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ANTIGENS OF THE ERYTHROCYTIC STAGES OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM* DETECTED BY MONOCLONAL ANTIBODIES

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A range of 22 mouse anti-*P. falciparum* monoclonal antibodies have been characterized by indirect immunofluorescence and immunoprecipitation. On the basis of these studies, 5 groups of antibodies and 6 classes of antigen were defined. Group I antibodies give, bright, uniform, generalised staining of all blood stages including gametocytes. Three of these antibodies precipitate a metabolically labelled molecule(s) of 35 kDa. One precipitates a 50 kDa antigen. Group II antibodies, which give strong localised immunofluorescence in merozoites, and a weak diffuse pattern in earlier stages, precipitate biosynthetically labelled molecules of 160 kDa. Group III antibodies react with all asexual stages. With merozoites they produce intense staining around the perimeter, both in fixed and unfixed preparations. They precipitate biosynthetic molecules of 190 kDa. Group IV antibodies are identical to Group III except they are stage restricted to schizonts and merozoites. They also precipitate 190 kDa antigens. These, however, in contrast to group III, are readily accessible to ^{125}I-lactoperoxidase labelling. One antibody also precipitates a set of smaller peptides. Finally, Group V antibodies produce very bright ill-defined staining of pigment-containing parasites, as well as of inclusions in the red cell. They precipitate a series of molecules of 160, 60 and 35 kDa which are readily accessible to ^{125}I. The 160 kDa molecule is also labelled by [^{35}S]methionine. These results are discussed in the context of the development of a malaria vaccine and immunodiagnostic tests.

Key words: Malaria; *Plasmodium falciparum*; Hybridomas; Monoclonal antibodies; Malaria antigens.

INTRODUCTION

Antigens of the malaria parasite are currently an intensive area of study. The development of a malaria vaccine, as well as immunodiagnostic techniques, relies upon the precise biochemical characterisation of relevant macromolecules. To these ends, two complementary approaches are being actively pursued at present.

First, monoclonal antibodies (McAbs) have been raised against the erythrocytic [1–7]

Abbreviations: McAbs, monoclonal antibodies.

and sporozoite [8, 9] stages, and used to identify protective components of the parasite [2–4, 7–9]. Second, immune and non-immune sera from infected mice [10, 11] and man [12–15] have been characterised in attempts to identify those components peculiar to the immune host. These studies have demonstrated that a large (⩾ 200 kDa) protein(s), associated with the surface of merozoites and schizonts in *P. yoelii* [7], *P. berghei* and *P. chabaudi* [10], *P. knowlesi* [3], and *P. falciparum* [12, 13] are important. In particular, it has been demonstrated that such a protein, when purified, can elicit a protective immune response [7]. Other components with apparent M_r of 41, 82 and 140 kDa [2] and 96 kDa [14] of *P. falciparum* and 66 kDa of *P. knowlesi* [4] have also been demonstrated to be important.

In this report, we present a characterisation of *P. falciparum* antigens, defined by a series of McAbs raised against the erythrocytic stages [16]. We demonstrate that the antigens can be classified into 6 major groups on the basis of immunofluorescence and immunochemical analyses. This preliminary study forms the beginning of a long term project to define relevant protective and immunodiagnostic parasite components. Because of the limitations of the in vitro culture system [17], we are currently developing gene libraries [18] in order that the relevant components can eventually be produced on a large scale in bacteria.

MATERIALS AND METHODS

Parasites. K1, a Thai isolate of *P. falciparum* [19] was used in the present experiments; in addition, cross-reactivities of monoclonal antibodies were tested against a panel of 27 different isolates of the parasite [16]. The parasites for immunization of mice and for indirect immunofluorescence assays were cultured in human erythrocytes by the method of Trager and Jensen [17]. The parasites for isotope-labelling experiments were grown in the modified medium of Zolg et al. [20]. The cultures were asynchronous and contained parasites at all stages of the erythrocytic cycle.

Preparation of saponin- and glycerol-treated parasites. Parasites were prepared by saponin-treatment modified from Zuckerman et al. [21]. We lysed infected erythrocytes with 0.1% saponin (15 min at 37°C). This treatment has been found to give a preparation of parasites retained within permeable erythrocyte ghosts [22]. For some experiments human red cells were lysed by osmotic shock using glycerol treatment: 0.4 ml of packed washed infected erythrocytes were resuspended in 10 ml of 7% (v/v) glycerol in RPMI 1640 for 20–30 min at room temperature, repacked by centrifugation, the supernatant was removed and the cells were lysed on *rapid* resuspension in 10 ml of RPMI 1640. This procedure resulted in preparations containing free parasites, parasites trapped in red cell ghosts and small numbers of unlysed red cells.

Total numbers of parasites and the content of schizonts in the above preparations were determined on an aliquot diluted in ethidium bromide (0.00001%, w/v, in phosphate-buffered saline) using a haemocytometer and a fluorescence microscope.

Production of McAbs. The procedure was as described earlier [16]. Briefly, female BALB/c mice, bred and maintained in the animal unit of the Department of Zoology, were immunised with saponin-treated parasites of the K1 strain. The mice were injected intraperitoneally (i.p.) 2–4 times with parasites incorporated in Freund's incomplete adjuvant (100–200 μg protein per injection), and 2–6 weeks after the last injection challenged intravenously (i.v.) with freshly prepared or frozen (-20°C) parasites in saline (100 μg protein). The animals were killed three days later and their spleen cells were fused with NS-1 myeloma cells [23] using 50% (w/v) polyethylene glycol 1500 [24]. Between 10 and 14 days after fusion hybridoma cultures producing antiplasmodial antibodies were identified by indirect immunofluorescence, and selected hybrids were purified by limiting-dilution cloning on thymocyte feeder layers. Spent medium from in vitro grown cloned hybrid cultures or ascites fluids from pristane-primed (2,6,10,14-tetramethylpentadecane, Aldrich Chemical Co., U.S.A.) BALB/c mice bearing the hybridomas were used as the source of McAbs. The isotypes of immunoglobulins secreted by the cloned hybridomas were determined by Ouchterlony double diffusion in agar using concentrated culture supernatants reacted against antisera specific for mouse Ig heavy chain classes and subclasses (Bionetics, Kensington, MD).

