Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5121–5125, August 1985
Immunology

A blood stage antigen of *Plasmodium falciparum* shares determinants with the sporozoite coat protein

(malaria/recombinant DNA/cDNA expression/colony immunoassay/nucleotide sequence)

ROSS L. COPPEL*, JENNY M. FAVALORO*, PAULINE E. CREWTHER*, TOM R. BURKOT†, A. EDWARD BIANCO*, HANS D. STAHL*, DAVID J. KEMP*, ROBIN F. ANDERS*, AND GRAHAM V. BROWN*

*The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia; and †Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

Communicated by G. J. V. Nossal, April 1, 1985

ABSTRACT **A cDNA clone expressing a *Plasmodium falciparum* blood-stage antigen in *Escherichia coli* was identified by colony immunoassay using immune human sera. Antibodies affinity-purified on extracts of this clone reacted with both asexual blood stages and sporozoites of *P. falciparum*, recognizing a M_r 23,000 protein in the blood stages. The nucleotide sequence of the cDNA revealed a signal peptide and an internal hydrophobic sequence typical of transmembrane anchor sequences. Located 3′ to the putative anchor are two tetramers, Asn-Ala-Asn-Pro and Asn-Ala-Asp-Pro, which are closely related to the repeats of the circumsporozoite protein of *P. falciparum*. The blood stage protein is conserved amongst several isolates of *P. falciparum*, and antibodies against it are common in the sera of individuals living in the area where the parasite is endemic.**

Plasmodium falciparum, the major cause of human malaria, has a complex life cycle. Sporozoites, injected by the mosquito vector, rapidly enter liver cells and disappear from the peripheral circulation within minutes (1). After a period of nuclear division within the hepatocyte, many merozoites are released to commence the erythrocytic cycle, which lasts days or weeks in the absence of treatment. Immunization with irradiated sporozoites gave substantial protection in mice and humans against subsequent sporozoite challenge (2, 3) but not against challenge with asexual blood stages (4). Sera collected from the immunized individuals reacted in an immunofluorescence assay with sporozoites but not blood stages (5). These findings were taken to discount the possibility of cross protection between these stages and implied slight or no cross-reaction between surface antigens.

Hope *et al.* (6) have recently described a monoclonal antibody raised against blood stages of *P. falciparum* that recognized a protein of M_r 23,000. Immunofluorescence studies on fixed preparations showed that this monoclonal antibody reacted with asexual blood stages and sporozoites, suggesting that at least one epitope was shared between stages.

P. falciparum sporozoites have a dominant coat protein (7), the circumsporozoite protein (CSP), the sequence of which has recently been determined (8, 9). The CSP contains many repeats of a tetrameric sequence. Preincubation with monoclonal antibodies directed against this repeat abrogated the infectivity of sporozoites (7).

We have recently reported the production of *P. falciparum* proteins in *Escherichia coli* (10–12). Many distinct antigens were detected by reaction of cloned proteins with sera from immune individuals. We report the detailed characterization of one such antigen identified in this way, a M_r 23,000 polypeptide that shares sequences with the repeat peptide of the CSP of *P. falciparum*. We show that human antibodies that react with this polypeptide also react with sporozoites.

MATERIALS AND METHODS

Preparation of Affinity-Purified Antibodies Against Ag61 Protein. Induced 50-ml cultures of *E. coli* carrying cDNA clone Ag61 and expression vector λAmp3 were prepared. The pelleted bacteria were sonicated in 100 mM sodium phosphate buffer, pH 6.8 (P_i)/10 mM dithiothreitol followed by mixing at room temperature with 1% $NaDodSO_4$. The soluble bacterial proteins were equilibrated with P_i/1 mM dithiothreitol/0.1% $NaDodSO_4$ by passage through Sephadex G-10 and conjugated to CNBr-activated Sepharose (Pharmacia, Sweden) at room temperature. A pool of human sera collected from individuals in Papua New Guinea was clarified by centrifugation, diluted with an equal volume of phosphate-buffered saline (P_i/NaCl), and preabsorbed on a λAmp3-Sepharose absorbent before being passed over the Ag61 absorbent. Nonspecifically bound proteins were removed by repeated wash cycles of 100 mM sodium borate/500 mM NaCl/0.05% Tween 20, pH 8.5, followed by P_i/NaCl. Bound antibodies were eluted with 100 mM glycine/150 mM NaCl, pH 2.6, and immediately neutralized with 2 M Tris·HCl, pH 8.0.

