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Characterization of P0, a Ribosomal Phosphoprotein of *Plasmodium falciparum*

ANTIBODY AGAINST AMINO-TERMINAL DOMAIN INHIBITS PARASITE GROWTH*

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A cDNA expression clone of the human malarial parasite *Plasmodium falciparum*, λPf4, which was reactive only to the immune sera and not to the patient sera, has recently been found to be the *P. falciparum* homologue of the P0 ribosomal phosphoprotein gene. A Northern analysis of the P0 gene revealed the presence of two transcripts, both present in all the different intraerythrocytic stages of the parasite life cycle. A 138-base pair amino-terminal domain of this gene was expressed as a fusion protein with glutathione *S*-transferase in *Escherichia coli*. Polyclonal antibodies raised against this domain immunoprecipitated the expected 38-kDa P0 protein from the ^{35}S-labeled as well as ^{32}P-labeled *P. falciparum* cultures. Monospecific human immune sera affinity-purified using the expression clone λPf4 also immunoprecipitated the same size protein from [^{35}S]methionine-labeled *P. falciparum* protein extract. Purified IgG from polyclonal antibodies raised against the amino-terminal domain of P0 protein completely inhibited the growth of *P. falciparum in vitro*. This inhibition appears to be mainly at the step of erythrocyte invasion by the parasites.

It has been documented that people living in malaria-endemic areas acquire immunity to *Plasmodium falciparum* after repeated infections. The nature of this immunity is poorly understood at the molecular level. It is apparent from studies involving passive transfer of IgG from immune adults to the non-immune subjects that circulating antibodies do play an important role (1, 2). The specificity of these protective antibodies is as yet unknown. It has been shown that the antibodies present in immune adults recognize domains that are conserved in different strains of *P. falciparum* (3). However, it has also been documented that many malarial antigens possess repetitive protein domains, which evoke a strong antibody response. Many of these antibodies are non-protective, and the corresponding malarial epitopes are postulated to be immune-evasive or smokescreen domains (4, 5). Thus, to search for pan-specific and possibly protective antibodies, a differential immunoscreening of an erythrocytic stage-specific cDNA expression library of *P. falciparum* was carried out using malaria-immune and acute patient sera. This resulted in the identification of several novel cDNA clones, which reacted exclusively and yet extensively with immune sera samples (6). The clone λPf4, which was reactive to the largest number of immune sera (80 out of 92), has been cloned and sequenced recently (7). This was found to be the *P. falciparum* gene homologue of the ribosomal phosphoprotein P0 (PfP0).[1]

Ribosomal phosphoprotein P0 is considered to be related to the family of the acidic ribosomal phosphoproteins P1 and P2, because of the highly homologous carboxyl-terminal domain (8). Antibodies against this domain coprecipitate all three P proteins (9). P0 could be cross-linked to P1 and P2 protein in *Artemia salina* ribosomes (10), and these data, along with that from yeast cells (11), strongly indicate the existence of a $(P1)_2$·P0·$(P2)_2$ protein complex in the eukaryotic ribosomes. This complex has been compared with the bacterial complex L10·$(L7/L12)_2$, which forms the stalk of the large subunit at the GTPase domain along with the 23 S ribosomal RNA (12, 13). It has been documented that P0 protein is absolutely required for the ribosomal activity and cell viability in yeast (14). The conserved carboxyl-terminal domain of the P proteins is very antigenic and found to be the main antigenic target for sera reactivity of about 10–15% of patients of the autoimmune disorder systemic lupus erythematosus (15). Antibodies to this domain have also been detected in patients of suffering from diseases caused by protozoan parasites such as Chagas' heart disease (16) and leishmaniasis (17). In this paper we report the characterization of this protein from *P. falciparum*, and we show for the first time that P0 is indeed a phosphoprotein. We also show that antibodies raised against the amino-terminal domain of this protein inhibits *P. falciparum* growth *in vitro*.

EXPERIMENTAL PROCEDURES

Materials—All reagents, unless otherwise specified, were purchased from Sigma. [α-^{32}P]dATP, [^{32}P]orthophosphoric acid were provided by the Board of Radiation Technologies, India. [^{35}S]Methionine and [^{35}S]cysteine were purchased from Amersham International (Buckinghamshire, England).

