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Immunology

Human antibodies to a M_r 155,000 *Plasmodium falciparum* antigen efficiently inhibit merozoite invasion

(malaria/growth inhibition/immunofluorescence/erythrocyte surface/immunoblotting)

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ABSTRACT **IgG from a donor clinically immune to *Plasmodium falciparum* malaria strongly inhibited reinvasion *in vitro* of human erythrocytes by the parasite. When added to monolayers of glutaraldehyde-fixed and air-dried erythrocytes infected with the parasite, this IgG also displayed a characteristic immunofluorescence restricted to the surface of infected erythrocytes. Elution of the IgG adsorbed to such monolayers gave an antibody fraction that was 40 times more efficient in the reinvasion inhibition assay (50% inhibition titer, <1 μg/ml) than the original IgG preparation. The major antibody in this eluate was directed against a parasite-derived antigen of M_r 155,000 (Pf 155) deposited by the parasite in the erythrocyte membrane in the course of invasion. A detailed study of IgG fractions from 11 donors with acute *P. falciparum* malaria or clinical immunity revealed the existence of an excellent correlation between their capacities to stain the surface of infected erythrocytes, their titers in reinvasion inhibition, and the presence of antibodies to Pf 155 as detected by immunoblotting. No such correlations were seen when the IgG fractions were analyzed for immunofluorescence of intracellular parasites or for the presence of antibodies to other parasite antigens as detected by immunoprecipitation of [^{35}S]methionine-labeled and NaDodSO$_4$/PAGE-separated parasite extracts. The results suggest that Pf 155 has an important role in the process of erythrocyte infection and that host antibodies to this antigen may efficiently interfere with this process.**

In view of the dramatic increase during the past 15 years of *Plasmodium falciparum* malaria, the most lethal of the human malarias, efforts are now being made to define parasite antigens that may be suitable candidates for a malaria vaccine. By means of a modified immunofluorescence (IF) assay we have recently identified some novel antigens in the membrane of human erythrocytes (RBCs) infected with *P. falciparum* (1, 2). These antigens are released from bursting schizonts or merozoites and are deposited in the RBC membrane in the course of invasion. Antibodies recognizing these antigens are of high prevalence in sera from residents of a holoendemic area of Africa (Liberia) but are also present in many sera of acutely infected patients from different parts of the world. A polypeptide of M_r 155,000 (Pf 155) appears to be primarily responsible for this RBC membrane IF. A similar antigen of the same apparent molecular weight has also been found recently by two other groups (3, 4). To further investigate the function of these antigens and the possible significance of the immune response against them, we have now tested the antibodies reacting with Pf 155 and related minor components for their capacity to inhibit RBC invasion by merozoites *in vitro*.

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MATERIAL AND METHODS

Parasites. Parasites were from a Tanzanian strain of *P. falciparum* (F 32) isolated in 1978 (5) and cultured *in vitro* in blood group O$^+$ RBCs (6).

Immune Sera. Five sera (Kinon, IS-6 to -8, X-12) were from Liberians, >15 years old (except X-12, from a 12-year-old boy), living in a *P. falciparum* holoendemic area in which in adult life clinical illness is rare and parasitemia is kept at a low-grade, mostly subpatent, level despite high sporozoite inoculation rates (7). These donors, designated as clinically immune, had not taken any antimalarial drug for the last 6 weeks (X-12) or longer. The 6 remaining donors had acute *P. falciparum* infections. Four donors (ASF, MV, JOR, FG) were South Americans from an endemic area of Colombia. Two Swedish patients (YC, HP) were suffering from a first infection acquired in Kenya. IgG was prepared by ammonium sulfate precipitation and ion-exchange chromatography on DEAE-Sephadex (Pharmacia). Immunoglobulin concentrations were determined by ELISA.

