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Cloning and expression in *Escherichia coli* of a surface antigen of *Plasmodium falciparum* merozoites

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A complementary DNA library was constructed from mRNA purified from asexual blood forms of *Plasmodium falciparum*. Among the members of this library we have identified a plasmid (pMC31-1) coding for a polypeptide exposed at the surface of merozoites, the invasive stage of the asexual cycle. This plasmid was identified by direct expression using both polyclonal and monoclonal antibodies specific for a schizont polypeptide of 200 kd which has been shown to be processed to an 83-kd polypeptide expressed at the surface of merozoites. The cDNA portion of the pMC31-1 plasmid hybridizes with DNA from three isolates of *P. falciparum*. Antisera raised against extracts of *Escherichia coli* harbouring pMC31-1 react with surface and internal structures of schizonts and with the surface of merozoites from all the isolates of *P. falciparum* examined. These results suggest that plasmid pMC31-1 encodes an antigen of value for the development of a vaccine against malaria.

Key words: Plasmodium falciparum merozoites/surface antigen/expression/*Escherichia coli*

Introduction

The multiplication of the asexual blood forms of plasmodia is responsible for the pathological manifestations of malaria in man. During this stage of the infection, the plasmodia and in particular *Plasmodium falciparum*, the most lethal species in man, develops within the host erythrocytes into three successive stages called rings, trophozoites and schizonts. Mature schizonts rupture the erythrocytes, releasing merozoites which after a short extracellular period invade new erythrocytes. Experimental evidence including passive transfer of immune human globulin (Cohen *et al.*, 1961), *in vitro* inhibition of parasite growth (Perrin and Dayal, 1982) and immunization of monkeys (Siddiqui, 1977; Mitchell *et al.*, 1977; Reese *et al.*, 1978) with whole merozoites suggest that merozoites are the target of a protective immune response. Recent experiments have shown that immunization of Saimiri monkeys with small amounts of a 200-kd schizont and merozoite specific polypeptide induces at least a partial protective immunity (Perrin *et al.*, 1984a,1984b). This polypeptide has been characterized by several groups over the last 4 years and has the following properties; its mol. wt. varies between 190 and 200 kd for different *P. falciparum* isolates (Perrin and Dayal, 1982; Holder and Freeman, 1982; Hall *et al.*, 1984); it shows a marked inter-isolate antigenic diversity (McBride *et al.*, 1982); it is expressed at the surface of schizonts (Howard *et al.*, 1984) and finally, a processed product of 83 kd is exposed at the surface of merozoites (Freeman and Holder, 1983; TDR/IMMAL/SWG 6, 1984) Here we describe the isolation and expression of a portion of a *P. falciparum* gene encoding a product with antigenic properties similar to those of the 190–200 kd polypeptide which is exposed on the surface of merozoites.

Results

Isolation of cDNA clones

Polyadenylated mRNA was prepared from *in vitro* cultured SGE2 isolates of *P. falciparum* enriched in schizonts and merozoites (Perrin *et al.*, 1984a), converted to double-stranded cDNA, inserted into the *Pst*I site of plasmid p31A by homopolymer tailing, and transformed into *Escherichia coli* C600 harbouring the pCI857 plasmid (Remaut *et al.*, 1981,1983). Bacterial transformants were grown at 30°C on nitrocellulose filters, and after induction at 42°C, lysed *in situ* with SDS (65°C for 30 min). SDS was removed and the filters incubated with a 1:500 dilution of serum from a rabbit immunized with affinity purified *P. falciparum* schizont and merozoite-specific antigens (Perrin *et al.*, 1984a). This rabbit serum reacted mainly with the 200- and 140-kd schizont and merozoite antigens (Perrin and Dayal, 1982). colonies binding rabbit antibodies were detected with [^{125}I]protein A. Multiple positive colonies were detected, and in this report we analyze one of these isolates, MC31-1.

Antigenic characterization of the fusion protein

Upon heat induction, clone MC31-1 makes large amounts of a 30-kd polypeptide instead of the 14-kd MS-2 polypeptide encoded by the parent plasmid (Figure 1A, lanes 1–4). Western blot analysis (Burnette, 1981) showed that the rabbit antiserum used in the initial screening recognizes the 30-kd polypeptide (Figure 1B). The Western blot analysis was repeated with sera from malaria-infected humans and with a pool of six monoclonal antibodies specific for the 190–200 kd schizont polypeptide with identical results (data not shown).