Immunoblots. *In vitro* cultures of *P. falciparum* were synchronized by treatment with 5% sorbitol (13). Protein extracts of cultures of *P. falciparum* were prepared and fractionated on 10% polyacrylamide/$NaDodSO_4$ gels. Proteins from the gels were transferred electrophoretically to nitrocellulose (14) and incubated in 5% nonfat milk powder in P_i/NaCl before reaction with affinity-purified human antiserum. The filters were incubated with ^{125}I-labeled staphylococcal protein A and autoradiographed.

Immunofluorescence. Human antibodies affinity purified on immunoabsorbents of Ag61 protein were allowed to react with fixed (90% acetone/10% methanol, vol/vol) thin blood films of asynchronous cultures of *P. falciparum* isolate FCQ27/PNG (FC27). Fluorescein-conjugated sheep anti-human Ig was used as the second antibody. Nuclei were counterstained with propidium iodide and the slides were mounted in 80% (vol/vol) glycerol in P_i/NaCl containing *p*-phenylenediamine prior to photography. Immunofluorescence assays were performed on sporozoites obtained from the salivary glands of mosquitoes captured in the village of Betelgut (Madang Province, Papua New Guinea), air-dried on microscope slides, and stored at −20°C until required. Sporozoites were first allowed to react with P_i containing 10% normal human serum, then with the dilutions of test sera.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CSP, circumsporozoite protein; CRA, circumsporozoite protein-related antigen; RESA, ring-infected erythrocyte surface antigen.

After 20-min incubation at room temperature the slides were washed in P_i and allowed to react with fluorescein-conjugated rabbit antibodies to human IgG (Commonwealth Serum Laboratories, Melbourne, Australia) for 20 min. After washing in P_i the slides were mounted in 90% glycerol in P_i and examined. The species of sporozoite was determined by reaction with typing monoclonal antibodies (15) specific for *P. falciparum* (1G3.4) and *Plasmodium vivax* (1A3.3) at a dilution of 1:50.

Nucleotide Sequence Determination. The dideoxy chain termination method (16) was employed for sequence determinations. The Ag61 cDNA insert and fragments generated from it by restriction enzyme digestion were cloned into phages M13mp8 and -9 (17).

Southern Hybridization. *P. falciparum* DNA was digested with restriction endonucleases and fractionated on 1% agarose gels. The DNA was transferred to nitrocellulose and prepared for hybridization by the method of Southern (18). Hybridization probes were prepared by nick-translation of DNA fragments purified by agarose gels electrophoresis. Filters were washed in 0.30 M NaCl/0.03 M sodium citrate at 65°C.

RESULTS

Identification of the *P. falciparum* Antigen Corresponding to Ag61. Clone Ag61 (Ag61) was one of 78 clones identified by screening a *P. falciparum* cDNA library in the expression vector λAmp3, as described (10, 11, 19). To identify the *P. falciparum* antigen expressed by clone Ag61, sera collected from adults living in the Madang province of Papua New Guinea were depleted of antibodies to *E. coli* by passage over a λAmp3 absorbent before affinity purification on clone Ag61. They were then used to characterize the corresponding antigen of *P. falciparum* by immunoblotting and immunofluorescence.

Highly purified populations of rings, trophozoites, schizonts, and merozoites from *P. falciparum* isolate FC27 were analyzed for the presence of polypeptides corresponding to Ag61. As shown in Fig. 1*A*, a dominant M_r 23,000 polypeptide was detected in all of the asexual stages. In the merozoite preparation two high molecular weight polypeptides (M_r 143,000 and 70,000) were detected, as were several smaller forms ranging in molecular weight from 18,000 to 23,000. It would appear from the nucleotide sequence data (see below) that the larger polypeptides are cross-reactive antigens unique to the merozoite, rather than precursors of the smaller polypeptides. When other isolates of *P. falciparum* were examined (K1, Thailand; V1, Vietnam; and NF7, Ghana) the same dominant M_r 23,000 polypeptide was present (data not shown).

