INFECTION AND IMMUNITY, Feb. 1994, p. 651–656
0019-9567/94/$04.00+0

Vol. 62, No. 2

# Novel Proteins of *Plasmodium falciparum* Identified by Differential Immunoscreening Using Immune and Patient Sera

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Received 26 January 1993/Returned for modification 9 March 1993/Accepted 9 November 1993

**A differential serological screen of a λgt11 cDNA expression library of *Plasmodium falciparum* was performed in an attempt to identify novel and putative host-protective antigens of the parasite. The screening was done with two categories of sera: (i) acute-phase sera obtained from smear-positive acutely infected *P. falciparum* patients from various regions in India and (ii) immune sera taken from healthy, permanent adult residents of *P. falciparum*-endemic rural districts of Orissa in eastern India. These adults had not suffered from any clinical malarial symptoms for at least the previous 3 years at the time of serum collection. Sixty-five clones obtained by screening the λgt11 library with two immune serum samples were analyzed extensively with a total of 70 acutely infected patient serum samples. Eight of these clones failed to react with any of the patient sera. Each of these eight clones, when tested individually with 92 serum samples from the immune group, reacted with a minimum of 43% of the samples from this category of sera. Thus, these eight epitopes may encode host-protective elements since they are not recognized by antibodies in the patient sera but react exclusively and extensively with the clinically immune set. Sequence analysis of two of these clones reveals that they are novel *Plasmodium* genes.**

Immunity to malaria develops gradually, after several attacks and over many years (12). It has long been known that malaria induces a detectable humoral response in humans. The successful passive transfer of malarial immunity in human subjects by means of gamma globulin from immune West Africans (3) focused attention on the protective nature of at least some malarial antibodies and implied that humoral mechanisms constituted a significant effector component of human immunoresponsiveness. A major thrust in malaria has thus been to identify these host-protective antibodies and their target antigens.

An unusual feature of almost all malarial antigens studied to date is the extensive array of tandemly repeating amino acid sequences (10). These repeats are immunodominant in the sense that much of the human antibody response is directed towards them. It has been observed that many nonimmune individuals have very high levels of antibodies to many repetitive antigens, and it has been suggested that this hyperstimulation of irrelevant B cells leads to a less effective immune response against critical protective epitopes; thus, the repeats are acting as a "smokescreen" (1). In addition to the repetitive epitopes, *Plasmodium falciparum* encodes an enormous number of cross-reactive epitopes, which are also postulated to be responsible for immune evasion (1). Therefore, it is desirable to identify those epitopes which may be protective but are perhaps obscured by the dominant immune response to the repetitive and the cross-reactive epitopes.

Screening of *P. falciparum* expression libraries with immune sera from protected individuals has been performed earlier, and a number of antigens have been identified, but indeed, most of these were found to be antigens containing repetitive domains (1, 4, 5, 10). No comparisons between sera from malaria-susceptible and -immune persons have been made to date. Thus, in an attempt to target antigenic determinants which react exclusively with the immune sera, we performed a differential screening of an expression library of the parasite with sera from healthy, adult residents of malaria-endemic areas who had not shown malarial symptoms for at least the previous 3 years and with sera from malaria patients actively presenting malarial symptoms at the time of serum collection. In this article, we present the results of such a screen, which yielded eight expression clones that react exclusively but extensively with the immune sera.

## MATERIALS AND METHODS

**Expression library.** The λgt11 cDNA expression library was a gift from G. N. Godson, New York University Medical Center. It was constructed from total parasite RNA isolated from the asexual erythrocytic stages of the FCR-3 strain of *P. falciparum* (18).

**Sera. (i) Acute-phase patient sera.** Thirty serum samples were collected from patients attending general hospitals in Surat, Gujarat (western India). Another 12 samples were obtained from patients visiting malaria clinical centers in Sonapur, Assam (eastern India). Patient sera were also collected from 23 patients admitted to hospitals in Cuttack, Phulbani, and Keonjhar, Orissa (eastern India). Five serum samples were collected from *P. falciparum*-infected individuals in Bombay. All of these sera were obtained from smear-positive individuals of both sexes and include samples collected from children as well.