A rabbit antiserum raised with a lysate of induced MC31-1 reacted with a *P. falciparum* schizont polypeptide of 200 kd and additional peptides which are derived from the same polypeptide (Freeman and Holder, 1983; Holder and Freeman, 1982) (Figure 2A). On Western blot analysis a similar reactivity was found using unlabeled schizonts and merozoites (Figure 2B). In the schizont preparation (lane 4) the anti-MC31-1 serum recognized the 200-kd polypeptide and related products, while in the purified merozoite preparation (lane 5) an 83-kd polypeptide (processed product of the 190–200 kd polypeptide) was recognized. The technique of indirect immunofluorescence (O'Neil and Johnson, 1970) was also used to test the rabbit antiserum against intact *P. falciparum* asexual blood stages grown *in vitro*. The antiserum reacted with the surface and internal structures of fixed schizonts and with the surface of both fixed and (with lower intensity) unfixed merozoites of all isolates of *P. falciparum* tested. These included SGE2 (Zaire), FUP Palo Alto, FCR3 clone A2, FCC2 (China) and M23 (Honduras) (Figure 3).

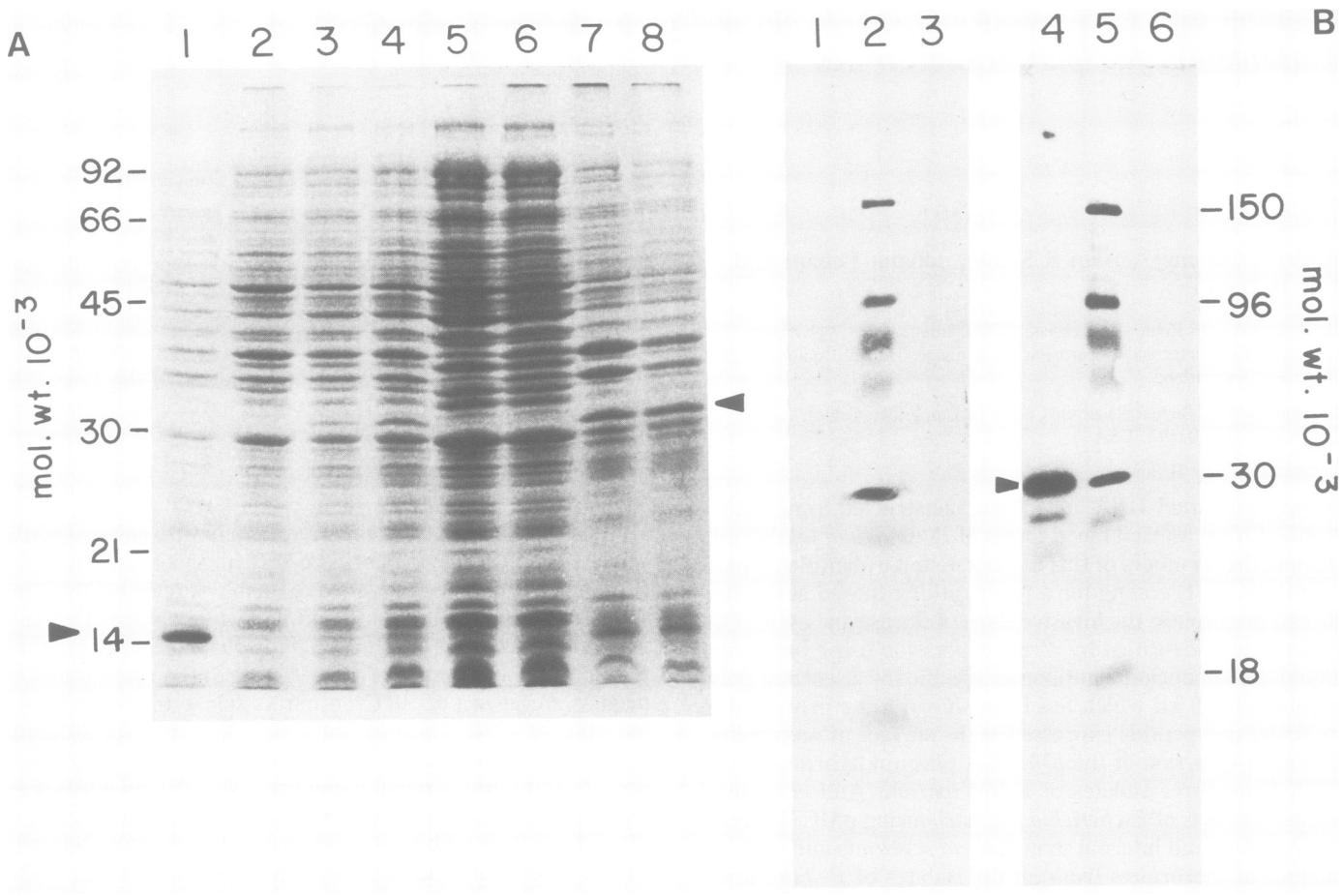

Fig. 1. (A) SDS-PAGE profile of recombinant clone MC31-1 polypeptides. SDS-PAGE was performed as described by Laemmli (1970). **Lane 1** is *E. coli* C600 containing the parent plasmid, p31A and pC1857 after induction at 42°C for 3 h; **lanes 2, 3** and **4** are MC31-1 before, after 1.5 h and after 3 h induction at 42°C, respectively. An aliquot of the culture was sonicated and centrifuged. The pellet was washed with PBS (supernatant 2, **lane 6**), and extracted twice with 6 M urea (**lanes 7** and **8**). **(B)** Autoradiograph of Western blot analysis (Burnette, 1981) of the MC31-1 chimeric protein. Bacterial samples prepared as described in **A** were subjected to SDS-PAGE and then electrophoretically transferred from the gel to nitrocellulose filters. Immunodetection of malarial antigens with rabbit antiserum was essentially as described by Burnette (1981). **Lanes 2** and **5** are mol. wt. standards; **lanes 1** and **4** are lysates of MC31-1 induced for 3 h at 42°C; and **lanes 3** and **6** are lysates of bacteria carrying p31A similarly induced. **Lanes 1–3** were treated with pre-immune rabbit serum. **Lanes 4–6** were treated with rabbit antiserum directed against schizont and merozoite antigens as described in the text.

DNA and protein sequence

