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Immunology

Characterization of an S antigen synthesized by several isolates of *Plasmodium falciparum*

(malaria/cloned parasites/Papua New Guinea/immunoblotting)

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ABSTRACT **The S antigen of a Papua New Guinean isolate of *Plasmodium falciparum* was identified by immunoblotting as the dominant antigen in culture supernatants. An antigen identical in molecular weight (M_r 220,000), isoelectric point (pI 4.2), and immunoreactivity with sera from individuals exposed to malaria was expressed by four Papua New Guinean isolates and one isolate of unknown origin. The M_r 220,000 antigen was not detected in culture supernatants derived from two isolates from Thailand and one from Ghana. The M_r 220,000, pI 4.2 S antigen may characterize a subpopulation of parasites common to many isolates of *P. falciparum*, which is selected for by continuous culture *in vitro*. A variant S antigen, 30 kilodaltons larger but with similar immunoreactivity, was expressed by 1 of 26 clonal populations derived by limit-dilution culture from one of the Papua New Guinean isolates of *P. falciparum*. The characteristics of the S antigen, defined by immunoblotting, allowed it to be identified in two-dimensional separations of [^{35}S]methionine-labeled parasite proteins, thus confirming the parasite origin of the antigen.**

Plasmodium falciparum, the major cause of morbidity and mortality in malaria infections of man, exhibits considerable antigenic heterogeneity, which may be a major problem in developing an effective vaccine against malaria. The most extensive studies of antigenic heterogeneity in *P. falciparum* have concerned a system of soluble heat-stable antigens known as the S antigens. The S antigens were first demonstrated in extracts of infected placentas but are also found in the sera of some infected individuals. The S antigens, which have been the subject of a series of detailed studies by Wilson and colleagues (1–9), have the following characteristics: (*i*) stability to heating at 100°C for 5 min; (*ii*) serological complexity with individual isolates expressing more than one specificity; (*iii*) poor immunogenicity—no heterologous antiserum has been produced and there is a marked age-dependent acquisition of antibodies in man; (*iv*) presence in the medium when *P. falciparum* is maintained in culture *in vitro*; and (*v*) molecular weight and charge heterogeneity.

Techniques such as two-dimensional gel electrophoresis of biosynthetically labeled immunoprecipitates (10) and immunofluorescence with monoclonal antibodies (11) have provided new approaches to the direct demonstration of antigenic heterogeneity in *P. falciparum*. However, these techniques have not been directly applied to an analysis of the S antigens. A major limitation to the more detailed analysis of S antigens has been the failure to label the antigens with radioactive metabolites (12). Thus, to date, analyses of the S antigen have been dependent on relatively insensitive gel precipitation techniques and have been restricted to sources in which antibodies and antigens are sufficiently abundant to give visible precipitates.

To circumvent the apparent problems of biosynthetic labeling S antigens, we have used the technique of "immunoblotting" to identify the presumptive S antigen in the culture supernatants of several isolates of *P. falciparum*. The S antigen appears to be far more abundant than any other antigen in culture supernatants. It has a M_r of 220,000 and was detected in all Papua New Guinean (PNG) isolates examined and in an isolate of unknown origin but not in one African and two Thai isolates examined.

MATERIALS AND METHODS

Parasites and Culture Supernatants. The following isolates of *P. falciparum* were used in these studies: FCQ2/PNG (FC2), FCQ27/PNG (FC27), FCQ29/PNG (FC29), and FCQ46/PNG (FC46) (all established from Papua New Guineans living in the Madang Province of PNG); T9-94 (a cloned line established from the Thai isolate T9); K1 (Thailand); NF7 (Ghana); and NF36 (established from an airport worker in Amsterdam but otherwise of unknown origin). T9-94, K1, NF7, and NF36 were generously made available to us by D. Walliker (University of Edinburgh). Cloned lines were derived from isolate FC27 by limit-dilution culture. All isolates were maintained in Hepes-buffered RPMI 1640 medium containing 10% serum by using the method of Trager and Jensen (13).