**(ii) Immune sera.** A total of 92 serum samples were obtained from healthy adult residents of *P. falciparum*-endemic areas of the state of Orissa, India. Of these, 67 samples were collected from the Phulbani and Keonjhar districts, while 25 serum samples were collected from a tribal population of the Rourkela district. None of these individuals exhibited malarial

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symptoms at the time of serum collection nor had they done so for the previous 3 to 12 years, as ascertained from an extensive questionnaire answered by the subjects as well as their records in the primary health centers. Of the donors, 83% were in the age group of 25 to 50 years, 4% were younger than 25 years, and 13% were older than 50 years. The percentages of male and female donors were 54% and 46%, respectively. About 67% of the donors had not had an attack of malarial fever for the previous 5 to 10 years, whereas 25% showed an absence of attack for the previous 3 to 5 years. The remaining 8% had been immune to malaria for more than 10 years. The blood samples of all of the immune adults were tested for *P. falciparum* at the time of collection, and only 2 of the 94 donors were found to be smear positive, with low levels of parasitemia. Both of these samples were collected from the Rourkela district.

All sera were extensively purified of anti-*Escherichia coli* antibodies by several cycles of absorption with filter lifts of wild-type λgt11 on Y1090 and used at a dilution of 1:100.

**Immunological screening of the library.** Immunological screening was done essentially by the method of Young and Davis (23). Briefly, 10,000 plaques were plated on each 87-mm NZ plate, and the plates were incubated at 42°C for 4 h and then at 37°C for 2 h. Isopropyl-β-D-thiogalactopyranoside (IPTG)-saturated filters were then placed on the phage to induce the expression of the fusion proteins and left standing overnight. The filters were blocked in 5% nonfat milk for 1 h. They were incubated in the *E. coli*-adsorbed immune human sera (diluted 1:100) at 4°C for 16 h. After the filters were washed in Tris-buffered saline–Tween, they were incubated with goat anti-human immunoglobulin G conjugated to horseradish peroxidase (Cappel, Organon Teknika, Durham, N.C.) for 1 h. These filters were then extensively washed, and positive reactions were detected by using diaminobenzidine and hydrogen peroxide as substrates. Immunoreactive plaques were purified to homogeneity by two to three cycles of infection with Y1090 and plating for single plaques as described above.

**Immunodot blots of phage.** Plaques which were positive with the immune sera were spotted in a grid on a lawn of Y1090. They were grown and induced as described above. The filters were then treated with the respective serum samples, and immunoreactivities were visually scored on a scale of − to +++ in increasing order of signal intensity.

**ELISA.** The enzyme-linked immunosorbent assay (ELISA) used for the determination of serum reactivity among immune serum samples was performed as follows. An asynchronous culture of parasite strain FCR-3 was harvested at a parasitemia of about 8%, and the parasite pellet was obtained by saponin lysis (24). The pellet was sonicated for 3 min in 0.15 M phosphate-buffered saline (PBS; pH 7.4). Two hundred microliters of a 5-μg/ml solution of the extract described above was added to microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) and left standing overnight at 4°C. After being washed with 0.05% (vol/vol) Tween 20 in PBS (PBST), the plates were coated with 200 μl of PBST containing 7% sheep serum for 1 h at 37°C. All subsequent steps were carried out at 37°C. The wells were incubated for 2 h with 200 μl of 20 random samples of immune sera from the Keonjhar and Phulbani districts, diluted 1:100 in PBST. After extensive washing of the wells with PBST, the binding of serum antibodies was detected with horseradish peroxidase-conjugated anti-human immunoglobulin G (Cappel), diluted 1:2,000 in PBST for 1 h. After the wells were washed three times with PBST, 200 μl of substrate solution [10 mg of 2,2′-azino-di(3-ethyl-benzylthiozoline)sulfonic acid 6 (ABTS; Boehringer GmbH, Mannheim, Germany)] in 50 ml of $NaHPO_4$ buffer (pH 4.0), with 125 μl of 10% $H_2O_2$, was dispensed into each well. The color was allowed to develop for 30 min at room temperature (25 ± 2°C), and the $A_{405}$ values were read.