The cDNA portion of plasmid pMC31-1 was released by digestion with restriction enzymes *Bam*HI and *Bgl*II. The DNA sequence was determined (Maxam and Gilbert, 1980) and the amino acid translation of the cDNA sequence is presented in Figure 4. The amino acid sequence is divided into three regions: 44 amino acids of unique sequence, a region composed of repeating units of three amino acids (Ser-Gly-Gly six times and Ser-Val-Ala three times), and a second region of 20 residues of unique sequence. The repeated sequences are composed exclusively of uncharged amino acids while the flanking sequences are acidic in character. Our sequence differs from previously published sequences of malarial repeats (Ozaki *et al.*, 1983; Coppel *et al.*, 1983,1984; Kemp *et al.*, 1983; Koenen *et al.*, 1984). We see variation in the second position of the codon leading to an amino acid change (e.g., GGT/gly→GTT/val) rather than third position variation conserving the amino acid sequence.

Genomic blotting analysis

Since the 190–200 kd protein has been implicated in inter-isolate antigenic diversity (McBride *et al.*, 1982), we looked for DNA sequence homology between clone MC31-1, derived from SGE2 (Zaire) and isolates M23 (Honduras) and FCC2 (China). These isolates have been selected on the basis of their antigenic differences at the level of the 190–200 kd polypeptide as detected by using monoclonal antibodies (L.Perrin, unpublished data). DNA extracted (Wallach, 1982) from each of these strains was digested separately with five restriction enzymes (*Bam*HI, *Bcl*I, *Eco*RI, *Hind*III and *Pvu*II), separated on a 0.7% agarose gel, transferred to a nitrocellulose filter (Southern, 1975), and probed with a riboprobe derived from the cDNA portion of pMC31-1 (Figure 5). Under stringent conditions the MC31-1 sequence hybridizes with equal efficiency to DNA from the China, Honduras and Zaire isolates of *P. falciparum*. The restriction patterns of the China and Zaire isolates are similar for these five enzymes and give fragments of the following sizes: *Bam*HI, 8.7 kb; *Bcl*I, 1.8 kb; *Eco*RI, 4.2 kb; *Hind*III, 3.1 kb; *Pvu*II, 6.7 kb. The Honduras isolate is polymorphic for *Bcl*I (8.0 kb), *Eco*RI (4.0 kb) and *Hind*III (6.5 kb). The DNA sequence of the cDNA portion of pMC31-1 contains a *Hind*III site and a *Pvu*II site separated by 81 bp. Southern blotting experiments indicate that these two restriction sites are present in all three isolates and are separated by the same interval (data not shown).

