The EMBO Journal vol.7 no.1 pp.225-230, 1988

Major surface antigen p190 of *Plasmodium falciparum*: detection of common epitopes present in a variety of plasmodia isolates

Reiner Gentz, Ulrich Certa, Bela Takacs, Hugues Matile, Heinz Döbeli, Richard Pink, Martin Mackay[1,2], Neil Bone[1,3] and John G.Scaife[1]

Central Research Units, F.Hoffmann-La Roche and Co. Ltd, 4002 Basel, Switzerland and [1]Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK

Present addresses: [2]Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex RH12 4AB and [3]Department of Zoology, University of Cambridge, Cambridge, CB2 3EJ, UK

Communicated by M.Noll

***Plasmodium falciparum* merozoites are covered with polymorphic proteins that are processed from a 190 kd (p190) precursor protein. These are candidates for an antimalarial vaccine. We cloned and expressed a number of DNA fragments, comprising almost the entire p190 gene of the K1 isolate, in *Escherichia coli*. Pooled human endemic-area sera and rabbit antibodies raised against p190 protein isolated from K1 parasites react with only a limited number of the recombinant proteins. From these studies we could select two antigenic polypeptides containing conserved amino acid stretches of the otherwise highly polymorphic protein. Rabbits and mice injected with the purified recombinant proteins produce antibodies reacting differentially with various isolates of *P. falciparum*. We obtained antibodies detecting all isolates tested and a monoclonal antibody specific for isolates containing a K1 type allele of the p190 gene.**

Key words: malaria/merozoite surface antigen/non-variable sequences/vaccine

Introduction

Merozoites of the human malaria parasite *Plasmodium falciparum* multiply in erythrocytes of the host. From a single blood cell 8-24 new merozoites are released into the bloodstream to infect new erythrocytes.

The major components of the merozoite's surface are processed products of the polymorphic schizont antigen p190 (Perrin *et al.*, 1980; Freeman and Holder, 1983; Holder and Freeman, 1982, 1984). The function of the protein and its processed products is unknown, but injection of purified p190 protein isolated from blood stage parasites into monkeys results in partial (Hall *et al.*, 1984b; Perrin *et al.*, 1984; Cheung *et al.*, 1985) or complete (Siddiqui *et al.*, 1987) protection against *P. falciparum* malaria after parasite challenge. Studies with monoclonal antibodies and cloning of p190 genes from a number of parasite isolates revealed that this antigen is polymorphic (Hall *et al.*, 1983; Holder *et al.*, 1985; Mackay *et al.*, 1985; McBride *et al.*, 1985; Lyon *et al.*, 1987; Weber *et al.*, 1986; Howard *et al.*, 1986; Tanabe *et al.*, 1987; Certa *et al.*, 1987). A detailed sequence analysis of four cloned genes suggests that the gene consists of only two prototype sequences (Tanabe *et al.*, 1987). Antigen diversity is most likely created by multiple intragenic crossover events resulting in polymorphic sequences (Tanabe *et al.*, 1987; Certa *et al.*, 1987). In addition, isolate-specific tripeptide repeats typical of many plasmodium antigens contribute to protein polymorphism (Holder *et al.*, 1985; Mackay *et al.*, 1985; Kemp *et al.*, 1986; Weber *et al.*, 1986; Howard *et al.*, 1986; Certa *et al.*, 1987).

Parts of the p190 gene have been expressed in *Escherichia coli*, with a view to using the product(s) in an antimalarial vaccine. Earlier attempts (Holder *et al.*, 1985; Lyon *et al.*, 1987) relied on cloning gene fragments into vectors which produced the parasite protein fused to a bacterial polypeptide (e.g. β-galactosidase). Fusion proteins containing a segment of a bacterial protein complicate interpretation of immunological assay; they were avoided in this study.

We selected two segments derived from non-variable parts of the gene (Tanabe *et al.*, 1987) which were well expressed and immunogenic.

Evidence is presented that these recombinant proteins and the p190 protein isolated from parasites elicit a similar immune response. The antibodies against the recombinant proteins react with every parasite isolate tested, suggesting that they contain non-polymorphic epitopes. Monoclonal antibodies raised against these conserved polypeptides of p190 react differently with the parasite isolates tested. Therefore our results extend the genetic data available for p190 polymorphism (Tanabe *et al.*, 1987) and suggest that the conserved amino acid stretches present in the otherwise polymorphic polypeptide may have important functions.

Finally, we discuss the importance of these epitopes in the development of the humoral response to parasite infection.