Parasite Cultures—Asexual stages of FCR3 (Gambia) and the FCK2 (India) strains of *P. falciparum* were cultured *in vitro* at 37 °C in the presence of human erythrocytes of serological type O+ in complete medium (RPMI 1640 medium containing 28 mM $NaHCO_3$, 25 mM HEPES, and supplemented with either 10% human serum or 0.5% Albumax (Life Technologies, Inc.) and 80 μg/ml Gentamycin sulfate) in sterile Petri dishes using the candle-jar method or sealed flasks flushed with 5% O_2, 5% CO_2, and 90% N_2 gas mixture (18). For stage-specific RNA preparation and *in vitro* parasite growth inhibition assays, cultures were synchronized by sorbitol treatment according to the method of Lambros and Vanderberg (19). Intracellular parasites from each of the substages were liberated from infected erythrocytes by saponin lysis

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[1] The abbreviations used are: PfP0, *P. falciparum* P0 protein; GST, glutathione *S*-transferase; PfP0-N, recombinant fusion protein of GST with the 46-amino acid peptide from the amino-terminal domain of P0 protein; kb, kilobase(s); IFA, indirect immunofluorescence assay; bp, base pair(s).

(20) for total RNA and genomic DNA extraction. The gametocytic stages of the parasite (NF54 strain) used for the immunofluorescence studies were kindly provided by Dr. Nirbhay Kumar, Johns Hopkins University (21).

Preparation of Nucleic Acids—Total cellular RNA was extracted using a single step method described by Chomczynski and Sacchi (22). Genomic DNA was extracted from total erythrocytic stages of the parasite as described in detail elsewhere (6).

Southern and Northern Hybridization—Southern and Northern hybridization were performed with the radioactively labeled [α-^{32}P]dATP 251-bp λPf4 cDNA fragment as well as the 700-bp L-4-7 (carboxyl-terminal fragment of PfP0 protein) with specific activity of 2×10^8 cpm by following the membrane manufacturer's protocol (Amersham International) in the presence of 50% formamide. Briefly, 2 μg of parasite genomic DNA cut with appropriate restriction enzymes (New England Biolabs Inc.) was electrophoresed in a 1.0% agarose gel. In the case of Northern hybridization, total RNA from the parasite was electrophoresed in formaldehyde containing 0.8% agarose gel. 0.69–9.44-kb RNA markers (Life Technologies, Inc.) were run and stained separately with ethidium bromide before transfer of the gel onto the membrane. Gels were transferred to Hybond N+ membrane (Amersham International) by capillary blotting following membrane manufacturer's protocol. Air-dried blots were then UV cross-linked in a UV cross-linker (Bio-Rad) for 2 min and pre-hybridized at 42 °C for 2–3 h. Hybridization was carried out at 42 °C for 18–20 h. Blots were washed twice at 65 °C for 15 min each with 2 × saline/sodium/phosphate/EDTA, 0.1% (w/v) SDS followed by washes with 1 × saline/sodium/phosphate/EDTA, 0.1% SDS and 0.1 × saline/sodium/phosphate/EDTA, 0.1% SDS, and exposed to Fuji x-ray film for autoradiography. The quantitation was performed using the gel-documentation system (Ultraviolet Products Inc.) as per manufacturer's protocol by estimating the volume of the signal.

Bacterial Expression of Amino-terminal Domain of P0 Protein as a GST-fusion Protein—A GST reporter-based vector (pGEX-1) was used as an expression vector (23). A *Hin*dIII-*Eco*RI restricted 138-bp amino-terminal fragment of PfP0 (46–184 bp of GenBank™ accession number U56663) (7) was flushed at both ends with Klenow and subcloned in pGEX-1 restricted with *Sma*I. The *Escherichia coli* cells harboring the vector pGEX-1 and the recombinant containing PFP0-N insert were induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 2 h at 37 °C (23). The total cell lysates were run on a 12% denaturing SDS-polyacrylamide gel electrophoresis and stained with Coomassie Blue to show the fusion protein (PfP0-N). The polyacrylamide gel piece containing the fusion protein band was cut out and crushed in liquid nitrogen and dissolved in phosphate-buffered saline (8 g/liter NaCl, 0.2 g/liter KCl, 1.44 g/liter Na_2HPO_4, 0.24 g/liter KH_2PO_4, pH adjusted to 7.4 with NaOH). The resulting slurry containing an estimated amount of 100 μg of protein was injected into two rabbits. Five boosts were given to each animal to generate antibodies with reasonable titer (>1000 as checked by enzyme-linked immunosorbent assay).