Indirect immunofluorescence assay. The specificities of McAbs were determined by indirect immunofluorescence [16] using acetone-fixed infected blood as antigen. Cultured parasites were harvested when between 0.1 and 1.0% of red cells were infected with mature schizonts, the blood was washed 3 times and diluted in RPMI 1640 to contain at least 10^4 mature schizonts and 10^5–10^6 other stages per 20 μl aliquots, which were dried onto the wells of multispot glass slides (Hendley-Essex Ltd., U.K.). The slides were stored at -20°C in sealed containers with dessicant until required. The indirect immunofluorescence assay test was carried out at room temperature in a moist chamber. After fixation in acetone (20 s) each well was treated with 50μl of tested antibody for 30 min, the slides were washed and stained for a further 30 min with the fluorescein isothiocyanate conjugate of immunoadsorbent-purified polyvalent rabbit anti-mouse immunoglobulin (FITC-anti-Ig, Miles-Yeda Ltd., Israel). The slides were counterstained with Evans blue (0.1% in phosphate-buffered saline for 5 min), mounted in 50% glycerol and examined at 500 times magnification by fluorescence microscopy.

For photography, immunofluorescence was performed on thin smears of infected blood which had been resuspended in foetal calf serum. Photographs were taken with an Olympus OM2 camera on Kodak Tri-X Pan film.

To determine whether the monoclonal antibodies reacted with antigens on the surface of intact infected red cells or of unfixed parasites the indirect immunofluorescence assay staining was carried out at 4°C in presence of 0.01% sodium azide as follows: washed erythrocytes containing 1–5 × 10^4 schizonts, or 1–2 × 10^6 parasites prepared by lysis of red cells by saponin or glycerol, were packed in the wells of microtitre plates by centrifugation, fluid was removed and the cells were resuspended in 100 μl of antibody. The rest of the procedure, examination and photography were as described above.

[^{35}S]Methionine labelling of saponin-treated parasite cultures. Asynchronous *P. falciparum* cultures (5–10% parasitaemia) were harvested and lysed with 0.1% saponin (15 min at 37°C) [21, 22]. The parasites were centrifuged (3000 rpm, 10 min, room temperature), washed twice in serum free RPMI and once in a methionine free incorporation medium [25]. Pellets were resuspended (2–5 X 10^9 parasites ml^{-1}) and incubated (2 h; 37°C) in incorporation medium supplemented with 10% foetal calf serum, and 300 μCi ml^{-1} [^{35}S]methionine (spec. act. 42 TBq $mmol^{-1}$, Amersham International). This method results in the incorporation of 10–50 X 10^6 trichloroacetic acid-precipitable cpm per 10^9 parasites.

^{125}I-labelling of saponin-treated parasite cultures. Parasite cultures lysed by saponin, were washed three times in phosphate-buffered saline (pH 7.3), and resuspended (2–5 X 10^9 cells ml^{-1}) in same. They were iodinated by the lactoperoxidase method [26] using 1 mCi ml^{-1} of carrier free sodium iodide (spec. act. 592 MBq ^{125}I μg^{-1} iodine, Amersham International). We routinely obtained 50 X 10^6 trichloroacetic acid-precipitable cpm per 10^9 cells.

Antigen solubilization. Saponin lysed, labelled parasites were extracted in 5 to 10 vol. of 1% Nonidet P40 (NP40) in 50 mM sodium phosphate, pH 8.0, 0.15 M NaCl, 1 mM phenylmethylsulphonylfluoride, 1% BSA, for 1 h at 4°C. 50–80% of the incorporated label was released into the NP40 supernatant. The extract was centrifuged (10 000 X *g*, 30 min, 4°C) and the supernatant analysed directly by SDS-PAGE or by immunoprecipitation.

Immunoprecipitation. Parasite extract (1–5 X 10^5 cpm) was incubated either with 5–20 μl of ascitic fluid containing antibody, or with 10–50 μg of antibodies from culture supernatants prebound to Protein A-Sepharose (Pharmacia), for 2 h at 22°C, in 0.1 M sodium phosphate, pH 8.0, 0.1% NP40, 1 mM EDTA, 1% BSA (PNEB) in a volume of 150 μl. After incubation with ascitic fluid antigen-antibody complexes were collected either on heat killed, formalin fixed *Staphyloccus aureus* (Cowan I strain) [27] or on Protein-A-Sepharose for 30 min at 22°C. Pellets were washed extensively as follows: twice in PNE (as PNEB but without BSA), once in the same buffer containing 1% NP40, once in PNE containing 0.5 M NaCl, and once in 0.01 M sodium phosphate, pH 8.0. Bound material was released by incubation with Laemmli sample buffer (2% SDS, 10% glycerol, 0.0625 M Tris pH 6.8, 0.01% Bromophenol blue, 10% β-mercaptoethanol) and analysed by SDS-PAGE on 10% gels [28]. The gels were processed for fluorography as described by Bonner and Laskey [29] dried and autoradiographed for 3 days to 1 month by exposure to Dupont Cronex X-ray film.

RESULTS

Hybridomas and patterns of immunofluorescence produced by monoclonal antibodies. In five fusions using the K1 isolate of *P. falciparum*, 590 hybrid cultures were obtained of which 137 secreted antiplasmodial antibodies detectable by indirect immunofluorescence assay. Since our hybridomas were raised against a mixture of developmental stages of the parasite, the antibodies produced by them were expected to recognise a range of different antigens.

A number of different indirect immunofluorescence assay reactivity patterns were distinguished during the primary screening, and 22 hybrids producing antibodies representing five such patterns were successfully cloned.

Individual McAbs produced characteristic patterns of indirect immunofluorescence staining on acetone-fixed smears of *P. falciparum.* Some could be further distinguished by the stage-specificity of the reaction. The pattern produced by any given antibody was the same on all parasite isolates which reacted with that McAb regardless of whether culture supernatants or ascitic fluids were used. Five staining patterns were recognised and the antibodies were classified into five corresponding groups (Table I, Fig. 1).