Sera. Sera were obtained from blood donors and villagers in the Madang Province during the course of a longitudinal seroepidemiological study of malaria in this region. Blood was allowed to clot at room temperature, and serum was removed after centrifugation and stored in Madang at −20°C for up to 3 months. Subsequently, the sera were transported to Melbourne on dry ice, where they were stored at −70°C. At the time of bleeding, thick and thin blood films were prepared and stained with Giemsa for subsequent microscopic examination for malaria parasites. Control sera were obtained from healthy adults living in Melbourne, where there is no transmission of malaria.

Gel Electrophoresis and Isoelectric Focusing (IEF). Culture supernatants were electrophoresed on 7.5% $NaDodSO_4$/polyacrylamide gels (14). Culture supernatants, which in some experiments had been heated at 100°C for 5 min and centrifuged (12,000 × *g* for 10 min) to remove heat-denaturable antigens, were usually diluted 1:10 in sample buffer and heated at 100°C for 2 min, and 40 μl of the final solution was added to each gel lane.

IEF was performed in 0.5-mm thick slab polyacrylamide gels by using an LKB 2112 multiphor unit. The gels incorporated

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Abbreviations: PNG, Papua New Guinea; IEF, isoelectric focusing; RIA, radioimmunoassay.

ampholines covering the pH range 4–6 at a final concentration of 5%. Samples were diluted 1:5 in distilled H_2O and 20-μl aliquots were applied to filter paper wicks towards the cathodal end of the gel. Focusing was performed at 4°C for 2 hr with the initial current of 25 mA and a final voltage of 2 kV.

Transfer of Proteins to Nitrocellulose and Detection of Antigens. After one-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis, culture supernatant proteins were electrophoretically transferred to nitrocellulose sheets (15). After transfer, nitrocellulose sheets were incubated in 10 mM Tris·HCl, pH 7.4/0.15 M NaCl/3% bovine serum albumin (Tris/NaCl/albumin) for 1 hr at room temperature before being probed for 90 min with sera diluted (most commonly 1:500) in the same solution. The nitrocellulose sheets were washed twice in Tris/NaCl and twice in Tris/NaCl/0.05% Triton X-100 and then were reacted for 30 min with ^{125}I-labeled protein A (^{125}I-protein A) (250,000 cpm/ml in Tris/NaCl/albumin). The protein A was labeled to a specific activity of 40 μCi/μg (1 Ci = 3.7×10^{10} Bq) by using the Chloramine-T method (16). The nitrocellulose sheets were washed again by using the same procedure and air dried, and autoradiographs were prepared by exposure to Agfa Curix RP-2 film employing DuPont Lightning Plus intensifying screens. Exposure times required for Figs. 1–5 ranged from 3 to 24 hr.

After IEF, culture supernatant proteins were transferred to nitrocellulose sheets by passive blotting (17). The nitrocellulose transfers were then processed in the same way as the electrophoretic transfers to visualize the bound antigens.

Biosynthetic Labeling and Immunoprecipitation. Parasites were biosynthetically radiolabeled by growing in the presence of [^{35}S]methionine. This procedure and the methods used to immunoprecipitate the labeled antigens and to analyze them by two-dimensional electrophoresis were the same as used previously (10).