Preparation of Monospecific Human Sera—λPf4 cDNA clones in λgt11, as well as wild type λgt11, were grown on *E. coli* strain Y1090 to lytic phase (6). Hybond-C membrane (Amersham International) soaked in isopropyl-β-D-thiogalactopyranoside was overlaid on these phage plates and grown for an additional 12–16 h. The membranes were washed three times with 1 × TBS-T (200 mM Tris, 50 mM NaCl, 0.05% Tween 20, pH 7.5). A pool of six reactive human immune sera (1:100 dilution) used for the differential immunoscreening (6) was first incubated overnight at 4 °C with membrane saturated with the wild type λgt11 lysate. Then the sera was incubated overnight with several membranes containing the lysate of recombinant λPf4. Monospecific antibodies against λPf4 were eluted with 5 mM glycine from the membrane, neutralized with 1 M Tris, and then dialyzed overnight with three changes of TBS-T. Resulting monospecific antibody solution against λPf4 was concentrated using an Amicon concentrator (Amicon Inc.), reconstituted to the original volume of the human sera with 1 × TBS-T, and used for immunoprecipitation.

Preparation of [^{35}S]Methionine, [^{35}S]Cysteine, and ^{32}P-Labeled Proteins from P. falciparum and Immunoprecipitation Studies—Asynchronous cultures of *P. falciparum* containing 10–12% parasitemia were washed twice with methionine- and cysteine-free RPMI 1640 medium and resuspended at a final hematocrit of 5% in the same medium supplemented with 10% human serum. 100 μCi/ml of both radiolabeled methionine and cysteine were added to the culture medium and incubated for 4 h at 37 °C under normal culture conditions with occasional shaking. After labeling, the cells were washed and extracted as described elsewhere (24). In the case of ^{32}P-labeling, 1 mCi/ml radiolabeled orthophosphoric acid was neutralized using 1 N NaOH added to the washed parasites at 5% hematocrit (about 10% parasitemia) in the RPMI 1640 medium supplemented with 0.5% Albumax (Life Technologies, Inc.), incubated for 4 h at 37 °C, and the rest of the steps were the same as above. Monospecific human immune sera against λPf4 as well as rabbit polyclonal antibody against PfP0-N were used to precipitate the ^{35}S- and ^{32}P-labeled parasite proteins (24). The immunoprecipitated samples were run on 12% denaturing gels, which were stained with Coomassie Blue for 1 h and destained with destaining solution (30% methanol, 10% acetic acid) for 2 h. ^{35}S-Containing gels were soaked with 1M sodium salicylate for 30 min and washed with water for another 30 min. In the case of ^{32}P-containing gels, the destaining was continued overnight with several changes of the destaining solution. The gels were then dried using Gel dryer (Hoefer Scientific Co.) and exposed to Fuji x-ray film for autoradiography.

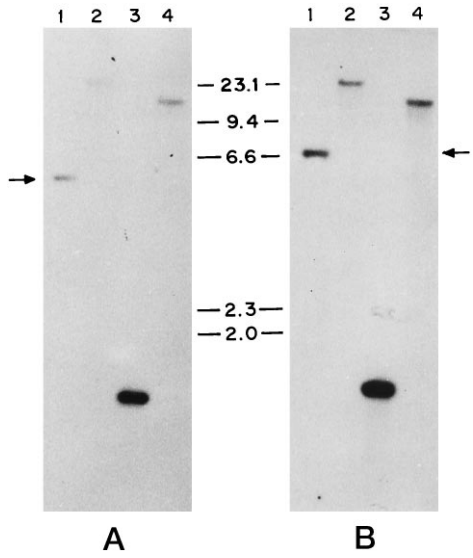

FIG. 1. **Southern blot analysis.** *Panel A*, Southern blot analysis of genomic DNA of the HB3 strain of *P. falciparum* cut with different restriction enzymes (*Eco*RI (*lane 1*), *Cla*I (*lane 2*), *Dra*I (*lane 3*), and *Hae*III (*lane 4*), respectively, and hybridized with radiolabeled 251-bp λPf4 fragment. *Panel B*, Southern analysis of the same blot with radiolabeled carboxyl-terminal L-4-7 fragment of P0, as described under "Experimental Procedures." The *arrows* indicate the two different genomic *Eco*RI fragments containing the P0 gene.

