JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1987, p. 2347–2354
0095-1137/87/122347-08$02.00/0

Vol. 25, No. 12

Absence of Antigenic Diversity in Pf155, a Major Parasite Antigen in Membranes of Erythrocytes Infected with *Plasmodium falciparum*

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Received 22 June 1987/Accepted 17 August 1987

Pf155 is a merozoite-derived polypeptide antigen which the parasite *Plasmodium falciparum* deposits in the membranes of erythrocytes at invasion. Eleven laboratory strains or clones of *P. falciparum* and a large number of isolates obtained from patients from different parts of the world were studied for antigenic diversity in Pf155. Immunoglobulin G antibodies from different serum samples from *P. falciparum*-infected donors were affinity purified on monolayers of glutaraldehyde-fixed and air-dried erythrocytes infected with *P. falciparum* of different origins and tested in different combinations by immunoblotting, reinvasion inhibition, and a modified immunofluorescence procedure in which the membranes of recently infected erythrocytes were stained. Similar experiments were performed with monoclonal and oligoclonal antibodies specific for different epitopes in the C-terminal region of Pf155. No strain- or isolate-associated antigenic diversity or size variation of Pf155 was detected, indicating that the immunodominant regions of this antigen are highly conserved throughout the world.

Pf155 is a merozoite-derived, heat-stable polypeptide (M_r, 155,000) which the human malaria parasite *Plasmodium falciparum* deposits in the membranes of erythrocytes at invasion (28). It is identical with ring-infected erythrocyte surface antigen (RESA) which has been described recently by others (11). Like several other *P. falciparum* antigens, Pf155 contains stretches of tandemly repeated amino acid sequences which are strongly antigenic in naturally infected humans (24, 27). The appearance of anti-Pf155 antibodies correlates well with the acquisition of clinical immunity (38), and these antibodies also very efficiently inhibit reinvasion of erythrocytes by *P. falciparum* merozoites in vitro (39). Thus, Pf155 may be an important candidate for a vaccine against the asexual blood stages of this malaria parasite. This is also supported by results of a recent vaccination trial in *Aotus* monkeys (10).

To be used for a vaccine, the immunodominant structures of an antigen should be invariant. Several of the plasmodial antigens detected on the surface of infected erythrocytes or on schizonts and merozoites exhibit a strain- and isolate-associated diversity (25, 27) or even antigenic variation (20, 21), probably obviating their usefulness as vaccine components. Results of preliminary serological studies of Pf155 have given no indications of antigenic diversity (28). Similarly, results of limited studies at the DNA level also suggest that the major antigenic structures of this immunogen are conserved in different *P. falciparum* strains (18). Results of more extensive experiments establishing the absence of antigenic diversity of Pf155, however, have not heretofore been reported. Here we report our results of an investigation of the reaction of Pf155 from a large number of *P. falciparum* strains, clones, or fresh isolates with a variety of antibodies. No strain- or isolate-associated antigenic diversity or size variation of Pf155 was detected.

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MATERIALS AND METHODS

Parasites. The parasites (*P. falciparum*) used in these experiments either were from cultured laboratory strains (34) or were isolates obtained from patients. These isolates were prepared for microscopic investigation without in vitro culturing. The following laboratory strains or cloned lines were used: F32 (Tanzania [23]); K1 (Thailand [31]); Thai1 (Thailand; established in 1985 from a chloroquine-resistant isolate of a Swedish patient (patient N) by A. Björkman, Stockholm); T9, clone 96 (Thailand [33]); CDC1, clone HB3 (Honduras [6]); IMTM22, clone 7G8 (Brazil [16]). Three additional strains (Honduras, Kenya, and Tanzania) were kindly provided by R. Nussenzweig, New York, N.Y. Two strains, FCR1/FVO (Vietnam) and FCR3/FMG (Gambia [35]), were obtained from the blood of *Aotus trivirgatus* monkeys. Isolates from patients were obtained from 11 Liberian and 9 Colombian donors who had repeated *P. falciparum* infections. Six isolates were from Swedish donors who had acquired their infections in Kenya ($n=3$), Zaire ($n = 1$), India ($n = 1$), and Thailand ($n = 1$). Freshly drawn blood (parasitemias, usually 2 to 10%) was washed three to four times with Tris-buffered Hanks solution, diluted to 1% suspensions, and layered on eight-well multitest slides for microscopic examination (28).

Immune sera. Human immune sera were obtained from Swedish or South American patients who had acute *P. falciparum* infections or from African blood donors living in an area of Liberia with high levels of *P. falciparum* transmission (7). Most donors had a history of repeated *P. falciparum* infections or were clinically immune to the disease.