**Phage DNA preparation and sequencing of the *Plasmodium* inserts.** Phage DNA was prepared by standard protocols (16). The DNA was cut with *Eco*RI, and inserts were sized by using standard molecular weight markers. The inserts were subcloned into pGEM 3Z(f$^-$) (Promega, Madison, Wis.). Plasmid sequencing was done by using standard dideoxy chain termination protocols (17) with Sequenase version 1 from United States Biochemicals (Cleveland, Ohio) and [$^{35}$S]dATP from Amersham (Buckinghamshire, United Kingdom).

**Genomic dot blots.** Genomic DNA was prepared from the FCR3-FMG strain of *P. falciparum*. Parasites were liberated from infected erythrocytes by saponin lysis (24) and digested in digestion buffer (10 mM Tris, 10 mM NaCl, 10 mM EDTA, proteinase K) at 50°C for 18 h. After extensive phenol-chloroform extractions, the DNA was precipitated with ethanol and resuspended at a concentration of 1 μg/μl. DNA extracted from *Drosophila melanogaster* was used as the negative control. DNA was denatured and spotted onto nitrocellulose paper (Hybond-C; Amersham) and probed with the respective probes labelled with [$^{32}$P]dATP to a specific activity of 2 × $10^8$ cpm/μg and hybridized in a mixture of 50% formamide, 5× SSC, 1× Denhardt's solution, and 200 μg of salmon sperm DNA per ml at 45°C for 16 h. (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The blots were washed extensively in 0.1× SSC at 65°C and exposed to Fuji (Tokyo, Japan) AIF-RX film for 16 to 72 h at −70°C.

**Nucleotide sequence accession numbers.** Two cDNA insert sequences, Pf 4 and Pf 10, have been recorded in the EMBL data base under accession numbers X71408 and X71409, respectively.

## RESULTS

An ELISA of 20 of the immune serum samples from the Phulbani and Keonjhar districts was performed to determine serum reactivity of this set of serum samples. Two serum samples, with the highest absorbance values (0.47 and 0.42, respectively), were pooled and used to screen the *P. falciparum* cDNA λgt11 library. Approximately 100 positives were selected from 20,000 plaques for subsequent differential screening. Of these, 65 clones with distinct positive signals for the immune sera persisted. These were then subjected to a screening with 47 patient serum samples in pooled lots. The results of this immunoscreening are shown in Table 1. Ten of these clones (subsequently designated Pf 1 to Pf 10) did not react with any of the patient sera, while the other 55 clones reacted with the patient sera in different ways. The 10 clones were then plaque purified and rechecked for nonreactivity with the patient sera (Table 2). All 10 clones remained nonreactive with these sera after plaque purification. Another 23 serum samples from malarial patients living in the same endemic region as the donors of the immune sera were used for further screening, and 2 of the 10 clones showed positive reactivities with single serum samples (Table 2). A representative screening with patient and immune sera is shown in Fig. 1. In all, 70 patient serum samples were used to test these clones, and eight of the expression clones remained nonreactive to this lot.

To have any significance regarding clinical protection, the epitopes encoded by these clones should be recognized by the bulk of the immune sera. Table 3 presents the results of such an analysis, which used 92 serum samples. All clones were found to react with more than 43% of these samples.

