Humoral Immune Responses of Africans to Cysteine Protease–Related Antigens of Plasmodium falciparum

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The Plasmodium falciparum serine repeat antigen (SERA) and serine repeat protein homologue (SERPH) contain highly conserved domains that appear to encode cysteine proteases or related proteins. Humoral immune responses against the protease domains of SERA and SERPH were evaluated. Malaria-immune Africans, but not nonimmune controls, demonstrated potent humoral responses against the protease domains. As the SERA and SERPH protease domains are likely accessible to circulating antibody, these results suggest that humoral responses to the domains may contribute to antimalarial immunity.

Despite extensive efforts, the development of a broadly effective malaria vaccine remains an elusive goal. Contributing to difficulties in vaccine development, malarial proteins that are exposed to the human immune response are often antigenically diverse, and immunologic pressure appears to elicit antigenic variation [1]. It seems appropriate, therefore, to consider as vaccine components malarial proteins that are targets of the host immune response but that are antigenically conserved. Among such proteins should be essential enzymes that are being studied as potential vaccine components [1]. SERA appears to be located in the parasitophorous vacuole surrounding the intraerythrocytic schizont [3] and on the surface of free merozoites [4], so it is likely accessible to circulating antibodies and other mediators of the host immune response. Supporting the consideration of SERA as a vaccine component, immunization with intact SERA [5] and amino-terminal SERA fragments [6] induced protective immunity in monkeys. The role of the carboxy-terminal portion of SERA in immune responses has been relatively little studied, although a hybrid vaccine that included carboxy-terminal SERA fragments also induced protective immunity in monkeys [7], and rat antisera produced against this portion of SERA inhibited the growth of malaria parasites in vitro [8]. In addition, in studies of carboxy-terminal fragments of SERA homologues from the murine parasite Plasmodium vinckei, mice immunized by serial infection and drug cure demonstrated humoral and cellular immune responses against the fragments, and naive mice immunized with one of the fragments were partially protected against lethal malaria [9]. Sequence comparisons of SERA [2] and a related schizont protein, the serine-rich protein homologue (SERPH) [10], show


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that an ~30-kDa domain of each protein located near the carboxy terminus has homology with cysteine proteases, particularly near highly conserved active site residues. This observation suggests that one or both of these late schizont/merozoite proteins act as a protease involved in the processing of malarial proteins in mature schizonts and/or the cleavage of host cytoskeletal proteins during erythrocyte rupture and invasion. The protease domains of SERA and SERPH are well-conserved; their amino acid sequences are 56% identical, although in SERA the predicted active site cysteine has been replaced by a serine [10]. The SERA protease domain is also highly conserved among \( P. falciparum \) isolates; the amino acid sequences of 10 independent isolates were identical, except for a single conservative substitution in some field isolates [11]. As the conserved protease domains appear to be exposed to circulating antibody at the time of erythrocyte rupture and invasion and as they may mediate essential enzymatic functions, antibodies directed against the SERA and SERPH protease domains, if present, may contribute to antimalarial immunity. Human humoral responses to the carboxy-terminal portions of SERA have not previously been studied. To determine if antibodies directed against the SERA and SERPH protease domains are produced in response to malarial infection, we compared immune responses against the protease domains in persons living in areas in which falciparum malaria is endemic with responses in nonimmune controls.

**Materials and Methods**

**Parasite culture.** \( I \)-strain \( P. falciparum \) parasites were cultured and, extracted of \( P. falciparum \) soluble proteins were prepared by saponin lysis of erythrocytes as previously described [12].

**Amplification of SERA and SERPH protease domains.** \( P. falciparum \) genomic DNA was purified by standard methods, and DNA encoding the protease domains of SERA and SERPH was amplified by polymerase chain reaction. The primers used were 5′-TGTAAATAAGAATTTGGA-3′ and 5′-GTGAAATAAGTTAAAATGACA-3′ for SERA, encoding a 746-bp construct, and 5′-TATGAAATATTTGGAATGTTGAC-3′ and 5′-GAGTATTTGTATTCTCCACAG-3′ for SERPH, encoding a 1007-bp construct that was homologous to the SERA protease domain but included an additional ~250 bp downstream from the highly conserved protease domain.