Rabbit antiserum to the synthetic dimer of the tetrapeptide Glu-Glu-Asn-Val, a sequence that is repeated approximately 30 times in the C-terminal end of Pf155 (14, 18), were obtained by immunizing rabbits with this peptide coupled to keyhole limpet hemocyanin, as described elsewhere (3). A mouse monoclonal antibody (immunoglobulin G1 [IgG1]) to

the octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala, another major C-terminal repeat of Pf155, was obtained by immunization of a BALB/c mouse with a peptide-keyhole limpet hemocyanin conjugate (W. Ruangjirachuporn, B. Wåhlin, H. Perlmann, J. Carlsson, K. Berzins, M. Wahlgren, R. Udomsangpetch, H. Wigzell, and P. Perlmann, submitted for publication). Human, rabbit, or mouse immunoglobulins were purified by ammonium sulfate precipitation and DEAE-Sephadex ion-exchange chromatography. A human monoclonal antibody (IgM; 33G2) was obtained from a cloned B-cell culture derived from Epstein-Barr virus-transformed B-lymphocytes of a *P. falciparum*-hyperimmune donor. Preparation and purification of these antibodies have been described previously (36).

Affinity purification of antibodies on monolayers of infected erythrocytes. For enrichment of antibodies to Pf155, serum or IgG fractions were adsorbed to monolayers of glutaraldehyde-fixed and air-dried erythrocytes in Petri dishes (parasitemias, 5 to 10%). The supernatants with nonadsorbed IgG were added sequentially to fresh monolayers in some experiments until they were free of Pf155 antibodies as detected by indirect immunofluorescence on monolayers of glutaraldehyde-fixed and air-dried *P. falciparum*-infected erythrocytes. After the plates were washed, the antibodies that adsorbed to the membranes of infected erythrocytes were isolated by acid elution (pH 2.8) and immediately neutralized (28).

Determination of immunoglobulin. Immunoglobulin in serum or in various antibody preparations was determined by a sandwich enzyme-linked immunosorbent assay (17). Anti-$F(ab')_2$ antibodies from a rabbit anti-human immunoglobulin serum sample were used to coat the microplates. Serial dilutions of the test samples and a known IgG standard (five dilutions) were then added as described previously (40). Alkaline phosphatase-conjugated rabbit antibodies specific for human γ or μ chains were used as indicator antibodies. For analysis of mouse or rabbit IgG, the plates were coated with rabbit anti-mouse or sheep anti-rabbit immunoglobulin. Indicator antibodies were alkaline phosphatase-conjugated anti-mouse or anti-rabbit IgG reagents. All determinations were done in duplicate. The variation coefficient (weighted standard deviations from means of independent determinations) was 19%, corresponding to an error of 27% for single determinations.

EMIF assay. A detailed description of the erythrocyte membrane immunofluorescence (EMIF) assay has been given previously (28). In brief, monolayers of erythrocytes (parasitemias, 2 to 10%) in the wells of eight-well multitest slides (Flow Laboratories, Inc., McLean, Va.) were glutaraldehyde fixed (1% glutaraldehyde in phosphate-buffered saline for 10 s), air dried, and treated with various dilutions of human serum or immunoglobulin fractions. In endpoint titration experiments, the antibodies were added in fivefold dilution steps. The titers given in Tables 1 to 4 were reproducible within one dilution step. For immunofluorescence staining, affinity-purified and biotinylated goat anti-human immunoglobulin and avidin conjugated with fluorescein isothiocyanate were used (Vector Laboratories Inc., Burlingame, Calif.). The immunofluorescence of erythrocytes given by mouse or rabbit antibodies was assayed similarly with biotinylated anti-mouse or anti-rabbit IgG reagents (Vector Laboratories).

In vitro reinvasion inhibition. The in vitro reinvasion inhibition assay was performed as described previously (39). In brief, *P. falciparum* cultures were set up in microculture plates (200 μl per well) at a 2% hematocrit and a starting parasitemia of about 0.5%; this consisted primarily of late trophozoites and early schizonts. After 20 h of incubation at 37°C in complete medium, which was supplemented with different dilutions of the various purified antibody preparations or normal IgG, the microcultures were washed by centrifugation. Glutaraldehyde-fixed and air-dried erythrocyte monolayers were prepared on multitest slides. The parasites were stained with acridine orange, and the percentage of newly infected erythrocytes was scored in a fluorescence microscope. Reinvasion inhibition was calculated as 100 × (percent parasitemia in control − percent parasitemia in test):(percent parasitemia in control). All cultures were set up in quadruplicates, and the values given are the means ± standard deviation from the 40,000 erythrocytes that were screened. The 50% inhibition titers were calculated from titration curves obtained by using at least four different concentrations of the inhibitory immunoglobulin.

Immunoblotting. Merozoite-enriched fractions of in vitro-cultured parasites were prepared from spent culture medium as described previously (28). The polypeptides of merozoite lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, electrophoretically transferred to nitrocellulose, and probed with the various antibody preparations. Bound antibodies were detected with alkaline phosphatase-conjugated anti-immunoglobulin reagents and by staining for alkaline phosphatase (3, 5, 28). Molecular weight markers were obtained from Bio-Rad Laboratories, Richmond, Calif.