This differential screen was expected to identify novel pro-

TABLE 1. Serological classification of clones obtained with different categories of sera

| No. of clones[a] | Immunoreactivity with serum samples from[b]: | | | | | |
|---|---|---|---|---|---|---|
| | Surat pool | | Assam pool | | General pool (5) | Orissa pool (2) |
| | 1 (12)[c] | 2 (18) | 1 (6) | 2 (6) | | |
| 4 | − | − | − | − | − | + |
| 5 | − | − | − | − | − | ++ |
| 1 | − | − | − | − | − | +++ |
| 2 | + | − | + | − | − | ++ |
| 1 | ++ | − | ++ | − | − | ++ |
| 1 | + | + | + | − | − | + |
| 1 | + | − | + | + | + | +++ |
| 1 | ++ | + | ++ | + | − | +++ |
| 1 | + | + | + | − | + | ++ |
| 1 | ++ | + | ++ | − | + | ++ |
| 38 | + | + | + | + | ND[d] | + |
| 9 | ++ | ++ | ++ | ++ | ++ | ++ |

[a] The total number of expression clones tested was 65.
[b] Immunoreactivity was scored as described in Materials and Methods.
[c] The number of serum samples used in the pool is shown in parentheses.
[d] ND, not determined.

tective determinants of *P. falciparum*. About 2 μg of the DNA from the 10 clones was checked for cross-hybridization with a pool of nine previously cloned and studied genes of *P. falciparum*. These included the S-antigen, MSA-1 (from two strains), HRP-1, HRP-2, HRP-3, RESA, MESA, and SERA. None of the 10 clones reacted with probes made from these genes (Fig. 2A). In a control reaction, the same probe was shown to light up 3.5 ng of the S-antigen DNA.

Inserts of five of the eight differential cDNA clones were used for cross-hybridization studies with the other clones. No cross-hybridization was obtained when such a study was performed. This showed that at least five of the eight clones coded for unique epitopes. A representative blot, using the insert from Pf 10, is shown in Fig. 2B, where a specific signal is seen only with DNA from phage Pf 10.

TABLE 2. Reactivity of the 10 λgt11 clones with patient sera

| Serum category (no. of samples) | Reactivity of Pf clone[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | PC[b] | NC[c] |
| Pools of patient sera from Assam and Surat and general pool[d] | − | − | − | − | − | − | − | − | − | − | ++ | − |
| Individual serum samples | | | | | | | | | | | | |
| Assam (3) | − | − | − | − | − | − | − | − | − | − | ++ | − |
| Surat (15) | − | − | − | − | − | − | − | − | − | − | ++ | − |
| Orissa (18) | − | − | − | − | − | − | − | − | − | − | +++ | − |
| Orissa (3) | − | − | − | − | − | − | − | − | − | − | − | − |
| Orissa (1) | − | − | − | − | + | − | − | − | − | − | +++ | − |
| Orissa (1) | − | − | − | − | − | + | − | − | − | − | + | − |

[a] Reactivity was scored as described in Materials and Methods.
[b] PC, positive control (a recombinant expression clone that reacted with most sera).
[c] NC, negative control of λgt11 phage.
[d] As described in Table 1.

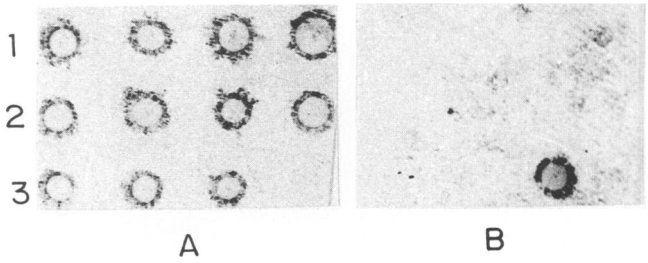


FIG. 1. A representative picture of the immunoscreening of the 10 λgt11 clones with a pool of two immune serum samples (A) and a pool of 14 patient serum samples, 6 from Assam, and 8 from Surat (B). The plaques were grown in the following order from left to right: row 1, Pf 1 to 4; row 2, Pf 5 to 8; row 3, Pf 9, 10, PC (a clone which reacted extensively with most of the patient as well as immune sera), and λgt11.