Indirect Immunofluorescence Assay (IFA)—IFA studies were done with *P. falciparum* asexual (FCR3 strain) as well as gametocytic stages (NF54 strain) with polyclonal antibodies against PfP0-N as described earlier (25). Briefly, asynchronous asexual and sexual stage cultures of *P. falciparum* were coated on glass slides as smears and air dried for overnight. The slides were fixed with methanol for 30 s at room temperature, blocked with 100 μg/ml bovine serum albumin solution for 2–3 h at room temperature, and washed at room temperature with 1 × TBS-T for 10 min each for three times. Slides were incubated with respective antibodies for 3–4 h at room temperature. FITC-conjugated anti-rabbit IgGs (Cappel, Organon Teknika, Durham, NC) at 1:80 dilution were used as the secondary antibody. The slides were observed under a Zeiss (Axioplan) microscope using × 100 Neofluor phase contrast objective and Zeiss filter FT500/600. All antibodies were cleared with GST and *E. coli* crude proteins and used at 1:100 dilution.

In Vitro Inhibition of P. falciparum Growth—IgG from rabbit sera were purified by ammonium sulfate precipitation followed by batch purification with DEAE-cellulose at pH 6.5 (26). *P. falciparum* growth inhibition assays were performed in triplicates in 24-well sterilized tissue culture plates (Nunc, Roskilde, Denmark) in a total culture volume of 1 ml. The effect of IgG on parasite growth was assessed by starting with ring stage-synchronized parasites at a 0.2–0.5% initial parasitemia and monitoring growth over 48 h without any medium replacement. Blood mononuclear cells from healthy donors were separated on Ficoll-Hypaque density gradient (Pharmacia Biotech Inc.). The adherent monocytes were selected in 24-well plates and counted (2). In the wells containing the adherent monocytes, *P. falciparum* cultures were added at a ratio of approximately 200 red blood cells/monocyte. Control wells consisted of (*a*) culture alone, (*b*) culture and monocytes alone, (*c*) culture and control IgG, and (*d*) culture, control IgG, and monocytes. Test and control IgG were added at a concentration of 1.0–1.5 mg/ml of final culture volume. Parasitemia was monitored every 6 h by preparing thin smear slides from each well and by micro-

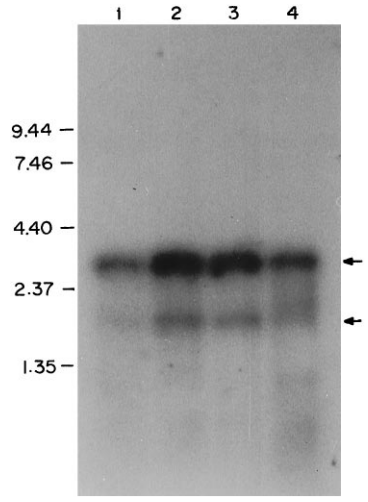

FIG. 2. **Northern blot analysis of total RNA from different intra-erythrocytic stages of *P. falciparum* with radiolabeled λPf4.** *Lane 1*, 100% rings; *lane 2*, early trophozoites (20% rings, 50% early trophozoites, 25% late trophozoites, and 5% schizonts); *lane 3*, late trophozoites (60% late trophozoites and 40% early schizonts); *lane 4*, schizonts (20% late trophozoites, 60% early schizonts, and 20% segmented schizonts). An equal amount of total RNA (10 μg), as determined by the absorbance value at 260 nm, was loaded in each *lane*. *Arrows* show the two P0 transcripts at 2.0 and 3.0 kb.