Preparation of S Antigen-Rich Fraction from Culture Supernatants. An S antigen preparation free of significant amounts of other antigens was required for use in a solid-phase radioimmunoassay (RIA) to measure levels of antibody to S antigen in sera. Supernatants (200 ml) from cultures of FC27 were heated at 100°C for 5 min and the heat-aggregated protein was removed by centrifugation at 12,000 × *g* for 20 min. The supernatant was subjected to $(NH_4)_2SO_4$ precipitation and the protein precipitating between 30% and 40% saturation was collected. The precipitate was dissolved in a minimal volume of distilled H_2O and loaded onto a Sepharose CL-6B gel filtration column (90 × 2.5 cm). The column was equilibrated with 0.1 M Tris·HCl, pH 8.0/0.5 M NaCl and was eluted with the same buffer at a flow rate of 30 ml/hr. Fractions containing the S antigen were identified by immunoblotting, pooled, concentrated ≈5-fold in an Amicon-stirred cell by using a YM-10 membrane, equilibrated with 0.025 M histidine·HCl at pH 6.2 by passing over a PD-10 dialyzing column (Pharmacia), and then further fractionated by chromatofocusing. This procedure employed a 1 × 12 cm column of Polybuffer exchanger (PBE94, Pharmacia) initially equilibrated at pH 6.2 with 0.025 M histidine·HCl and eluted at 40 ml/hr with 9 column vol of Polybuffer 74-HCl at pH 4.0. S antigen remained bound to the PBE94 after the completion of this procedure but was recovered from the column by eluting with a further 2 column vol of Polybuffer 74-HCl (pH 4.0) made 1 M with NaCl. The fractions containing S antigen were pooled, aliquoted, and stored at −80°C. It was estimated that the antigen was purified ≈300-fold by this procedure. However, it constituted no more than 5% of the final preparation, which contained serum albumin as a major component.

RIA for Antibodies to the S Antigen of FC27. The S antigen preparation was diluted to 3 μg/ml in phosphate-buffered saline (P_i/NaCl) and 50-μl amounts were added to the wells of a flexible polyvinyl chloride microtiter plate (Dynatech, Alexandria, VA). After 2 hr of incubation in a moist box at room temperature, the plates were washed three times with P_i/NaCl/0.5% Tween 20 and twice with H_2O and duplicate 1:100 and 1:1,000 dilutions (in 0.5% albumin/P_i/NaCl/Tween 20) of serum samples were added to the wells. On each plate duplicate serial 2-fold dilutions (from 1:100 to 1:6,400) of a standard positive serum were included. This standard serum was arbitrarily assigned a level of 1,000 units/ml of antibody to S antigen. Serum dilutions were incubated in the wells at room temperature for 2 hr, after which time they were removed by aspiration and the plate was washed as before. Bound antibodies were detected by a 60-min incubation with ^{125}I-protein A (20,000 cpm in 50 μl per well) diluted in albumin/P_i/NaCl/Tween 20. The washed plates were cut up by using a hot wire, and radioactivity in the wells was counted on an automatic gamma spectrometer. The units of antibodies to S antigen in each serum were determined by reference to a standard curve constructed from the data obtained for dilutions of the standard serum on the same plate.

RESULTS

Identification of a Presumptive S Antigen in Culture Supernatants. The antigens in culture supernatants, fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose, were initially probed with a 1:500 dilution of a pool of sera prepared from 10 adult Papua New Guineans having high antimalarial antibody titers. A major antigen, M_r 220,000, was detected in the culture supernatant of PNG isolate FC27 (Fig. 1*A*, lane 1) but not in the supernatant of K1, an isolate from Thailand (Fig. 1*A*, lane 3). Consistent with this antigen being an S antigen, heating the supernatants at 100°C for 5 min before dilution in the electrophoresis sample buffer had no effect on the intensity of the antigen detected on the immunoblot (Fig. 1*A*, lane 2).

Subsequently, long-term culture supernatants from a variety of *P. falciparum* isolates were examined by the same procedure by using either the pool of high-titer sera as a probe (as in Fig. 1*A*) or, alternatively, the one serum in the pool that had a significant level of antibodies to the antigen in FC27 culture supernatants. Both probes detected a heat-stable antigen of similar size in the supernatants of the four PNG isolates examined and in the supernatant of NF36, the isolate of unknown origin established from an Amsterdam airport worker. The superna-