When sequenced, two of the cDNA clones, Pf 4 and Pf 10, revealed open reading frames of 238 and 531 bp in frame with *lacZ* of λgt11 (Fig. 3 and 4). The A+T contents of Pf 4 and Pf 10 inserts were 71 and 54%, respectively. The sequences were analyzed by the FASTA program of GenBank (13) to check for homologies to other known sequences. They both appear to be novel sequences with no significant homologies with any of the known genes of *P. falciparum* or with any other gene from other organisms. Clone 4 was reported to have a slight homology to the KAHRP of *P. falciparum*. The FASTA score for this homology was 85. Neither clones showed any repeat sequences, either overt or cryptic, when put through the dot plot program. A genomic dot blot shows a specific hybridization of the insert of clone 10 to the DNA from the FCR3-FMG strain of *P. falciparum* (Fig. 2C). The same result was obtained with the inserts of clones 9, 4, and 2 (data not shown), thus confirming that these four clones indeed have a *P. falciparum* origin. A Southern blot analysis revealed that at least two of the clones, Pf 4 and Pf 3, were not the same genes, as they hybridized to different bands in the genomic blot (8a).

A protein homology search was performed on the predicted amino acid sequences by using the FASTA program with these two sequences and the EMBL data bank (13). The only significant matches observed for Pf 4 and Pf 10 are shown in Fig. 5. Pf 4 showed 53.8% identity in a 52-amino-acid overlap, with the 60S ribosomal phosphoprotein $P_o$ of human origin, while Pf 10 showed about 30% identity over a 30-amino-acid overlap with the $M_2$ matrix protein of influenza virus of various

TABLE 3. Percent reactivity of the 10 λgt11 clones with the immune sera

| Expression clone no. | % of serum samples reacting with clone | | |
|---|---|---|---|
| | From Phulbani and Keonjhar (67)[a] | From Rourkela (25) | Total (92) |
| Pf 1 | 58 | 52 | 57 |
| Pf 2 | 57 | 60 | 58 |
| Pf 3 | 46 | 35 | 43 |
| Pf 4 | 88 | 85 | 87 |
| Pf 5 | 60 | 80 | 65 |
| Pf 6 | 51 | 50 | 50 |
| Pf 7 | 63 | 40 | 57 |
| Pf 8 | 63 | 45 | 58 |
| Pf 9 | 69 | 63 | 67 |
| Pf 10 | 66 | 63 | 65 |

[a] The total number of serum samples tested is shown in parentheses. Each serum sample was tested individually as described in Materials and Methods.

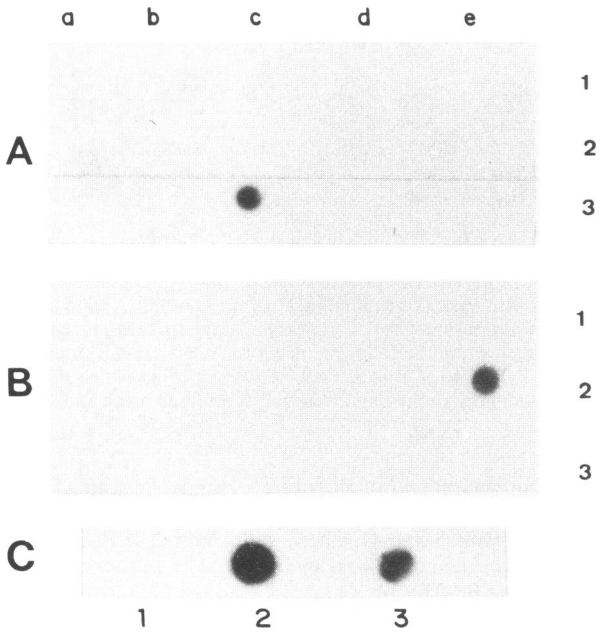


FIG. 2. (A) DNA from the 10 λgt11 clones probed with a pool of nine known genes of *P. falciparum*. The probe consisted of 50 ng of each of the plasmid DNAs containing the following antigens: S-antigen (8), MESA (6), RESA (7), MSA-1 (Camp strain), MSA-1 (FC27 strain) (20), KAHRP (19), HRP-11 and HRP-111 (22), and SERA (21). Two micrograms of each Pf clone DNA was loaded in the following order from left to right: row 1, Pf 1 to 5; row 2, Pf 6 to 10; row 3, S-antigen (3.5 ng; column c) and λgt11 (2 μg; column d). In row 3, the positions in columns a, b, and e were left empty. (B) The same blot as that of panel A but probed with the insert of clone Pf 10. (C) Blots: 1, 2 μg of *Drosophila* Schneider cell DNA; 2 and 3: 1 and 0.5 μg, respectively, of genomic DNA from the FCR3-FMG strain of *P. falciparum* probed with the insert of clone Pf 10.