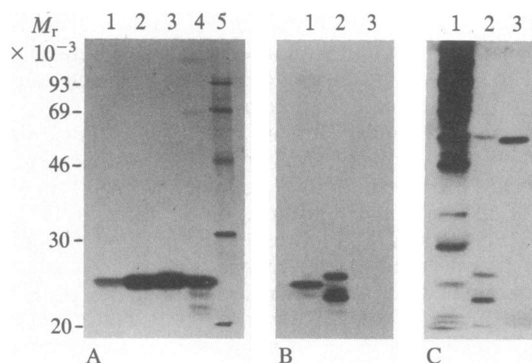


FIG. 1. Immunoblots with human antibodies to Ag61 protein. (*A*) Detergent extracts of a synchronized *in vitro* culture of the FC27 isolate of *P. falciparum* (20). Tracks 1–4 were prepared from rings, trophozoites, schizonts, and merozoites, respectively. Track 5, molecular weight markers. (*B* and *C*) Detergent extracts of an asynchronous *in vitro* culture of the FC27 isolate of *P. falciparum* (track 1) and of induced cultures of *E. coli* infected with Ag61 (track 2) and with λAmp3 (track 3). In *A* and *B* the probe was affinity-purified human anti-Ag61, and in *C* the probe was serum collected from an adult living in Madang, Papua New Guinea.

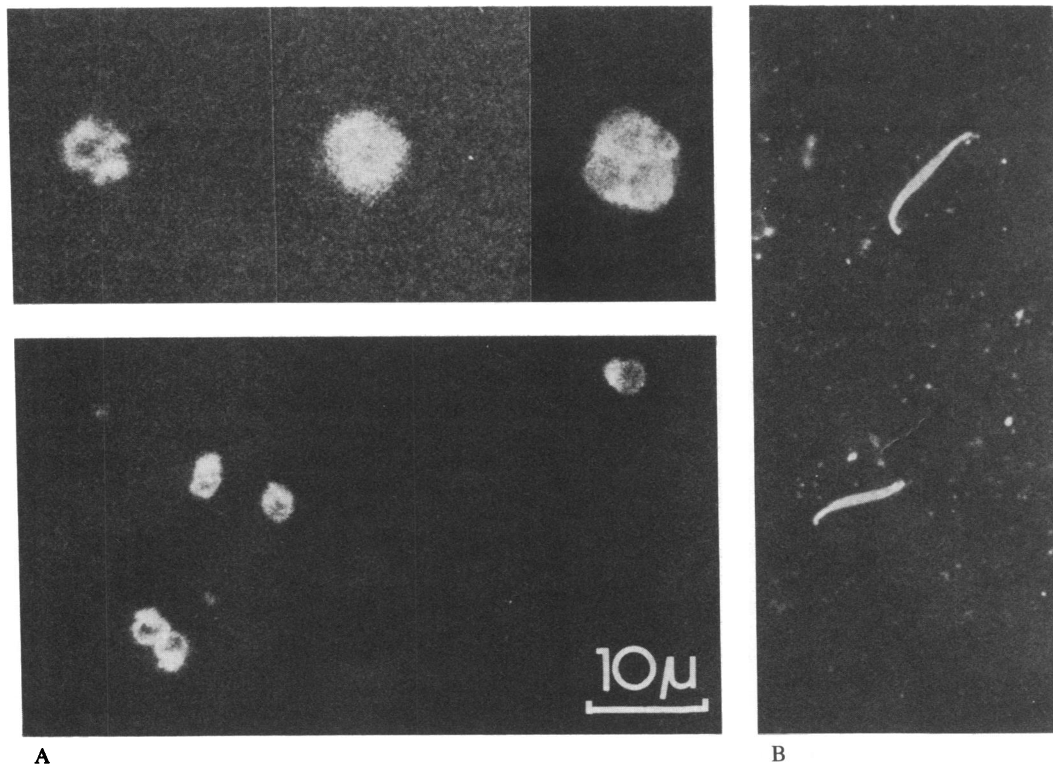
Immunoblots on protein extracts from Ag61 with these purified human antibodies revealed that the antigen was expressed in *E. coli* in two major forms, M_r 25,000 and M_r 21,000 (Fig. 1*B*). Long exposures show that a M_r 25,000 form is also present in *P. falciparum*. It is therefore likely that the M_r 25,000 form is a full-length precursor in both *E. coli* and *P. falciparum* but the processing differs. These precursor forms are also detectable by using whole human sera (Fig. 1*C*).