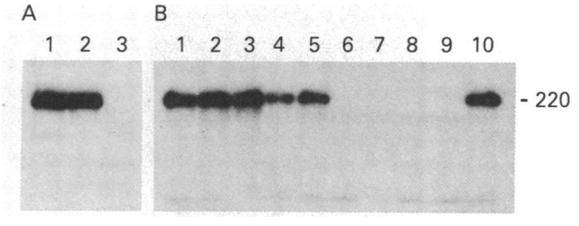

FIG. 1. Immunoblot of one-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis separation of culture supernatants from various isolates of *P. falciparum* probed with a pool of PNG sera (*A*) and probed with a single serum (*B*). The serum in each case was diluted 1:500. Unheated culture supernatants were from four PNG isolates: FC27 (*A*, lane 1, and *B*, lane 2), FC2 (*B*, lane 4), FC29 (*B*, lane 5), and FC46 (*B*, lane 1); two isolates from Thailand: K1 (*A*, lane 3, and *B*, lane 6) and T9-94 (*B*, lane 8); one isolate from Ghana: NF7 (*B*, lane 9); and one isolate of unknown origin: NF36 (*B*, lane 10). Heated supernatants were from the PNG isolate FC27 (*A*, lane 2, and *B*, lane 3) and the Thailand isolate K1 (*B*, lane 7). Molecular weight is shown as $M_r \times 10^{-3}$.

tants of the two isolates from Thailand and the Ghanaian isolate lacked an antigen (of equivalent abundance) reactive with either the PNG serum pool or the individual serum of high reactivity (Fig. 1*B*).

Identification of the S Antigen in Immunoblots of IEF Gels. As each of the PNG isolates and NF36 had a dominant antigen in the culture supernatant identical with respect to apparent molecular weight and reactivity with one serum, we examined the isoelectric points of the antigen in each of the supernatants. Focused culture supernatants transferred to nitrocellulose by direct blotting were probed with the same PNG serum pool and single serum as was used for probing the electrophoretic blots of the $NaDodSO_4$/polyacrylamide gel electrophoresis separations. By using a narrow pH range of ampholines (pH 4–6), a major antigen with a pI close to 4.2 was detected that had the same isolate distribution as the antigen detected on blots of the $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 2). There appeared to be some heterogeneity of the S antigen as one or two minor bands focusing just cathodally to the major antigen were present in each sample (Fig. 2). The heavily labeled material at the cathodal end of the transfer that was reactive with the ^{125}I-protein A probe was presumed to be immunoglobulin from the serum in the culture supernatant.

S Antigen in Culture Supernatants of Cloned Populations of *P. falciparum*. Studies of the S antigens produced by individual cloned populations derived from FC27 have confirmed that parasites in one isolate can produce different S antigens. The culture supernatants of 26 different cloned lines, which were selected from FC27 by limit-dilution culture, were examined by immunoblotting of one-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis separations. In 25 cases, a dominant antigen was seen that was identical in apparent molecular weight with the antigen present in supernatants from cultures of FC27, the parental isolate. In one case (clone E12), the M_r 220,000 antigen was absent but an equally dominant larger antigen (M_r ≈ 250,000) was present (Fig. 3*A*, lane 4).

Immunoblots of IEF gels established that the S antigen characterizing clone E12 also differed in isoelectric point, focusing slightly cathodally relative to the dominant S antigen in the parental isolate and other clones (Fig. 3*B*). The antigen in the clones

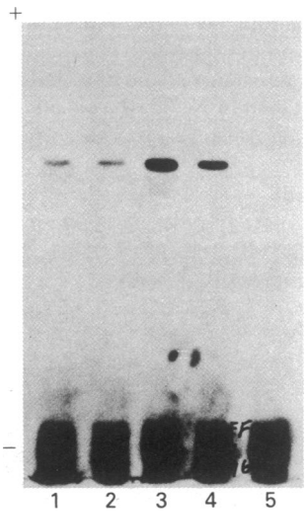

FIG. 2. Immunoblot of IEF gel on which *P. falciparum* culture supernatants were fractionated. Three samples were from PNG isolates: FC27 (lane 1), FC2 (lane 2), and FC29 (lane 3); one of unknown origin: NF36 (lane 4); and one from Thailand: K1 (lane 5). The transfer was probed with a 1:500 dilution of the same serum used to obtain the results depicted in Fig. 1*B*.

