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Comparison of asexual blood-stage antigens of *Plasmodium falciparum* recognized by antibody reagents from nine laboratories*

R. DAYAL,[1] C. DECRIND,[2] & P. H. LAMBERT[3]

Several laboratories have reported a large number of plasmodial antigens that may be implicated in the immune-mediated protection against the asexual blood stage of Plasmodium falciparum. *In order to establish relationships between the antigens reported by different laboratories, a comparative testing of 37 antibody reagents was carried out at the Reference Laboratory for Malaria Antibodies and Antigens. Based on the results of the comparative testing, these antibodies were divided into seven groups according to similarities in the stage-specificity, the subcellular localization and the apparent molecular mass (M_r) of the antigens they recognized.*

Twenty-two antibody reagents were grouped together on the basis that all gave a characteristic grape-pattern in indirect immunofluorescence (IIF) and precipitated a schizont/merozoite antigen of M_r 190 000 to 205 000. Another group consisted of one antibody which reacted with a M_r 90 000 protein expressed only in mature trophozoites and young schizonts. A third group consisted of three antibodies which reacted with all blood stages of the parasite and recognized a M_r 36 000 protein. The fourth group contained one antibody which recognized a M_r 14 000 protein and gave an IIF staining of schizonts. The fifth group consisted of an antiserum raised against a fusion protein. This antiserum reacted with schizont and ring-stage parasites and precipitated a M_r 75 000 antigen. One rabbit antiserum raised against a different fusion protein and one monoclonal antibody (Mab) were put together in the sixth group on the basis that both gave a cytoplasmic IIF staining of schizonts and recognized a strain-specific, M_r 250 000 antigen from one P. falciparum *strain. A seventh group was made up of 8 antibodies on the basis that all gave a similar two-dot IIF staining pattern of segmented schizonts and free merozoites, although these antibodies recognized four different sets of antigens.*

These results were presented to a subcommittee of the Scientific Working Group on the Immunology of Malaria (SWG-IMMAL) during a workshop held at the reference laboratory. The P. falciparum *proteins recognized by the antibody reagents used for this workshop represent only some of the different types of asexual blood-stage antigens that have been described in the literature..*

Following a request by the Steering Committee of the Scientific Working Group on the Immunology of Malaria (SWG-IMMAL), the Reference Laboratory for Malaria Antibodies and Antigens in Geneva, Switzerland, carried out a comparative testing of the antibody reagents being used in different laboratories for the identification of asexual blood stage antigens of *Plasmodium falciparum*. The Steering Committee considered it essential to carry out this comparison in order to determine the relationships between the antigens being studied in different laboratories. Scientists who were interested in participating in this study submitted their antibody reagents to the reference laboratory. The antibodies were tested by indirect immunofluorescence (IIF) on acetone-fixed parasites and by immunoprecipitation of ^{35}S-methionine-

* From the WHO Immunology Research and Training Centre, Department of Pathology, University of Geneva, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland. This paper presents the results of a workshop held in conjunction with the Sixth Meeting of the Scientific Working Group on the Immunology of Malaria. The participants whose reagents were used in the workshop are R. F. Anders, G. H. Campbell, A. A. Holder, J. Lyon, J. M. McBride, L. Pereira da Silva, M. E. Perkins, L. H Perrin, and A. Saul.

[1] Scientist, WHO Immunology Research and Training Centre. Requests for reprints should be sent to this author.

[2] Technician.

[3] Professor and Head of Department.

labelled *P. falciparum* proteins and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) to compare the asexual blood stage, subcellular localization, and apparent molecular mass (M_r) of the antigens they recognized. The results of these tests on 37 submitted reagents were presented to a subcommittee of the SWG-IMMAL during a workshop held at the reference laboratory in conjunction with the Sixth SWG-IMMAL Meeting in 1984. This paper reports on the results of this workshop.

MATERIALS AND METHODS

Antibody reagents

Thirty-seven reagents were submitted and used for the workshop. These antibodies were directed against the asexual blood stage antigens of *P. falciparum*. The 37 reagents included 31 monoclonal antibodies (Mabs), 2 rabbit antibodies raised against affinity-purified proteins, 3 antisera raised against cloned gene products (fusion proteins), and 1 immune serum pool from monkeys vaccinated with a purified *P. falciparum* antigen. The 37 antibody reagents used are listed in Table 1.

Parasites

P. falciparum strains M25 from Zaire and FCQ-27 from Papua New Guinea (PNG), and clones FCR-3.A-2 from Gambia (*1*) and T9.94 from Thailand (*2*) were used. The parasites were cultured in 100 mm Petri dishes in a candle jar (*3*). Cultures were initiated at 1% parasitaemia in a 5% suspension of A+

Table 1. Antibody reagents used for the workshop

Participant/prefix and institute	Antibody reagents		
	Name	No.	Type
R. Anders/**A** Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia	A/α-Ag16 A/α-Ag63	1 1	Rabbit antiserum Mouse antiserum
G. Campbell/**B** Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA, USA	B/23-5.6, B/12-110.3	2	Mouse Mabs
A. Holder/**C** Wellcome Research Laboratories, Beckenham, Kent, England	C/89.1	1	Mouse Mab
J. Lyon/**D** Walter Reed Army Institute of Research, Washington, DC, USA	D/1A7.6, D/3B10.1, D/7B2.11, D/7H10.19, D/7B11.17	5	Mouse Mabs
J. McBride/**E** Department of Zoology, University of Edinburgh, Edinburgh, Scotland	E/2.2-7, E/9.8-4, E/9.5-1, E/13.3-3, E/7.5-1, E/7.3-7, E/1.2.2-1, E/2.13-19, E/12.8-2, E/9.2-6, E/17.2-3, E/7 12-6	12	Mouse Mabs
L. Pereira da Silva/**F** Unité de Parasitologie expérimentale, Institut Pasteur, Paris, France	F/XIV/7	1	Mouse Mab
M. Perkins/**G** The Rockefeller University, New York, USA	G/5B1.A8 G/α-200 000	1 1	Mouse Mab Rabbit antiserum
L. Perrin/**H** Division of Haematology, University of Geneva, Geneva, Switzerland	H/70C18, H/80C18, H/80C110, H/31C13, H/α-41 000, H/α-fusion protein pMC 31-1, H/α-41 000	4 2 1	Mouse Mabs Rabbit antisera Immune monkey serum
A. Saul/**I** Queensland Institute of Medical Research, Brisbane, Australia	I/3G7-18, I/3E10-18, I/1G3-34, I/3E2-34, I/3A10-11	5	Mouse Mabs