Localization of the antigen in the parasite was studied by indirect immunofluorescence microscopy on slides prepared from fixed asynchronous cultures of FC27. Fluorescence was prominent in the trophozoite and schizont, where it appeared diffuse and granular and not apparently localized to membranes or organelles (Fig. 2*A*). There was also fluorescence detectable on the ring stage, the fluorescence appearing in a rim around the parasite (Fig. 2*A*). This contrasts with the characteristic location of the ring-infected erythrocyte surface antigen (RESA), which is localized at the membrane of the ring-infected erythrocyte (21). Immunofluorescence studies on the other *P. falciparum* isolates gave results similar to those with FC27 (data not shown).

Determination of the Nucleotide Sequence of Ag61. DNA was prepared from clone Ag61 and digested with *Eco*RI to release the *P. falciparum* insert. Examination of digestion products by agarose gel electrophoresis revealed that four distinct DNA segments had been ligated into the λAmp3 phage, as observed for some other clones (11). To identify the expressed DNA segment, each segment was recloned separately in λAmp3 and tested for expression of Ag61 sequences by reaction with affinity-purified human antibodies to Ag61 protein. The expressing insert was then subcloned in an M13 vector and sequenced by the dideoxy method (16). The nucleotide sequence and derived amino acid sequence are presented in Fig. 3. The insert is 770 nucleotides long and consists of the entire coding sequence of the gene together with untranslated regions.

The deduced coding region commences with an ATG codon at nucleotide 159 preceded by a region of high A+T content with stop codons in all frames. The Ag61 sequence is out of phase with β-galactosidase and therefore could not be expressed as a fused polypeptide. The one open reading frame continues until a termination codon commencing at nucleotide 645—i.e., 486 nucleotides later. The predicted amino acid sequence of 162 amino acids has several noteworthy features. Immediately 3′ to the initiation codon is a lysine residue followed by a hydrophobic region of 15 amino acid residues consistent with a signal sequence. Presumably the M_r 25,000 product seen in *E. coli* and to a lesser extent in *P. falciparum* (Fig. 1 *B* and *C*) represents the full-length molecule before removal of this signal sequence. Cleavage therefore probably occurs at different points in the two organisms. The predicted 28 amino acid sequence from nucleotide 384 to nucleotide 467, which is very hydrophobic and flanked by several basic residues, is typical of transmembrane anchor sequences found in integral membrane proteins.

A surprising feature is the presence of a set of related amino acid residues, clearly evident in a homology plot (Fig. 4*A*), located between nucleotides 498 and 569. Although this region does not contain any of the exact tandem repeats that are characteristic of other *P. falciparum* antigens, it is closely related to the tandemly repeating peptide unit found in the circumsporozoite protein of *P. falciparum* (8, 9) as



A B

FIG. 2. Immunofluorescence with affinity-purified human antibodies to Ag61. (*A*) Composite of fixed asynchronous cultures of FC27; mature parasites (upper panels) and ring stages (lower panel) are shown. (*B*) *P. falciparum* sporozoites isolated from salivary glands of wild mosquitoes from the Madang region of Papua New Guinea. (Original magnification was ×1000, with oil immersion; final magnification, ×1600.) There was no detectable fluorescence when fixed parasites were allowed to react with antibodies affinity purified with λAmp3-Sepharose.