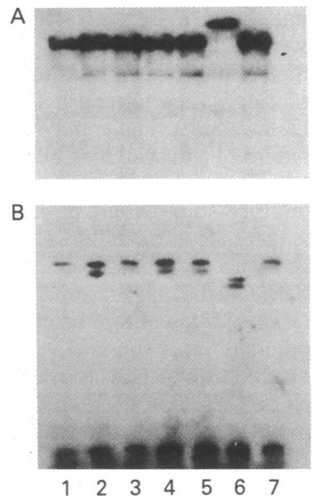

FIG. 3. (*A*) Immunoblot of $NaDodSO_4$/polyacrylamide gel electrophoresis separation of culture supernatants from clonal populations of *P. falciparum* (lanes 1–6) derived from PNG isolate FC27 (lane 7). (*B*) Immunoblot of isoelectric focusing gel on which the same culture supernatants examined in *A* were fractionated. Both transfers were probed with a 1:500 dilution of a single PNG serum.

focused as a doublet as did the antigen in the parental isolate. Thus, this charge heterogeneity does not represent different S antigens produced by different parasite subpopulations.

Prevalence of Antibodies to FC27 S Antigen. A number of sera from Papua New Guineans (all having high titers of antimalarial antibodies) were screened by RIA for reactivity against the M_r 220,000 antigen semipurified from FC27 culture supernatants. The majority of the sera had negligible levels of antibodies to this antigen, but ≈10% of the sera had readily detectable levels. Several of the sera were also screened for reactivity against concentrated (30-fold), heated (100°C for 5 min) FC27 culture supernatant in immunodiffusion tests and for ability to detect the M_r 220,000 antigen on immunoblots (Table 1). There was a clear correlation between a high value in the RIA, ability to react in immunodiffusion, and reactivity with the M_r 220,000 antigen on immunoblots, indicating that all three assays de-

Table 1. Correlation between ability of sera to detect FC27 S antigen in RIA, immunodiffusion, and immunoblots

Serum sample	Antimalaria ELISA titer	Antibodies to S antigen of FC27 in RIA, units/ml	Immunodiffusion test*	Immunoblot†
1	1:195,000	3,120	+	+
2	1:7,290	1,030	−	+
3	1:7,290	13	−	−
4	1:65,000	112	−	+
5	1:270	9	−	−
6	1:7,290	92	−	−
7	1:21,870	12	−	−
8	1:21,870	11	−	−
9	1:2,430	56	−	−
10	1:7,290	86	−	−
11	1:65,000	2,800	+	+
12	1:2,430	14	−	−

ELISA, enzyme-linked immunosorbent assay.

* Sera that generated a precipitin band visible at 48 hr without staining were scored positive.

† Sera used in a dilution of 1:500 that detected the M_r 220,000 antigen with an overnight exposure of the autoradiograph were scored positive.

tected antibodies of the same specificity—i.e., antibodies to FC27 S antigen.

Identification of the S Antigen as a Parasite Product. The identification of a dominant and minor variant S antigen in clones of FC27 allowed the equivalent proteins in two-dimensional gels of [^{35}S]methionine-labeled parasite lysates to be identified. In FC27 a protein complex (labeled S in Fig. 4*A*) having the approximate pI and size of the S antigen was absent from the two-dimensional gels of both E12 (the variant clone of FC27) and K1 (Fig. 4 *B* and *C*). However, in E12 a unique protein was present (S′ in Fig. 4*B*), having a similar charge to protein S in FC27 but with an $M_r \approx 30,000$ larger.

Further evidence that the ^{35}S-labeled proteins S and S′ correspond to the respective S antigens was obtained from immunoprecipitation experiments. Sera lacking detectable reactivity in the RIA to S antigen of FC27 failed to precipitate either of these proteins but sera positive in the RIA did precipitate protein S (Fig. 5). Interestingly, protein S seen in the two-dimensional gels of immunoprecipitates (Fig. 5 *B* and *C*) displays both charge and molecular weight heterogeneity, neither of which is accounted for by the homogeneous spot S′ (Fig. 4*B*) presumed to be the S antigen in E12, the cloned line derived from FC27. It is possible that the different spots in the S complex represent S antigen specificities characterizing different clonal populations of *P. falciparum* within the FC27 isolate.