erythrocytes in RPMI 1640 medium containing 10% human serum. The medium was changed daily and, after 4 days, asynchronous parasitaemia in culture reached 10–15%. Asynchronous cultures containing 5–7% schizonts were used for the experiments.

Indirect immunofluorescence assay

Asynchronous cultures at 5–10% parasitaemia were used to make smears on multiwell glass slides.[a] The slides were wrapped individually in tissue paper, sealed and stored at −75 °C; they were thawed progressively at −20 °C and +4 °C before use. Smears were fixed in acetone at +4 °C for 10 min, air-dried and then incubated with the test antibodies in a humid chamber at room temperature for 30 min. After several washings with phosphate-buffer saline (PBS), pH 7.4, the smears were incubated with FITC (fluorescein isothyocyanate)-conjugated antiglobulins. The FITC conjugates used were absorbed with infected erythrocytes from *P. falciparum* cultures. The slides were mounted in 50% glycerol in PBS, pH 8.0, and examined at 100× magnification by fluorescence microscopy. The IIF staining patterns were recorded for antibodies which reacted with the *P. falciparum* strains and clones used (*4*).

Metabolic labelling of P. falciparum *parasites in culture*

P. falciparum strains M25/Zaire and FCQ-27/PNG and clone FCR-3.A-2/Gambia were metabolically labelled with ^{35}S-methionine, and the labelled antigen preparations were used for immunoprecipitation experiments. Asynchronous cultures at 10–15% parasitaemia were labelled when 5–7% of the infected erythrocytes were at the trophozoite and young schizont stage. The cultures were labelled for 6–8 hours with 50 μCi/ml of ^{35}S-L-methionine[b] (specific activity, 800 μCi/mmol, 37 MBq/mCi) in methionine-free MEM medium[c] containing 10% human serum. These conditions allow for a maximum incorporation of ^{35}S-methionine into *de novo* synthesized parasite proteins (*5*). Microscopic examinations of Giemsa-stained smears of the labelled cell preparations showed morphologically healthy parasites. Parasitaemias were essentially the same as recorded in cultures at the beginning of labelling. The labelled cultures contained all asexual blood stages, with more than 5% of the infected erythrocytes at the segmented schizont stage.

Extraction of labelled parasite proteins

^{35}S-methionine-labelled proteins were extracted with Nonidet P40 (NP40). Labelled cultures were harvested by centrifugation at 1500 *g* for 10 min and washed 3 times with cold RPMI 1640 without serum. The cell pellets were extracted at room temperature in 20 times their volume of 1% NP40 in Tris buffer saline pH 7.2 (TBS) containing a cocktail of protease inhibitors: 5 mmol/l EDTA, 0.2 mmol/l each of tosyl-phenyl-chloromethylketone (TPCK) and tosyl-lysyl-chloromethylketone (TLCK), 1 mmol/l *o*-phenanthroline, 2 mmol/l phenylmethylsulfonyl-fluoride (PMSF)[d] and 50 μg/ml each of antipain, leupeptin[e] and chymostatin.[f] The extracts were centrifuged at 400 *g* for 10 min to remove the nuclei and then at 40 000 *g* for 1 hour at 4 °C to remove the NP40-insoluble material. The supernates containing the NP40-soluble, labelled parasite proteins were used for immunoprecipitation experiments. The amount of radioactivity incorporated into macromolecules was determined by TCA precipitation. On the average, more than 60% of the incorporated radiolabel was associated with NP40-soluble macromolecules.

Purification of trophozoite and schizont-infected erythrocytes

Trophozoites and schizonts were purified from cultures of FCQ-27/PNG for Western blot analysis. A 10% cell suspension of FCQ-27 culture was layered over an equal volume of 60% isotonic Percoll[g] and centrifuged at 1000 *g* for 15 min. Infected erythrocytes containing trophozoites and schizonts remained on top of the Percoll layer; young forms and uninfected erythrocytes were pelleted to the bottom of the tube. The trophozoite and schizont-enriched preparation was washed twice with cold RPMI 1640 without serum and the cell pellet extracted with 1% NP40 in TBS containing protease inhibitors. Nuclei were removed by centrifugation at 400 *g* for 10 min. The extracts containing both NP40 soluble and insoluble proteins were used for Western blot analysis.

Immunoprecipitation

Samples of NP40 supernates containing $1–2 \times 10^5$ counts/min of TCA-precipitable material were mixed with 5–10 μl of the test antibody reagents (ascitic fluids, antisera, immune sera) in a final

[a] Cooke slides from Dynatech, Kloten, Switzerland.

[b] Amersham International Ltd, Amersham, Buckinghamshire, England.

[c] Gibco, Basle, Switzerland.

[d] Serva, Heidelberg, Federal Republic of Germany.

[e] Boehringer-Mannheim, Mannheim, Federal Republic of Germany.

[f] Sigma, St. Louis, MO, USA.

[g] Pharmacia Fine Chemicals, Uppsala, Sweden.

volume of 100 μl and incubated for 3 hours at room temperature or overnight at 4 °C. Two types of second reagents were used to precipitate the antigen-antibody complexes formed.