**Expression and purification of protease domains.** The DNAs encoding the protease domains were ligated into pET-30 expression vectors (Novagen, Madison, WI), using NeoI and HindIII cleavage sites. BL21(DE3)pLysS strain \( E. coli \) were transformed with these plasmids and, for the expression of control thioredoxin, the pET-32 plasmid, which contains a thioredoxin gene. Protein expression was induced by incubating log-phase bacterial cultures with isopropyl-\( \beta \)-D-thiogalactoside (1 mM) for 5 h before harvesting the bacteria. Expressed polypeptides were affinity-purified on a nickel nitrilo-triacetic acid resin column (Qiagen, Chatsworth, CA), as previously described [9].

**Blood samples.** Serum or plasma samples were provided from healthy adults residing in two regions of Africa in which falciparum malaria is highly endemic. Twenty samples were from persons (ages 18–69; equal numbers of men and women) residing in Brefet, The Gambia, an area with heavy seasonal transmission of malaria, and 15 samples were from persons (all age >18) residing in Kampala, Uganda, an area with year-round high rates of transmission. Control sera were collected from 7 adults in the United States with either no history or only an uncertain, very distant history of malaria.

**Characterization of humoral responses.** IgG responses directed against SERA and SERPH protease domains were assessed by ELISA. Purified SERA and SERPH protease domains (10 \( \mu \)g/mL in 0.1 M sodium bicarbonate, pH 8.3; 50 \( \mu \)L/well), purified control thioredoxin (10 \( \mu \)g/mL), and a soluble extract of \( P. falciparum \) trophozoites (20 \( \mu \)g/mL) were fixed to microtiter plates (Immunon 4; Dynatech, Chantilly, VA) by overnight incubation. The plates were washed with PTE (PBS, 0.05% Tween 20, 1 mM EDTA) and blocked with 5% nonfat dried milk in PTE, and individual sera from Africans and controls were incubated with the antigens overnight at 4°C in PTE. After washing with PTE, the plates were then incubated with 1:1000 dilutions (in PTE) of second antibody (goat anti-human IgG; Pierce, Rockford, IL) for 1 h, washed again, and developed by the addition of \( p \)-nitrophenyl phosphate. Absorbance at 405 nm was read after an equal period of development for each sample in a given experiment. To determine appropriate dilutions for sera in these studies, pooled serum samples from Africans and controls were evaluated as discussed above at serial dilutions of 1:100–1:3200. The steepest slopes of the titration curves were generally at dilutions of 1:100–1:800. The dilution of 1:800 was therefore chosen for evaluations of individual sera.

To study the subclass specificity of humoral responses to \( P. falciparum \) protease domains, the presence of antibodies against the protease domains was evaluated in pooled sera, diluted at 1:100 and 1:800, by ELISA, as discussed above, with second antibodies (Sigma, St. Louis) directed against each of the four human IgG subclasses.

**Results**

**Evaluation of SERA and SERPH protease domains.** Sequencing of \( I \)-strain DNAs encoding the protease domains of SERA and SERPH showed that they were identical to the previously published sequences from other strains [2, 11] except for one silent (not changing the amino acid sequence) substitution in some field isolates [11]. As the protease domains are well-conserved; the amino acid sequences of 10 independent isolates were identical, except for a single conservative substitution in some field isolates [11]. As the conserved protease domains appear to be exposed to circulating antibody at the time of erythrocyte rupture and invasion and as they may mediate essential enzymatic functions, antibodies directed against the SERA and SERPH protease domains, if present, may contribute to antimalarial immunity. Human humoral responses to the carboxy-terminal portions of SERA have not previously been studied. To determine if antibodies directed against the SERA and SERPH protease domains are produced in response to malarial infection, we compared immune responses against the protease domains in persons living in areas in which falciparum malaria is endemic with responses in nonimmune controls.