DISCUSSION

The S antigens of *P. falciparum* are heat-stable antigens showing remarkable serologic diversity among different isolates of *P. falciparum*. Despite extensive studies of the prevalence and distribution of the antigens and antibodies to them (1–9), they remain poorly characterized in molecular terms. The studies described here provide evidence that the S antigen released into culture supernatants by several isolates of *P. falciparum* adapted to continuous culture *in vitro* is an acidic protein (pI 4.2) of M_r 220,000. The immunoblotting technique used here has provided a simple means of detecting the antigen in unconcentrated culture supernatants and, with sera of appropriate specificity, will enable S antigens of diverse isolates of *P. falciparum* to be characterized.

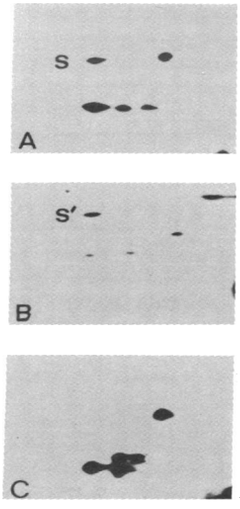

FIG. 4. Two-dimensional gel separations of [^{35}S]methionine-labeled whole infected cell lysates of the PNG isolate FC27 (*A*), the clonal population derived from FC27 that has a large S antigen, E12 (*B*), and an isolate from Thailand, K1 (*C*). Each panel encompasses only those proteins with M_rs > 160,000 and with pIs < 4.5.

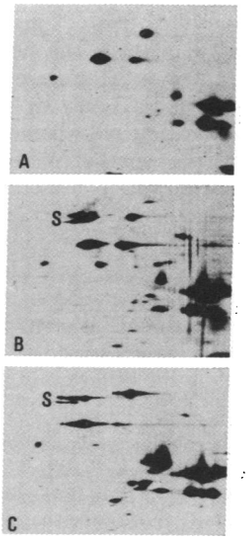

FIG. 5. Two-dimensional gel separations of [^{35}S]methionine-labeled antigens immunoprecipitated from lysates of erythrocytes infected with FC27 by using sera from individuals chronically exposed to malaria. (*A*) Immunoprecipitates generated with a serum lacking antibodies to the S antigen. (*B* and *C*) Immunoprecipitates generated with sera with antibodies to the S antigen of FC27.

In addition to the acidic nature and molecular size (18), there are a number of other characteristics that have led us to conclude that the M_r 220,000 antigen described here is the S antigen of Wilson *et al.* (1–9): antigenic activity stable to heating at 100°C for 5 min; abundance in culture supernatants; and stable expression of the antigen in isolates cultured *in vitro* for long periods. Furthermore, a minority of individuals exposed to endemic malaria has a high level of antibodies to the antigen.

Wilson *et al.* (8, 9) did not observe geographical restriction of S antigen serotypes. This contrasts with our finding that an antigen identical in size, charge, and immunoreactivity characterized four PNG isolates but was absent from three non-PNG isolates (NF36, which also had the identical antigen, is of unknown origin). One explanation for this finding could be cross-contamination during parasite culture with the result that one parasite population has overgrown the other isolates. This is an unlikely explanation because other isolates cultured under the same conditions have maintained their antigen "negative" status. A more likely explanation is that long-term isolates are a selected subset of parasites and therefore express a limited number of S antigen specificities. Supporting this explanation is the report by Wilson (9) that there is restricted expression of S antigen serotypes in isolates of *P. falciparum* adapted to growth in the *Aotus* monkey.

The results of S antigen analysis of clonal populations of parasites derived from FC27 clearly established that despite the stable expression of the M_r 220,000, pI 4.2 S antigen over a prolonged period of *in vitro* culture, parasites persist within the isolate capable of expressing other specificities. Only one alternative antigen ($M_r \approx 250,000$, pI 4.3) has been identified so far, but the heterogeneity revealed on two-dimensional gel analysis of FC27 suggests that there may be others. The antigen characterizing E12 (i.e., the M_r 250,000 S antigen) must be closely related structurally to the M_r 220,000 antigen, as no serum has been found that reacts with one but not the other (data not shown).