The technique described by Kessler (*6*) was used with slight modifications. The antigen-antibody mixtures were incubated with 100 μl of a 50% suspension of protein A-sepharose[g] in TBS containing 5 mmol/l EDTA and 0.5% each of NP40 and bovine serum albumin (BSA).[h] Since some mouse IgG subclasses do not bind strongly to protein A, each mixture containing mouse antibodies was incubated with 20 μg of rabbit antibodies to mouse immunoglobulins prior to the incubation with the protein A-sepharose beads. The protein A-sepharose beads were collected from the mixtures by centrifugation at 2500 *g* for 5 min. The beads were washed several times with TBS-EDTA containing 0.5% each of NaCl, BSA and NP40; then with the same buffer containing only 0.25% NP40 to remove the non-specifically bound antigens. The specific antigen-antibody complexes bound to protein A-sepharose beads were eluted with 60 μl Laemmli sample buffer (*7*) containing 2% SDS and 3% 2-mercaptoethanol.

In other experiments, the antigen-antibody mixtures were incubated overnight with 300 μl of sheep antiserum to mouse immunoglobulins. The precipitated immune complexes were collected from the mixtures by centrifugation at 500 *g* for 5 min and washed several times as described above. The precipitates were solubilized in a minimum volume of Laemmli sample buffer. Samples containing immunoprecipitated antigens were boiled for 3 min and analysed by SDS-PAGE and fluorography.

SDS-PAGE and fluorography

Electrophoresis was performed in 8%, 10% and 6–20% gradient polyacrylamide gels as described by Laemmli (*7*). The standard proteins used were myosin, M_r 200 000; β-galactosidase, M_r 116 250; phosphorylase B, M_r 92 500; bovine serum albumin, M_r 66 200; ovalbumin, M_r 45 000; carbonic anhydrase, M_r 31 000; soybean trypsin inhibitor, M_r 21 500; and lysozyme, M_r 14 400.[i] Proteins were stained with 0.1% Coomassie blue. After destaining, the gels were treated with En^{3}Hance[j] for 2 hours and then rinsed with water. Dried gels were exposed to X-ray films at −75 °C for 5–20 days.

Western blotting

Samples of the NP40 extract of purified FCQ-27/PNG trophozoites and schizonts containing 2–5 × 10^6 infected erythrocytes were subjected to SDS-PAGE in 8% gels. The resolved proteins were electrophoretically blotted onto nitrocellulose paper at 5 V/cm for 16 hours in Towbin's buffer containing 0.01% SDS and 10% methanol (*8*). The blots were incubated for 1 hour in 0.2% gelatin (EIA grade)[i] in TBS to saturate the free protein-binding sites on the nitrocellulose paper. They were incubated overnight at 4 °C or at room temperature for 3 hours with the test antibodies diluted in TBS-gelatin. After several washings in TBS containing 0.05% Tween 20, they were incubated with horseradish peroxidase (HRP) conjugated antiglobulins.[k] The antigens recognized by the test antibodies were revealed with HRP substrate chloronaphtol.[l] The standard proteins and the total extract were stained with 0.1% amido black.

RESULTS AND DISCUSSION

The antibody reagents submitted for the comparative testing had been produced against asexual blood-stage antigens of several *P. falciparum* strains and clones from different geographical regions. The 37 reagents used for the workshop (Table 1) were tested with asexual blood-stage parasites of *P. falciparum* strains M25 from Zaire and FCQ-27 from Papua New Guinea (PNG), and clones FCR-3.A-2 from Gambia and T9.94 from Thailand.

The antibodies were tested by indirect immunofluorescence on acetone-fixed parasites and by immunoprecipitation using metabolically-labelled, NP40-soluble antigens and SDS-PAGE analysis. The different antibodies were compared according to the stage-specificity, the subcellular localization, and the M_r of the antigens they recognized. A summary of these results is given in Table 2. Although these results have permitted the grouping of antibody reagents according to the similar properties of the antigens they recognize, additional testing of the antibodies, for example in two-site and competition immunoassays, is necessary to make conclusions regarding the identity of the antigens recognized by each group.

Antibodies to a major high M_r *schizont/merozoite antigen*

Twenty-two antibody reagents from 7 laboratories were classified in this group on the basis that they all gave a characteristic grape-like IIF staining pattern of segmented schizonts and free merozoites in acetone-

[h] Calbiochem, San Diego, CA, USA.

[i] Bio-Rad, San Diego, CA, USA.

[j] New England Nuclear, Dreieich, Federal Republic of Germany.

[k] Dakopatts a/s, DK-2600 Glostrup, Denmark.

[l] Merck, Darmstadt, Federal Republic of Germany.

Table 2. Summary of the workshop results obtained with antibodies to asexual blood-stage antigens of *P. falciparum*

Group	IIF staining	M_r of target antigen	Antibodies: Name[a]	Antibodies: Total number	Figure No.: IIF	Figure No.: SDS-PAGE	Characteristics and references
1.	Rim staining of schizonts/ merozoites (SZ/MZ)	190–205 000	B/23-5-6, C/89.1, D/3B10-1, D/7B2-11, D/7H10-19, E/2.2, E/9.8, E/7.5, E/17.2, E/12.8, G/5B1.A8, H/80C110, I/3E2-34 (2 samples), G/α-200 000, H/α-fusion protein pMC31-1, D/3B.11, E/9.5, E/7.3, E/12.2, E/9.2, H/80C18	22	1A & B	2	MZ/SZ surface (*9, 11, 12*); glycoprotein (*11*); processed in MZ (*15, 16*); strain-specific (*4, 9, 10, 13, 14*)
2.	Trophozoites (TZ) and young SZ	90 000	F/XIV/7	1	1C & D	3	Parasite surface and/or parasitophorous vacuole (*17*)
3.	All blood	36 000	E/13.3-3, H/70C18, I/3G7-18	3	1E	3	E/13.3 reacts with lactate dehydrogenase (*18*)
4.	SZ diffuse staining of the parasite	14 000	I/1G3-34	1	1F	3	—
5.	SZ and rings	75 000	A/mouse α-Ag63	1	—	3	(*19*)
6.	Granular staining of SZ and infected cell cytoplasm	250 000	I/3A10-11, A/rabbit α-Ag16	2	1G & H	4	Soluble, heat-stable S antigen; strain-specific (*20, 23*); repeat unit (*21*)
7.	Two-dot staining of segmented SZ and of free MZ	145/135 000	B/12-110.3	1	1I & J	5	Rhoptry organelles by EM (*23, 24*)[b]
		145/135 000	E/2.13, E/7.12	2			(*4, 9*)
		82/41 000	D/1A7.6, H/31C13	2			(*5, 25*)
		41 000	H/rabbit α-41 000	1			(*26*)
		41 000	H/monkey α-41 000	1			(*26*)
		36 000	I/3E10-18	1			—

[a] The prefixes A to I in each name refer to the nine participants (see Table 1).

