that the analysis of the polymorphism of* P. falciparum *isolates in different geographical areas is a preliminary stage before the final drawing of an universal vaccine against malaria can be reached.*

Key words: malaria - *Plasmodium falciparum* - serine rich antigen - polymorphism

Malaria is still nowadays one of the most important problems of public health in endemic areas. As a result and because of the emergence of resistance of both the parasite and the mosquito vector to drugs and insecticides, respectively, a malaria vaccine is one of the most powerful potential tools to be added to those classically used to control malaria transmission. Consequently, different antigens expressed during the asexual cycle of the malaria parasite and their encoding genes have been characterized in the last years (Ferreira et al. 1998, Sallenave-Sales et al. 2000, Magesa et al. 2002). The gene codifying to the serine rich antigen (SERA), a protein also known as p126 or serine-rich protein (SERP), which is located in the parasitophorous vacuole of trophozoites and schizonts, has been also target of interest to several groups. SERA ranks as a candidate antigen for inclusion as a subunit in a polyantigen malaria vaccine because: (i) specific monoclonal and polyclonal antibodies against SERA can inhibit the in vitro growth of the parasite (Chulay et al. 1987, Bzik et al. 1988, Knapp 1989); (ii) immunization with SERA can induce partial protection against parasite challenge in *Saimiri* and *Aotus* monkeys (Perrin et al. 1984, Delplace et al. 1985, Inselburg et al. 1991, Enders et al. 1992, Knapp et al. 1992); and iii) a positive association between infection induced antibody response and the degree of protective immunity has been reported (Banic et al. 1998, Okech et al. 2001). The SERA gene, firstly isolated from the genomic DNA of FCR3 strain, is localized in the chromosome 2 of the *P. falciparum* genome (Biggs et al. 1989).

Although SERA showed a quite conserved sequence in *P. falciparum* isolates from different geographical origins including Asia, Africa, and South America (Bhatia et al. 1987, Delplace et al. 1988), two regions of polymorphism have been observed in different *P. falciparum* laboratory samples. The registered polymorphism comprises events of deletions/insertions in two repetitive regions of the protein: one in the polyserine region/serine repeats (SR) and one other in the amino-terminal region/octamer repeats (OR) that could comprise 5 instead of 6 octamer units (Fig. 1) (Bizik et al. 1988, Knapp et al. 1989, Morimatsu et al. 1997, Safitri et al. 2003). Basically, the number of OR, containing 8 amino acids each one, characterizes the polymorphism: the allele I, with 5 repeats, and the allele II, with 6 repeats (Li et al. 1989). This later region was showed to be very immunogenic (Banic et al. 1998) and is involved in the induction of protective immunity in non-human primates (Inselburg et al. 1993a,b, Suzue et al. 1997). Consid-

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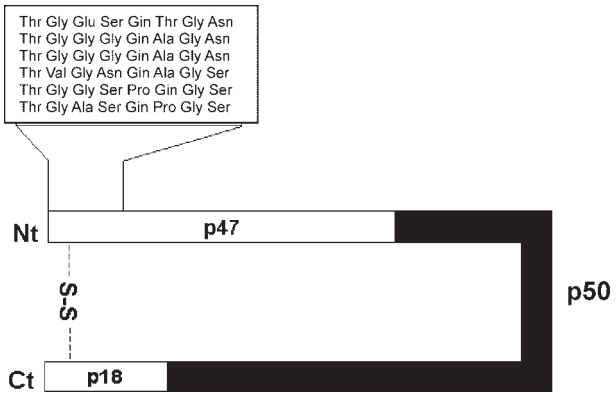

Fig. 1: sequence of the amino-terminal region of the serine rich antigen (SERA) of *Plasmodium falciparum.* At the end of asexual blood stage SERA is proteolytically processed into a 47 kDa N-terminal domain (p47/repeat motifs), a 50 kDa central domain (p50), and a 18kDa C-terminal domain (p18).