***In Vitro* Growth Inhibition Assay.** Infected RBCs from *P. falciparum* cultures (5–10% parasitemia, ≈70% schizonts) were diluted with normal O$^+$ RBCs to a parasitemia of 0.5%. They were adjusted to 2% hematocrit with Hepes-buffered (20 mM) RPMI-1640 medium (GIBCO) containing 15% normal human serum, 2 mM glutamine, 25 μg of gentamycin per ml, and 0.2% $NaHCO_3$ [complete tissue culture medium (TC medium)]. In some experiments, synchronized parasite cultures were used. In this case, the original cultures (5–10% parasitemia) were adjusted to 10% hematocrit and layered on top of 2.5 ml of 60% Percoll (Pharmacia) diluted in complete TC medium in conical centrifuge tubes. After centrifugation for 15 min at 1500 × *g* at room temperature, the bands formed by 50–100% parasitized RBCs (late trophozoites and schizonts) were pooled and washed twice in complete TC medium by centrifugation for 10 min at 250 × *g*. They were diluted with non-infected RBCs to 1% parasitemia and 2% hematocrit. Four-milliliter aliquots were cultured for 48 hr in tissue culture flasks (no. 3013, Falcon). During this time, parasitemia increased approximately three times and the cultures were highly synchronized (>95% schizonts).

For the assay, 100-μl aliquots of nonsynchronized or synchronized cultures were seeded in 96-well flat-bottomed microculture plates (Sterilin, Teddington, Middlesex, England) with 100 μl of complete TC medium and various dilutions of IgG. After 20 hr at 37°C, the RBCs from each well were separately transferred to tubes, washed twice by centrifugation with Tris-buffered Hank's solution (TH solution) at pH 7.2, and diluted to a 1% suspension in TH solution. Monolayers of infected RBCs were formed in the wells of 8-well multitest slides coated with 0.06 M bicarbonate buffer at pH 9.6 (2).

Abbreviations: Pf 155, *P. falciparum* polypeptide of M_r 155,000; RBC, erythrocyte; IF, immunofluorescence.

Each test sample was set up in duplicate (i.e., 8 wells per sample). The monolayers were fixed by two treatments for 10 sec with 1% glutaraldehyde. The slides were washed with distilled water and air dried. They were stored at −20°C until analyzed in the microscope. To distinguish infected from noninfected RBCs, the parasites were stained by adding one drop of acridin orange per well (10 μg/ml). After a few seconds the slides were washed with distilled water, mounted, and scored under incident ultraviolet light in a fluorescence microscope. Twenty five microscope fields (≈200 RBCs per field) were screened for each well. The percentages of parasitemia given are the mean values from 40,000 RBCs (8 wells) screened. In some experiments, the percentage of different parasite stages was assessed by differential counting.

Elution of Antibodies from RBC Monolayers. Elution with 0.2 M glycine buffer at pH 2.8 was performed as described. The eluates were neutralized with 0.1 ml of 2 M Tris base and dialyzed overnight against TC medium (2).

Indirect IF. Indirect IF of unfixed or glutaraldehyde-fixed and air-dried monolayers was performed by treating the slides sequentially with various dilutions of immune IgG, biotinylated goat antibodies to human IgG, and avidin conjugated with fluorescein isothiocyanate (2).

Biosynthetic Labeling, Solubilization, and Immunoprecipitation of Parasite Antigens. Nonsynchronized *P. falciparum* cultures were labeled for 48 hr with [^{35}S]methionine (25 μCi/ml of methionine-free medium, 1.4 Ci/mmol; 1 Ci = 37 GBq; Radiochemical Centre) as described elsewhere (8). Extraction of schizont-enriched preparations and immunoprecipitation with a polyvalent rabbit anti-human Ig serum were essentially as described (9).

NaDodSO$_4$/PAGE. Immunoprecipitated antigen–antibody complexes were analyzed by NaDodSO$_4$/PAGE under reducing conditions (10, 11). The slab gels were stained with Coomassie brilliant blue R (Sigma) in fixative and treated with EN^{3}HANCE (New England Nuclear) before drying. For fluorography at −70°C, Kodak X-Omat RP film (Kodak) was used.

Immunoblotting. After NaDodSO$_4$/PAGE under reducing conditions, polypeptides from merozoite-enriched preparations were electrophoretically transferred to nitrocellulose strips and treated with immune serum diluted 1:100–1:1000 as described (12, 13).