[b] See footnote *m* in the text (p. 408).

fixed smears of *P. falciparum* (Fig. 1A & B). Some of these antibodies (E/7.3 (*4, 9, 10*), D/7H10-19 (*11*) and G/5B1.A8 (*12*)) have been shown to give a rim fluorescence staining of saponin-lysed schizonts and of merozoites in suspension, suggesting that the antigen recognized is located on the parasite's surface. The surface localization of the antigen recognized by antibodies D/7H10-19 (*11*) and G/5B1.A8 (*12*) has been confirmed by immune electron microscopy. Fourteen Mabs and two rabbit antisera, G/α-200 000 (*12*) and H/α-fusion protein pMC31-1 (*13*), were found to react with the tested strains. The remaining 6 Mabs were directed against strain-specific epitopes not present on this antigen from all strains tested (Table 2, group 1). Strain-specific epitopes of this antigen have been identified in cultured *P. falciparum* isolates from different geographical regions using a panel of Mabs (*4, 9, 10*). Using this panel, eleven serotypes were identified in the malaria parasite population of two small endemic areas in Gambia (J. McBride, personal communication, 1984).

These antibodies recognized a ^{35}S-methionine-labelled schizont antigen which varied in size between M_r 190 000 and 205 000 in different *P. falciparum* strains and clones (Fig. 2). In strain M25/Zaire the antigen recognized was represented as a doublet at M_r 195 000–200 000 (Fig. 2, lane 2), the lower band being more intensely labelled than the upper band. The analogous antigen recognized from clone FCR-3.A-2/Gambia was a strongly labelled band which co-migrated with the lower band of M25 (Fig. 2, lane 1). In FCQ-27/PNG the antibodies recognized an antigen which migrated as a single band to a position close to that of the upper band of M25 (Fig. 2, lane 3). Fig. 2 shows the antigens precipitated by two strain-specific Mabs (lanes 4 & 6) and one antibody which reacted with all tested strains of *P. falciparum* (lane 5).

Antibody to a stage-specific antigen of trophozoites and young schizonts

One Mab (XIV-7) (*17*) which reacted with the four tested strains gave an IIF staining of trophozoites and young schizonts (Fig. 1C & D). The IIF staining was localized to the parasite surface and/or parasitophorous vacuole (*17*). The antibody precipitated a ^{35}S-methionine-labelled antigen of M_r 90 000 from the four strains tested. The NP40 supernate of methionine-labelled M25/Zaire and the antigen precipitated by antibody F/XIV-7 are shown in Fig. 3 (lanes 1 & 2).

Antibodies to an antigen common to all blood stages

Three Mabs (E/13.3, H/70CL8 and I/3G7-18) gave an IIF staining of all development stages of blood parasites (Fig. 1E). The antibodies reacted with intracellular parasites of the four strains tested. These antibodies recognized a methionine-labelled antigen of M_r 36 000 (Fig. 3, lane 5). The M_r 36 000 antigen recognized by antibody E/13.3 has been shown to be lactate dehydrogenase (*18*).

Antibodies to a schizont antigen of M_r *14 000*

One Mab (I/1G3-34) gave an IIF staining of schizonts (Fig. 1F). The antibody stained the intracellular parasite and reacted with all four strains tested. This antibody recognized a ^{35}S-methionine-labelled antigen of M_r 14 000 (Fig. 3, lane 6).

Antibody to an antigen of ring and schizont stages

A mouse antiserum (*19*), raised against a fusion protein Ag63 expressed by an asexual blood-stage cDNA insert of FCQ-27/PNG, gave an IIF staining of schizonts and ring forms. This antiserum reacted with the intracellular parasites of all four tested strains. It precipitated a ^{35}S-methionine-labelled antigen of M_r 75 000 (Fig. 4, lane 3).

Antibodies to the S antigen of strain FCQ-27/PNG

Two antibody reagents, one Mab I/3A10-11 (*20*) and one rabbit antiserum to a fusion protein Ag16 (*21*), reacted specifically with schizonts by IIF on acetone-fixed smears (Fig. 1G & H). This antibody gave a granular IIF staining of schizonts with bright fluorescence accumulations in the parasite, within and surrounding the infected erythrocyte. Both antibodies reacted only with FCQ-27. The antigens recognized were determined by immunoprecipitation and by Western blot analysis (Fig. 4). The antibodies recognized a FCQ-27 antigen of M_r 250 000. Fig. 4 shows the NP40 supernate of methionine-labelled FCQ-27 (lane 4) and the antigen recognized by the Mab I/3A10-11 (lane 5). The autoradiogram was overexposed to reveal the labelled M_r 250 000 band since this antigen incorporated very low levels of ^{35}S-methionine. By Western blotting the antibody reacted with one protein band which migrated to the same position as the methionine-labelled M_r 250 000 antigen (Fig. 4, lane 8).