graphically demonstrated in a homology plot (Fig. 4*B*). There is one occurrence of the major tetramer repeat of the CSP, namely Asn-Ala-Asn-Pro, and a sequence Asn-Ala-Asp-Pro closely related to the alternate repeat of the CSP Asn-Val-Asp-Pro (8). Additionally, the related sequences of Ser-Ser-Asp-Pro and Asn-Gly-Glu-Pro are present.

```
GAATTCCGAAAAATATTTAATTATCTAAATAAATTTAATTAAAAATTTTTATAACATATTTTATTTAAGATTTTATAATAATTAAGTTT   89

                                                                                 O
                                                                          MetLysIleLeuSerValPhe
TAATTTCTTTTGATCCAAAGTTTTTAATAATTAAATTTGTAGATTTTTAATTTATTTAATATATTCAAAATGAAAATCTTATCAGTATTT  179

                        O
PheLeuAlaLeuPhePheIleIlePheAsnLysGluSerLeuAlaGluLysThrAsnLysGlyThrGlySerGlyValSerSerLysLys
TTTCTTGCTCTTTTCTTTATCATTTTCAATAAAGAATCCTTAGCCGAAAAAACAAACAAAGGAACTGGAAGTGGTGTTAGCAGCAAAAAA  269

LysAsnLysLysGlySerGlyGluProLeuIleAspValHisAspLeuIleSerAspMetIleLysLysGluGluGluLeuValGluVal
AAAAATAAAAAAGGATCAGGTGAACCATTAATAGATGTACACGATTTAATATCTGATATGATCAAAAAAGAAGAAGAACTTGTTGAAGTT  359

                        Δ
AsnLysArgLysSerLysTyrLysLeuAlaThrSerValLeuAlaGlyLeuLeuGlyValValSerThrValLeuLeuGlyGlyValGly
AACAAAAGAAAATCCAAATATAAACTTGCCACTTCAGTACTTGCAGGTTTATTAGGTGTAGTATCCACCGTATTATTAGGAGGTGTTGGT  449

          Δ                                         □
LeuValLeuTyrAsnThrGluLysGlyArgHisProPheLysIleGlySerSerAspProAlaAspAsnAlaAsnProAspAlaAspSer
TTAGTATTATACAATACTGAAAAAGGAAGACACCCATTCAAAATAGGATCAAGCGACCCAGCTGATAATGCTAACCCAGATGCTGATTCT  539

                           □
GluSerAsnGlyGluProAsnAlaAspProGlnValThrAlaGlnAspValThrProGluGlnProGlnGlyAspAspAsnAsnLeuVal
GAATCCAATGGAGAACCAAATGCAGACCCACAAGTTACAGCTCAAGATGTTACACCAGAGCAACCACAAGGTGACGACAACAACCTCGTA  629

SerGlyProGluHis***
AGTGGCCCTGAACACTAAACAGCTGTAAACTTTTTTGTTAATGGGTTTTTTTGAAACACGTGAAAATAATTTTTATTTATGATTATATTA  719

TATATATTGCTATTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAACGGAATTC                                       770
```

FIG. 3. Nucleotide sequence and derived amino acid sequence of Ag61. Coding region commences at nucleotide 159 and terminates at the codon marked by asterisks. The putative signal peptide is the region shown bounded by Os, while the putative anchor sequence is delineated by Δs. The region homologous to the CSP is bounded by □s.

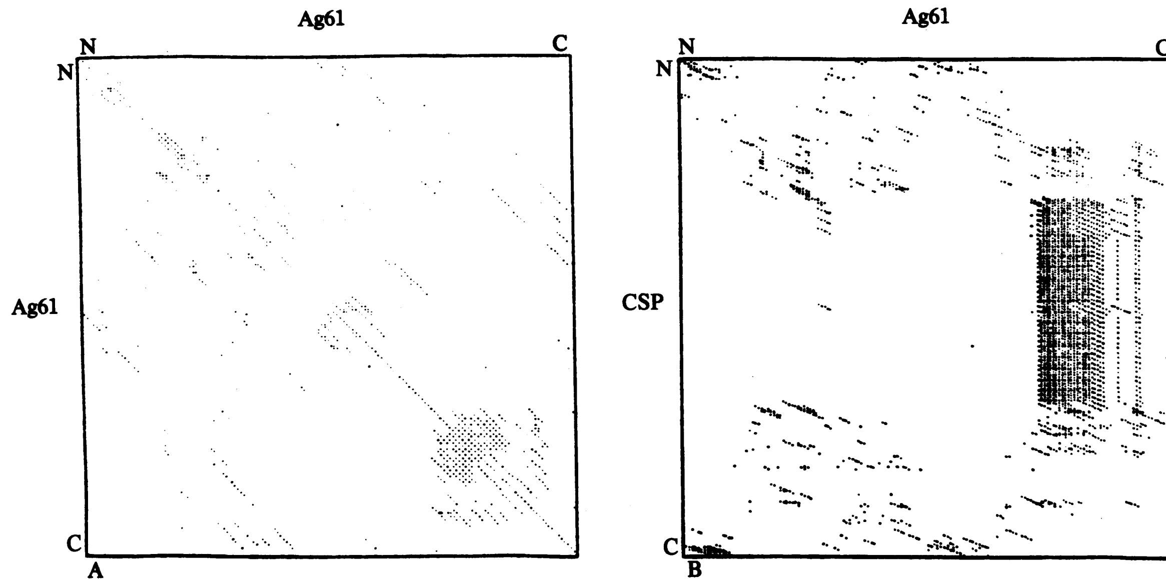


FIG. 4. Homologies in the amino acid sequence of Ag61. In *A* the amino acid sequence of Ag61 is compared and aligned for best fit with itself according to the DBUTIL programs of Staden (22). The regions of diagonal plot correspond to regions of homology in the sequence. In *B* the amino acid sequence of Ag61 is compared to that of the CSP of *P. falciparum*. The homologous region extends over the whole repeat region of the CSP protein. The NH_2 and COOH terminals of the proteins are indicated by N and C.