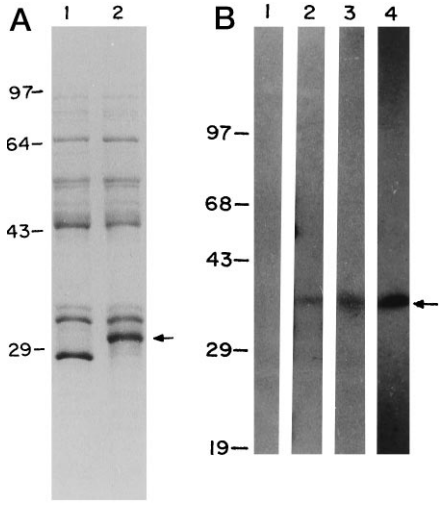

FIG. 3. *Panel A*, expression of the recombinant PfP0-N in *E. coli* as a fusion protein with GST. Total lysates of *E. coli* induced with 1 mM isopropyl-β-D-thiogalactopyranoside were run on a 12% SDS-polyacrylamide gel electrophoresis and stained with Coomassie Blue. Lysates of cells transfected with *lane 1* is the vector pGEX and *lane 2* is the recombinant-containing PfP0-N insert. The fusion protein GST-PfP0-N is marked with an *arrow*. *Panel B*, immunoprecipitation studies using immunoprecipitation of [^{35}S]methionine, [^{35}S]cysteine-labeled (*lanes 1*, *2*, and *4*), and ^{32}P-labeled (*lane 3*) *P. falciparum* asexual stage proteins. The antibodies used for immunoprecipitation were monospecific human immune sera affinity-purified against wild type λgt11 (*lane 1*), monospecific human immune sera affinity-purified against λPf4 (*lane 2*), and rabbit polyclonal antibodies against PfP0-N (*lanes 3* and *4*). The *arrow* marks the 38-kDa P0 protein.

scopic examination of >10,000 red blood cells. In each experiment, the number of rings, trophozoites, and schizonts were counted separately. Total parasitemia was estimated as the sum total of the rings, trophozoites, and schizonts.

RESULTS

A Southern blot of genomic DNA from the HB3 strain of *P. falciparum*, probed with the 251-bp λPf4 insert, showed that it hybridizes with the 6.1-, 13-, 1.5-, and 11-kilobase pair band when the DNA was restricted with *Eco*RI, *Cla*I, *Dra*I, and

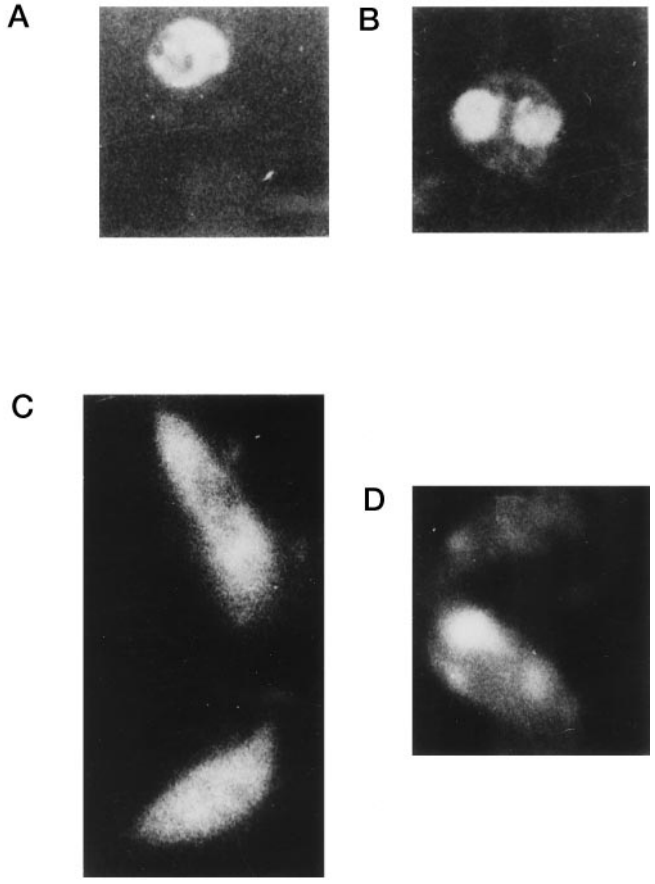

FIG. 4. **IFA with rabbit anti-PfP0-N antibody-indirect immunofluorescence assays of erythrocytic and gametocytic stages of *P. falciparum* were performed with polyclonal antibodies against PfP0-N.** *Panels A* and *B*, the erythrocytic stages; *panels C* and *D*, the gametocytic stages.

*Hae*III, respectively (Fig. 1, *panel A*). The gene has an internal *Eco*RI site, and this was demonstrated by probing the same blot with a 700-bp fragment, L-4-7, representing the carboxyl-terminal part of the protein (7), which lit up the same bands for all restriction digests except that of *Eco*RI, where it showed a 7.0-kilobase pair band (Fig. 1, *panel B*). Southern analysis of genomic DNA from two other *P. falciparum* strains, FCR3 and NF54, was also performed with these two probes, and no significant restriction fragment length polymorphism was observed. These results show that the PfP0 is coded by a single gene and is well conserved in different strains of the parasite.