These antibodies have been reported to react with a strain-specific M_r 220 000 antigen of several PNG strains (*20*, *21*) and Mab I/A10-11 has also been shown to react with some *P. falciparum* isolates from other geographical regions (*23*). A cloned parasite population of the FCQ-27 strain, however, was reported to synthesize a M_r 250 000 protein antigenically related to the M_r 220 000 antigen (*21*). It could be that the major parasite population of the FCQ-27/PNG strain that we have been maintaining in Geneva consists of parasites which synthesize the M_r 250 000 strain-specific antigen. Alternatively, it could be that slight differences between the SDS-PAGE conditions in different laboratories may lead to significant differences in the apparent M_r obtained for some malaria antigens owing to their unusual structure containing repeated and/or highly acidic regions.

Antibodies recognizing stage-specific antigens at the merozoite apex

Eight antibody reagents submitted by five laboratories gave a two-dot IIF staining of segmented schizonts and merozoites in acetone-fixed parasites. These antibodies stained the apical end of merozoites in segmented schizonts and of merozoites released after schizont rupture (Fig. 1I & J). They reacted with all tested strains of *P. falciparum*. The antibodies were divided into four subgroups according to the M_r of the antigens they recognized.

Three Mabs (B/12-110.3 (*23*),[m] E/7.12 and E/2.13 (*4*, *9*) precipitated ^{35}S-methionine-labelled antigens of M_r 145 000 and 135 000. Fig. 5 (lane 7) shows the labelled band pattern obtained with antibody B/12-110.3. This antibody has been shown by immune electron microscopy to bind to the rhoptry organelles at the merozoite apex.[m] By comparison, the two other antibodies from a different laboratory appear to recognize the same rhoptry antigens.

Two Mabs (H/31C13 (*5*, *25*) and D/1A7.6[m]) precipitated ^{35}S-methionine-labelled antigens at M_r 82 000, M_r 69 000 and a doublet at M_r 41 000 (Fig. 5, lane 3). The antibody H/31C13 has been shown to inhibit the *in-vitro* multiplication of *P. falciparum* (*5*,

[m] *Report of the Sixth Meeting of the Scientific Working Group on the Immunology of Malaria*. Unpublished WHO document, TDR/IMMAL/SWG(6)/84.3 (1984).

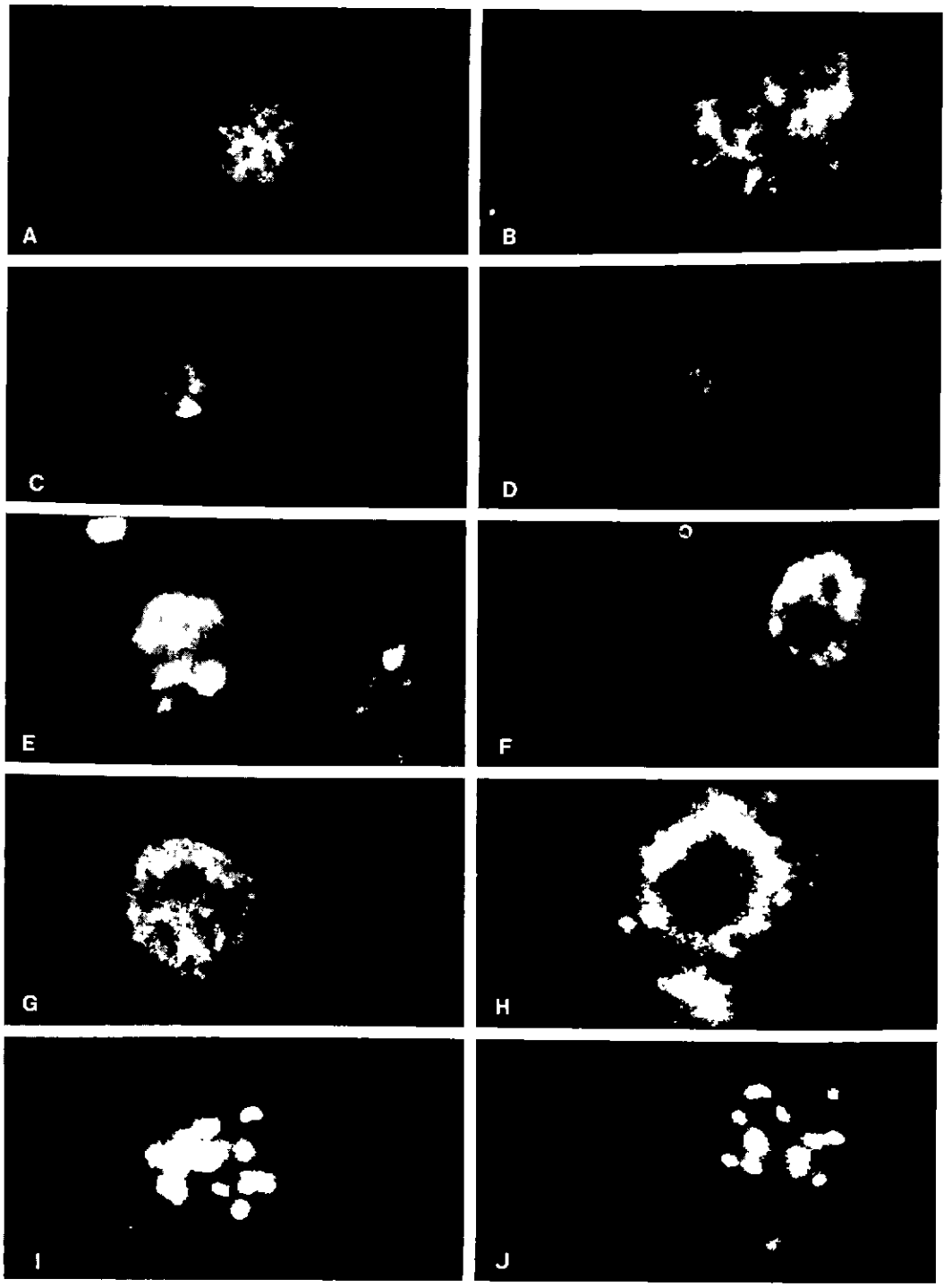

Fig. 1. Different staining patterns obtained with the antibody reagents tested by IIF on acetone-fixed parasites from *in vitro* culture of *P. falciparum*.