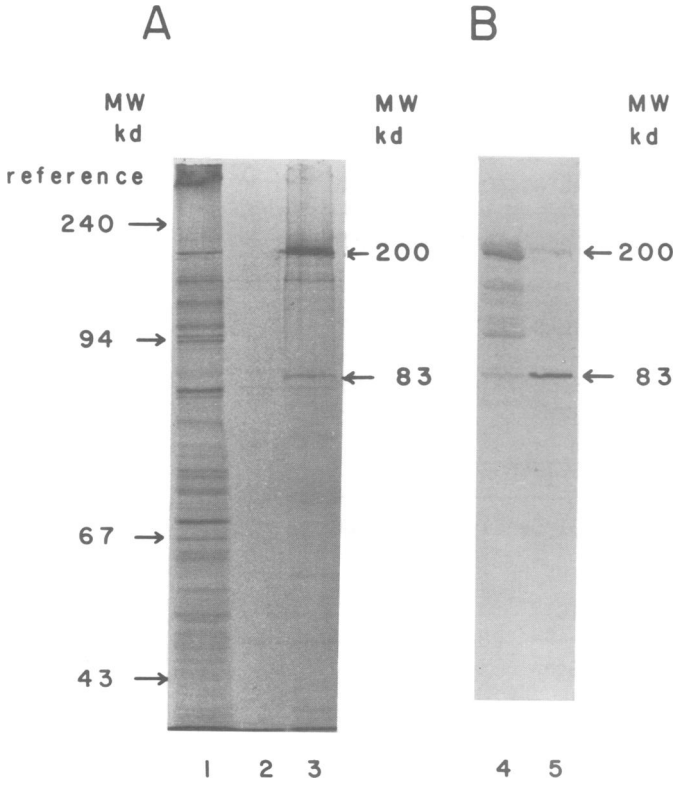

Fig. 2. (A) Autoradiograph of *P. falciparum* [^{35}S]methionine-labeled polypeptides immunoprecipitated by rabbit antiserum against MC31-1. 10 μl of serum from a rabbit with an antibody titre of 1:8000, as measured by indirect immunofluorescence, was incubated with 500 000 c.p.m. of an extract of *P. falciparum* (isolate SGE2), metabolically labeled for 6 h with 100 μCi/ml [^{35}S]methionine (Perrin *et al.*, 1984a). Protein A was used as second reagent in the immunoprecipitation reaction **2**. **Lane 1** is the total *P. falciparum* extract. **Lanes 2** and **3** are immunoprecipitates obtained using serum from the rabbit after and before immunization. Reference mol. wts. are indicated. **(B)** Identification of polypeptides recognized by the anti-MC31-1 rabbit antiserum. For this experiment, lysates of unlabeled schizonts **(lane 4)** and merozoites **(lane 5)** prepared as previously reported (Perrin *et al.*, 1984a; Freeman and Holder, 1983; Mrema *et al.*, 1982) were run on 8% SDS-PAGE and transferred to nitrocellulose. The sheet was incubated overnight with a 1/50 dilution of rabbit anti-MC31-1 serum, washed, and subsequently incubated with 40 ml of ^{125}I-labeled affinity purified goat anti-rabbit IgG at a concentration of 100 000 c.p.m./ml (New England Nuclear). After extensive washing (Burnette, 1981), the nitrocellulose sheet was autoradiographed for 48 h.