A Northern blot of stage-specific total RNA from the asexual stages of *P. falciparum*, probed with the 251-bp λPf4 fragment, showed a dominant 3.0-kb-size fragment in every substage (Fig. 2). However, a second band, about 2.0 kb in size, was also seen in all stages. A quantitative determination of the ratio of these transcripts showed that the 3.0-kb message was 2.1 ± 0.26-fold as abundant as the 2.0-kb message in each of the stages. However, the 3.0-kb transcript was about 2.0- and 1.5-fold greater in abundance in the trophozoites compared with the rings and the schizont stages, respectively. The coding region of the P0 gene is 957 bp. However, the gene seems to possess a long 5′-untranslated region of about 1.4 kb in length, as observed by the size of cDNA clones isolated (7). Polymerase chain reaction studies using primer sequences within the coding region amplify the same size of fragments when genomic DNA is used as a template (data not shown), and therefore, there are no introns within the coding sequence. However, the presence of introns in the 5′-untranslated region is yet to be determined.

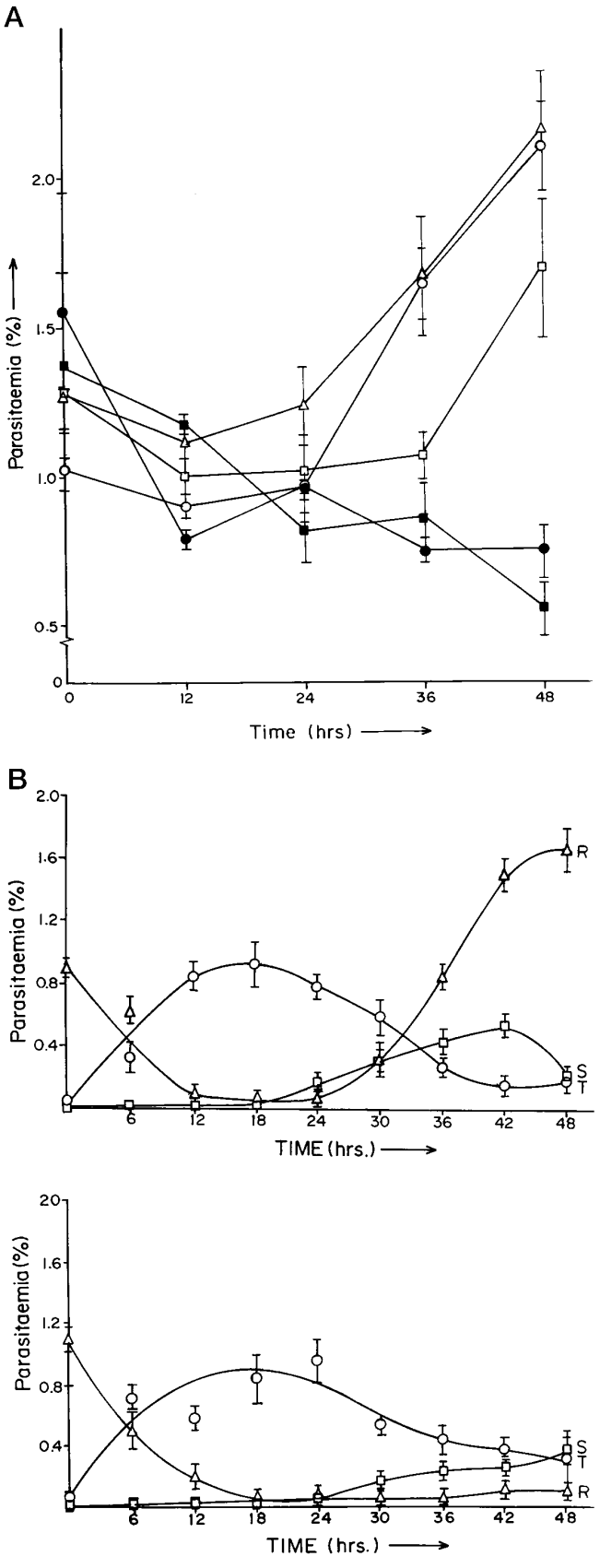

FIG. 5. ***In vitro* growth inhibition assay.** *Panel A* shows the effect of purified IgG from rabbit polyclonal antibodies against PfP0-N either in the presence (■) or absence (●) of human monocytes on the growth of 48-h *in vitro* culture of *P. falciparum* FCK2 strain. Preimmune sera with (□) or without (○) human monocytes and an antibody against irrelevant GST-fusion protein (△) were used as controls. *Panel B* shows the comparison of the percentage of rings (△), trophozoites (○), and schizonts (□) present at different time points during the same *in vitro* growth inhibition assay described in *panel A*, and in the presence of monocytes, The top *panel* shows the distribution of substages in the presence of control preimmune sera, and the *lower panel* shows the substages in the presence of rabbit anti-PfP0-N antibody.