Antibodies of Group 1 produce uniformly bright generalised staining of all blood forms including gametocytes on fixed preparations (Fig. 1.I). None of these McAbs react with unfixed parasites from saponin-lysed cells (data not shown), and it is assumed that they react with parasite cytoplasmic antigen(s). Group II antibodies react strongly with both free merozoites and those within schizonts, and weakly with the earlier asexual forms. In contrast to the faint diffuse reaction with the earlier stage parasites the most intense staining is localised to an area of merozoites, smaller than and distinct from the nucleus, suggesting that the antigen(s) are concentrated in an organelle (or a group of organelles) which is not well defined in the other stages (Fig. 1.II). Since these McAbs do not react with most unfixed saponin- or glycerol-treated parasites (even when these contain over 50% of schizonts reactive on fixed slides – data not shown), the antigen(s) are assumed to be predominantly intraparasitic. Group III and IV antibodies produce an intense staining around the perimeter of individual merozoites within developing schizonts and after their rupture (Fig. 1.III and 1.IV) and since, with the exception of McAb 7.10, they also react with unfixed parasites (Fig. 2 a–c), we propose that these McAbs recognise antigen(s) on or close to the parasite surface. The two groups are however, clearly distinguished by their stage-specificity. Those of Group IV become detectable only in segmenting schizonts containing several nuclei and a large aggregate of pigment, while the antigen(s) of Group III are present in all asexual stages. Pigment-containing parasites were stained brightest by Group V antibodies. In addition, these antibodies stained pleiomorphic inclusions located within the infected erythrocytes, but external to the parasite (Fig. 1.V). The intensity of the staining increases with maturation of the parasite but unambiguous reactions with merozoites have not been observed. Glycerol-treated parasites of variable size show a typical pattern of membrane fluorescence (Fig. 2d); we suggest that these antigens are associated with the parasitophorous vacuole and parasite-derived inclusions in the red cell cytoplasm. None of the 22 McAbs react with intact infected erythrocytes indicating that the respective antigenic determinants are not exposed on the red cell surface.

TABLE I – Summary of anti-*P. falciparum* (isolate K1) monoclonal antibodies

Antibody	Isotype		Immunoprecipitated products labelled by		Indirect immunofluorescence reactions			
			[^{35}S]Met	^{125}I	Pattern group[a]	Reactive blood stages	Unfixed free parasites[b]	Strain distribution[c]
2.30	IgG1	P50	+	-	I	all asexual and gametocytes	-	27
2.32		P35	+	-	I		-	27
6.3	IgG1		-	NT	I		NT[g]	27
7.2	IgG1	P35	+	-	I		-	27
7.8	IgG1	P35	+	-	I		-	27
2.13	IgG1	P160	+	-	II	(trophozoites) schizonts, merozoites	±	27
2.15	IgG1		-	-	II		±	27
2.22	IgG1		-	NT	II		±	27
2.29	IgG1		-	-	II		±	27
6.6	IgG1		-	-	II		NT	27
6.10	IgG1		-	-	II		NT	27
7.12	IgG1	P160	+	-	II		±	
8.1	IgG1		NT	NT	II		±	27
2.2	IgG1	P190a	+	+	III	all asexual	+	27
7.5	IgG1	P190a	+	-	III	all asexual	+	27[d]
6.1	IgG1		NT	-	IV	schizonts, metozoites	+	10
7.1	IgG1		NT	-	IV		+	22[e]
7.3	IgG2a	P190b (+ peptides)	+	+	IV		+	10
7.6	IgG1	P190b	+	+	IV		+	10
7.10	IgG1		NT	NT	IV		-	27
5.1	IgG1	160,93,60,35[f]	-	+	V	trophozoites, schizonts	+	24
7.7	IgG1	160,93,60,35	160kDa	+	V	trophozoites, schizonts	+	27

a For a description of patterns see text.
b Released from erythrocytes lysed by saponin or glycerol treatment.
c Number of positively reactive parasite isolates out of 27 tested. See [9] for details.
d Eleven of the positive strains gave subnormal dull reactions.
e Twelve of the positive strains gave subnormal dull reactions.
f The 93 kDa protein only appears in some extracts.
g Not tested.

Antibodies assigned to the five different groups obviously recognise different antigens distinguished by their subcellular locations and distributions within the erythrocytic cycle of the parasite.

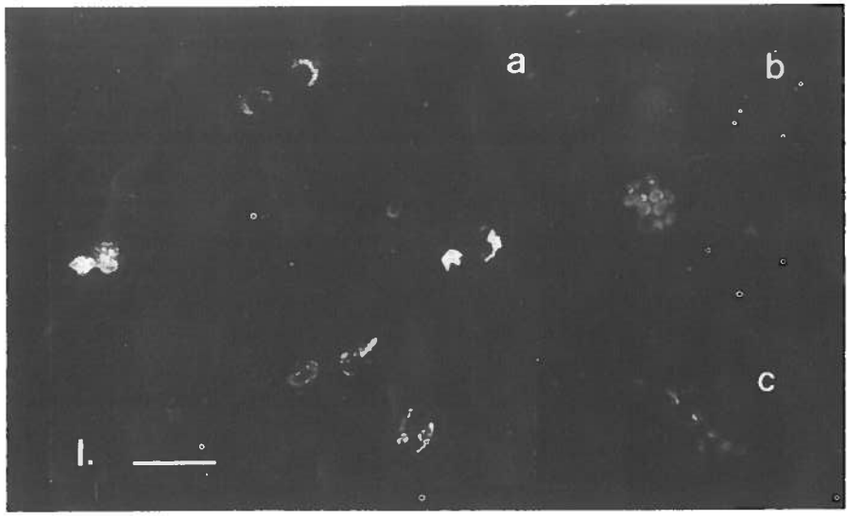
SDS PAGE analysis of [^{35}S]methionine (biosynthetically) labelled parasite antigens. To determine which antigens were proteins we labelled saponin-treated parasites with [^{35}S]-methionine, extracted them with non-ionic detergent and tried to immunoprecipitate the antigens from the extracts. Ten McAbs precipitated proteins detectable by this method.

