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## *Plasmodium falciparum* Serine Repeat Protein, a New Target of Monocyte-Dependent Antibody-Mediated Parasite Killing

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**Using monoclonal antibodies and human affinity-purified antibodies specific to the *Plasmodium falciparum* 126-kDa serine-rich protein, SERP, we found that these antibodies have no direct effect upon merozoite invasion at the concentrations tested but can cooperate with blood monocytes to strongly inhibit *P. falciparum* in vitro growth.**

Since the original observation that immunization with a P140 protein purified from cultured *P. falciparum* parasites could confer protection in *Saimiri* monkeys (15), there has been a sustained interest in investigating the vaccine potential of this molecule, a 126-kDa serine-rich protein called SERA, SERP, or P126 (1) (referred to here as SERP). Further studies were carried out using two recombinant proteins of 262 (positions 24 to 285) and 483 (positions 24 to 506) amino acids from the N-terminal region of SERP which were used to immunize *Aotus* monkeys with Freund's adjuvant, i.e., under immunizing conditions which cannot be used in humans but which are optimal to induce strong immune responses (8). Challenge with blood-stage parasites revealed that a high degree of protection was obtained in four of six immunized monkeys (i.e., a 1,000-fold reduction in peak parasitemia), a level of protection among the highest reported for artificial immunization against malaria (10). Attempts to correlate the level of protection with immune responses were not conclusive. High antibody titers, as measured by enzyme-linked immunosorbent assay, were detected in three of the protected monkeys, but similar levels of antibody titers were also found among those less or not protected (8). Since a SERP-specific monoclonal antibody (MAb), 43E5, which reacts with the N-terminal region of the molecule was found to directly inhibit the growth of *P. falciparum* in vitro (2), the *Aotus* sera were studied for growth inhibition. However, sera containing high levels of SERP antibodies from immunized *Aotus* were not able to inhibit *P. falciparum* multiplication in vitro (J. Inselburg et al., unpublished data).

It was previously demonstrated that protection in humans was mediated by antibodies which have no significant effect upon merozoite invasion but which in contrast inhibited growth indirectly by cooperating with blood monocytes (3). This mechanism, called antibody-dependent cell inhibition (ADCI), is mediated by soluble components released by monocytes which block the division of intraerythrocytic parasites and

is triggered by merozoite surface components (4). This has led us to identify a new merozoite surface protein, MSP3, as a main target of ADCI-effective antibodies (11). We recently demonstrated that natural antibodies specific to another parasite antigen, glutamate-rich protein, can also inhibit *P. falciparum* multiplication through ADCI (17). We therefore thought it of interest to investigate whether the antibodies raised against SERP were implicated in ADCI. To this end we tested a panel of human and mouse antibodies in parallel assays of direct and ADCI-mediated parasite growth inhibition.

The direct merozoite invasion inhibition and ADCI assays were performed as previously described (3, 9). Blood mononuclear cells from healthy donors, separated on Ficoll-Hypaque, were distributed into 96-well flat-bottom plates (TPP, Trasadingen, Switzerland) at a rate of  $2 \times 10^5$  monocytes per well. After 1 h of incubation at 37°C in a 5% CO<sub>2</sub>-air mixture, nonadherent cells were removed by washings with RPMI. Monocyte viability was estimated by the nonspecific esterase stain. *P. falciparum* cultures at the schizont stage were added at a ratio of 200 red blood cells per monocyte. The culture medium (RPMI plus 10% Albumax) was supplemented with each of the antibodies to be tested, including positive control immunoglobulin G (IgG) from African adults and negative control IgG from European donors, in wells with and without monocytes. Starting parasitemia was 0.5% with 2% hematocrit. Only assays in which the final parasitemia reached  $\geq 10\%$  were kept for analysis. The specific growth-inhibitory index (SGI) was calculated as follows:  $100 \times \{1 - [( \text{percent parasitemia with monocyte and test antibody} / \text{percent parasitemia with test antibody without monocytes} ) / ( \text{percent parasitemia with negative control antibody with monocytes} / \text{percent parasitemia with negative control antibody without monocytes} )]\}$ .

We first studied two MAbs, 24C6 and 23D5, which are specific for two distinct epitopes derived from the N-terminal region of SERP (6, 7) and which recognize the 126-kDa polypeptide on Western blots of *P. falciparum* asexual blood-stage extracts. Neither antibody showed a significant direct inhibitory effect in the absence of monocytes at the dilutions tested (data not shown). In contrast, ADCI-mediated parasite