Results

Expression of the K1 p190 gene in E. coli

In order to identify the epitopes responsible for malaria protection in the monkey model (Perrin *et al.*, 1984; Siddiqui *et al.*, 1987) we decided to express the whole p190 protein or parts of it in *E.coli* and analyse the recombinant proteins with rabbit antibodies against p190 protein from parasites and human endemic sera. Initially, we attempted to express the full-length protein. However, instability of the large insert fragment (5.4 kb) made this approach unlikely to succeed (data not shown). We consequently divided the sequence in several subclones, with the exception of the signal peptide, tripeptide repeats and anchor sequences (Mackay *et al.*, 1985). For initial expression experiments overlapping fragments were cloned into the pUC plasmids (Vieira and Messing, 1982) in which the foreign polypeptide is fused to the N-terminus of β-galactosidase under *lac* repressor control. Two fragments encoding conserved parts of the gene

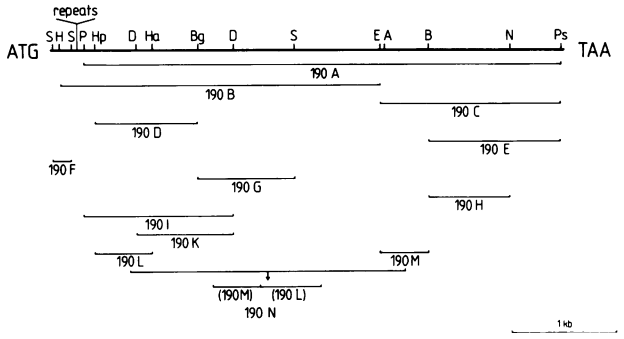

Fig. 1. Subcloning strategy of the p190 gene in expression vectors. The DNA came from genomic fragments, p190-3 and p190-4, cloned in phage λ (Mackay *et al.*, 1985). The relevant restriction sites appear in the top line; the fragments cloned and nomenclature appear below. The vectors used, properties of the expressed proteins and their reactivity in Western blots are summarized in Table I. For simplicity the recombinant proteins expressed were named alphabetically 190A to 190N. The plasmids encoding them have the prefix p. (A, *Alu*I; B, *Bam*HI; Bg, *Bgl*II; D, *Dra*I; E, *Eco*RI.; H, *Hin*dIII; Ha, *Hae*III; Hp, *Hpa*I; N, *Nde*I; P, *Pst*I; S, *Sau*3A). The position of the tripeptide repeats is indicated. Fragments sizes are given in kilobase (kb) of DNA. 190N is a fusion protein of 190L and 190M (see text).

Table I. Expression of p190 gene fragments in *E.coli*

Polypeptide	Plasmid	Size[a]	Degrad.[b]	Coom.[c]
190A	pUC	ND	ND	−
190B	pUC	54 + 58[d]	+	−
190C	pUC	81	−	−
190D	pDS	42	+	−
190E	pDS	45	+	−
190F	pUC	21	−	−
190G	pUC	54	+	−
190G*	pDS	31	+	+
190H	pDS	42	+	−
190I	pUC[e]	42	−	−
190K	pBR-tac	41	−	−
190L	pDS	25	−	+
190M	pDS	22	−	+
190N	pDS	43	+	+

The recombinant proteins analysed except 190A gave a positive reaction in Western blots with rabbit anti-p190 serum (Hall *et al.*, 1984). 190F and 190G proteins were fused to the N-terminus of β-galactosidase encoded by the pUC-plasmids (Vieira and Messing, 1982). 190G* is encoded by the same DNA insert but not fused to any foreign protein. The location of the DNA encoding the polypeptides 190A−190N is given in Figure 1. ND, not determined.
[a] Apparent mol. wt on SDS-gels (kd).
[b]Appearance of multiple bands on Western blots.
[c]High-level expression detectable after Coomassie-blue staining of total bacterial lysates on SDS−gels.
[d]Presumed cleavage products of a larger precursor.
[e]Fused to a part of β-galactosidase.

were cloned into pDS plasmids for high level expression. These vectors contain the regulatable promoter/operator element $P_{N25*/0}$ (Stüber *et al.*, 1984) and the synthetic ribosomal binding site RBSII (Certa *et al.*, 1986). Due to the high efficiency of the expression signals the vectors can only be stably maintained when the plasmid pDMI,1, which expresses elevated levels of *lac* repressor, is present in the cells (Certa *et al.*, 1986).