RESULTS

Fig. 1*A* shows the distinct staining by indirect IF of the surface of glutaraldehyde-fixed and air-dried RBCs infected with early developmental stages of *P. falciparum* (RBC-surface IF). The monolayers were stained with diluted IgG from the serum of a hyperimmune Liberian donor (Kinon). Under these conditions, the intracellular parasites were not stained. However, this was the case when the same IgG was added to air-dried but unfixed monolayers (Fig. 1*B*). To separate antibodies directed to the parasite antigens in the RBC membrane, IgG was eluted from glutaraldehyde-fixed monolayers (2). The yield of the Kinon IgG recovered from infected RBCs was 4% of the input. The yield of the Kinon IgG eluted from non-infected RBCs was 0.1–0.3%. The eluate from infected RBCs gave an RBC-surface IF that was indistinguishable from that of the original IgG fraction.

IgG fractions from the same donor were tested in the growth inhibition assay (Fig. 2). The total IgG strongly inhibited parasite growth, with a 50% inhibition titer of 30 μg/ml. The two independently prepared eluates were more efficient, with 50% inhibition titers of 0.55 and 0.85 μg/ml, respectively. The inhibition titers of these preparations compared favorably with their titers in the RBC-surface IF. These were 2 μg/ml for whole IgG and 0.1 μg/ml for the eluates. Eluates of normal IgG from infected RBCs and of immune IgG from non-infected RBCs were inactive in both RBC-surface IF and growth inhibition.

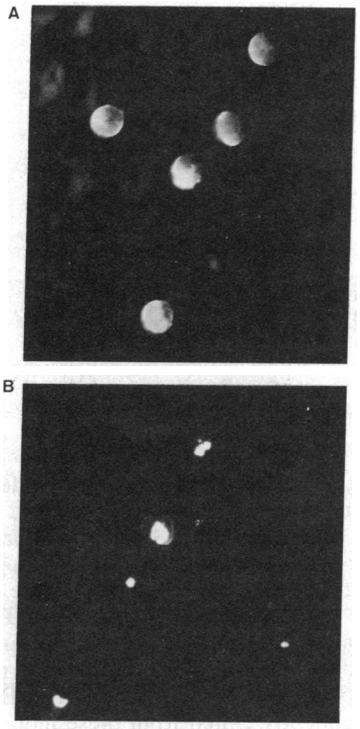

FIG. 1. Indirect IF of monolayers of *P. falciparum*-infected RBCs (5% parasitemia) exposed to IgG (2 μg/ml) from the immune donor Kinon. (*A*) IF of the surface of infected RBCs after glutaraldehyde fixation and air drying. (*B*) IF of intracellular parasites in unfixed and air-dried RBCs.

The *P. falciparum* cultures used in the growth inhibition assay of Fig. 2 were not synchronized. Very similar results were obtained with synchronized cultures (Table 1). Differential counts of the parasites at the conclusion of the experiments revealed a significant drop in the relative numbers of rings and early trophozoites and a marked increase in the relative numbers of merozoites in the test samples as compared to the controls. In the test samples a conspicuous number of merozoites was attached to the surface of noninfected RBCs. Differential counts of nonsynchronized cultures gave similar figures. The results indicate that inhibition was primarily due to blockade of RBC reinvasion.

Fig. 3 shows an inhibition experiment with total IgG from 11 different sera. Five of these IgGs were completely or almost completely inactive at concentrations up to 8 mg/ml.

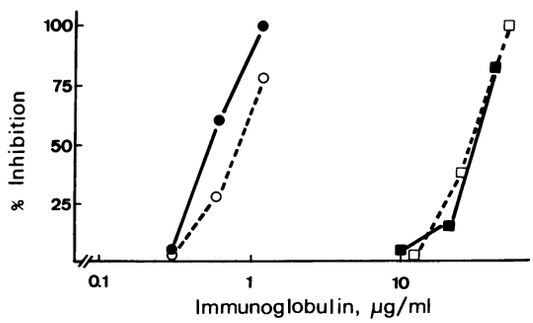

FIG. 2. Growth inhibition *in vitro* of *P. falciparum* in nonsynchronized cultures by total IgG fraction (□, ■) or by antibodies eluted from monolayers of infected RBCs (○, ●). IgG donor: Kinon. Percent inhibition (ordinate) after 20 hr was calculated as shown in Table 1. The results of two independent experiments (solid and broken lines, respectively) are shown.