Four of the antibodies from Group I react with proteins. Three of them (2.32, 7.2 and 7.8) precipitate molecules which in 10% polyacrylamide gels migrate as a protein of 35 kDa (eg. McAb 7.2, Fig. 3, track 4). The fourth McAb (2.30) reacts with a protein equivalent to 50 kDa (Fig. 3, track 11). Two group II antibodies (2.13, 7.12) precipitate a protein(s) migrating as 160 kDa (Fig. 3 tracks 2 and 3). The antibodies of Group III (2.2 and 7.5) precipitate a single high molecular weight protein migrating at 190 kDa (Fig. 3 tracks 5 and 6).

Of the five Group IV antibodies we have succeeded in precipitating proteins with two (7.3 and 7.6). Both antibodies react with a large protein (190 kDa) but unlike 7.6, McAb 7.3 also reproducibly precipitates a set of lower molecular weight products (Fig. 3, tracks 10 and 9).

Sequential immunoprecipitation studies indicate that although McAbs 7.3 and 7.6 have different precipitating properties they nevertheless react with the same molecule. Labelled parasite extract was reacted with antibody 7.3 and the antigen-antibody complexes bound to *Staph. aureus* were removed by centrifugation. A portion of the remaining supernatant was readsorbed with the same antibody to test whether all detectable 7.3-specific antigen had been removed. Another aliquot of the depleted supernatant was reacted with McAb 7.6 to see whether McAb 7.3 had also removed the antigen recognised by 7.6. The three precipitates were analysed in parallel (Fig. 4). As expected McAb 7.3 reacted with a protein of 190 kDa (Track 2). The depletion was efficient since none of the protein could be recovered by repeated precipitation with 7.3 (track 3). When the McAb 7.3-depleted supernatant was challenged with McAb 7.6 no antigen was found in the precipitate (track 4) implying that the two antibodies reacted with the same protein. We are confident that McAb 7.6 was active because it did precipitate the antigen from the unadsorbed extract (track 5). The reciprocal experiment (data not shown) also supported the conclusion that both McAbs, 7.3 and 7.6, recognise antigenic sites on the same 190 kDa protein. We suspect that the antigenic *sites* may be different, since unlike McAb 7.6, antibody 7.3 precipitated a set of additional smaller peptides. However, this result may be explained in different ways. For example, antigens complexed with 7.3 (IgG2a) may be more efficiently precipitated than those complexed with 7.6 (IgG1) and the minor peptides may go undetected.

Finally, we tested the two antibodies in Group V (McAbs 5.1 and 7.7) and could only precipitate a faint band at 160 kDa with McAb 7.7 (data not shown). The results present-



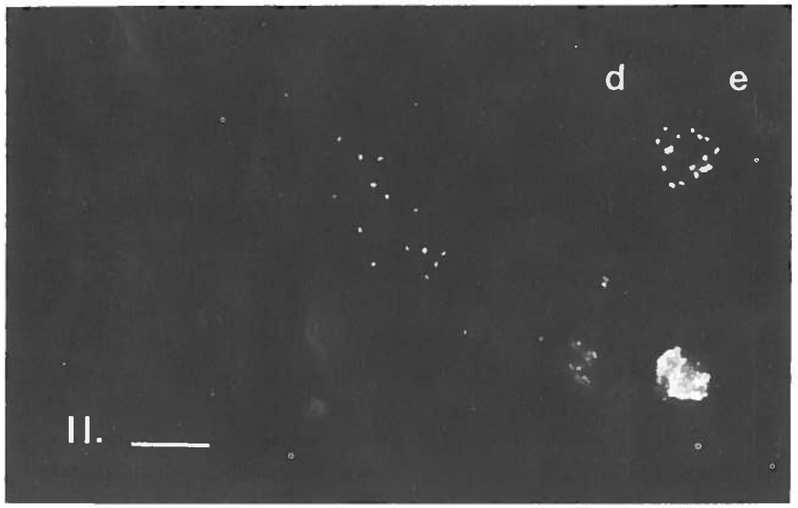
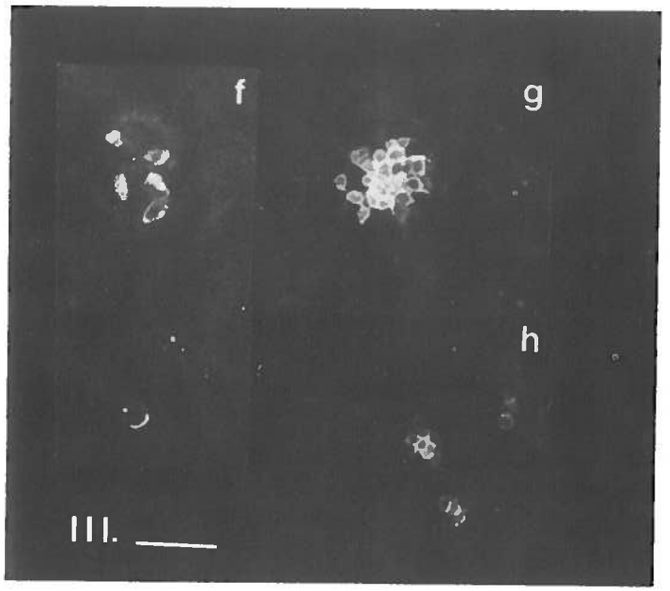


Fig. 1. Different patterns of indirect immunofluorescent assay (IFA) staining produced by monoclonal antibodies on acetone-fixed blood films of *P. falciparum*. I: IFA staining with Group I antibodies: a, trophozoites including ring-forms (McAb 7.8); b, schizont (McAb 7.2); c, merozoites (McAb 7.8). II: IFA staining with Group II antibodies: d, merozoites and ring-forms (McAb 7.12); e, mature (top) and immature (bottom) schizonts (McAb 2.13). III: IFA staining with Group III antibodies: f, ring-forms; g, schizont; h, merozoites (McAb 7.5). IV: IFA staining with Group IV antibodies: i and k, schizonts (McAbs 7.1 and 7.3, respectively); j, ruptured schizont and released merozoites (McAb 6.1). V: IFA staining with Group V antibodies of pigment-containing parasites: l, McAb 5.1; m, McAb 7.7. EB: asexual blood forms stained with ethidium bromide; r, ring-forms; t, trophozoites; s, schizonts; m, merozoites from a ruptured schizont. Bar = 10 μm.