The selected fragments appear in Figure 1 and their properties in *E.coli* are summarized in Table I. Note that the largest fragment encoding 190A has not been successfully expressed. All others are expressed in *E.coli* and are detected in Western blots probed with rabbit anti-p190 serum. However, as others have observed (Holder *et al.*, 1985), some of them are subject to proteolysis. In our hands, protease-deficient strains (Trisler and Gottesmann, 1984) did not significantly improve the expression yield or stability of these proteins.

From these experiments we selected the polypeptides 190L and 190M for immunological studies. In contrast to the majority of the proteins expressed these polypeptides are made at high levels with no or little protein degradation detectable in Western blots (Figure 2, panel A, track 1 and 2). In addition, their amino acid sequences are almost identical in the K1 and MAD-20 isolates (Tanabe *et al.*, 1987). Encouraged by these findings we constructed the plasmid p190N where 190L and 190M are jointly expressed. As expected the fusion protein 190N is recognized by the rabbit serum to the same extent as the unfused polypeptides (Figure 2, panel A, track 3).

Metal chelate columns provide an efficient means of purifying polypeptides containing tandem histidine residues (Hochuli *et al.*, in press). We therefore designed an expression vector encoding two histidine residues upstream and in frame with the insert. The practical value of this approach is illustrated in Figure 5. At least a 50-fold enrichment of the product is obtained in a single step.

We have begun to study the biological properties of these products. We thought it important to discover whether the polypeptides 190L, 190M or 190N contain epitopes exposed to the human immune system during natural *P. falciparum* infection. We probed the antigens in Western blots with a pool of human endemic sera from Nigeria (Hall *et al.*, 1984b). Interestingly only 190L and the fusion protein (190N) are recognized by these sera (Figure 2, panel B). When the same antigens are assayed by ELISA using 36 sera obtained from malaria immune donors living at the Ivory Coast, 90% of these sera detect 190N and about 60% react with 190M at serum dilutions of 1:100 or more (data not shown).

In summary, our results show that epitopes in the polypeptides depicted are antigenic and their epitopes are probably exposed during a natural infection.

Testing different parasite isolates with antibodies raised against polypeptide 190N

The available genetic data from four isolates (Tanabe *et al.*, 1987) suggest that 190L and 190M contain conserved amino acid stretches. We thus expected that antibodies raised against the recombinant protein should recognize the native p190 from a wide range of parasite isolates. For practical reasons we immunized rabbits with the purified fusion protein 190N instead of the unfused protein 190L and 190M. Fourteen of the sixteen isolates were tested with the anti-190N serum on Western blots and by immunfluorescence and appeared positive in both. They came from East (RO-58, RO-59, FCH 5-C2, Palo Alto) and West Africa (R-FCR 3, RO-33, Geneva No. 13, RO-53), Central (H-B 3) and South America (542), Asia (K1, CPG-1, T9/94, NT 112), and the South Pacific (MAD-20). All isolates tested reacted positively with the anti-190N serum in Western blots (Figure 3, panel A), in immunofluorescence (Table II) or in both tests.

In the Western blot analysis of the parasite extracts (Figure 3, panel A) we see several bands recognized by anti-190N