RESULTS

Titers of different human serum samples by the EMIF assay. Pf155 in the membranes of ring-infected erythrocytes is most easily detected by indirect immunofluorescence after short glutaraldehyde fixation and drying of erythrocyte monolayers (28). The results of a typical experiment in which 13 different serum samples from malaria-exposed donors were analyzed in the EMIF assay on erythrocytes

TABLE 1. Titers determined by EMIF of different serum samples from malaria-exposed donors

Serum donor[a]	Titers with *P. falciparum* of the following origin[b]:		
	Tanzania (F32)	Liberia (patient)	Colombia (patient)
1			
A	62,500	62,500	12,500
B	500	500	500
C	500	20	100
2			
D	12,500	12,500	12,500
E	2,500	500	100
F	2,500	500	500
G	100	100	100
H	100	20	100
3			
I	12,500	2,500	500
J	2,500	2,500	2,500
K	500	100	20
L	500	100	100
M	500	20	100

[a] 1, Liberians; 2, Colombians; 3, Swedish patients who acquired infection in Tanzania (donors I and K), Nigeria (donor J), Kenya (donor L), or Thailand (donor M).

[b] Reciprocals of highest dilution giving a positive result by the EMIF assay (see text). *P. falciparum* was laboratory strain F32 or was freshly isolated from patients.

infected with the Tanzanian laboratory strain F32 or on erythrocytes isolated from either a Liberian or a Colombian patient with acute *P. falciparum* infection are shown in Table 1. All serum samples contained anti-Pf155 antibodies, as established by prescreening at a dilution of 1:100 in the EMIF assay on F32-infected erythrocytes. The serum samples varied considerably in their endpoint titers both within and between the three different parasite isolates used for the test. However, no variations related to the geographic origin of either parasites or serum samples were detected. Similar results were obtained with an additional 21 serum samples, which were titrated on human erythrocytes infected with the laboratory strains IMTM22/7G8 or K1 or on *Aotus* erythrocytes infected with strains FCR-1/FVO or FCR-3/FMG (data not shown).

Activity of affinity-purified human IgG fractions. Antibodies to Pf155 can be enriched by adsorbing IgG from malarial serum to glutaraldehyde-fixed and air-dried monolayers of ring-infected erythrocytes and subsequent acid elution (28). IgG from one Liberian (donor A) and one Colombian donor (donor D), both with high Pf155 titers in the EMIF assay (Table 1), was affinity purified on erythrocytes infected with any one of the three different parasites F32, K1, or IMTM22/7G8. When tested by immunoblotting with SDS-PAGE-separated merozoite lysates of F32, all six antibody preparations gave the banding pattern previously described, with Pf155 being the strongly dominating antigen (Fig. 1). All serum samples also reacted significantly with a polypeptide with an M_r of 31,000, which is believed to be a cross-reacting antigen encoded by a different parasite gene (D. Mattei, K. Berzins, M. Wahlgren, R. Udomsangpetch, P. Perlmann, H. W. Griesser, A. Scherf, B. Müller-Hill, M. Guillotte, G. Langsley, L. Pereira da Silva, and O. Mercereau-Puijalon, J. Immunol., in press) but which is not seen in the EMIF assay. The results indicate that antibodies reacting with F32 Pf155 were present in both serum samples and were eluted in apparently similar amounts from the three different parasites. In the reverse experiment, antibodies from a Liberian donor (donor A) were eluted from F32-infected erythrocytes and used for immunoblotting of F32, K1, or IMTM22/7G8 merozoite lysates. Pf155 derived from these parasites did not significantly vary in size (Fig. 2).

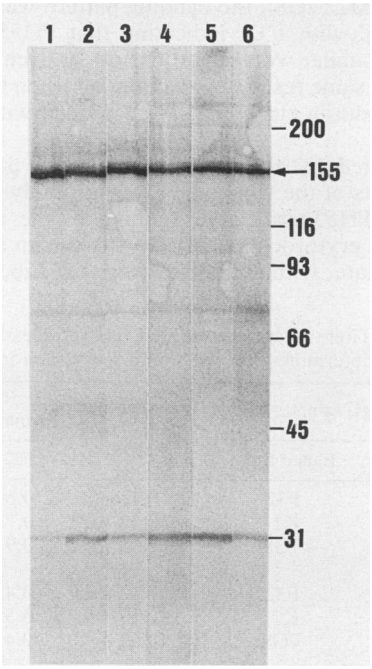

FIG. 1. Immunoblotting analysis of F32 merozoite-enriched lysates probed with different human antibody preparations. Lanes 1 to 3 (donor A), IgG affinity purified by elution from erythrocytes infected with F32 (Tanzania), K1 (Thailand), or IMTM22/7G8 (Brazil), respectively, and diluted 1:3; lanes 4 to 6 (donor D), IgG treated as described above for donor A in lanes 1 to 3, respectively. Numbers to the right of the gels indicate relative molecular weights (10^3). The arrow shows the position of Pf155.