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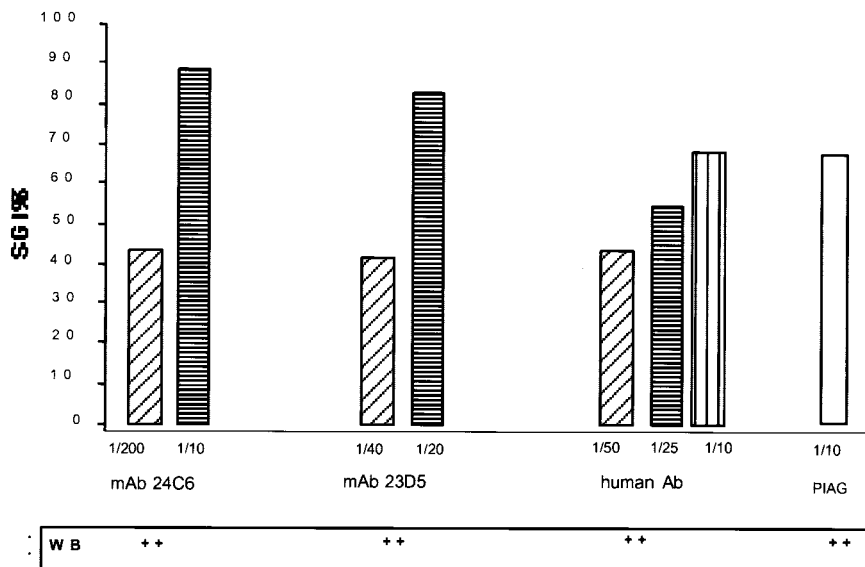


FIG. 1. ADICI results obtained using anti-SERP antibodies. Results were obtained with two SERP MABs and human antibodies affinity purified with the recombinant antigen SE47, at various concentrations (volume/volume in RPMI medium) in the ADICI assay. Results are expressed as the SGI as described in the text; values of >30% are significant. PIAG, positive control from the pool of Ivory Coast adult sera used in passive transfer in humans (3). WB, Western blotting; ++, positive.

killing was clearly observed, and parasite growth inhibition was dependent on antibody concentrations (Fig. 1). Results were reproducible with *P. falciparum* strains NF54, T9-96, and FCIP-150. We then investigated the activity of naturally acquired antibodies specific to SERP. A recombinant protein, SE47', corresponding to amino acid residues 17 to 382 of the N-terminal domain (16) was used to affinity purify specific antibodies from sera of African (Ivory Coast) immune adults (3) as described in reference 5. These antibodies, which were SERP specific (Fig. 2), had no direct effect on parasite growth but were able to exert a strong inhibitory ADICI-mediated effect in a dose-dependent manner (Fig. 1). Similar results (not shown) were obtained with affinity-purified antibodies from hyperimmune adults from Uganda (Fig. 2).

These results, obtained with mouse and human anti-SERP antibodies, establish SERP as a novel target for the antibody-dependent monocyte-mediated mechanism of inhibition of *P. falciparum*. This role of SERP in protection is in addition to the direct inhibition of growth obtained at high antibody concentrations (13, 14). Recent immunoepidemiological investigations carried out with sera from children living in an area of holoendemicity in Uganda support ADICI as a natural defense mechanism, since they revealed that higher levels of naturally induced IgG3 cytophilic antibodies against this antigen correlate with lower levels of peripheral parasitemia and an absence of malaria fever (12). The ADICI monocyte-dependent effect can be obtained at antibody concentrations which do not exert any direct inhibition of growth. Finally, our results reinforce the role of ADICI in the acquisition of immunity against malaria and broaden the range of parasite antigens implicated in this defense mechanism. We therefore suggest that ADICI assays should be used to assess the functional role of naturally induced antibodies and to monitor and optimize the protective efficacy of artificial immunization against malaria.

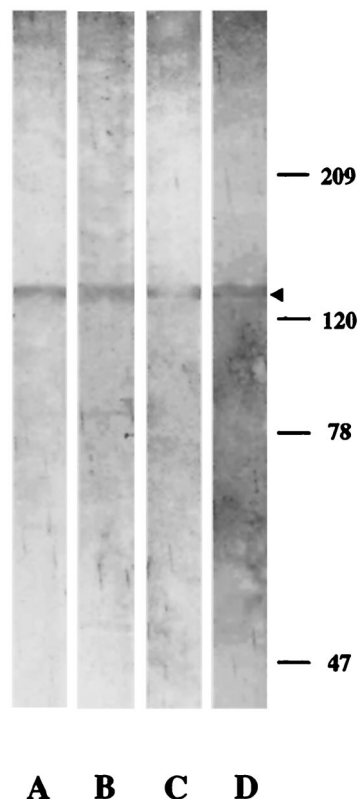


FIG. 2. Western blot analysis of *P. falciparum* schizont protein extract using anti-SERP antibodies. Parasite proteins were resolved by sodium dodecyl sulfate-8% polyacrylamide gel electrophoresis under denaturing conditions, electroblotted onto nitrocellulose, and probed with anti-SERP MABs 24C6 (lane A) and 23D5 (lane B) and affinity-purified human anti-SERP antibodies from hyperimmune sera from residents of Ivory Coast (lane C) and Uganda (lane D). Numbers on the right indicate the positions of molecular weight markers (in thousands). The arrowhead shows the position of SERP.

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#### ADDENDUM

We obtained 12 sera corresponding to the pre- and postimmunization samples from the six *Aotus* monkeys immunized with the SERP antigen, as described in reference 8. We found that only four of these *Aotus* sera were positive in Western blots of parasite extracts, showing a single polypeptide with a size similar to that of the protein revealed by the two anti-SERP MAbs. The same four sera, but not the remaining eight sera, exerted a strong ADCI effect (70 to 80% inhibitory effect).

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