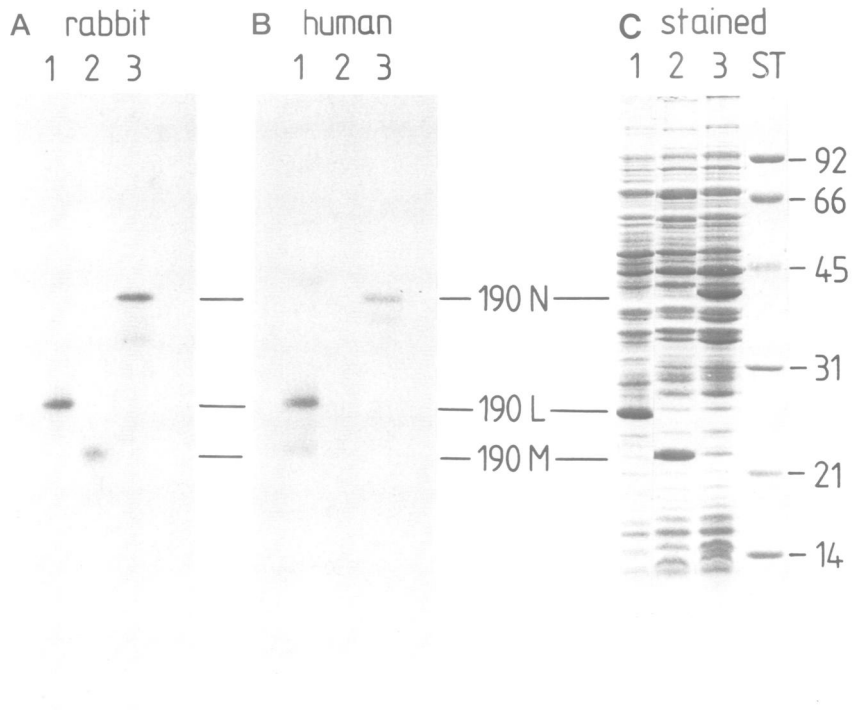

Fig. 2. Western blot and protein analysis of 190L, 190M and 190N. **Panel A** shows a Western blot of total bacterial lysates expressing 190L, 190M and 190N (**lanes 1, 2** and **3**) using rabbit anti-p190 serum. **Panel B** shows the same samples reacted with pooled human sera from endemic areas. The rabbit serum reacts with all three antigens whereas the human sera panel B react only with 190L and 190N. Antigen–antibody complexes were visualized by the horseradish peroxidase method. **Panel C** shows a Coomassie-Blue stained SDS-gel of the samples. The positions of the recombinant proteins are marked. ST indicates a mol. wt standard. Sizes are given in kd mol. wt.

serum, representing processing and perhaps breakdown products of the precursor. Similar patterns appear in blots containing identical samples probed with rabbit antibodies raised against the whole parasite p190 protein (Figure 3, panel B). Strikingly, the anti-190N serum detects at least as many products as the control antibody, showing that the fusion protein (190N) contains many of the epitopes important in the immune response in rabbits to the native protein.

These results are confirmed by immunofluorescence tests on fixed whole parasites (Table II). Even in the case (Figure 3, panel A and B, track 13) where P190 recognition is poor, a clear positive signal is seen in immunofluorescence (Table II, isolate no. 13).

Our results thus greatly extend the conclusions drawn from DNA sequence studies of cloned genes and confirm that the constant regions discovered by Tanabe *et al.* (1987) are present in p190 proteins of all parasites tested.

Monoclonal antibodies against 190L and 190M

To begin to understand the antigenic structure of conserved parts of p190 we have raised a panel of mouse monoclonal antibodies against 190L and 190M. The antigens were purified by NTA-affinity chromatography and their N-terminal sequences were determined (for details see Materials and methods). As expected from the results above, one monoclonal antibody (22-2) raised against a constant region of p190 near the N-terminus (190L) recognizes all the parasite isolates tested (Figure 4, panel A). The isolates RO-33, RO-59 and no. 13 react weakly in the immunoblot with this monoclonal antibody. However, when assayed by immunofluorescence these isolates react with 22-2 at high dilutions and are indistinguishable from the other parasites tested (data not shown). The other monoclonal antibody (34-5), raised against a more variable polypeptide derived from the C-terminus of p190 (190M; see also Figure 1) detects only six out of the 14 isolates in Western blots (Figure 4, panel B). In the more sensitive immunofluorescence assays 34-5 reacts strongly with these six parasites plus the no. 13

Table II. Quantitative immunofluorescence of different *Plasmodium falciparum* isolates using rabbit anti-190N serum

Isolate	Titer	Allele	Western	Track[a]
K1	20.480	K1	+++	14
no. 13	5120	K1	+	13
R-FCR-3	5120	K1	+++	12
Palo Alto	5120	–	++	11
CPG-1	10.240	–	+++	10
T9/94	2560	–	+++	9
HB-3	2560	K1	++	8
542	1280	MAD-20	+	7
RO-59	1280	–	+	6
RO-53	40.960	K1	++	5
MAD-20	2560	MAD-20	++	4
RO-33	640	MAD-20	+	3
FCH-5C2	640	MAD-20	+++	2
NT 112	2560	–	+	1
RO-56	2560	K1	NT	–
RO-58	5120	K1	NT	–

The titres indicate the serum dilution where a positive signal was still detectable. Controls using sporozoites or gametocytes has titre values of 40 (data not shown). Western blot data were included from Figure 3 (this paper) and Southern hybridization data from Berta *et al.* (1987). –, allele unknown; NT, not tested.
[a]Track numbers from Figure 3.