origins. Pf 10 also showed a 17% identity in a 126-amino-acid overlap with the major capsid protein of varicella-zoster virus (data not shown).

## DISCUSSION

This study describes an attempt to isolate novel, putative protective antigens of *P. falciparum*. As shown in Table 1, 47 of 65 expression clones reacted with all sets of serum samples tested. These, therefore, encode epitopes that are possibly the immunodominant and cross-reactive epitopes of *P. falciparum* (4, 10). There were eight clones that reacted with certain pools and not with others. These may be antigenically diverse epitopes of *P. falciparum*, the positive responses indicating common molecular epitopes between the FCR-3 strain and certain Indian strains. However, the differential responses of these eight clones are more likely to be due to the differential immune response of individuals since no definitive correlation was observed for sera obtained from a particular locality. The possibility of antigenic diversity, or a genetically restricted immune response causing the exclusive reaction of the 10 clones with the immune sera collected from Orissa, was eliminated by the use of a set of patient serum samples from the same endemic area, i.e., Orissa (Table 2). Two determinants, Pf 5 and Pf 6, were indeed found to react with single serum samples obtained from patients from Orissa. These may be the candidates for antigenically diverse epitopes. However, eight of the expression clones did not react with any of the 70 patient serum samples tested, whereas they were immunopositive with at least 43% of the 92 immune serum samples tested (Table 3).

Approximately 40 genes coding for various surface antigens of *P. falciparum* have been cloned and sequenced at least in part. Many of them have been isolated by screening expression libraries with clinical sera or monoclonal antibodies (1, 4, 5, 9, 10). This study shows that the differential serological screen that we employed resulted in the identification of 10 expression clones that were different from nine common antigens of *P. falciparum* (Fig. 2A). Further analysis showed that two of these are indeed novel epitopes, without any repetitive regions (Fig. 3 and 4). Pf 4 is of special interest because it is the most widely occurring epitope among the immune serum samples. Attempts to affinity purify the specific antibody corresponding to the clones Pf 4 and Pf 10 and subsequently detect the parasite antigen on a Western blot (immunoblot) met with limited success (11a). It is possible that the antigens may be minor constituents of the parasite proteins or may be transitionally expressed epitopes. The β-galactosidase fusion proteins from the original λgt11 clones, as well as other fusion proteins of these epitopes constructed in other expression systems in *E. coli*, were found to be unstable.