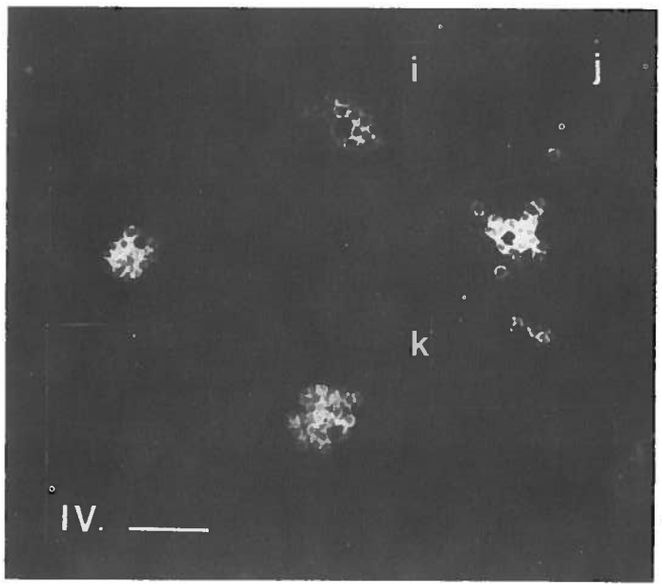
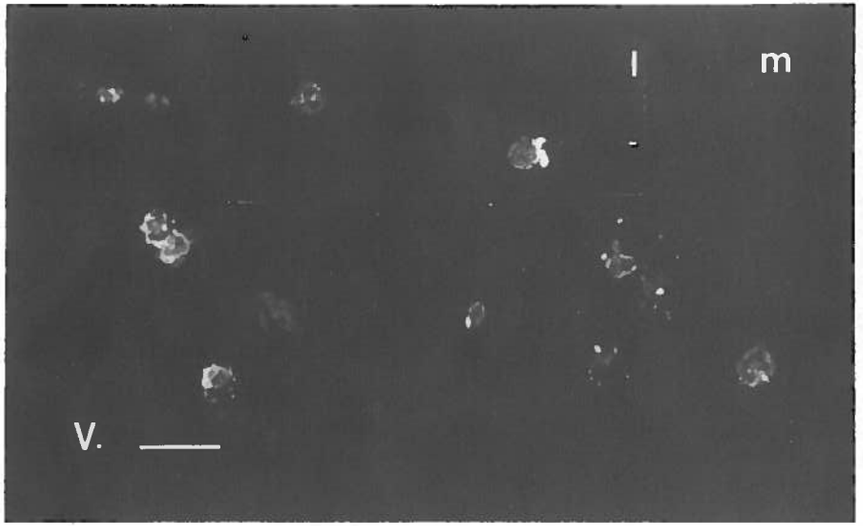


Fig. 1. III and 1. IV, legend opposite.



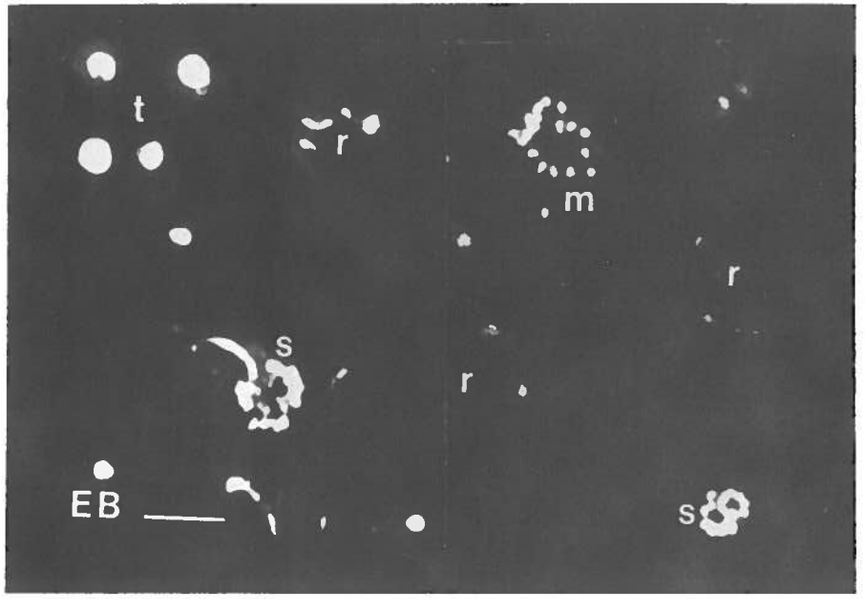


Fig. 1. V and 1. EB. For legend see page 254.