A & B: rim staining of segemented schizonts and merozoites with Mab C/89.1 to the M_r 190 000 to 205 000 surface antigen (group 1)

C & D: staining of trophozoites and young schizonts with Mab F/XIV/7 to the M_r 90 000 antigen (group 2)

E: staining of all blood stage parasites by Mab E/13 3-3 (group 3)

F: schizont staining with Mab I/1G3-34 to the M_r 14 000 antigen (group 4)

G & H: granular staining of schizont and infected cell cytoplasm with Mab I/3A10-11 to the M_r 250 000 S antigen of FCQ-27 (group 6)

I & J: two-dot staining pattern of segmented schizonts and free merozoites with Mab H/31C13 to M_r 82 000/41 000 antigens and with Mab B/12-110.3 to the M_r 145 000/135 000 antigen (group 7).

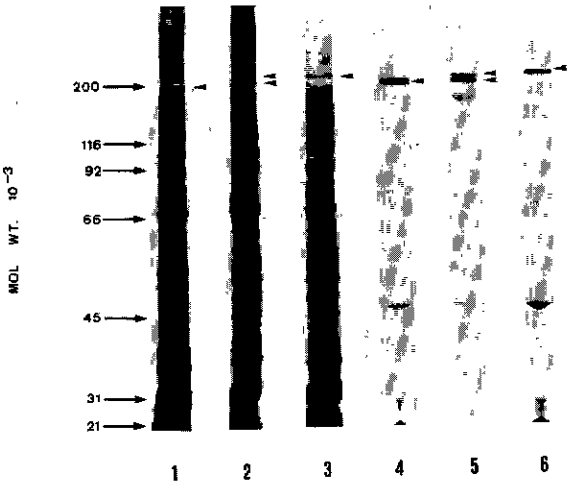

Fig. 2. SDS-PAGE analysis and fluorography of ^{35}S-methionine-labelled asexual blood-stage antigens of *P. falciparum* strains and clones.

NP40 supernates of lane 1: FRC-3.A-2 from Gambia; lane 2: M25 from Zaire; and lane 3: FCQ-27 from Papua New Guinea.

NP40-soluble antigens immunoprecipitated with monoclonal antibodies which give a rim IIF staining of schizonts and merozoites (group 1). Lane 4: antibody E/7.5 with A-2; lane 5: antibody C/89.1 with M25; and lane 6: antibody H/80C18 with FCQ-27.

(Arrow heads point to the antigens recognized by these antibodies; M_r of standard proteins are indicated on the left.)

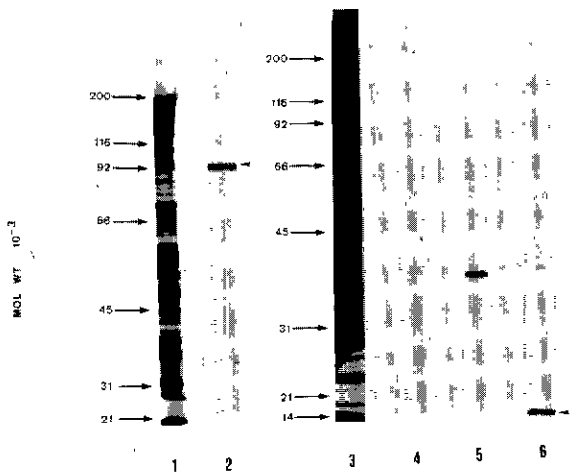

Fig. 3. SDS-PAGE analysis and fluorography of ^{35}S-methionine-labelled proteins of M25 from Zaire, recognized by antibodies to asexual stage antigens.

Lane 1: NP40 supernate resolved in a 8% SDS gel; lane 2: M_r 90 000 antigen precipitated by Mab F/XIV/7 reacting with trophozoites and young schizonts (group 2).

Lane 3: NP40 supernate resolved in a 10% SDS gel; lane 4: control immunoprecipitate using normal mouse serum; and lane 5: Mab H/70C18 against the M_r 36 000 antigen present in all blood forms (group 3).

Lane 6: Mab I/1G3-34 against a M_r 14 000 schizont antigen (group 4).

(Arrow heads point to the antigens recognized by these antibodies; M_r of standard proteins are indicated on the left.)

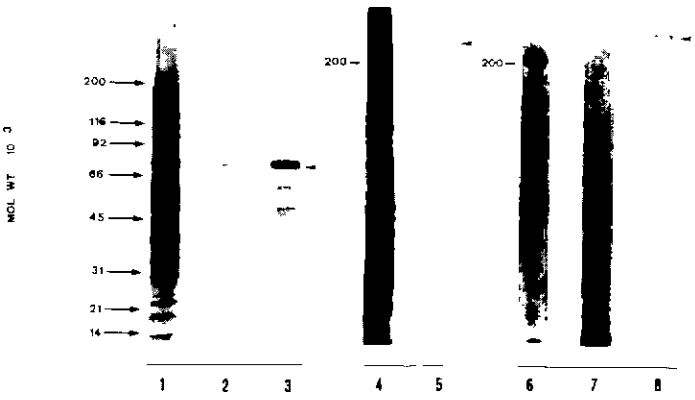

Fig. 4. SDS-PAGE analysis and fluorography of ^{35}S-methionine-labelled asexual blood-stage antigens of FCQ-27 from Papua New Guinea.

Lane 1: NP40-soluble proteins resolved in a 6–20% gradient gel, lane 2: control immunoprecipitate using normal mouse serum; lane 3: M_r 75 000 band precipitated by a mouse antiserum A/α-Ag63 raised against a fusion protein (group 5).