It is possible that, in such a differential screen, we are missing certain epitopes that may be relevant to protection. It is known that adults of endemic area are often protected. Some

```
                12                  24                  36                  48                  60
GAA TTC CTT TTT CGC GCA TTG TTC CCA TTA TAT TTT AAT TTA AAT ATT ATT TTA TTT TTT
Glu Phe Leu Phe Arg Ala Leu Phe Pro Leu Tyr Phe Asn Leu Asn Ile Ile Leu Phe Phe

                72                  84                  96                  108                 120
AAA AAA AAG GCG AAA TTA TCC AAG CAA CAA AAA AAG CAA ATG TAC ATT GAG AAG CTT AGC
Lys Lys Lys Ala Lys Leu Ser Lys Gln Gln Lys Lys Gln Met Tyr Ile Glu Lys Leu Ser

                132                 144                 156                 168                 180
TCT CTC ATT CAA CAA TAT TCC AAA ATA TTA ATT GTG CAT GTA GAC AAT GTG GGA TCT AAT
Ser Leu Ile Gln Gln Tyr Ser Lys Ile Leu Ile Val His Val Asp Asn Val Gly Ser Asn

                192                 204                 216                 228                 240
CAA ATG GCT AGT GTT CGT AAA AGT TTA AGA GGA AAG GCT ACA ATA TTG ATG GGA AAA AAT
Gln Met Ala Ser Val Arg Lys Ser Leu Arg Gly Lys Ala Thr Ile Leu Met Gly Lys Asn


ACA AGA ATT C
Thr Arg Ile
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FIG. 3. Nucleotide and predicted amino acid sequences of the entire cDNA insert of Pf 4 encoding an open reading frame of 250 bp, starting and ending with the λgt11 *Eco*RI sites.

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            12                  24                  36                  48                  60
GAA TTC AGC TAT GCA TCC AAC GCG TTG GGA GCT CAT CGC AAA ATT TTT CAG GCC TAC AGA
Glu Phe Ser Tyr Ala Ser Asn Ala Leu Gly Ala His Arg Lys Ile Phe Gln Ala Tyr Arg

            72                  84                  96                  108                 120
GAT GCG CCG TTA TTG ACG ATG CGA GTT TTC TAC CAA ATT ATG AAT ATG CTG CTG CGT ATT
Asp Ala Pro Leu Leu Thr Met Arg Val Phe Tyr Gln Ile Met Asn Met Leu Leu Arg Ile

            132                 144                 156                 168                 180
CCA AAT GTA ATA ATA TTC CAC TGT ATT CCA TTT TTC GGA TCA GAC CAT CAT CAG TTT TTC
Pro Asn Val Ile Ile Phe His Cys Ile Pro Phe Phe Gly Ser Asp His His Gln Phe Phe

            192                 204                 216                 228                 240
TCA ACA TCA GGA TAC TGT TTT ATT TTT GGT CCT CCA TAC CAT TCT TCG GAT CAG ATC ATC
Ser Thr Ser Gly Tyr Cys Phe Ile Phe Gly Pro Pro Tyr His Ser Ser Asp Gln Ile Ile

            252                 264                 276                 288                 300
ACC AAT TTT TTA GTA CCT CAG GAT ACT GTT TCT TTG TCT TTT AGA CAT TCT TTG AAC ATC
Thr Asn Phe Leu Val Pro Gln Asp Thr Val Ser Leu Ser Phe Arg His Ser Leu Asn Ile

            312                 324                 336                 348                 360
ACA GTG CCG TCG TTG TTA TGT ACG CAA TCA TGC TTC TGC CAT GCC TAT GCA CGG CTG TAT
Thr Val Pro Ser Leu Leu Cys Thr Gln Ser Cys Phe Cys His Ala Tyr Ala Arg Leu Tyr

            372                 384                 396                 408                 420
ACT GTA GAT TGT GCA TCT GTG GCA CAT CTC CTT GCA TGC ACA TTC TTG CTG CTG CGT GCT
Thr Val Asp Cys Ala Ser Val Ala His Leu Leu Ala Cys Thr Phe Leu Leu Leu Arg Ala

            432                 444                 456                 468                 480
CAC GTC CTC AAC CTA CTA CTG GGC TGC TTC CTA ATG CAG AGT CGC ATA AGG GAT GAC TGT
His Val Leu Asn Leu Leu Leu Gly Cys Phe Leu Met Gln Ser Arg Ile Arg Asp Asp Cys

            492                 504                 516                 528                 540
CGA CCT GCA GGC GGC GGC CGC ACT AGT GAT ATC CCA CAT GGC GGC CGG GAG CAT GCA GAA
Arg Pro Ala Gly Gly Gly Arg Thr Ser Asp Ile Pro His Gly Gly Arg Glu His Ala Glu

TTC
Phe
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FIG. 4. Nucleotide and predicted amino acid sequences of the entire cDNA insert of Pf 10 encoding an open reading frame of 543 bp, starting and ending with the λgt11 *Eco*RI sites.

of the patient serum samples used in our screening were obtained from adults living in an endemic area and may contain antibodies against epitopes that are important for protection. Indeed, serum samples that reacted with Pf5 and Pf6 might belong to this category.