ering the potential inclusion of SERA in a malaria vaccine together to the fact that repeat motifs in the amino-terminal region of SERA contain epitopes recognized by parasite-inhibitor antibodies (Fox et al. 2002), the study of the genetic diversity of this protein is mandatory, since sequence variation in exon II may represent one of the parasite's immune-evasion strategies. By combining previously published sequences, it has been observed that FCR3 type alleles predominated in *P. falciparum* field isolates from Indonesia, Brazil, and Solomon Islands but have not been found in Myanmar and Africa (Safitri et al. 2003). Here we have investigated the frequency of polymorphism in exon II of SERA gene, which encodes most of the amino-terminal region of the antigen in *P. falciparum* field samples. The blood samples were colleted from *P. falciparum* infected individuals in three areas of the Brazilian Amazon: Porto Velho, at the state of Rondônia; Belém and Marabá, at the state of Pará; and Manaus, at the state of Amazonas. The patients were assisted at the Centro de Medicina Tropical de Rondônia in Porto Velho (n = 29), at the Instituto Evandro Chagas of the Secretaria de Vigilância em Saúde in Pará (n = 8), and at the Fundação de Medicina Tropical do Amazonas in Manaus (n = 15). Patients were invited to participate through the "Term of Post-informed Consent", informed of the objectives, and the role of their participation in the study and written consents were obtained. After submitting to each patient a questionnaire, which included personal history and epidemiological data, a venous blood sample (5 ml) was collected from each individual using vacutainer tubes containing EDTA. After centrifugation, packed red blood cells (RBC) were separated for DNA extraction and PCR analysis, and all the samples were cryopreserved in glycerolyte (w/v) in duplicate and stored in liquid nitrogen tank, until use. By using PCR it was possible to characterize two fragments (Fig. 2): one of 175 bp corresponding to the repeat region with 5 octamer units (allele I) and one other of 199 bp corresponding to the 6 repetitive octamer units (allele II) of SERA protein. As shown in the Table, the 199 bp fragment was the predominant one in all the studied areas. The higher frequency of this fragment could be explained by an immunological selection of the plasmodial population in the infected individuals under study (Daubersies et al. 1994). We should emphasize that mixed infections have not been observed in any of the isolates studied. In conclusion, if the here reported sequence polymorphism affects the immune recognition of SERA, the present re-

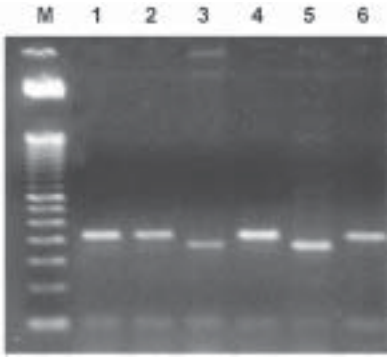

Fig. 2: analysis of polymerase chain reaction (PCR) products of DNA from *Plasmodium falciparum* infecting individuals living in three endemic Brazilian states. For PCR procedures, 1 ml of blood samples was suspended in 10 ml in a buffer solution (BPS), 100 µl EDTA (0.5M pH 8.0), and 100 µl saponin (15%). After centrifugation (300 x g – 10 min) the pellet was suspended in 300 µl NET buffer pH 7.5 (0.15 M Nacl, 0.01 M EDTA, 0.05 M Tris), 3 µl proteinase K (20 mg/ml), and 3 µl sarcosyl (10%). After incubation at 42°C for 24 h, DNA was extracted once with phenol/chloroform/isoamilyc alcohol (25:24:1) and then with chloroform/isoamilyc alcohol (24:1). The DNA was precipitated by the addition of 3M sodium acetate (1/10), 2 volumes ETOH (–20°C). After incubation –20°C for 16 h the DNA was centrifuged (30 min – 14,000 g) and the pellet was suspended in 1 ml cold ethanol (70% v/v). Before use centrifuged pellet was suspended in 100 µl TE buffer. For PCR, we used 5 µl of DNA extracted from blood samples. The sequences of primers used in the nested PCR were the following: A: $^{5'}$ AAT GAA GTC ATA TAT TTC CTT G $^{3'}$; B: $^{5'}$ CAA TGT TGT TCT TAA TTC GAT A $^{3'}$; C: $^{5'}$ GTG TTA TAT TTA ACA AAA ATG $^{3'}$; D: $^{5'}$ CTT ACA GGA TTG CTT GGT TCG $^{3'}$. DNA samples were amplified by double or nested PCR (Wataya et al. 1993). We firstly used the set of oligonucleotides A and B and for the second round C and D. A program of 35 cycles was used, in that each cycle corresponds to 1 min for 94°C, 1 min for 47°C and 2 min for 72°C. Distilled water was used as negative control. Electrophoresis was carried out through a 2% agarose gel in 0.5% Tris-Borate-EDTA (TBE) buffer. DNA bands were visualized by staining with ethidium bromide (0.5 µg/ml) and photographed. Oligonucleosomal fragments appeared as ladders of bands whose molecular sizes are approximate of 175 and 199 bp. Molecular size marker of 100 bp is shown on the left (M). Lanes 3 and 5 represents the fragments of 175 bp and lines 1, 2, 4, and 6 the fragments of 199 bp.

TABLE

Frequency of polymerase chain reaction (PCR) fragments of the repetitive region of serine rich antigen gene

	PCR products	
Samples (n)	199 bp (%)	175 bp (%)
Rondônia (29)	27 (93.1)	2 (6.9)
Pará (8)	5 (62.5)	3 (37.5)
Amazonas (15)	14 (93.3)	1 (6.6)
Total (52)	46 (88.5)	6 (11.5)

sults indicate that the analysis of the polymorphism of *P. falciparum* isolates is a fundamental stage before the final drawing of an universal vaccine against malaria can be reached.

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