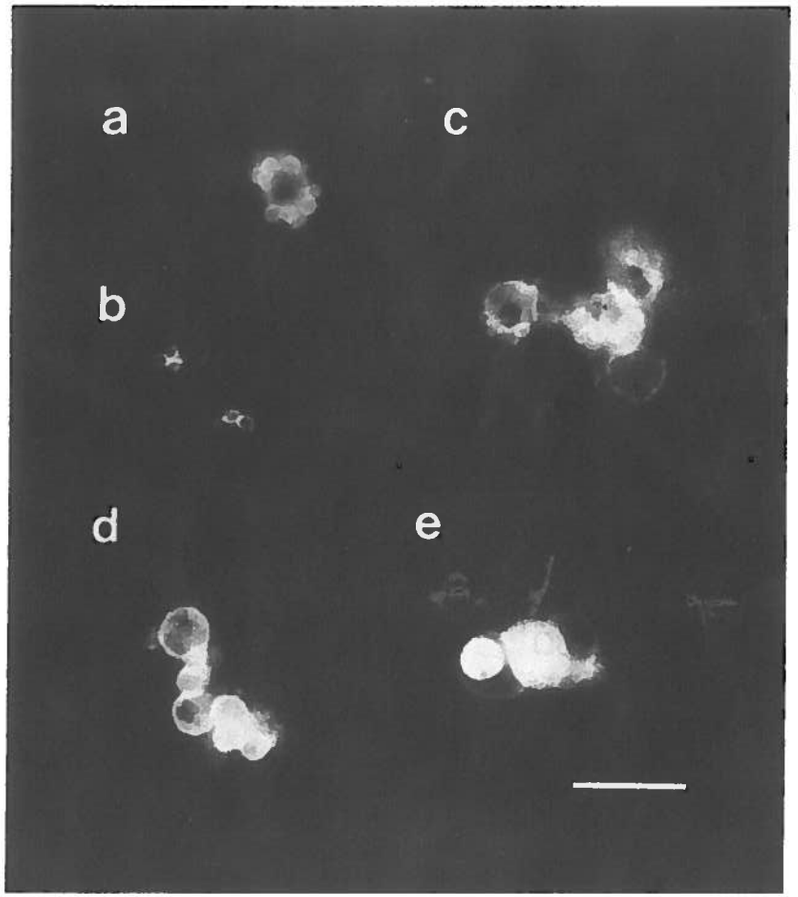


Fig. 2. Surface indirect immunofluorescence staining produced by monoclonal antibodies on *P. falciparum* parasites freed from red cells lysed with glycerol. a, schizont and b, merozoites stained with Group IV antibody 7.6. c, staining of agglutinated schizonts and large trophozoites by Group III antibody 7.5. d, parasites of variable size reacted with Group V antibody 5.1. e, erythrocytes stained with a monoclonal antibody to normal human red cell membrane; the two cells on the left contain immature trophozoites counterstained with ethidium bromide for comparison of size. Bar = 10 μm.

ed in the next section, however, show more clearly that their antigens are associated with proteins.

^{125}I-labelling of parasite antigens. Radioiodination of saponin-treated parasites can, firstly, identify antigens likely to be located at the parasite surface and, secondly, identify some proteins not labelled during the growth in [^{35}S]methionine either because they are not synthesised during the pulse or (less likely) because they lack methionine.

^{125}I-labelled parasites were detergent-extracted, reacted with the antibodies and anal-

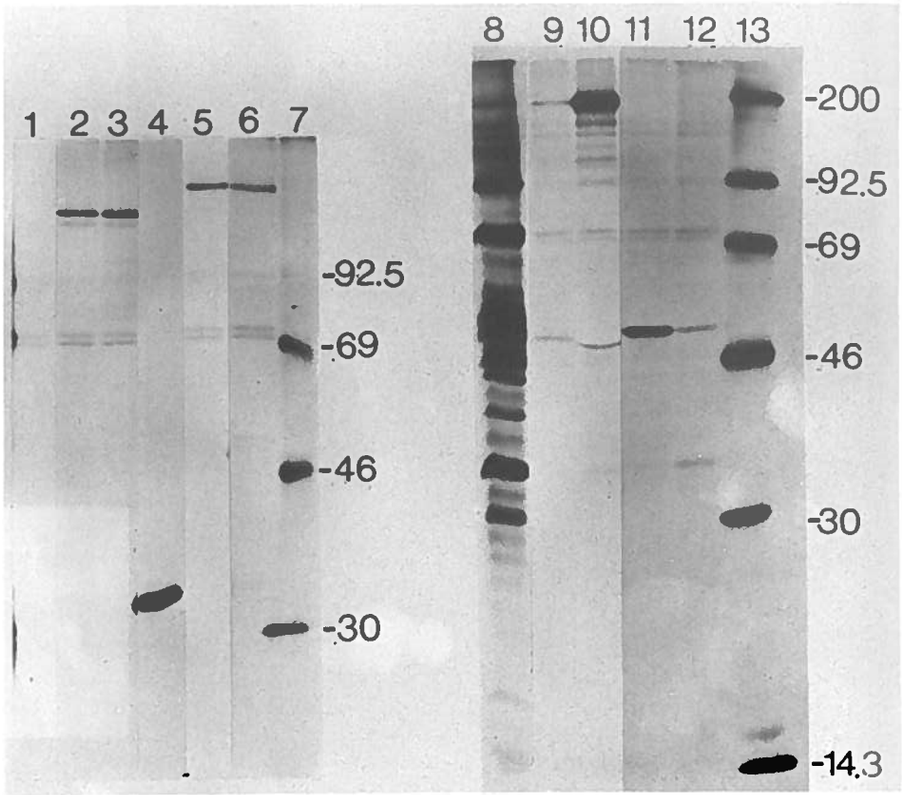


Fig. 3. Autoradiograms of 10% SDS-PAGE analyses of [^{35}S]methionine-labelled *P. falciparum* antigens. Total proteins (track 8) were mixed (Methods) with various monoclonal antobodies in hybridoma supernatants (tracks 2–6 and 9–11) or parent myeloma supernatant (tracks 1 and 12) and protein A sepharose (Pharmacia). The antibodies are as follows: Group I (McAb 7.2), track 4 and (McAb 2.30) track 11; Group II (McAbs 2.13 and 7.12), tracks 2 and 3; Group III (McAbs 2.2 and 7.5), tracks 5 and 6; Group IV (McAbs 7.6 and 7.3), tracks 9 and 10. Tracks 7 and 13 are ^{14}C-labelled marker proteins (Amersham International): 200 kDa, myosin; 92.5 kDa, phosphorylase b, 69 kDa; bovine serum albumin; 46 kDa, ovalbumin; 30 kDa, carbonic anhydrase; 14.3 kDa lysozyme.

ysed by SDS-PAGE as before. As expected from the serological studies, the Group I (Fig. 5a, track 9, McAb 7.2) and Group II McAbs (Fig. 5a, tracks 7 and 8, McAbs 2.13 and 7.12), which are probably directed to predominantly internal antigens, did not precipitate any material labelled with ^{125}I. Interestingly, the 190 kDa protein(s) of Group III (McAbs 2.2 and 7.5), which by indirect immunofluorescence assay appear to be associated with the parasite surface, are nevertheless not readily detectable after ^{125}I-labelling (Fig. 5a, tracks 4 and 5). In some experiments McAb 2.2 does precipitate a faint 190 kDa ^{125}I-labelled band (data not shown). By contrast, the 190 kDa protein common to McAbs 7.3 and 7.6 (Group IV) was strongly precipitated from the iodinated extract (Fig. 5a, tracks 3 and 6). In addition, smaller peptides precipitated by McAb