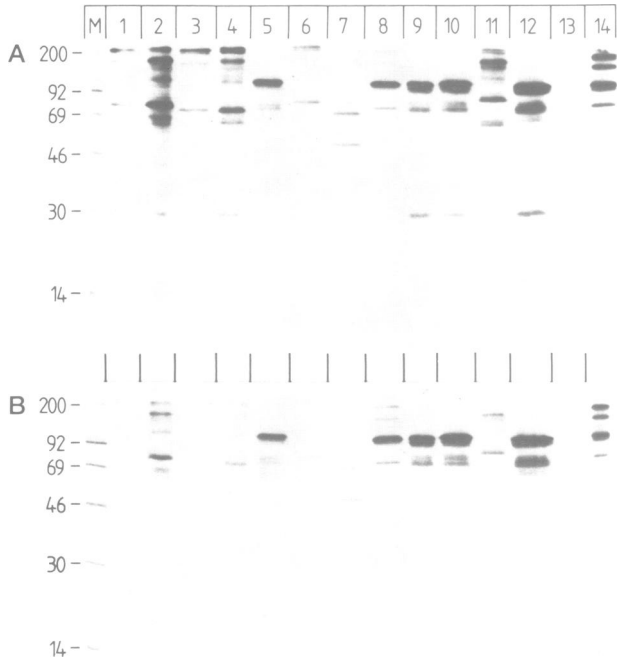

Fig. 3. Western blot analysis of 14 total parasite lysates using serum against full-length parasite protein and 190N recombinant protein. Washed parasites were lysed in SDS-sample buffer (Laemmli, 1970) and electrophoresed on SDS-gels. The samples were then transferred to nitrocellulose (Takacs and Staehli, 1987) and reacted either with rabbit polyclonal serum against 190N recombinant protein (**panel A**) or against parasite p190 (**panel B;** Hall *et al.*, 1984a). Apart from minor differences, the detection patterns are virtaully identical. Differential reaction is most likely due to unsynchronized parasite cultures and partial degradation (compare with Table II). Protein amounts per track were calibrated on a Coomassie-Blue stained gel with the same samples. The ST lane contains radioactive size standards. **Lane 1** contains isolate NT 112, **2**- FCH-5-C2, **3**-RO-33, **4**- MAD-20, **5**- RO-53, **6**-RO-59, **7**- 542, **8**- HB-3, **9**- T9/94, **10**- CP6-1, **11**- Palo-Alto, **12**- R-FCR-3, **13**- no. 13, **14**- K1 (control). These data are summarized and compared with immunofluorescence in Table II.

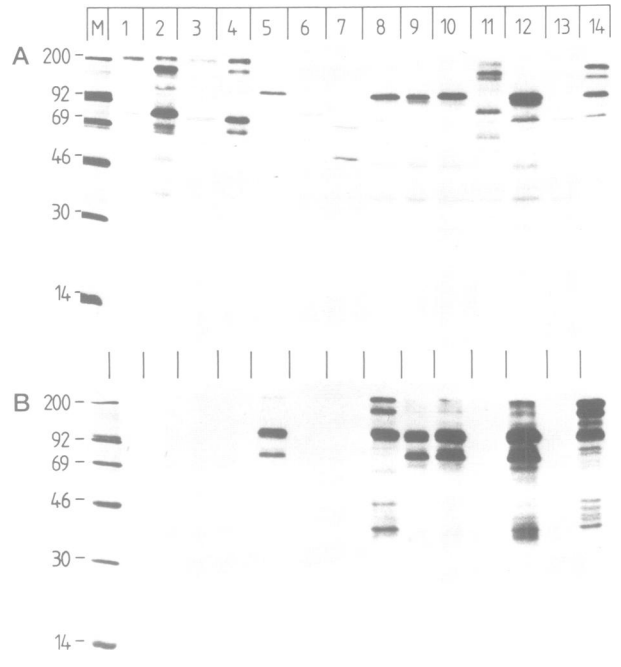

Fig. 4. Western blot analysis of 14 total parasite lysates using monoclonal antibodies raised against 190L and 190M. The same samples used in Figure 3 were probed with monoclonal antibodies raised against 190L (mAb 22-2; **panel A**) and 190M (mAb 34-5; **panel B**). mAb 22-2 reacts with all isolates tested whilst mAb 34-5 detects only 6 out of 14 isolates. The **M lane** contains molecular weight markers (kd). Note that the isolates RO-33 (**lane 3**), RO-59 (**lane 6**) and no. 13 (**lane 13**) give a weak reaction with the monoclonal antibody 22-2 consistent with the results shown in Figure 3.

isolate, but very weakly or not at all with the remaining isolates tested (data not shown). Strikingly, the 190M antigen contains stretches of amino acids encoded only by K1-type p190 genes (Tanabe *et al.*, 1987). The isolates positive with 34-5 all contain K1 type p190 genes (Certa *et al.*, 1987) supporting our assumption that the antibody recognizes a K1-specific epitope.