Conservation of Restriction Sites Flanking the Ag61 Gene. The genomic context of Ag61 was examined in three parasite isolates—namely, FC27 from Papua New Guinea, K1 from Thailand, and NF7 from Ghana. DNA from each isolate was digested with either *Eco*RI or *Hin*dIII, electrophoretically separated on an agarose gel and transferred to nitrocellulose (18), and probed with radioactively labeled Ag61 DNA. The size of hybridizing bands, 21.5 kilobases (kb) for *Eco*RI and 5.9 kb for *Hin*dIII, is constant for each isolate (Fig. 5), suggesting that the genomic context of this gene is preserved in isolates from widely separated regions.

Specificity and Prevalence of Antibodies to Ag61 Protein. The unexpected finding of sequences within the coding sequence of Ag61 that are shared with the CSP suggested that antibodies purified on lysates of Ag61 would react with *P. falciparum* sporozoites. Accordingly, affinity-purified antibodies to Ag61 protein were allowed to react with air-dried sporozoites of *P. falciparum* and *P. vivax*. Anti-Ag61 antibodies reacted with *P. falciparum* sporozoites (Fig. 2*B*) but not with *P. vivax* sporozoites, with positive fluorescence still readily detectable at a titer of 1:1200.

Sixteen sera from individuals living in an area in which *P. falciparum* is endemic were tested in the colony immunoassay for antibodies directed against Ag61 protein. Eight sera (50%) had detectable levels of antibody as measured in this relatively insensitive assay (data not shown).

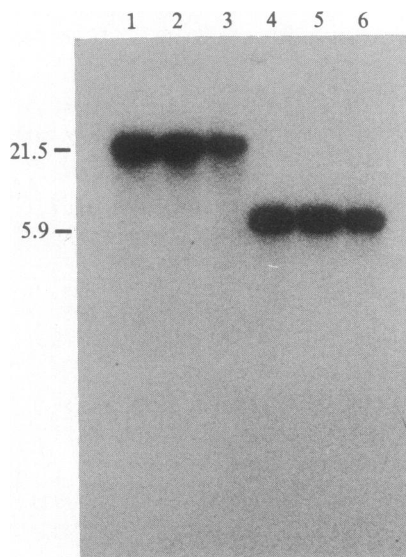


FIG. 5. Southern hybridization of Ag61 to *P. falciparum* chromosomal DNA from several isolates. DNAs from *P. falciparum* isolates FC27 (tracks 1 and 4), K1 (tracks 2 and 5), and NF7 (tracks 3 and 6) were digested with either *Eco*RI (tracks 1–3) or *Hin*dIII (tracks 4–6), separated electrophoretically on an agarose gel, and blotted to nitrocellulose. The filter was probed with ^{32}P-labeled Ag61 and washed with 0.3 M NaCl/0.03 M sodium citrate at 65°C. Indicated sizes are in kilobases.

DISCUSSION

We have presented here the complete nucleotide sequence of a *P. falciparum* asexual blood stage antigen. It is unusual among *P. falciparum* antigens already described (12, 21, 23, 24) in that it is a small protein, it does not contain any tandemly repeating peptides, and there is no polymorphism in size of this protein amongst isolates. These features are linked, as the large size and polymorphism of *P. falciparum* antigens such as the S antigen, RESA, and the falciparum interspersed repeat antigen are contributed to by the presence of these repeats (12, 21, 23).