Lane 4: NP40-soluble proteins resolved in a 8% gel; lane 5: Mab I/3A10-11 against strain-specific M_r 250 000 S antigen of FCQ-27 schizonts (group 6). Lanes 4 & 5 are overexposed to reveal the M_r 250 000 antigen since it incorporates a very low amount of methionine.

Western blot analysis using purified FCQ-27/PNG schizonts resolved in a 8% SDS gel. Lane 6: high M_r standard proteins stained with amido black; lane 7: 2×10^6 schizonts stained with amido black; and lane 8: M_r 250 000 antigen recognized by the same Mab as in lane 5.

(Arrow heads point to the antigens recognized by the tested antibodies; M_r of standard proteins are indicated on the left.)

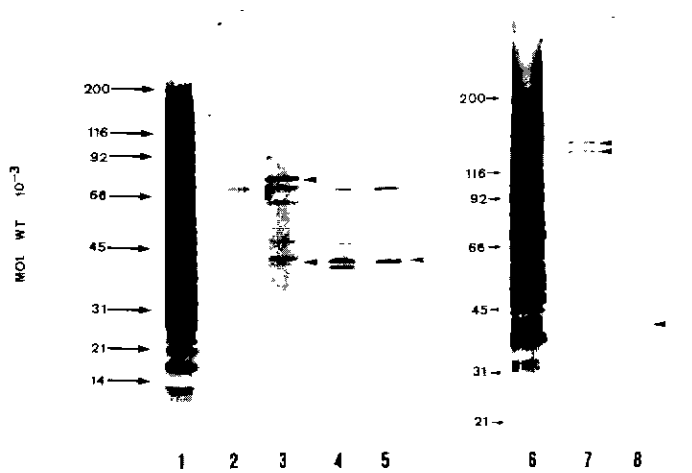

Fig. 5. SDS-PAGE analysis and fluorography of ^{35}S-methionine-labelled asexual blood-stage antigens of clone FCR-3.A-2/Gambia. Antigens immunoprecipitated with five antibody reagents which gave a two-dot IIF staining of segmented schizonts and free merozoites (group 7).

Lane 1: NP40 supernate resolved in a 6–20% SDS gel; lane 2: control immunoprecipitate using normal mouse serum; lane 3: M_r 82 000 and 41 000 antigens precipitated by Mab H/31C13; lane 4: M_r 41 000 (doublet) antigen precipitated by an immune monkey serum H/α-41 000 from animals vaccinated with the purified M_r 41 000 protein; lane 5: M_r 41 000 (upper band) precipitated by a rabbit antiserum raised against the same antigen.

Lane 6: NP40 supernate resolved in a 8% SDS gel; lane 7 M_r 145 000 and 135 000 antigens recognized by Mab B/12-110.3; lane 8: M_r 36 000 merozoite antigen precipitated by Mab I/3E10-18.

(Arrow heads point to the antigens recognized by these antibodies; M_r of standard proteins are indicated on the left.)

25). A rabbit antiserum H/rabbit α-41 000 (*26*) raised against the purified M_r 41 000 component gave the same two-dot IIF staining of segmented schizonts and merozoites and precipitated the upper band of the M_r 41 000 doublet (Fig. 5, lane 4). Monkeys immunized with the same preparation of the M_r 41 000 antigen developed a partial protection against asexual blood parasites (*26*). A serum pool from the immune monkeys gave the characteristic two-dot IIF staining pattern and recognized the ^{35}S-methionine-labelled doublet at M_r 41 000 (Fig. 5, lane 5).

One Mab (I/3E10-18), which also gave a two-dot IIF staining of segmented schizonts and merozoites, reacted with a ^{35}S-methionine-labelled antigen of M_r 36 000 (Fig. 5, lane 8). This antibody reacted with all four strains tested.

The relationships between the four groups of antigens recognized by antibodies which give a similar two-dot IIF staining of segmented schizonts and merozoites is not known.

CONCLUSION

The 37 antibody reagents used for the workshop were characterized by indirect immunofluoresence using acetone-fixed smears and by immunoprecipitation using ^{35}S-methionine-labelled asexual blood-stage antigens of *P. falciparum*. These antibodies were classified into seven groups according to the stage-specificity, the subcellular localization, and the M_r of the antigens recognized (Table 2). Based on these results an attempt was made to find analogies between the seven groups and the antibody groups I to V described by McBride et al. (*4*) and Hall et al. (*9*).

One group of 22 antibody reagents which reacted with the M_r 190 000–205 000 schizont/merozoite surface antigen (*4*, *9–12*, *14*, *15*) had the highest proportion of strain-variant antibodies. These antibodies had similar properties to the group IV antibodies. The second group containing one Mab, which reacted with the stage-specific M_r 90 000 antigen at the surface of trophozoites and young schizonts (*17*), represented a new group since these properties were not shared by any of the group I to group V antibodies. Three antibodies which reacted with all blood forms and recognized a M_r 36 000 methionine-labelled protein were similar to group I antibodies. One reagent, which recognized a schizont antigen of M_r 14 000, did not fall into any of the groups described by McBride and coworkers. This was also the case for one reagent which reacted with schizont and ring-stage parasites and recognized a M_r 75 000 antigen. The IIF and M_r characteristics of the two strain-specific antibodies which reacted with the M_r 250 000 schizont antigen of FCQ-27 (*20*, *21*) differ from those of the group I to group V antibodies. The eight antibody reagents in the seventh group which react with four different sets of ^{35}S-methionine-labelled antigens gave a two-dot fluorescence staining of merozoites similar to that described for group II antibodies (*23*, *24*).[m]

The antibodies tested have been assigned to group I, group II and group IV antibodies described by McBride et al. (*4*) and Hall et al. (*9*). In addition to these, four new groups of antibodies have been described on the basis of their IIF pattern and the M_r of the antigens recognized. It is expected that there will be additions and modifications to this nomenclature in the future. Also, the antigens recognized by the 37 antibody reagents used for the workshop represent only some of the different asexual blood-stage proteins described in the literature. Additional analysis of these reagents in two-site assays will be necessary to determine the identity between reagents which are classified into these groups. This work is continuing at the reference laboratory. Antibodies supplied are tested similarly and classified into the existing groups or into new groups. Interested scientists are invited to contribute reagents and to collaborate in this work.