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also demonstrated antibody responses directed against the SERA and SERPH protease domains (Figure 1). In general, the magnitude of responses to the two antigens was similar for a given individual. Responses of nonimmune controls to both plasmodial extract and the protease domains were much lower than those of Africans. To control for nonspecific hyperreactivity of the immune sera, responses were also tested against bovine serum albumin (BSA; not shown) and the E. coli protein thioredoxin, which was expressed in E. coli and purified exactly as were the SERA and SERPH protease domains. Responses of the immune sera to thioredoxin were higher in Africans than in controls and somewhat higher than those to BSA, suggesting that some of the reactivity of African sera against E. coli–expressed antigens was due to nonspecific hyperreactivity against E. coli proteins. However, reactivity to SERA and SERPH was consistently higher than that against the controls, particularly with the Gambian sera, in which reactivity with thioredoxin was low.

**Subclass specificity of humoral responses to SERA and SERPH protease domains.** Samples from nonimmune controls and immune populations were pooled, and the presence of antibodies against the protease domains was evaluated by ELISA with second antibodies directed against each of the four human IgG subclasses (Figure 2). In analyses using 1:100 and 1:800 (not shown) dilutions of antisera, antibodies directed against P. falciparum extracts and the SERA and SERPH protease domains were primarily of the IgG1 subclass.

**Discussion**

The *P. falciparum* proteins SERA and SERPH contain conserved protease domains that are likely accessible to circulating antibodies and that may mediate critical functions in mature erythrocytic parasites. We hypothesize that antibodies directed against these domains inhibit key functions and thus contribute to antimalarial immunity. To begin to test this hypothesis, we evaluated humoral responses against the protease domains of SERA and SERPH. Persons from two regions of Africa in which falciparum malaria is highly endemic, but not nonimmune controls, demonstrated potent humoral responses against the protease domains.

Responses were quantitatively greater to SERPH than SERA. It is not clear whether this difference relates to a difference in the antigenicity of the two protease domains or to the fact that the SERPH construct included an additional 85 amino acids carboxy-terminal to the protease domain. Reactivity with the E. coli–expressed thioredoxin control was greater in the Ugandan than the Gambian sera, perhaps because of differences in infection rates with human immunodeficiency virus or other pathogens between the groups. The increased nonspecific reactivity of the Ugandan sera served to diminish the difference between responses to the control antigen and the SERA and SERPH protease domains, but quantitative responses to the protease domains in sera from the two regions were similar. Overall, the results clearly indicated that specific antibody responses were directed by malaria-immune Africans against the protease domains of SERA and SERPH.
In the Africans studied, antibodies directed against the SERA and SERPH protease domains were primarily of the IgG1 subclass. This subclass is cytophilic and relatively long-lived, suggesting that it is most likely, among available subclasses, to provide effective humoral protection against malaria [13]. In studies of other plasmodial antigens, persons demonstrating humoral responses to merozoite surface protein 1 also produced primarily IgG1 [14], but antibodies directed against merozoite surface protein 2 were primarily of the IgG3 subclass [15], which is relatively short-lived, and so may not provide long-term protection [13].

Our results are consistent with the hypothesis that circulating antibodies directed against the SERA and SERPH protease domains contribute to antimalarial immunity. Humoral responses to carboxy-terminal protease domains of both SERA and SERPH were seen in malaria-immune persons. There is, however, as yet no direct evidence that the protease domain–directed antibodies we identified (or additional antibodies recognizing conformational epitopes not present in the recombinant antigens) contribute to the protective responses of malaria-immune persons. Further studies are needed to evaluate the biologic roles of SERA and SERPH and the immunologic effects of antibodies directed against the protease domains of these proteins.

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References