Discussion

The aim of this work was to identify regions of the p190 protein which could be used in monkey protection studies and human trials to assess their value for a vaccine. We sought polypeptides which are made abundantly in *E.coli*, and are not degraded by bacterial proteases. The chosen polypeptides were antigenic in rabbits and mice and 190L and 190N were recognized by endemic human sera in Western blots.

As it turned out, our choice was limited. Some polypeptides were antigenic but were recovered in poor yield, others were abundantly made but elicited a weak immune response. In addition, we wished the chosen sequences to be conserved in all parasite isolates. These factors led us to two recombinant proteins. One, 190L, is part of the major N-terminal primary processing product (80 kd) which is believed to be exposed on the parasite surface (Lyon *et al.*, 1986). The other, 190M, is nearer the C-terminus but is probably shed during invasion (Holder *et al.*, 1985).

Consistent with a recent report (Holder *et al.*, 1987), 190M is not detected by certain human sera. Nevertheless we considered it important to include this polypeptide, since it could either elicit a humoral response blocked in a natural infection or a cellular response which we have not yet been able to measure. In this connection it is important to note that both 190L and 190M contain T-cell epitopes (Sinigaglia *et al.*, unpublished results).

Our data obtained with the antibodies raised against the recombinant proteins 190L, 190M and 190N confirm the conclusion of Tanabe *et al.* (1987) at the protein level. Two constant regions of p190 revealed by DNA sequencing are now be detected at the protein level by polyclonal and monoclonal antibodies. So far, parasite isolates lacking these conserved amino acid stretches have not been found, suggesting that they play an important role in parasite function.

For the first time the monoclonal antibody 34-5 detects the dimorphic nature of the p190 gene on the protein level. It reacts exclusively with parasites expressing the K1 allele of p190 (Certa *et al.*, 1987). The most economical explanation is that the epitope of 34-5 is in the C-terminal amino acid stretch of 190M which is different from MAD-20. The antibody is therefore a useful tool for detecting K1-type se-