Fig. 3*A* shows the expression of the GST-fusion protein of the amino-terminal domain of the *P. falciparum* P0 protein (PfP0-N) in the total *E. coli* cell lysate. The 46-amino acid stretch (17–62 amino acids) from the 138-bp *Hin*dIII-*Eco*RI fragment (7) produced the expected 31-kDa GST-fusion protein. Polyclonal rabbit antibodies, with a titer of >1000, were raised against PfP0-N and used for immunoprecipitation, immunofluorescence, and growth inhibition studies.

This antibody immunoprecipitated a single 38-kDa phosphoprotein from the ^{35}S- and ^{32}P-labeled *P. falciparum* proteins (Fig. 3*B*). Control antibodies such as rabbit preimmune sera and rabbit polyclonal antibodies raised against GST did not show any band (data not shown). To ascertain that the human immune sera originally used for the differential screen actually recognized the PfP0 protein domain in the λPf4 expression clone, monospecific human immune sera was affinity-purified using λPf4 expression clone and used for immunoprecipitation analysis. This also brought down the 38-kDa P0 protein from ^{35}S-labeled parasite proteins (Fig. 3*B*). Human immune sera affinity-purified against the control phage λgt11 did not show any reactivity with the parasite extract (Fig. 3*B*). Western blots of the recombinant PfP0-N protein with immune and patient sera samples showed that the recombinant protein was detected only by immune sera samples and not by patient sera samples (data not shown).

Rabbit antibodies raised against PfP0-N were used for IFA studies with different asexual stages (Fig. 4, *A* and *B*) as well as gametocytic stages of the parasite (Fig. 4, *C* and *D*). Rabbit preimmune sera and polyclonal antibodies against GST were used as control. The control antibodies showed no staining with parasites, whereas the anti-PfP0 antibody showed staining with all the different developmental stages of the parasite. In some of the gametocytic stages, intense staining of some subcellular domains were noticed (Fig. 4*D*), but the significance of this observation is not clear. The localization of the antigen was predominantly intracellular, however there appeared to be some staining at the surface as well. Preliminary experiments with biochemically fractionated parasite extracts do show antibody reactivity to the particulate fractions on Western blots (data not shown). When tested on *Toxoplasma gondii*, an apicomplexan parasite closely related to *Plasmodium,* it was observed to stain the surface, as confirmed by double staining with surface and internal markers of *Toxoplasma*.[2]

It has been reported that the protective human immune sera inhibits the *in vitro* growth of *P. falciparum* only in the presence of monocytes (2). However, these experiments were performed with mixed specificities of immunoglobulins. To ascertain whether anti-PfP0 antibody inhibits the growth of *P. falciparum* in the presence or absence of monocytes, *in vitro* inhibition studies using synchronized parasites starting from the ring stages were performed (Fig. 5). The antibodies raised against PfP0-N completely inhibited the growth of the parasites over a 48-h period (Fig. 5, *panel A*). Preimmune sera and sera raised against an irrelevant *P. falciparum* GST-fusion protein showed no inhibition in the growth of the parasites. Cultures without any IgG and with sera raised against GST protein showed normal growth (data not shown). 4–6 independent experiments were performed for each treatment, and

[2] A. Goswami and S. Sharma, unpublished data.

the figure represents an average of these experiments. The presence of monocytes were found to stimulate the parasite growth, but the inhibition of growth of *P. falciparum* by the antibody was independent of the presence of monocytes (Fig. 5*A*).

The growth inhibition studies were assayed by microscopically counting at least 10,000 red blood cells for each time point. The counting assay was performed rather than the hypoxanthine-uptake assay, because the uptake of hypoxanthine by the monocytes would cause errors in the assay. Also, starting with a synchronized culture and counting the different substages at every time point would show the development of the substages of the parasite with time. Cultures with synchronized ring stages were the starting point, and every 6 h the different substages of the parasite were counted. In the presence of the control antibody, the parasites progressed through the ring, trophozoite, and schizont stages in about 24 h, and subsequently there was an increase in the ring stages, indicating the invasion of fresh red blood cells (Fig. 5*B*, *top panel*). However, in the presence of anti-PfP0 antibody, even though the parasites developed from rings to trophozoite and schizont stages, fresh infection of red blood cells did not take place as the number of ring stages remained close to zero until 48 h in culture (Fig. 5*B*, *lower panel*). The same profile was observed for the distribution of the parasite substages irrespective of the presence (Fig. 5*B*) or absence (data not shown) of monocytes.