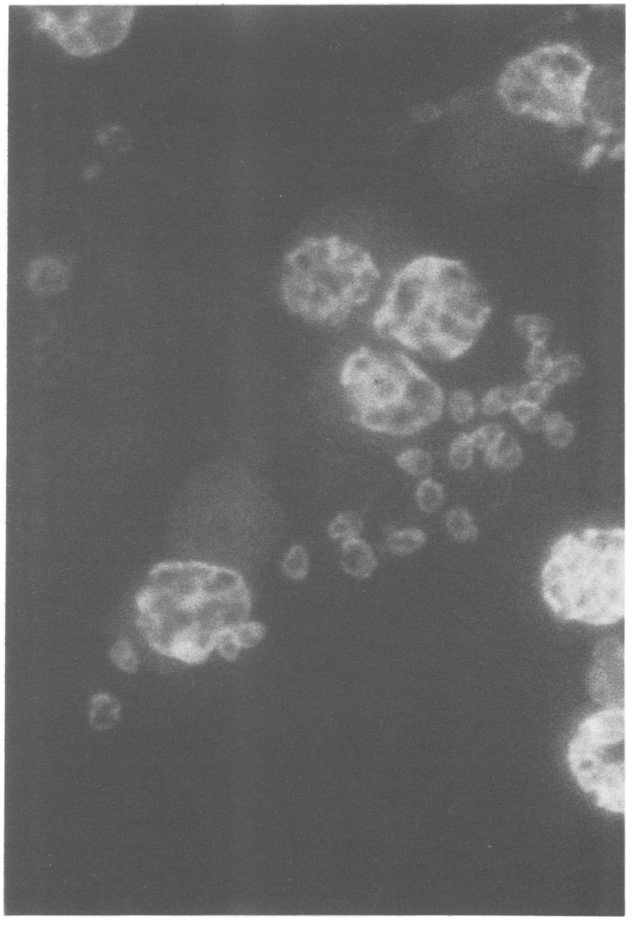

Fig. 3. Specificity of rabbit antibodies against MC31-1 visualized by indirect immunofluorescence. Acetone-fixed SGE2 schizonts and merozoites on a multispot slide were incubated with a 1/500 dilution of the rabbit serum followed by a 1/50 dilution of fluoresceinated affinity purified anti-rabbit antibodies. These antibodies are evenly distributed over the entire surface of the merozoites (upper center of figure) and also react with the membrane and internal structures of schizonts shown at various stages of the developmental cycle (magnification 2000x).

Discussion

Merozoites are the extracellular forms of *P. falciparum* during the asexual cycle, and the molecules expressed at their surface are obvious candidates for the development of a vaccine based on asexual blood stages (TDR/IMMAL SWG 6, 1984). One of the major polypeptides exposed at the surface of merozoites has a mol. wt. of 83 kd and has been shown to be a processed product of a shizont-specific polypeptide of 190 – 200 kd (Freeman and Holder, 1983). In this paper we report the cloning of a *P. falciparum* DNA sequence which codes for a portion of the 190 – 200 kd schizont and merozoite specific polypeptide. This clone was identified using, as a probe for expression of malarial peptides, polyclonal and pooled monoclonal antibodies directed against the 190 – 200 kd polypeptide. This polypeptide, like several other known plasmodial polypeptides (Ozaki *et al.*, 1983; Coppel *et al.*, 1983,1984; Kemp *et al.*, 1983; Koenen *et al.*, 1984), contains amino acid repeats as well as flanking portions of unique sequence. Recently, Odink *et al.* (1984) and Hall *et al.* (1984) have reported the molecular cloning of portions of a gene encoding a 190 – 200 kd polypeptide. It is possible that clone MC31-1 (this report), clone pFC17 (Odink *et al.*) and clone lambda p190-1 (Hall *et al.*) are different portions of the same gene. The sequence reported by Hall *et al.* (1984) corresponds to the 3′ end of the gene and encodes the epitope found on a variety of polypeptides processed from the 190-kd protein. Antibodies raised against the polypeptide encoded by pMC31-1 react with both the 190 – 200 kd schizont polypeptide and its 83-kd processed product, suggesting that the polypeptide contains epitopes exposed at the merozoite surface. Moreover the same antibodies react with the surface of merozoites by IFA.

The 190 – 200 kd polypeptide has been shown to be involved in inter-isolate diversity (McBride *et al.*, 1982); in this respect, antibodies raised against pMC31-1 react with the surface of merozoites and schizonts from all the *P. falciparum* isolates tested, suggesting that at least some of the epitopes of the polypeptide are present on several strains of *P. falciparum*. These investigations have recently been extended by immunizing rabbits with synthetic polypeptides corresponding to both unique and repeated sequences of the polypeptide encoded by pMC31-1. Antibodies from these rabbits gave the same IFA pattern on SGE2, FCC2, and M23 merozoites and schizonts as antibodies raised

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                                    HindIII
    TTATCAAGAACTTGTCAAAAAACTAGAAGCTTTAGAAGATGCAGTATTGACAGGTTATAG
1   ---------+---------+---------+---------+---------+---------+   60
     TyrGlnGluLeuValLysLysLeuGluAlaLeuGluAspAlaValLeuThrGlyTyrSe

                                                        PvuII
    TTTATTTCAAAAGGAAAAAATGGTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAAC
61  ---------+---------+---------+---------+---------+---------+  120
    rLeuPheGlnLysGluLysMetValLeuAsnGluGlyThrSerGlyThrAlaValThrTh


    TAGTACACCTGGTTCAGGTGGTTCAGTTACTTCAGGTGGTTCAGGTGGTTCAGTTGCTTC
121 ---------+---------+---------+---------+---------+---------+  180
    rSerThrProGlySerGlyGlySerValThrSerGlyGlySerGlyGlySerValAlaSe
                ->        ->        ->        ->        ->        ->

    AGTTGCTTCAGGTGGTTCAGGTGGCTCAGTTGCTTCAGGTGGTTCAGGTAATTCAAGACG
181 ---------+---------+---------+---------+---------+---------+  240
    rValAlaSerGlyGlySerGlyGlySerValAlaSerGlyGlySerGlyAsnSerArgAr
          ->        ->        ->        ->