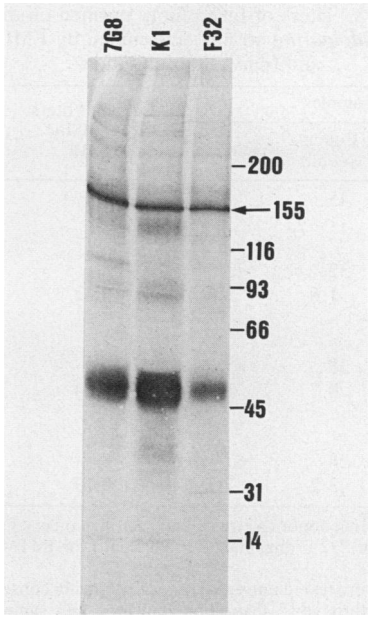

FIG. 2. Immunoblotting analysis of merozoite-enriched lysates of IMTM22/7G8 (Brazil), K1 (Thailand), or F32 (Tanzania) probed with donor A IgG affinity purified on F32-infected erythrocytes. For details see Fig. 1 legend and the text. The heavily stained band at M_r 45,000 represents human immunoglobulin heavy chains present in the antigen preparations.

The results of a typical experiment in which IgG of the same Liberian donor (donor A) was affinity purified on K1, IMTM22/7G8, or F32 and on fresh isolates derived from a Colombian and a Liberian patient are shown in Table 2. Results for an eluate from *Aotus* monkey erythrocytes infected with FCR-1/FVO are also shown in Table 2. The amounts of IgG antibody eluted from different parasites were

TABLE 2. Titers of IgG affinity purified on erythrocytes infected with *P. falciparum* of different origins determined by EMIF[a]

IgG eluted from the following parasites:	μg of IgG/ml of eluate	EMIF titers of eluates on the following parasites[b]		
		F32	K1	7G8
F32 (Tanzania)	0.31	0.10	0.31	0.10
K1 (Thailand)	0.37	0.04	0.12	0.04
7G8 (Brazil)	1.00	0.04	0.11	0.04
FCR-1/FVO (Vietnam)[c]	1.35	0.05	0.27	0.05
Colombian isolate[d]	0.15	0.05	0.15	0.13
Liberian isolate[d]	0.33	0.11	0.33	0.11

[a] IgG from Liberian donor A was affinity purified on infected erythrocytes.
[b] Lowest concentration of IgG (see text) giving a positive EMIF assay result on parasites F32, K1, or IMTM22/7G8.
[c] Infected erythrocytes were taken from *A. trivirgatus* monkey.
[d] Infected erythrocytes were taken from patients.

TABLE 3. Titers of IgG affinity purified on different *P. falciparum* strains determined by EMIF and reinvasion inhibition[a]

IgG samples[b]			Endpoint titers (μg/ml) by EMIF	50% reinvasion inhibition (μg/ml)[c]
Treatment	Concn (μg/ml)	Yield (%)[d]		
None	45		1.8	16 ± 1.1
F32				
Absorption	32	72	–	27 ± 2.1
Elution	1.6	3.6	0.32	0.5 ± 0.02
K1				
Absorption	38	85	–	30 ± 1.7
Elution	0.7	1.6	0.14	0.6 ± 0.04
7G8				
Absorption	28	63	–	22 ± 1.4
Elution	3.2	7.1	0.13	0.6 ± 0.06

[a] IgG from Liberian donor (A) was affinity purified on erythrocytes infected with *P. falciparum* F32 (Tanzania), K1 (Thailand), or IMTM22/7G8 (Brazil) and tested on F32.

[b] IgG samples were tested untreated (none) or after 10 consecutive passages over infected erythrocytes; absorption indicates IgG samples depleted of anti-Pf155 antibodies by adsorption to infected erythrocytes; elution indicates IgG samples enriched in anti-Pf155 antibodies by acid elution from erythrocytes.

[c] See text. At an assay time of 19 h parasitemias in controls were 1.46 ± 0.07%; at time zero parasitemias in controls was 0.43 ± 0.05%.

[d] Percent input (45 μg of IgG per ml).

similar. When erythrocytes infected with F32, K1, or IMTM22/7G8 were analyzed in the EMIF assay, no significant variation in endpoint titers related to parasite origin was seen between the different eluates.