DISCUSSION

Earlier we had reported the cloning of the full-length gene of PfP0 from the 7G8 strain of *P. falciparum* (7). The largest cDNA clone had a coding region 957 bases long, an unusually long 5′-untranslated region of at least ~1.4 kb, and a 3′-untranslated region of ~50 bases, making it a total of at least ~2.4 kb in size. The 3.0-kb transcript therefore matches with this size. It is not clear whether the second transcript of ~2.0-kb size is a processed transcript or a degradation product. The transcripts are twice as abundant in the trophozoite stages, indicating that the expression of this gene may be regulated.

The predicted molecular mass of the full-length gene sequence is 37.5 kDa. The rabbit sera raised against the recombinant protein domain of the P0 gene recognized a 38-kDa *P. falciparum* protein from both ^{35}S- and ^{32}P-labeled cultures, which matches with the expected size of the deduced P0 protein. The monospecific immune sera also recognized the same size protein. These immunoprecipitation studies showed that the antibodies raised against the PfP0-N recognize a protein of the predicted size, that it is indeed a phosphoprotein, and that antibodies against PfP0-N are present among malaria-immune people and not in patients.

The P0 protein was first identified as a member of the P family of proteins through coprecipitation studies using antibodies against the conserved carboxyl-terminal region (9, 15). Although definitive studies have been performed to demonstrate that P1 and P2 are phosphoproteins (27), there has been no direct evidence that P0 is a phosphoprotein. ^{32}P-Labeled cultures of yeast cells showed a large number of phosphoriboproteins (28), of which the 41-kDa protein has been assumed to be the P0 protein (9). Thus, for the first time, through immunoprecipitation studies using anti-P0 antibodies, we are demonstrating that P0 is indeed a phosphoprotein.

Autoantibodies against the conserved carboxyl-terminal domain of the P proteins have been found in 10–15% of patients with an autoimmune disorder, systemic lupus erythematosus (15). These autoantibodies have been implicated as a cause for the psychotic disorders among these patients. Immune response has also been documented against P0 protein in chronic Chagas' disease (16) and leishmaniasis (17). However, in each of these cases the reactivity has been reported against the carboxyl-terminal domain. The systemic lupus erythematosus patient sera have been tested for reactivity against the amino-terminal domain of the P2 protein but did not show any response (15). The reactivity of the malaria-immune sera to P0 is widespread (87% of immune sera samples) (6) and is directed toward the Pf4 region which is the amino-terminal domain of the PfP0 protein (7). Thus this immune response toward P0 in malaria-immune persons appears to be different from that observed for the systemic lupus erythematosus patients.

The mechanism of the passive clearance of the parasites in malaria patients with IgG of malaria-immune persons is not clearly understood. The IgG may be specifically interacting and blocking crucial parasite domains, or it may be attaching to the parasitized cells and allowing other cellular components of the immune system to clear the parasites. The latter has been supported by studies in which a pool of immune human sera inhibited the *in vitro* growth of *P. falciparum* only in the presence of monocytes (2). However, these experiments were performed with mixed specificities of immunoglobulins. Anti-PfP0-N antibody inhibits the growth of *P. falciparum* irrespective of the presence or absence of monocytes, indicating a direct block of parasite invasion. The mechanism behind the inhibition of the *P. falciparum* growth with anti-PfP0-N antibodies is unclear given the predominant cytoplasmic localization of the PfP0 protein. The inhibition is unlikely to be due to the inhibition of the *P. falciparum* ribosomal activity as the development from the ring to the schizont stages were nearly normal in the presence of anti-PfP0-N antibody over a period of 24 h. The growth inhibition by anti-PfP0-N IgG clearly seems to act at the erythrocyte-invasion step. While the IFA results do indicate a surface component in addition to the cytoplasmic localization of P0 in the trophozoites and gametocytic stages, whether there is a surface localization of this protein in the merozoite stage is yet to be determined. Interestingly, the presence of an antigenic determinant related to the carboxyl-terminal of P0 protein has been localized to the surface of hepatoma, neuroblastoma, and human fibroblast cells (29). What this surface determinant of P0 protein may be and what role it may play in the erythrocyte invasion by the malarial parasite remains to be established.

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