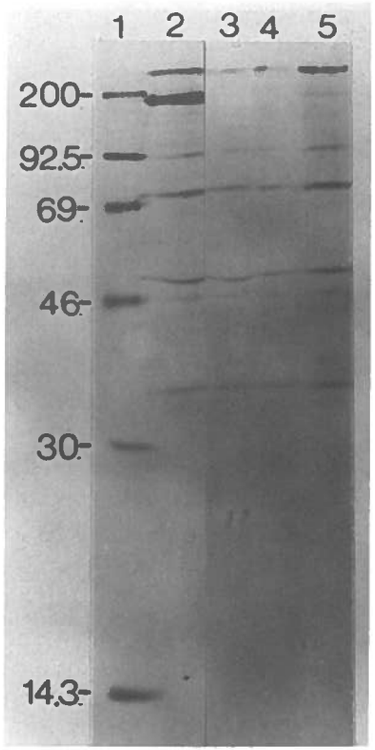


Fig. 4. Sequential immunoprecipitation between the group IV antibodies 7.3 and 7.6. [^{35}S]methionine-labelled proteins were precipitated with McAbs 7.3 (track 2), and 7.6 (track 5). The supernatant of the 7.3 precipitate was reprecipitated with 7.3 (track 3) and 7.6 (track 4). Track 1 shows the same markers as Fig. 3. The gel was 10% SDS-PAGE.

7.3 were also labelled with ^{125}I. One of these, a band migrating at the 88 kDa position was markedly more heavily ^{125}I-labelled than the other peptides (Fig. 5a, track 3).

This technique allowed us to detect the antigens of both Group V antibodies (McAbs 5.1 and 7.7). Both McAbs reacted reproducibly with radioiodinated products which migrated as proteins of 160, 60 and 35 kDa (Fig. 5b, tracks 3 and 4). A strong band at 93 kDa, appears in some labelled extracts (Fig. 5b, tracks 3 and 4).

Thus by combining these two labelling techniques we have isolated and partially characterised some of the antigen molecules corresponding to each of the five Groups proposed on the basis of serological findings. Moreover, combination of these biochemical approaches and of the indirect immunofluorescence serology provides an internally consistent way to group the antigens.

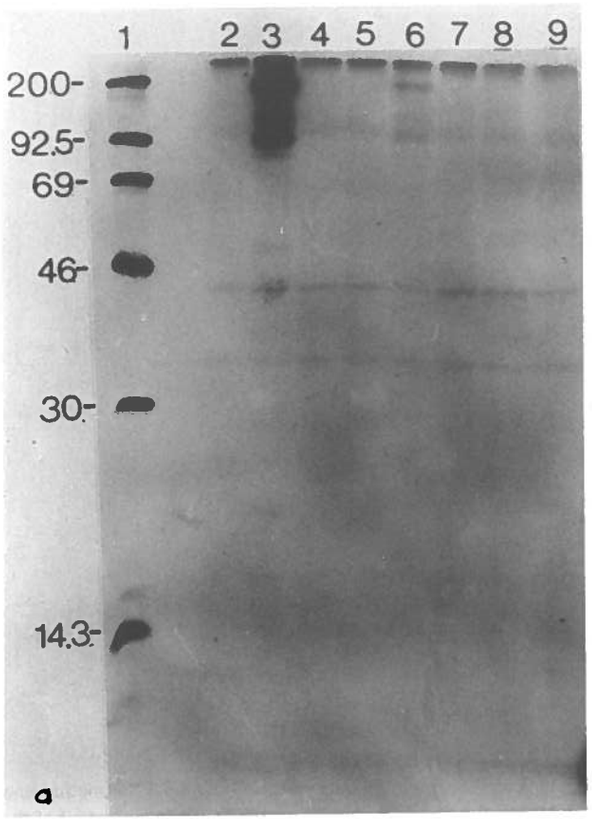


Fig. 5. Autoradiograms of 10% SDS-PAGE analyses of ^{125}I-labelled *P. falciparum* antigens (Methods). (a) Immunoprecipitation with antibodies of Groups I, II, III and IV as follows: Group I (McAb 7.2), track 9; Group II (McAbs 2.13 and 7.12), tracks 7 and 8; Group III (McAbs 2.2 and 7.5), tracks 4 and 5; Group IV (McAbs 7.3 and 7.6), tracks 3 and 6. Track 2 is a control using the parent myeloma supernatant and track 1 shows ^{14}C-labelled marker proteins as in Fig. 3. (b) Immunoprecipitation with antibodies of Group V: McAb 5.1 track 3 and McAb 7.7 track 4. Tracks 2 and 5 are control immunoprecipitates with the parent myeloma supernatant. Track 1 shows the total iodinated proteins. Markers as in Fig. 3.

DISCUSSION

Monoclonal antibodies produced by 22 mouse hybridomas were used to characterise antigens of the human malaria parasite *P. falciparum*. Since our antibodies were raised against a mixture of developmental stages of the erythrocytic cycle they were expected to recognise a number of different antigens.

Five groups of antibodies have been distinguished by their stage-specificity and subcellular locations by indirect immunofluorescence staining (Table I and [16]). The biochemical data presented here confirm and extend this classification.

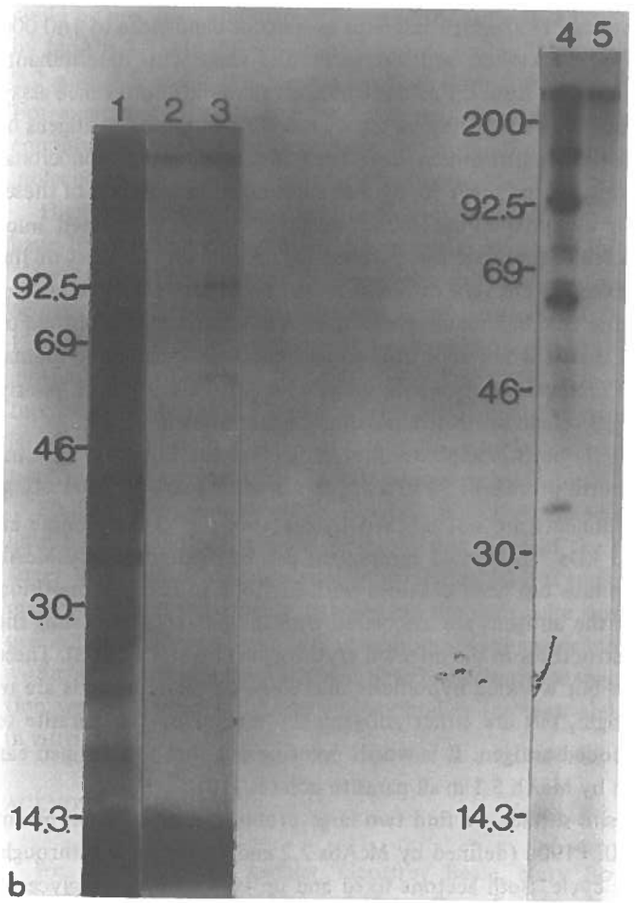


Fig. 5(b). Legend opposite.