Reinvasion inhibition by affinity-purified human antibodies. To establish the biological activity of the anti-Pf155 antibodies which bind to different parasites, IgG fractions were affinity purified and tested for reinvasion inhibition in vitro (39). When purified on F32, such antibody preparations have previously been shown to give complete and dose-dependent reinvasion inhibition of this parasite and to have much higher inhibitory titers than the total IgG fractions from which they were derived. Results of an experiment in which IgG from the serum of the Liberian donor A was affinity purified on erythrocytes infected with F32, K1, or IMTM22/7G8 and tested for reinvasion inhibition in the F32 system are shown in Table 3. Both the total IgG fraction and the three affinity-purified preparations completely inhibited reinvasion in a dose-dependent fashion (data not shown). The 50% inhibition titer of the affinity-purified antibodies was approximately 30 times higher than that of the original IgG fraction and was similar for all three preparations (Table 3), suggesting that the F32-inhibitory antibodies were of similar specificity. After absorption on erythrocytes infected with these different parasite strains, the IgG fractions retained a reinvasion-inhibitory activity, although this was approximately 50 times less than that of the affinity-purified antibodies. As these IgG fractions had lost their activity in the EMIF assay, they were probably depleted of anti-Pf155 antibodies, suggesting that their residual inhibitory activity was due to antibodies to other parasite antigens than Pf155.

The results of an experiment in which the inhibitory activity of affinity-purified antibodies of the Liberian donor A was compared with that of the Colombian donor D, whose total IgG also gave complete inhibition, are shown in Table 4. The results suggest that the inhibitory capacity of anti-Pf155 antibodies was unrelated to the geographic origin of the parasites giving rise to their formation. IgG fractions from the serum of the donors A or N were affinity purified on F32 (Table 4). The affinity-purified antibodies from both donors had the same high reinvasion inhibition titers when tested on either F32- or Thai1-infected erythrocytes. In the latter case IgG and parasites were derived from the same patient (donor N).

Activities of antibodies of restricted specificities. Although results of all the experiments described above suggest that Pf155 is antigenically invariant, they do not rigorously prove this, since the antibodies used were polyclonal and, with a few exceptions, were derived from donors who have had repeated *P. falciparum* infections. For this reason, similar experiments were performed with antibodies with restricted specificities. The following reagents were used: (i) a human monoclonal IgM antibody (33G2) that reacted with an epitope in the C-terminal repeat region of Pf155 (36); (ii) a mouse monoclonal antibody to the synthetic octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala conjugated with keyhole limpet hemocyanin (Ruangjirachuporn et al., submitted); (iii) an oligoclonal IgG preparation obtained from a rabbit immunized with a linear dimer of the synthetic peptide Glu-Glu-Asn-Val also conjugated with keyhole limpet hemocyanin (4). These amino acid sequences are major antigenic structures of Pf155 (1, 3, 4), in which they occur as tandemly arranged repeats in the C-terminal part of the molecule (14).

When tested by immunoblotting of SDS-PAGE-separated lysates of F32 merozoites or concentrated supernatants of F32 in vitro cultures, all these reagents give a strong staining of Pf155 and a weaker staining of a few faster-migrating bands believed to be breakdown products of Pf155 (3, 4, 36). When tested on merozoite lysates from additional strains (Thai1, IMTM22/7G8), the banding pattern was the same as that obtained with F32, indicating that Pf155 in all three lysates was similar with regard to both antigenicity and size (Fig. 3). The same results were obtained when lysates of F32 and the Honduran clone HB3 were probed with 33G2 (data not shown).

When tested by the EMIF assay with parasites from different parts of the world, all three antibody reagents give the typical Pf155-associated staining of the membrane of ring-infected erythrocytes (28). This is shown in Fig. 4 for a Liberian isolate, with antibodies from the Liberian donor A

TABLE 4. Titers of affinity-purified IgG samples from different donors determined by EMIF and reinvasion inhibition[a]

Expt	IgG samples		Endpoint titer (μg/ml) by EMIF[b]	50% reinvasion inhibition (μg/ml)[c]	
	Donor	Eluted from:		F32	Thai1
1	A	F32	0.18	0.6	ND
		K1	0.17	0.7	ND
		7G8	0.18	0.9	ND
	D	F32	0.12	0.4	ND
		K1	0.17	0.5	ND
		7G8	0.11	0.4	ND
2	A	F32	0.13	1.7	1.5 ± 0.07
	N	F32	0.16	1.9	1.7 ± 0.12

[a] IgG from Liberian donor (donor A), Colombian donor (donor D), or Swedish donor (donor N) affinity purified by elution from erythrocytes infected with F32 (Tanzania), K1 (Thailand), or IMTM22/7G8 (Brazil).

[b] Endpoint titers on F32- and Thai1-infected erythrocytes. (Thai1 parasites were isolated from patient N).

[c] See text; ND, not done.

included as a positive control. In essence all antibody reagents stained ring-infected erythrocytes in an equal manner when added at an optimal concentration. The results of similar experiments performed with 11 different laboratory strains of *P. falciparum* and 26 erythrocyte samples isolated from patients who acquired their infections in Africa, South America, or Asia are given in Table 5. With a few exceptions, all samples exhibited a similar bright membrane staining of all or most ring- and trophozoite-infected erythrocytes (Fig. 4). In only a few cases the staining was dim and less complete. This was mainly the case with the human monoclonal antibody 33G2, which may recognize an epitope that is partially susceptible to glutaraldehyde treatment.