This differential screen was designed to isolate expression clones of *P. falciparum* against which antibodies were present only in malaria-immune persons, and therefore, they may be putative protective antigens. However, these immune persons have been exposed to repeated attacks of malaria, and thus it was anticipated that along with antibodies relevant for the immune response, there may be antibodies against nonsurface proteins as well. Especially, autoantibodies against protein domains of *P. falciparum* which are conserved between humans and parasites may be raised in such a set of immune persons. If Pf 4 turns out to be the *P. falciparum* equivalent for the ribosomal protein $P_o$, it would belong to such a class of protein domain. To date, only the amino acid sequence of the human $P_o$ phosphoriboprotein is available (15), and therefore it is difficult to predict whether this homologous domain is conserved in all organisms. It would, nevertheless, be important to characterize this protein of *P. falciparum* since ribosomes form a prime target for drugs.

The matrix protein $M_2$ of the influenza virus is a protein that is expressed abundantly on the infected cell surface (11). $M_2$ is a type III membrane protein with 24 N-terminal residues in the lumen, 54 residues in the cytosol, and a single membrane-spanning segment (11). The homology of $M_2$ with Pf 10 starts from the membrane-spanning domain and continues in the cytosolic domain. $M_2$ has recently been shown to be an

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(A)
            10          20          30          40          50          60
Pf4   FLFRALFPLYEMLNIILFFKKKAKLSKQQKKQMYIEKLSSLIQQYSKILIVHVDNVGSN
                                  :  :. :.  :....:.: .X: .:::::.
HuPo                        MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSK

              70          80
Pf4   QMASVRKSLRGKATILMGKNT
      ::. .:.:::::..:::::X
HuPo  QMQQIRMSLRGKAVVLMGKNT


(B)
                        10          20          30          40          50
Pf10            SYASNALGAHRKIFQAYRDAPLLTMRVFYQIMNMLLRIPNVIIFHCI-PFF
                                   X:..     .  :.....:: . ...::X .::
M2H/U       MSLLTEVETPIRNEWGCRCNDSSDPLVVAASIIGILHLILWILDRLFFKCIYRFF
                    10          20          30          40          50
M2H/B
M2H/P                                 G     AI  N                     R
M2A/F                    T  G E           II                  N        RL
M2A/M                    T  G E K          I                           RL

                   60          70          80          90
Pf10          GSDHHQFFSTSGYCFIFGPPYHSSDQIITNFLVPQDTVSLSF
              .. .  :::.:     .   :.....:
M2H/U         EHGLKRGPSTEGVPESMREEYRKEQQSAVDADDSHFVSIELE
                 60          70          80          90
M2H/B         K                             N
M2H/P         KY   G         K                     G
M2A/F         KY                        Q         V  G   N
M2A/M         KY                        Q         V  G   N
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FIG. 5. (A) The homology observed between the protein coded by Pf 4 and the human 60S ribosomal phosphoprotein $P_o$ (15). (B) The predicted homology between the protein coded by Pf 10 and the $M_2$ proteins from different strains of influenza A virus. H/U, H/B, and H/P are three strains of human influenza virus, while A/F and A/M are avian strains (2). These homologies were determined by using the FASTA program (13), and X's denote the ends of the initial region found by FASTA.

amantadine-sensitive ion channel for monovalent cations (14). Further characterizations of Pf 10 will determine whether it plays an equivalent role in *P. falciparum*.

## ACKNOWLEDGMENTS

We thank N. Patel and D. S. Chowdhury for providing some of the human serum samples, G. N. Godson for providing the cDNA library of the parasite and for helping with the cDNA sequencing, Azmat Maskati for her help during the initial immunoscreening, and D. J. Kemp, J. L. Weber, and T. E. Wellems for providing us with the DNA probes for the various *P. falciparum* antigens.

This work was supported by a grant (SP/SO/D-72/89) from the Department of Science and Technology, India.

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