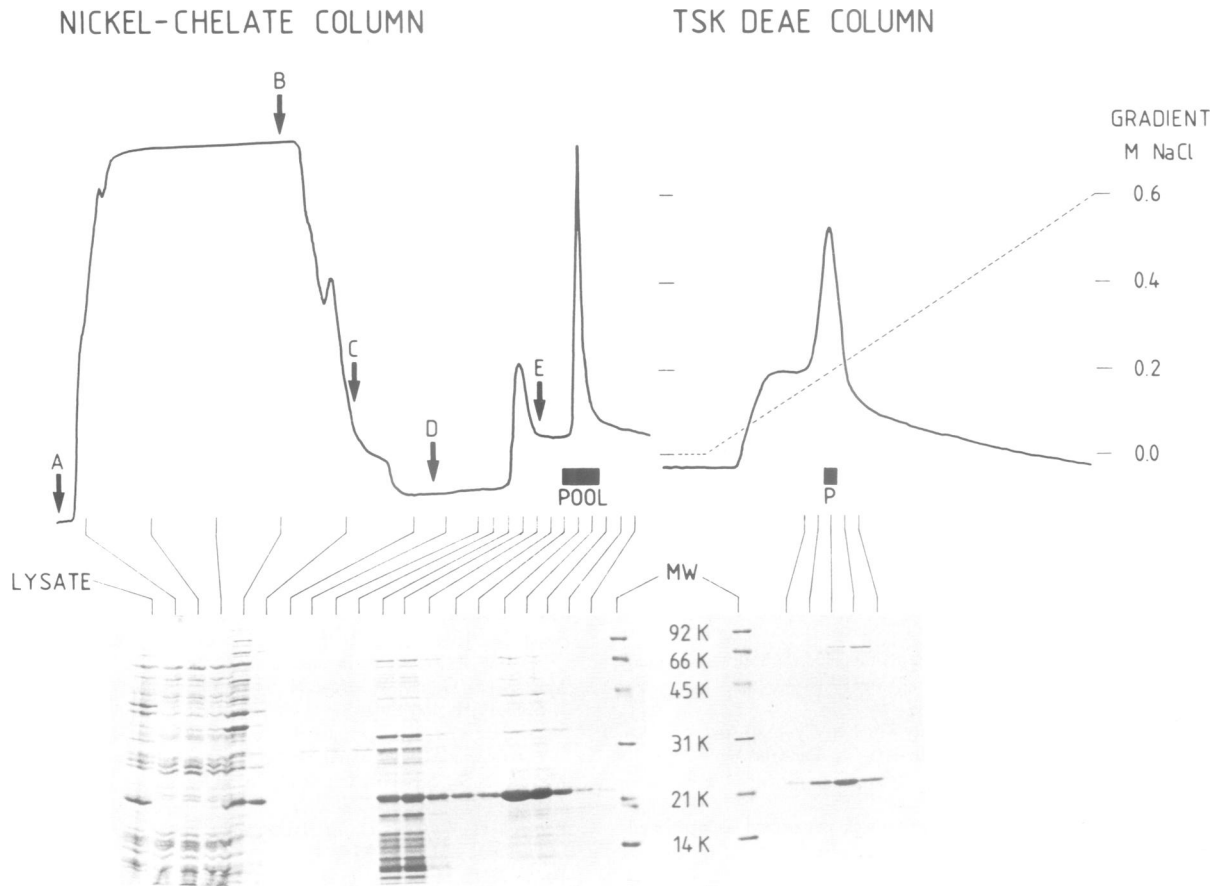

Fig. 5. Purification of 190M. Illustration of 190M purification by NTA and ion exchange chromatography. The left panel shows the purification of 190M on the NTA-column monitored by UV-absorbance and SDS-gel chromatography. The right panel shows further purification of the 190M containing fractions by ion exchange chromatography using TSK DEAE 650 (M) resin monitored by UV-absorbance and SDS-gel chromatography. Arrows in the UV-profile indicate the buffer changes described in Materials and methods. Sizes of marker proteins are given in kd (**lane M**).

quences in an unknown parasite isolate without any DNA analysis.

Although earlier studies using monoclonal antibodies (McBride *et al.*, 1985) suggested that polymorphism was an important feature of p190 our own data establish that constant parts of the protein can be found in all isolates tested. At least one polypeptide (190L) elicits a response in all malaria-immune individuals tested here. Together with the recent study showing a complete protection of Aotus monkeys by parasite p190 protein (Siddiqui *et al.*, 1987), our results encourage the view that these recombinant proteins could be active in a vaccine.

Materials and methods

Parasites

Sixteen isolates of parasites were used in this study (RO-33, Ghana; RO-56, Ethiopia; RO-59, Kenya; Geneva no. 13, Senegal; H-B3, Honduras; RO-53, Camerun; R-FCR 3, The Gambia; MAD-20, Papua New Guinea; 542, Brazil; RO-58, East Africa; FCH-5-C2, Tanzania; K1, CPG-1, T9/94, NT 112, Thailand; Palo Alto, Uganda). All strains bearing the initials RO were cultivated from tourists with malaria symptoms. Infected blood was cultured by the method of Trager and Jensen (1976) with some modifications described by Certa *et al.* (1987).

Bacteria

For DNA transformations the *E.coli* K12 strain HB 101 was used. Recombinant proteins were expressed in *E.coli* strains M15 (Certa *et al.*, 1986) or SG 200-50 (lon A^-; Trisler and Gottesmann, 1984).

DNA manipulations

DNA manipulations were performed by standard techniques (Maniatis *et al.*, 1982). Inserts of the genomic K1 phage clones p190.3 and p190.4 (Mackay *et al.*, 1985) were isolated and digested with *Sau*3A to completion. The individual fragments depicted in Figure 1 were electrophoresed through 1.2% agarose gels, isolated and cloned in frame into the *Bam*HI site of pUC8, pUC9 or pBR-Tac (Vieira and Messing, 1982; Amann *et al.*, 1983). Selected fragments were further cloned into pDS plasmids in order to improve expression yields (Stüber *et al.*, 1984). The reading frames of some inserts were adapted to the vector by filling in the restriction ends and adding the appropriate linkers.

Construction of p190N

Restriction analysis of p190M revealed that the *Bam*HI site downstream of the insert was lost. This circumstance allowed us to clone the *Bam*HI insert of p190M and p190L. The resulting clone p190N expresses a fusion protein if the insert is cloned in the correct orientation. The *Bam*HI insert of p190L was isolated and cloned into the unique *Bam*HI site of p190M. Transformants were analysed by SDS-gel electrophoresis for production of the fusion protein. The correct insert orientation was confirmed by restriction analysis and DNA sequencing (Sanger *et al.*, 1977) using end-labelled sequencing primers complementary to the ribosomal binding site of the vector. In Figure 2 we further tested the fusion protein with rabbit antibodies against parasite p190 protein.