The three antibody reagents have previously been shown to inhibit reinvasion of erythrocytes by F32 merozoites in vitro (4, 29, 36). In those experiments the inhibitory titer of the human monoclonal antibody 33G2 was 5 to 10 times lower than that of the polyclonal human preparations enriched in anti-Pf155 antibodies (36), while that of the antibodies against synthetic peptides was from 10 to 100 times lower but yet highly significant (3, 4). This also held true when 33G2 was tested for reinvasion inhibition of merozoites from two different *P. falciparum* strains (F32, Thai1; Fig. 5). Similarly, in a separate experiment, the mouse monoclonal antibody to the synthetic octapeptide had 50% reinvasion inhibition titers of 38 and 34 μg/ml when tested on F32 and IMTM22/7G8, respectively (data not shown).

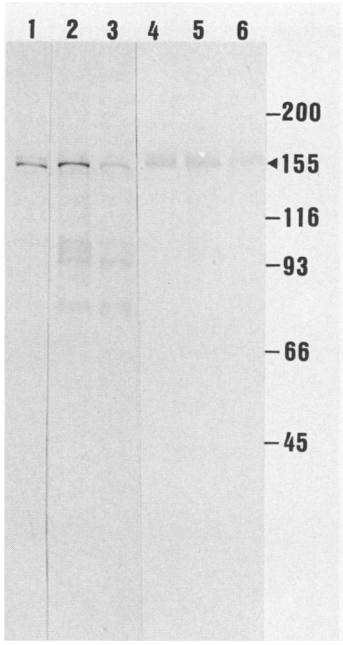

FIG. 3. Immunoblotting analysis of merozoite-enriched lysates F32 (Tanzania), IMTM22/7G8 (Brazil), or Thai1 (Thailand) probed with the mouse monoclonal antibody (ascites, diluted 1:100) to Glu-Glu-Asn-Val-Glu-His-Asp-Ala (lanes 1 to 3) or the human monoclonal antibody 33G2 (culture supernatant diluted 1:25; lanes 4 to 6). For details see Fig. 1 legend and the text.

DISCUSSION

In this investigation, no antigenic diversity was detected when fresh *P. falciparum* isolates as well as different laboratory strains, including a number of cloned lines, were tested for their reactivity with anti-Pf155 antibodies in human serum samples of malaria-exposed donors from different parts of the world. The major test to demonstrate this was a modified immunofluorescence test, in which Pf155 was detected in the membranes of recently infected erythrocytes. Although other plasmodial antigens than Pf155 may be expected to be present on the surface of such erythrocytes (19), Pf155 is the major antigen giving the typical erythrocyte surface staining seen in the EMIF assay (28, 39). Moreover, although antibodies to Pf155 are known to cross-react with a variety of plasmodial antigens encoded by different genes, these are not expressed on the surface of freshly infected and glutaraldehyde-fixed erythrocytes (13, 26; Mattei et al., in press).

Endpoint titrations of different immune serum samples on different isolates showed no variations between titers correlated to geographical origin. The titer variations seen in these experiments reflected differences in antibody concentrations and in antigen expression rather than antigenic diversity. Similar results were obtained by using antibody preparations strongly enriched in anti-Pf155 antibodies by adsorption to infected erythrocytes, followed by acid elution. Antibodies eluted from erythrocytes infected with different parasites had similar endpoint titers in the EMIF assay when tested reciprocally on different isolates. Moreover, the amounts of antibodies positive by the EMIF assay recovered in the different eluates were similar, and this was also the case when they were tested by immunoblotting SDS-PAGE-separated merozoite lysates. Results of these experiments also demonstrated that the anti-Pf155 antibodies are the predominating, although not the only, antibodies in the eluates. In a limited number of tests it was further shown that the Pf155 that was present in four different *P. falciparum* strains did not display any significant differences in size. Thus, Pf155 is distinct from the S antigens which are similar with respect to heat stability and solubility (28, 41) but exhibit considerable serological diversity as well as size variation (2, 22, 42), most likely reflecting the existence of allelic genes encoding antigens which differ in their immunodominant amino acid sequences (12, 15).

Antibodies to Pf155 have previously been shown to be very efficient inhibitors of reinvasion of erythrocytes by F32 merozoites in vitro (39). Fab fragments of these antibodies inhibited almost as efficiently as intact IgG, indicating that reinvasion inhibition is due to antibody blocking of some biologically important sites on the antigen rather than to merozoite agglutination (29). However, because the antigen is not readily accessible on the erythrocyte surface unless the cells are fixed and dried, inhibition must take place by interaction of the antibodies with Pf155 before it attaches to the erythrocyte membrane. Be this as it may, results of the present experiments with anti-Pf155 antibodies isolated from different parasite strains and tested in different combinations revealed no antigenic diversity, suggesting that some of the biologically important structures of Pf155 involved in invasion are antigenically invariant. This is in contrast to what has been seen in inhibition experiments involving other *P. falciparum* antigens (8, 9, 32, 37; B. Wåhlin et al., manuscript in preparation).