Although there are no authentic repeats in this antigen, the region bounded by nucleotides 498 and 569 contains a number of internal homologies—in particular, the two tetramers Asn-Ala-Asn-Pro and Asn-Ala-Asp-Pro. Surprisingly, the first of these related tetramers is the dominant repeat found in the CSP of *P. falciparum* (8, 9). The CSP is composed of 37 repeats of Asn-Ala-Asn-Pro and 4 repeats of Asn-Val-Asp-Pro. There are eight different combinations of nucleotides that code for the tetramer Asn-Ala-Asn-Pro in the CSP, all of which differ from the coding sequence found in Ag61. This suggests that the tetramer in Ag61 could not be derived from the CSP by shuffling of repeat sequences around the genome. Because of the presence of these tetramers we have designated this protein the circumsporozoite protein-related antigen (CRA).

The presence of a long hydrophobic stretch is consistent with an anchor sequence of an integral membrane protein.

The fluorescence pattern suggests that CRA may be associated with the surface of the ring stage parasite within the erythrocyte (see Fig. 1*B*).

Hope *et al.* (6) have described monoclonal antibodies that react with both blood stages and sporozoites and recognize a blood stage protein of M_r 23,000. We believe that CRA is the target antigen of that monoclonal antibody, because anti-CRA antibodies react with *P. falciparum* sporozoites in indirect immunofluorescence assays. The sporozoite is present in the circulation for a very short time, but asexual stages persist for long periods. The observation that many individuals in the area where these parasites are endemic have antibodies against sporozoites as measured by indirect immunofluorescence (25) may reflect antibodies originally raised against CRA. This raises the possibility that immune responses to CRA may act against the sporozoite, an event apparently counterproductive to successful parasitism, as it has been reported that antibodies directed against the repeats in the CSP of *P. falciparum* are protective (7, 8). Perhaps these sequences in CRA stimulate a form of concomitant immunity that prevents superinfection of the host by *P. falciparum*. While the host is parasitemic, anti-sporozoite responses are boosted, lessening the likelihood of successful new infection. When the host is nonparasitemic, these responses will wane, favoring successful invasion of hepatocytes by newly injected sporozoites. An alternative role for CRA is as an absorbent removing anti-CSP antibodies from the circulation. However the small number of repeats that could act as binding sites and the relative scarcity of CRA in blood stages as evidenced by cDNA hybridization studies (data not shown) leads us to discount this possibility.

The CRA is found in many parasite strains. In this study it was found in 4 of 4 strains. Studies by Hall *et al.* (28), using a monoclonal antibody that we believe to be directed against the same protein, found it in 22 of 27 strains studied. Antibodies to it are common, being present in 50% of sera that we assayed. We have examined 16 sera that had previously been assayed for their ability to inhibit the growth of the parasite *in vitro*. These sera were divided into two groups: those causing greater than 50% inhibition of growth, and those causing less than 25% growth inhibition. When these sera were assayed against a clone producing CRA in a colony immunoassay, there was a correlation between anti-CRA antibody and the ability to inhibit *in vitro* growth significant at $P < 0.05$ (data not shown). If purified anti-CRA antibodies are found to inhibit growth, it raises the possibility of a single unit malaria vaccine active against both sporozoites and blood stages.

After the completion of the work described in this paper Hope *et al.* (26) reported the nucleotide sequence of a protein that they designated Ag5.1. A monoclonal antibody that reacted with clone Ag5.1 also reacted with sporozoites, in accordance with our data on human antibodies. The sequence is identical to that of the CRA except for an A-to-G transition at codon 28, resulting in a change from glutamate to glycine. This conservation of sequence is interesting in view of the widely differing sequences reported for the S antigen of *P. falciparum* (27), the only other *P. falciparum* protein for which sequences from two strains are available.

We thank Wayne Thomas and Graham Mitchell for help and advice in the execution of this work and Kirstin Easton, Kathy Elkins, Sheevaun Carey, Marissa Keagan, and Mary-Lou O'Halloran for expert technical assistance and A. Kyne for assistance with computing. This work was supported by the Australian National Health and Medical Research Council, the Rockefeller Foundation Great Neglected Diseases Network, and the John D. and Catherine T. MacArthur Foundation.