    TACAAATCCTTCAGATAATTCAAGTGATTCAGATGCTAAATCTT
241 ---------+---------+---------+---------+----  284
    gThrAsnProSerAspAsnSerSerAspSerAspAlaLysSer
```

Fig. 4. Sequence of the cDNA portion of plasmid pMC31-1. The DNA sequence was determined by the method of Maxam and Gilbert (1980). The derived amino acid translation is shown below the DNA sequence. Landmark restriction sites (*Hind*III and *Pvu*II) are shown above the DNA sequence. Tripeptide repeats are indicated by arrows below the amino acid sequence.

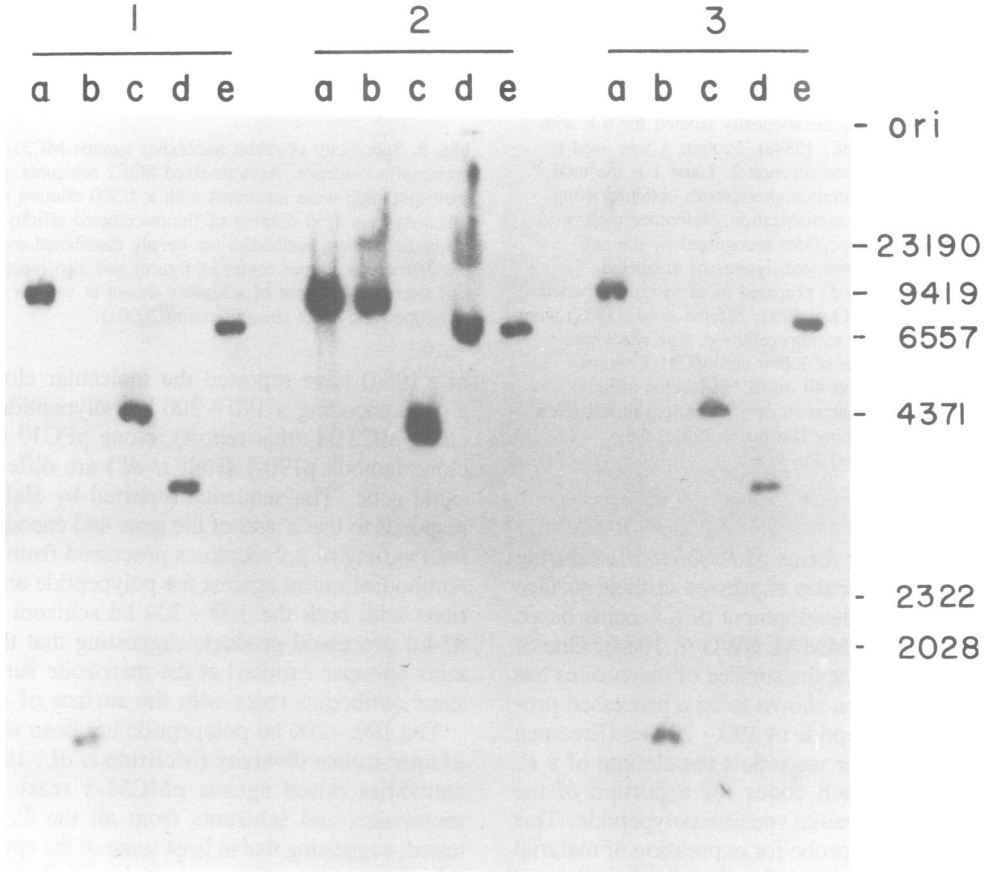

Fig. 5. Southern blot of restriction digests of DNA from China, Honduras, and Zaire isolates of *P. falciparum*. DNA was extracted (Wallach, 1982) from isolates FCC2 (China, **lane group 1**), M23 (Honduras, **lane group 2**) and SGE2 (Zaire, **lane group 3**). 4 μg of each DNA was digested with 20 units each of *Bam*HI **(a),** *Bcl*I **(b),** *Eco*RI **(c),** *Hind*III **(d),** and *Pvu*II **(e)** for 2 h and separated on a 0.7% agarose gel. The DNA was transferred to nitrocellulose (Souther, 1975) and probed with a riboprobe transcribed from a pSP64 vector (Promega Biotech) carrying the cDNA portion of pMC31-1. Hybridization was in 2 x SSC, 50% formamide at 45°C. Autoradiography was for 10 h at −80°C on Kodak XAR-5 film with DuPont Cronex Lightning Plus intensifying screens. The positions of size standards (lambda DNA digested with *Hind*III) are noted at the right margin.

against pMC31-1 (J.Leban, A.Shaw, A.Cheung and L.Perrin, in preparation) suggesting that the major part of pMC31-1 is represented in the 83-kd polypeptide exposed at the surface of merozoites.

The presence of the DNA sequence cloned in pMC31-1 in various isolates was also studied. Indeed, the pMC31-1 insert hybridizes with equal efficiency to DNA of all the isolates tested. In addition, the restriction patterns obtained with DNA of two isolates (FCC2 and SGE2) are similar for digests obtained using five different enzymes. However a difference was seen with three enzymes for the third strain tested. These results suggest that the pMC31-1 probe and other probes currently selected by hybridization to pMC31-1 on a cDNA library, may be of use in the typing of *P. falciparum* isolates and for further study of the structure of the gene for the 190–200 kd polypeptide.

In conclusion, the location of the MC31-1 antigen on the surface of merozoites, and its presence in isolates of *P. falciparum* from different endemic areas suggest the potential usefulness of the polypeptide encoded pMC31-1 for the development of a malaria vaccine against asexual blood stages.

Materials and methods

Parasites

P. falciparum schizonts and merozoites from three different isolates (SGE2 from Zaire, FCC2 from China and M23 from Honduras) were purified by differential centrifugation using as starting material *in vitro* cultures (Perrin *et al.*, 1984a). These schizonts and merozoites were subsequently used for DNA purification.

Metabolic labelling of asexual blood stages with [^{35}S]methionine, preparation of soluble extracts, and immunoprecipitation with protein A were done as previously described (Perrin *et al.*, 1984a).

Infected red cells from these isolates and from two additional isolates (FUP Palo Alto and FCR3 clone A2) were coated on multispot slides or used as 2.5% suspension for indirect immunofluorescent antibody (IFA) analyses (O'Neill and Johnson, 1970).