Table 1. Growth inhibition of synchronized *P. falciparum* cultures

Culture*	IgG, μg/ml†	% inhibition‡	% parasitemia	Stage, %§ R	Tr	S	Mz
Control	—	—	2.80	74	17	9	0
Whole IgG	42	84	1.07	55	12	10	23
Eluted IgG	1.2	100	0.26	52	8	2	34

*>95% early or late schizonts.
†IgG fractions from immune donor (Kinon).
‡[(% parasitemia in control − % parasitemia in test)/(% parasitemia in control − % parasitemia at time 0)] × 100; time of assay: 20 hr; % parasitemia at time 0: 0.74.
§At end of assay; R = rings; Tr = trophozoites; S = schizonts; Mz = merozoites.

The remaining 6 were inhibitory to different degrees, which could not be increased by increasing the dose of the inhibitor. With one exception (JOR), their inhibitory titers, here defined as the lowest amount of IgG giving optimal inhibition, were correlated to the maximal degree of inhibition achieved (Fig. 3). To elucidate the reasons for the lower efficiency of some of the IgGs in both RBC-surface IF and growth inhibition, three preparations of intermediate reactivity were separately adsorbed to infected RBCs. Antibodies were subsequently eluted from these monolayers as described (Table 2). The input of IgG was in accordance with their titers in RBC-surface IF. For two of the preparations (IS-7, MV) the yield of the eluted IgG was significantly lower than that obtained with the Kinon IgG. However, on a weight basis, the titers of these eluates were similar to those of the Kinon eluate in both RBC-surface IF and growth inhibition. Moreover, both eluates completely inhibited parasite growth. This was also the case for the third eluate (ASF), although its specific activity was significantly lower in both assays. In contrast, the yield of ASF IgG eluted from the monolayers was not significantly different from that of the Kinon IgG.

Different activities of the total IgGs from the 11 donors discussed above are summarized in Table 3. These donors had been selected to cover the whole range of reactivities in the RBC-surface IF. The five Liberian preparations were from clinically immune donors but with periodic asymptomatic parasitemias. All other preparations were from patients with acute malaria. There was an excellent correlation

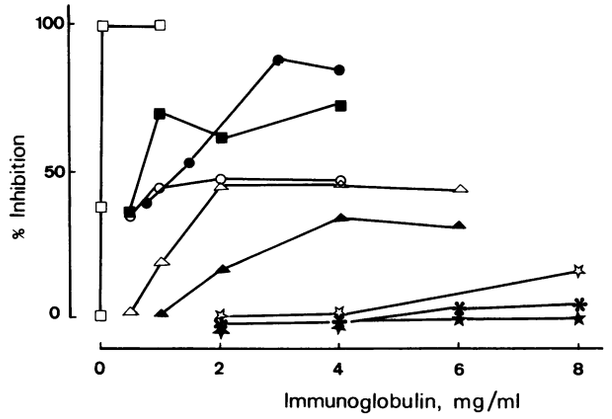

FIG. 3. Parasite growth inhibition *in vitro* in nonsynchronized cultures by total IgG fractions from 11 donors with acute *P. falciparum* malaria or clinically immune donors. ★, Donors IS-8, FG, HP; *, X-12; ☆, IS-6; ▲, YC; △, IS-7; ○, ASF; ●, JOR; ■, MV; □, Kinon; for further information on these donors, see Table 3. Percent inhibition after 20 hr was calculated as shown in Table 1.

Table 2. RBC-surface IF and parasite growth inhibition by IgG eluted from fixed and air-dried monolayers of infected RBCs

Source of IgG*	Yield, %†	IF, μg/ml‡	50% growth inhibition, μg/ml§
Kinon	4	0.1	0.4
IS-7	0.2	1.0	1.1
MV	<0.2	0.4	0.4
ASF	1.7	4.2	5.9

*For donors, see Table 3.
†Yield of IgG eluted as % of input, which was 0.05, 2.5, 1.25, 1.25, and 4.0 mg of IgG per ml for Kinon, IS-7, MV, ASF, and IgG control, respectively.
‡Endpoint titers.
§For calculation of % inhibition, see Table 1; time of assay: 20 hr; % parasitemia in control: 1.14; % parasitemia at time 0: 0.54. Controls with pooled IgG from normal donors or buffer (pH 2.8) without IgG were negative.

between RBC-surface IF titers and the endpoint titers in the inhibition assay. In contrast, the latter titers were only poorly correlated to the endpoint titers obtained in IF measuring reactivity with intracellular parasites.