A large fraction of the human anti-Pf155 antibodies is directed to epitopes included in the regions of the molecule composed of short amino acid sequences which are tandemly repeated many times. Human antibodies that were affinity purified on synthetic oligopeptides from the C-terminal repeat region of this antigen reacted with Pf155 in immunoblotting and gave the typical membrane staining in

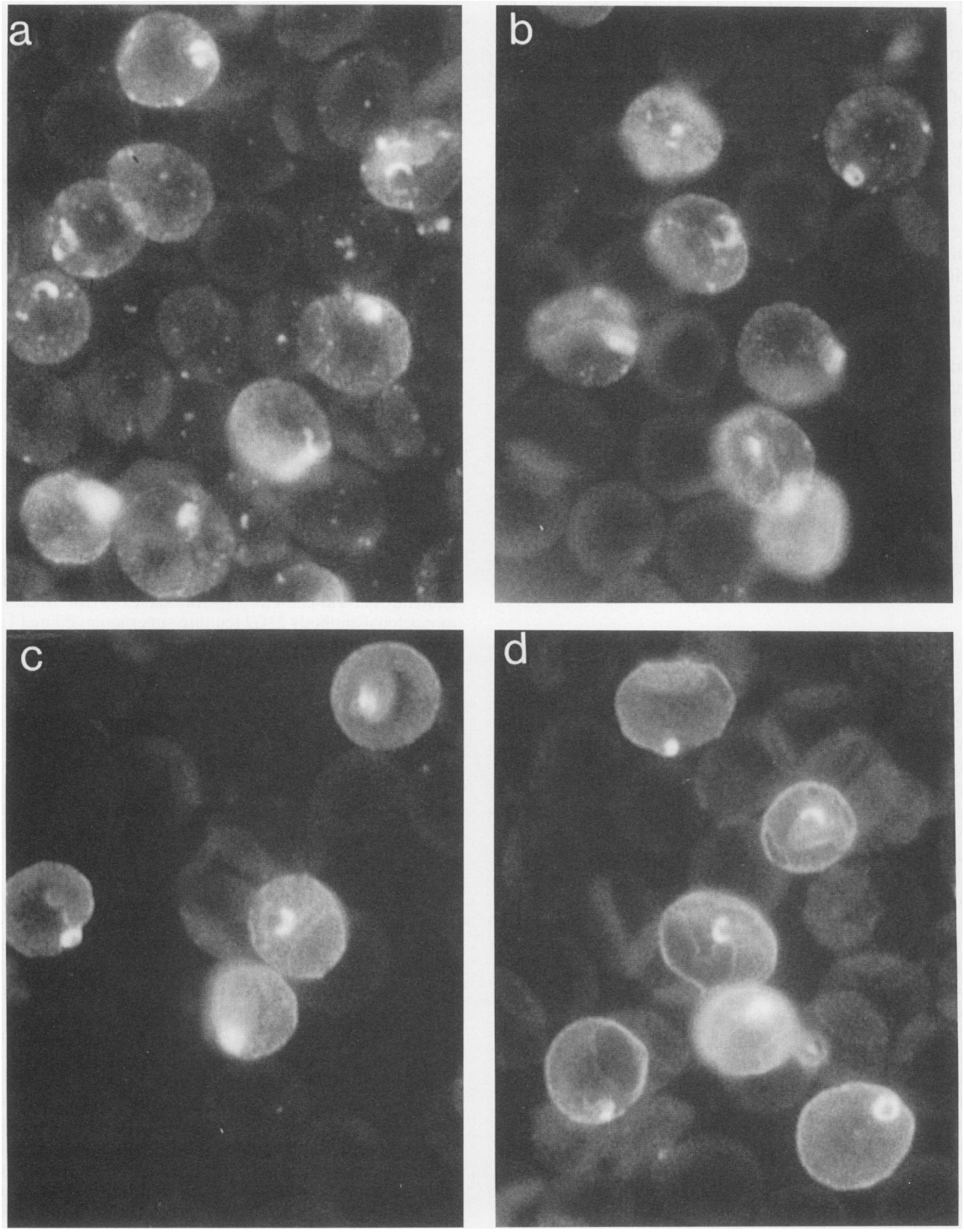

FIG. 4. EMIF assay of glutaraldehyde-fixed and air-dried monolayers of erythrocytes from a Liberian patient infected with *P. falciparum* and stained with IgG from Liberian donor A (a), human monoclonal antibody 33G2 (b), mouse monoclonal antibody to the synthetic octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala (c), or rabbit oligoclonal antibody to the synthetic octapeptide Glu-Glu-Asn-Val-Glu-Glu-Asn-Val (d). The parasite nuclei were counterstained with ethidium bromide. Scale, 1:2,000.

the EMIF assay. They also inhibited reinvasion by F32 merozoites at very high titers, indicating that the corresponding sequences are accessible for antibody binding by the antigen in its native state (3, 30). Similar results were obtained with a fraction of mouse or rabbit antibodies raised against synthetic oligopeptides of the same sequences (4; Ruangjirachuporn et al., submitted).