The size and charge characteristics of the S antigens established by immunoblotting of $NaDodSO_4$/polyacrylamide gel electrophoresis and IEF gels allowed identification of the

antigen on two-dimensional gels of biosynthetically labeled ([^{35}S]methionine) parasite proteins. The identification was based on the presence of a protein with the correct strain distribution, which was immunoprecipitated only by sera with significant titers of antibody to S antigen and which was slightly larger in E12. Thus, contrary to the report of Wilson (12), S antigen can be biosynthetically labeled with [^{35}S]methionine and therefore is of parasite rather than modified host origin.

Tait (19) has analyzed the ^{35}S-labeled proteins of eight isolates of *P. falciparum* and found variant forms of 14 proteins. The most variable (six variants among eight isolates) was a high molecular weight acidic protein. It seems highly probable that this protein (protein 1), which varied in charge and size (M_r 130,000 to >200,000) in the different isolates, is S antigen.

Serological diversity in *Plasmodium* species infecting man is believed to be a major factor in the slow development of protective immunity to malaria. This implies that host-protective immune responses are directed in part against antigens that exhibit serological diversity. Evidence that S antigens may be targets of host-protective immune responses is the finding that an inhibitory monoclonal antibody (20) appears to recognize the S antigen of FC27 (unpublished data). Paradoxically, there was no correlation between the titer of antibodies to the S antigen of FC27 and the inhibitory activity of different PNG antibody preparations for the growth of FC27 *in vitro* (unpublished data).

If antibodies to S antigens are protective, it seems likely that the release of large amounts of the antigen into the serum during acute infections is a mechanism designed to help the parasite evade this host response.

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1. Wilson, R. J. M., McGregor, I. A., Hall, P., Williams, K. & Bartholomew, R. (1969) *Lancet* **ii**, 201–205.
2. Wilson, R. J. M. (1970) *Trans. R. Soc. Trop. Med. Hyg.* **64**, 547–554.
3. McGregor, I. A. & Wilson, R. J. M. (1971) *Trans. R. Soc. Trop. Med. Hyg.* **65**, 136–145.
4. Wilson, R. J. M. & Voller, A. (1972) *Parasitology* **64**, 191–195.
5. Wilson, R. J. M., McGregor, I. A. & Wilson, M. E. (1973) *Int. J. Parasitol.* **3**, 511–520.
6. Wilson, R. J. M. & Bartholomew, R. K. (1975) *Parasitology* **71**, 183–192.
7. Wilson, R. J. M., McGregor, I. A. & Hall, P. (1975) *Trans. R. Soc. Trop. Med. Hyg.* **69**, 460–467.
8. Wilson, R. J. M., McGregor, I. A. & Williams, K. (1975) *Trans. R. Soc. Trop. Med. Hyg.* **69**, 453–459.
9. Wilson, R. J. M. (1980) *Nature (London)* **284**, 451–452.
10. Brown, G. V., Anders, R. F. & Knowles, G. (1983) *Infect. Immun.* **39**, 1228–1235.
11. McBride, J. S., Walliker, D. & Morgan, G. (1982) *Science* **217**, 254–257.
12. Wilson, R. J. M. (1974) *Int. J. Parasitol.* **4**, 537–547.
13. Trager, W. & Jensen, J. B. (1976) *Science* **193**, 673–675.
14. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
15. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
16. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496.
17. Glynn, P., Gilbert, H., Newcombe, J. I. A. & Cuzner, M. L. (1982) *J. Immunol. Methods* **51**, 251–257.
18. Wilson, R. J. M. & Ling, I. (1979) *Bull. WHO* **57**, Suppl. 1, 123–133.
19. Tait, A. (1981) *Mol. Biochem. Parasitol.* **2**, 205–218.
20. Schofield, L., Saul, A., Myler, P. & Kidson, C. (1982) *Infect. Immun.* **38**, 893–897.