Of the antibodies screened, protein associated antigens have been detected for twelve, using a combination of [^{35}S]methionine incorporation and ^{125}I-lactoperoxidase labelling. Several reasons can be suggested to explain why the remaining antigens have not been biochemically defined. For example they may simply not be proteins; or, they may be proteins which are methionine or tyrosine deficient, or which are present at levels too low to detect. Alternatively they may not be soluble in NP40.

We have identified two major protein antigens, P35 and P50, the apparent molecular weights of which are 35 000 and 50 000, respectively. At present, we assume that these molecules are located throughout the cytoplasm of the parasite. These antigens are present in all blood stages of the parasite and the marker determinants recognised by our McAbs are common to all isolates of the parasite tested so far. Thus being common to all blood forms as well as to (probably) all strains of the parasite these antigens may prove to be of value for immunodiagnosis of the disease.

A third intraparasitic protein, P160, which migrates as a major band close to 160 000 is most strongly expressed in merozoites, and our antibodies react with determinants which are common to all strains we tested. From the indirect immunofluorescence assay labelling it is tempting to infer that P160 may be associated with rhoptries. Antigens of very similar stage- and subcellular distribution have been identified using monoclonal antibodies to the rodent parasite *P. y. yoelli* [6, 5] and immunization with one of these, a merozoite-specific protein of apparent molecular weight 235 000, protected mice against infection with a virulent variant of the parasite [7]. Although the sizes of the human and rodent parasite antigens are very different it will be of interest to determine whether P160 has an analogous role in immune protection. A schizont-specific protein of 160 kDa, similar to P160 in that it is not accessible to lactoperoxidase catalysed iodination, has been described in *P. falciparum* by Perrin and Dayal [2]. This protein is poorly immunogenic in humans and its role in protective immunity is not known.

Two antibodies (5.1 and 7.7) both precipitate three molecules equivalent to proteins of 160, 60 and 35 kDa. A fourth protein of 93 kDa appears in some extracts. Interestingly the 93, 60, 35 kDa components are not labelled by metabolic [^{35}S]methionine incorporation. However a 160 kDa ^{35}S-labelled component can be precipitated by McAb 7.7. To date no such precipitate has been obtained with McAb 5.1. The immunofluorescence studies indicate that the antigens are associated with membrane(s) enclosing the parasite, and with irregular structures in the infected erythrocyte (Maurer's clefts). These findings are compatible with our working hypothesis that some of these proteins are of host rather than parasite origin, but are either antigenically altered by the parasite or complexed with a parasite-coded antigen. It is worth pointing out that this antigen can vary since it is not detectable by McAb 5.1 in all parasite isolates [16].

Associated with the parasite surface we find two large proteins, both of an apparent molecular weight of 190 000. P190a (defined by McAbs 2.2 and 7.5) is present throughout the asexual erythrocytic cycle. Both acetone-fixed and unfixed saponin- or glycerol-treated parasites have this antigen accessible for reaction with the antibodies. However, we do not observe strong labelling of this antigen by ^{125}I, suggesting that the target amino acid residues are inaccessible, or that the antigenic determinants recognised by the McAbs are destroyed during iodination (e.g. because of the location of a tyrosine at or near the antigenic site). The protein P190b defined by McAbs 7.3 and 7.6, differs from P190a in at least three ways. It can be readily ^{125}I-labelled, it is detectable only late in schizogony and it carries strain-specific determinants recognised by the two McAbs. Whilst McAbs 7.3 and 7.6 both react with P190b, McAb 7.3 also precipitates four other peptides from labelled extracts. We hope to determine whether these are cleavage products of the same parent molecule, different molecules with a common epitope or proteins forming a complex with P190b, which is not dissociated by NP40. In this context we recall that ^{125}I-labelling of these peptides gives a band (at 88 dKa) more heavily labelled than its larger and smaller neighbours (Fig. 5a, track 3). We would not normally expect this result if the peptides were cleavage products of a uniformly labelled parent protein.

At present we are assuming that P190a and P190b are different proteins, but they could be precursor and mature forms of the same gene product. The epitope(s) recognised by McAbs 7.3 and 7.6 would be acquired during the maturation, while those for McAbs 2.2 and 7.5 would be present on both forms. Both models make strong predictions. For example, if P190a and 190b are two forms of the same protein they should share common antigenic sites detectable by antibodies raised against either of the purified molecules.

High molecular weight schizont- and merozoite-specific antigens of *P. falciparum* have been described by other laboratories. Perkins [30] identified three proteins of around 200 000 as being on the merozoite surface and suggested that they may be homologous to the large polypeptides which are synthesised late in the erythrocytic cycle [2, 15] and are immunoprecipitated by immune sera [13]. It is possible that the P190b molecule identified here using McAbs 7.3 and 7.6 is the same as the above antigen(s). Interestingly McAbs 7.3 and 7.6 have been shown to be isolate-specific [16]. Thus the large surface protein which they recognise is subject to variation and, clearly, this variation could be important for the host-parasite relationship.

Similar large proteins of *P. y. yoelii* [6, 7], *P. chabaudi* [10] and *P. knowlesi* [3] have been implicated in immune protection. One of the *P. y. yoelii* proteins, although larger than P190b, has some similar properties: (i) it is apparently associated with parasite membranes and is probably exposed on the merozoite surface and (ii) McAbs against it precipitate smaller polypeptides in addition to the major molecule.

Since most of the large antigens have been shown or suggested to have a role in protection we are currently testing our antibodies for their ability to inhibit parasite growth in vitro.

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