Antibodies

Two rabbits were immunized with purified *P. falciparum* polypeptides (mainly 200, 140 and 83 kd) used previously in monkey immunization experiments (Perrin *et al.*, 1984a). The rabbits were immunized three times at 2-week intervals with 100 μg of antigen mixed with Freund's complete adjuvant the first time and Freund's incomplete adjuvant the second and third times. A pool of the sera had a titer of 1/10 000 by IFA. This pool was adsorbed for 1 h at 4°C with one volume of packed normal human red blood cells and subsequently with one volume of packed *E. coli* C600. This pool of sera immunoprecipitated 200-, 140- and 83-kd *P. falciparum* polypeptides.

Two rabbits were immunized three times with 3 mg of a lysate of induced MC31-1 according to the schedule described above. Serum from one of these rabbits had an end point titer of 1/8000 by IFA and was used for immunoprecipitation and Western blot analysis.

A pool of six monoclonal antibodies against the 190–200 kd polypeptide was selected on the basis of IFA reactivity and on the basis of reactivity by immunoprecipitation (Perrin and Dayal, 1982; McBride *et al.*, 1982).

Isolation of mRNA and DNA from P. falciparum

Total nucleic acid was isolated from cultured parasites by the method of Wallach (1982). Upon ethanol precipitation the high mol. wt. DNA formed a large aggregate which was removed with sterile forceps. Polyadenylated RNA was isolated from the remaining nucleic acid by oligo(dT)-cellulose chromatography (Maniatis *et al.*, 1982). Conversion of polyadenylated RNA to cDNA, insertion of cDNA into the plasmid vector and transformation of the bacterial host were all by standard methods (Maniatis *et al.*, 1982).

Bacteria and plasmids

Plasmid p31A, the kind gift of Dr. H. Kupper, is a derivative of pPLMC24 (Remaut *et al.*, 1981) bearing the ampicillin resistance gene from plasmid pUR1 (Ruther, 1980) lacking a *Pst*I restriction site. Plasmid p31A contains the lambda PL promoter followed by the coding sequence for the NH_2-terminal 99 amino acids of the bacteriophage MS-2 polymerase and a synthetic polylinker with *Eco*RI, *Bam*HI, *Pst*I, *Bgl*II, *Xba*I and *Hind*III restriction sites. cDNA sequences inserted in the *Pst*I site are expressed as MS-2 fusion polypeptides under the control of the PL promoter. The host for plasmid p31A, *E. coli* C600, contains a second plasmid, pcI857, which encodes kanamycin resistance and a temperature-sensitive lambda cI repressor protein which regulates the activity of the PL promoter.

Expression of cloned antigen

Bacterial colonies containing p31A:cDNA were transferred to nitrocellulose filters on L broth agar plates containing 40 μg/ml each of ampicillin and kanamycin. After growth overnight at 30°C the plates were incubated at 42°C for 4 h. The filters were then placed colonies up on 3MM (Whatman) paper soaked in 2% SDS for 15 min. The SDS was removed electrophoretically. Colonies producing *P. falciparum* antigens were detected by incubation with a 1:50 dilution of rabbit antiserum raised against purified schizont and merozoite polypeptides followed by washing and incubation with ^{125}I-labeled Staph protein A.