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RÉSUMÉ

COMPARAISON DES ANTIGÈNES DES STADES SANGUINS ASEXUÉS DE *PLASMODIUM FALCIPARUM* RECONNUS PAR DES RÉACTIFS ANTICORPS DANS NEUF LABORATOIRES

Plusieurs laboratoires ont reconnu un grand nombre d'antigènes plasmodiques qui pourraient être impliqués dans la protection immunitaire contre les stades sanguins asexués de *Plasmodium falciparum*. Afin de déterminer quelles sont les relations entre les antigènes mentionnés par différents laboratoires, un essai comparatif de 37 réactifs anticorps a été effectué au laboratoire de référence pour les anticorps et antigènes du paludisme. D'après les résultats de cet essai, les anticorps ont été divisés en sept groupes selon leurs analogies en matière de spécificité de stade et de localisation intracellulaire et selon la masse moléculaire apparente (M_r) des antigènes reconnus.

Vingt-deux réactifs anticorps ont été placés dans un premier groupe car ils donnent en immunofluorescence indirecte un aspect caractéristique en forme de grain de raisin et précipitent un antigène de schizonte/mérozoïte de M_r 190 000 à 205 000. Le deuxième groupe était constitué d'un seul anticorps réagissant avec une protéine de M_r 90 000 exprimée uniquement chez les trophozoïtes mûrs et les jeunes schizontes. Un troisième groupe comprenait trois anticorps réagissant avec tous les stades sanguins du parasite et reconnaissant une protéine de M_r 36 000. Le quatrième groupe contenait un anticorps reconnaissant une protéine de M_r 14 000 et donnant en immunofluorescence indirecte une coloration des schizontes. Le cinquième groupe consistait en un immunsérum dirigé contre une protéine de fusion, réagissant avec les schizontes et les stades annulaires du parasite et précipitant un antigène de M_r 75 000. Un immunsérum de lapin dirigé contre une autre protéine de fusion et un anticorps monoclonal ont été réunis dans le sixième groupe car ils donnent tous deux une coloration du cytoplasme des schizontes en immunofluorescence indirecte et reconnaissent un antigène de M_r 250 000 spécifique de souche, provenant d'une souche de *P. falciparum*. Le septième groupe était formé de 8 anticorps donnant en immunofluorescence indirecte un aspect identique en deux taches avec les schizontes segmentés et les mérozoïtes libres, bien qu'ils reconnaissent quatre séries différentes d'antigènes.

Ces résultats ont été présentés à un sous-comité du Groupe de travail scientifique sur l'immunologie du paludisme (SWG-IMMAL) au cours d'un atelier qui s'est tenu au laboratoire de référence. Les protéines de *P. falciparum* reconnues par les anticorps utilisés ne représentent que quelques-uns des différents types d'antigènes de stades sanguins asexués décrits dans la littérature.

REFERENCES

1. TRAGER, W. ET AL. *Proceedings of the National Academy of Sciences, USA*, **78**: 6527–6530 (1981).
2. THAITHONG, S. ET AL. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **78**: 242–245 (1984).
3. TRAGER, W. & JENSEN, J. B. *Science*, **193**: 673–675 (1976).
4. MCBRIDE, J. S. ET AL. *Science*, **217**: 254–257 (1982).
5. PERRIN, L. H. & DAYAL, R. *Immunological reviews*, **61**: 245–269 (1982).
6. KESSLER, S. W. *Journal of immunology*, **115**: 1617–1624 (1975).
7. LAEMMLI, M. K. *Nature*, **277**: 680–682 (1970).
8. TOWBIN, H. ET AL. *Proceedings of the National Academy of Sciences, USA*, **76**: 4350–4354 (1979).
9. HALL, R. ET AL. *Molecular and biochemical parasitology*, **7**: 247–265 (1983).
10. MCBRIDE, J. S. ET AL. *Journal of experimental medicine*, **161**: 160–180 (1985).
11. HOWARD, R. J. ET AL. *Molecular and biochemical parasitology*, **11**: 349–362 (1984).
12. PIRSON, P. J. & PERKINS, W. E. *Journal of immunology*, **134**: 1946–1951 (1985).
13. CHEUNG, A. ET AL. *The European Molecular Biology Organisation journal*, **4**: 1007–1012 (1985).
14. SCHOFIELD, L. ET AL. *Infection and immunity*, **38**: 893–897 (1982).
15. HOLDER, A. A. & FREEMAN, R. R. *Journal of experimental medicine*, **156**: 1528–1538 (1982).
16. LYON, J. A. & HAYNES, J. D. *Journal of immunology*, **136**: 2245–2251 (1986).
17. JENDOUET, M. ET AL. *Journal of immunology*, **134**: 1941–1945 (1985).
18. HYDE, J. E. ET AL. *Molecular and biochemical parasitology*, **10**: 269–285 (1984).
19. ANDERS, R. F. ET AL. *Molecular and biological medicine*, **2**: 177–192 (1984).
20. SAUL, A. ET AL. *Parasite immunology*, **6**: 39–50 (1984).
21. COPPEL, R. L. ET AL. *Nature*, **306**: 751–756 (1983).
22. SCHOFIELD, L. ET AL. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**: 493–494 (1985).
23. CAMPBELL, G. H. ET AL. *American journal of tropical medicine and hygiene*, **33**: 1051–1054 (1984).
24. HOLDER, A. A. ET AL. *Molecular and biochemical parasitology*, **14**: 293–303 (1985).
25. PERRIN. L. H. ET AL. *Nature*, **289**: 301–303 (1981).
26. PERRIN. L. H. ET AL. *Journal of clinical investigation*, **75**: 1718–1721 (1985).