To further investigate antigenic diversity of Pf155 from different isolates, we used a mouse monoclonal antibody to the octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala and an

TABLE 5. EMIF assay results with different *P. falciparum* strains or fresh isolates[a]

Parasite origin (no.)	Antibodies[b]							
	A		33G2		MoMAb		RaAb	
	+	(+)	+	(+)	+	(+)	+	(+)
Strains or clones[c] (11)	11	0	6	5	11	0	11	0
Liberian patients[d] (11)	11	0	8	3	10	1	11	0
Colombian patients[d] (9)	9	0	4	5	9	0	9	0
Swedish patients[d,e] (6)	6	0	5	1	5	1	6	0

[a] Indirect immunofluorescence with antibody containing immunoglobulin fractions concentrated by ammonium sulfate precipitation and used at optimal concentrations. Controls of uninfected erythrocytes or erythrocytes infected with *P. ovale* were negative throughout.

[b] A is Liberian donor A; IgG was affinity purified on F32-infected erythrocytes; 33G2 is human monoclonal antibody IgM; MoMAb, is mouse monoclonal antibody (IgG1) against synthetic octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala; RaAb is rabbit antibodies against synthetic peptide Glu-Glu-Asn-Val-Glu-Glu-Asn-Val. Abbreviations: +, Bright staining of most or all ring-infected erythrocytes; (+); dim staining of a large fraction (>50%) of ring-infected erythrocytes.

[c] Laboratory strains grown in culture (9) or obtained from infected *Aotus* monkeys (2); see text.

[d] Parasites from blood of *P. falciparum* patients.

[e] Infected in West or Central Africa or Asia.

oligoclonal rabbit antibody to the linear tetrapeptide dimer $(Glu\text{-}Glu\text{-}Asn\text{-}Val)_2$ as well as a human monoclonal antibody reacting with a thus far undefined epitope in the C-terminal repeat region of Pf155 (36). Although this latter antibody reacted strongly with Pf155 in immunoblotting and in the EMIF assay, it has been shown to cross-react with other genetically distinct *P. falciparum* polypeptides (Mattei et al., in press). Nevertheless, when tested by the EMIF assay, immunoblotting, and reinvasion inhibition on different *P. falciparum* strains and isolates, no antigenic diversity was detected. This reflects the high degree of conservation in the C-terminal amino acid repeats of this polypeptide, as is also indicated by studies of its DNA. The available evidence suggests that this gene occurs in different isolates in at least two allelic forms. The nucleotide sequences encoding the amino acid repeats appear to be conserved in both alleles, although the number of the C-terminal repeats may vary (18). This conservation of the immunodominant repeats in Pf155 is in contrast to the polymorphism found in immunodominant amino acid repeats in several other *P. falciparum* antigens (24, 27). However, results of neither the DNA studies nor the results reported here exclude the existence of a minor antigenic diversity in Pf155 structures outside the immunodominant repeats. Further investigations will be required to establish this.

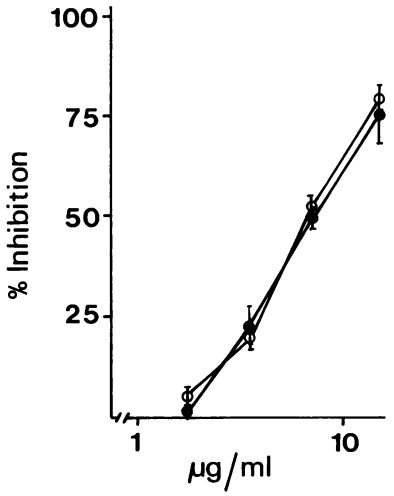

FIG. 5. Reinvasion inhibition in vitro by monoclonal human antibodies 33G2 of *P. falciparum* F32 (Tanzania) (●) or Thai1 (Thailand) (○). Abscissa, Micrograms of antibody per milliliter; ordinate, percent inhibition. At an assay time of 21 h, parasitemias in controls were 0.63 ± 0.04 or 0.92 ± 0.07% for F32 and Thai1, respectively; at time zero the respective values were 0.22 ± 0.02 or 0.36 ± 0.06%.

ACKNOWLEDGMENTS

This study was supported by grants from the Rockefeller Foundation Great Neglected Diseases Network, the Swedish Medical Research Council, the Swedish Agency for Research Cooperation with Developing Countries, and the United Nations Development Programme/World Bank/World Health Organization, Special Programme for Research and Training in Tropical Diseases.

We thank A. Björkman, Department of Infectious Diseases, Karolinska Institute, Stockholm, and E. M. Patarroyo, Department of Immunobiology, National University of Colombia, Bogota, Colombia, for the generous supply of blood samples and parasites. The skillful technical assistance of M. Hagstedt and I. Andersson is gratefully acknowledged.

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