Samples of chimeric antigens were prepared as follows. A bacterial culture was grown overnight (30°C) in L-broth supplemented with 40 μg/ml each of ampicillin and kanamycin. After diluting the overnight culture 1:4 into pre-warmed broth (42°C), the culture was incubated at 42°C for an additional 3 h with vigourous shaking. Cells were collected by centrifugation, washed once in phosphate-buffered saline and lysed by boiling in SDS mercaptoethanol (Laemmli, 1970).

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References

Burnette,W.N. (1981) *Anal. Biochem.*, **112**, 195-203.
Cohen,S., McGregor,I.A. and Carrington,S.C. (1961) *Nature*, **192**, 733-735.
Coppel,R.L., Cowman,A.F., Lingelbach,K.R., Brown,G.V., Saint,R.B., Kemp,D.J. and Anders,R.F. (1983) *Nature*, **306**, 751-756.
Coppel,R.L., Cowman,A.F., Anders,R.F., Bianco,A.E., Saint,R.B., Lingelbach,K.R., Kemp,D.J. and Brown,G.V. (1984) *Nature*, **310**, 789-792.
Freeman,R.R. and Holder,A.A. (1983) *J. Exp. Med.*, **158**, 1647-1653.
Hall,R., Hyde,J.E., Goman,M., Simmons,D.L., Hope,I.A., Mackay,M. and Scaife,J. (1984) *Nature*, **311**, 379-382.
Holder,A.A. and Freeman,R.R. (1982) *J. Exp. Med.*, **156**, 1528-1533.
Howard,R.J., Lyon,J.A., Diggs,C.L., Haynes,J.D., Leach,J.H., Barnwell,J.W., Aley,S.B., Aikawa,M. and Miller,L.H. (1984) *Mol. Biochem. Parasitol.*, **11**, 349-362.
Kemp,D.J., Coppel,R.L., Cowman,A.F., Saint,R.B., Brown,G.V. and Anders,R.F. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3787-3791.
Koenen,M., Scherf,A., Mercereau,O., Langsley,G., Sibill,L., Dubois,P., Pereira da Silva,L. and Muller-Hill,B. (1984) *Nature*, **311**, 382-385.
Laemmli,U.K. (1970) *Nature*, **227**, 680-685.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
Maxam,A. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 449-560.
McBride,J., Walliker,D. and Morgan,G. (1982) *Science (Wash.)*, **217**, 254-256.
Mitchell,Q.H., Butcher,G.A., Richards,W.G.H. and Cohen,S. (1977) *Lancet*, **i**, 1335-1341.
Mrema,J.E.K., Langreth,S., Jost,R.C., Reickmann,K.H. and Heidrich,H.G. (1982) *Exp. Parasitol.*, **54**, 285-295.
Odink,K.G., Lockyer,M.J., Nicholls,S.C., Hillman,Y., Freeman,R.R. and Holder,A.H. (1984) *FEBS Lett.*, **173**, 108-112.
O'Neill,P. and Johnson,G.D. (1970) *J. Clin. Pathol.*, **23**, 185-193.
Ozaki,L.S., Svec,P., Nussenzweig,R.S. Nussenzweig,V. and Godson,G.N. (1983) *Cell*, **34**, 815-822.
Perrin,L.H. and Dayal,R. (1982) *Immunol. Rev.*, **61**, 245-269.
Perrin,L.H., Loche,M., Dedet,J.P., Roussilhon,C. and Fandeur,T. (1984a) *Clin. Exp. Immunol.*, **56**, 67-72.
Perrin,L.H., Merkli,B., Loche,M., Chizzolini,C., Smart,J. and Richle,R. (1984b) *J. Exp. Med.*, **160**, 441-451.
Reese,R.T., Trager,W., Jensen,J.B., Miller,D.A. and Tantravali,R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 5565-5568.
Remaut,E., Stanssens,P. and Fiers,W. (1981) *Gene*, **15**, 81-93.
Remaut,E., Tsao,H. and Fiers,W. (1983) *Gene*, **22**, 103-113.
Ruther,U. (1980) *Mol. Gen. Genet.*, **178**, 475-477.
Siddiqui,W.A. (1977) *Science (Wash.)*, **197**, 388-390.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
TDR/IMMAL/SWG (6)/84.3 Geneva, Switzerland, March 26-28, 1984.
Wallach,M. (1982) *Mol. Biochem. Parasitol.*, **6**, 335-342.

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