1. Wernsdorfer, W. H. (1980) in *Malaria*, ed. Kreier, J. P. (Academic, New York), Vol. 1, pp. 1–93.
2. Vanderberg, J., Nussenzweig, R. & Most, H. (1969) *Mil. Med.*, Suppl., **134**, 1183–1190.
3. Clyde, D. F., McCarthy, V. C., Miller, R. M. & Woodward, W. E. (1975) *Am. J. Trop. Med. Hyg.* **24**, 397–402.
4. Clyde, D. F., Most, H., McCarthy, V. C. & Vanderberg, J. P. (1973) *Am. J. Med. Sci.* **266**, 169–177.
5. Vanderberg, J. P., Nussenzweig, R. S., Sanabria, Y., Nawrot, R. & Most, H. (1972) *Proc. Helminthol. Soc. Wash.* **39**, 514–525.
6. Hope, I. A., Hall, R., Simmons, D. L. I., Hyde, J. E. & Scaife, J. G. (1984) *Nature (London)* **308**, 191–193.
7. Nardin, E. H., Nussenzweig, V., Nussenzweig, R. S., Collins, W. E., Harinasuta, K. T., Tapchaisri, P. & Chomcharn, Y. (1982) *J. Exp. Med.* **156**, 20–30.
8. Dame, J. B., Williams, J. L., McCutchan, T. F., Weber, J. L., Wortz, R. A., Hockmeyer, W. T., Maloy, W. L., Haynes, J. O., Schneider, I., Roberts, D., Sanders, G. S., Reddy, E. P., Diggs, C. L. & Miller, L. H. (1984) *Science* **225**, 593–599.
9. Enea, V., Ellis, J., Zavala, F., Arnot, D. E., Asavanich, A., Masuda, A., Quakyi, I. & Nussenzweig, R. S. (1984) *Science* **225**, 628–629.
10. Kemp, D. J., Coppel, R. L., Cowman, A. F., Saint, R. B., Brown, G. V. & Anders, R. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3787–3791.
11. Anders, R. F., Coppel, R. L., Brown, G. V., Saint, R. B., Cowman, A. F., Lingelbach, K. R., Mitchell, G. F. & Kemp, D. J. (1984) *Mol. Biol. Med.*, in press.
12. Stahl, H.-D., Crewther, P. E., Anders, R. F., Brown, G. V., Coppel, R. L., Bianco, A. E., Mitchell, G. F. & Kemp, D. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 543–547.
13. Lambros, C. & Vanderberg, J. P. (1979) *J. Parasitol.* **65**, 418–420.
14. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
15. Burkot, T. R., Williams, J. L. & Schneider, I. (1984) *Am. J. Trop. Med. Hyg.* **33**, 783–788.
16. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
17. Messing, J. & Vieira, J. (1982) *Gene* **19**, 269–276.
18. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
19. Coppel, R. L., Anders, R. F., Brown, G. V., Cowman, A. F., Lingelbach, K. R., Saint, R. B., Mitchell, G. F. & Kemp, D. J. (1984) in *Molecular Parasitology*, ed. August, T. (Academic, New York), pp. 103–115.
20. Chen, P., Lamont, G., Elliot, T., Kidson, C., Brown, G., Mitchell, G., Stace, J. & Alpers, M. (1980) *Southeast Asian J. Trop. Med. Public Health* **11**, 435–440.
21. Coppel, R. L., Cowman, A. F., Anders, R. F., Bianco, A. B., Saint, R. B., Lingelbach, K. R., Kemp, D. J. & Brown, G. V. (1984) *Nature (London)* **310**, 789–791.
22. Staden, R. (1980) *Nucleic Acids Res.* **8**, 3673–3694.
23. Coppel, R. L., Cowman, A. F., Lingelbach, K. R., Brown, G. V., Saint, R. B., Kemp, D. J. & Anders, R. F. (1983) *Nature (London)* **306**, 751–756.
24. Koenen, M., Scherf, A., Mercereau, O., Langsley, G., Sibilli, L., Dubois, P., Pereira da Silva, L. & Muller-Hill, B. (1984) *Nature (London)* **311**, 382–385.
25. Nardin, E. H., Nussenzweig, R. S., McGregor, I. A. & Bryan, J. H. (1979) *Science* **206**, 597–599.
26. Hope, I. A., Mackay, M., Hyde, J. E., Goman, M. & Scaife, J. (1985) *Nucleic Acids Res.* **13**, 369–379.
27. Cowman, A. F., Saint, R. B., Coppel, R. L., Brown, G. V., Kemp, D. J. & Anders, R. F. (1985) *Cell* **40**, 775–783.
28. Hall, R., McBride, J., Morgan, G., Tait, A., Zolg, J. W., Walliker, D. & Scaife, J. (1983) *Mol. Biochem. Parasitol.* **7**, 247–265.