Analysis of recombinant proteins

Samples of uninduced and induced bacteria harbouring expression plasmids were analysed by SDS–PAGE as described (Certa *et al.*, 1986). Clones were further analysed by immunoblotting using either polyclonal rabbit (dilution 1:1000) or pooled endemic sera from African donors (dilution 1:1000; Takacs and Staehli, 1987). Antigen–antibody complexes were visualized by the horseradish peroxidase method using a commercial kit (Vector, Burlingame, California).

Protein purification from crude E.coli extracts by nickel-chelate chromatography

The pDS vector used encodes two histidine residues proceding the protein to be expressed. This label has a high affinity for nickel-nitrilo-triacetic acid (NTA) chelate resin (Hochuli *et al.*, 1987). Purification of 190L is illustrated in Figure 5.

E.coli cells expressing recombinant proteins were harvested by centrifugation and lysed for 1 h at 4°C in 6 M guanidinium hydrochloride. After centrifugation the supernatant was diluted 5-fold with 0.1 M Tris−HCl, pH 7.5, 0.2 M NaCl. The lysate was loaded on the NTA-column (Figure 5, step A) followed by one wash with 0.1 M Tris−HCl, pH 7.5, 0.2 M NaCl (B), a second wash with 0.1 M Tris−HCl, pH 6.0, 1 M $(NH_4)_2SO_4$ (C), a third wash with 0.1 M Tris−HCl, pH 4.5, 1 M $(NH_4)_2SO_4$ (D) and the recombinant protein was finally eluted with 0.1 M Tris−HCl, pH 4.5, 0.2 M NaCl (step E, Figure 5).

Ion exchange chromatography

The eluates of the NTA-column were diluted 10-fold with 50 mM sodium phosphate, pH 6.0 (190L) or 25 mM Tris−HCl, pH 7.5 (190M) and chromatographed on Fractogel TSK CM 650 (190M) or Fractogel TSK DEAE 650 (190L) columns (Merck, ϕ 1.6 cm, length 7.0 cm, flow rate 1.5 ml/min.). The proteins were eluted for 2 h with a linear NaCl gradient from 0 to 0.6 M. Fractions were analysed by gel chromatography (Figure 5). 190N was purified with a slightly modified protocol or by eluting the protein from preperative SDS-gels.

Protein sequencing

Edman degradation of the purified proteins revealed the following amino terminal sequences:

190L: Met-His-His-Ala-Pro-Gly-Ser-Gly-Thr-Leu-Cys-Asp-Asn
190M: Met-His-His-Ala-Pro-Ala-Glu-Ile-Ala-Glu-Thr-Glu-Asn

Immunofluorescence

Indirect immunofluorescence on parasites was performed as described (Ardeshir *et al.*, 1985).

Monoclonal antibodies

Monoclonal antibodies were produced (Fazekas *et al.*, 1980) from spleens and mesenteric lymph nodes of BALB/c mice given three injections at ~1 month intervals of 190L (50 μg in saline/injection) or 190M (200 μg, 200 μg and 50 μg). Priming injections (s.c. and i.p.) were in complete Freund's adjuvant, boosting (i.p.) in incomplete Freund's adjuvant. The fusions were made with the SP2-0/Ag14 cell line three days after the last injection. Hybridoma supernatants were screened by ELISA-assays on plates coated with 190L or 190M (2 μg/ml) using alkaline phsophatase-conjugated rabbit anti-mouse Ig (SIGMA) as detecting reagent. Positivity was confirmed by immunofluorescence on fixed K1 stage parasites. Culture supernatants were used undiluted in ELISA-assays and Western blots. Antibodies 22-2 and 34-5 are both IgG 1.

Acknowledgements

We wish to thank Heidi Taatjes, Annemarie Rijnbeek and Peter Jakob for excellent technical assistance and art work. We are further grateful to Dr D.Stüber for supplying plasmid constructs, Dr E.Hochuli for synthesis of nitrilo-triacetic acid chelate chromatography resin, Dr H.Etlinger for immunizing rabbits and A.Reber-Liske for some parasite isolates. Finally, we thank Dr H.Kocher for protein sequencing of 190L and 190M.

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Received on September 11, 1987; revised on October 26, 1987