To establish if the sera differed qualitatively in their capacity to precipitate different parasite antigens, 8 of them were also analyzed by immunoprecipitation and NaDodSO$_4$/PAGE of [^{35}S]methionine-labeled extracts of schizont-enriched parasite preparations (8). Fig. 4 shows the representative results obtained with an inhibitory and a non-inhibitory serum, respectively. In general, there was no difference in precipitation patterns between preparations that inhibited RBC reinvasion and those that did not. Thus, all preparations gave major precipitates of parasite polypeptides of M_rs 195,000, 150,000, 140,000, 130,000, 118,000, and 80,000 as well as some minor precipitates.

Pf 155 is poor in methionine and therefore difficult to detect in the immunoprecipitation test performed as described.

Table 3. Reactivities of serum or isolated IgG fractions from patients with acute *P. falciparum* infections or from immune donors

Donor*	IF titer† RBC surface Serum	μg of IgG per ml	Parasites, μg of IgG per ml	Anti-Pf 155‡	Growth inhibition§ μg of IgG per ml	%
Kinon^i	15,000	2	0.2	++	50	100
ASF^a (<1)	250	50	0.08	++	1000	44
MV^a (1–2)	625	50	0.08	++	1000	20
IS-7^i (0)	625	100	0.4	+	2000	45
JOR^a (<1)	125	150	0.08	+	3000	88
YC^a	125	200	2	±	4000	30
IS-6^i (0.001)	125	500	2	±	>8000	16
X-12^i (0.07)	5	3000	0.4	ND	>8000	4
IS-8^i (0.01)	25	Neg	2	±	>8000	0
FG^a (<1)	5	Neg	0.4	−	>8000	0
HP^a	<5	Neg	0.4	−	>8000	0

ND = not done.
*i = Immune; a = acute infection; numbers in parentheses = % parasitemia.
†Reciprocal of serum dilution or μg of isolated IgG per ml, endpoint titers; Neg = no IF at highest concentration tested (6.0 mg of IgG per ml).
‡Antibodies to Pf 155 probed by immunoblotting with serum at three dilutions (1:100, 1:300, 1:1000); ++ = strong staining; + = weak staining; ± = weak staining only seen at lowest serum dilutions; − = no staining.
§Micrograms of IgG per ml giving optimal inhibition (Fig. 3); % inhibition: see Table 1; time of assay: 20 hr; % parasitemia in control: 1.4; % parasitemia at time 0: 0.4.

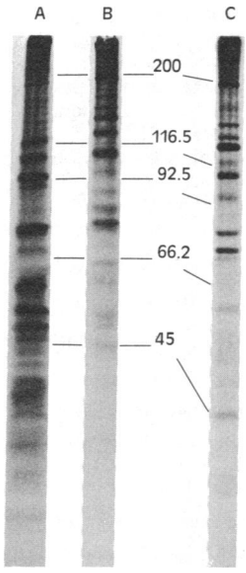

FIG. 4. Fluorographs of [^{35}S]methionine-labeled extracts of *P. falciparum* schizonts separated by NaDodSO$_4$/PAGE. Lane A, total extract; lanes B and C, immunoprecipitated parasite polypeptides obtained with growth inhibitory serum, donor MV (lane B), and with non-inhibitory serum, donor HP (lane C) (see Table 3). The numbers are relative molecular weights, shown as $M_r \times 10^{-3}$. Molecular weight markers were from Bio-Rad.

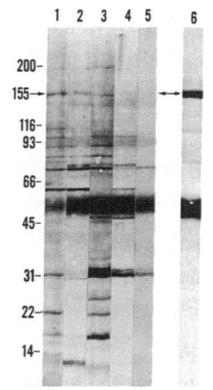

FIG. 5. Immunoblots of extracts of a merozoite-enriched fraction of *P. falciparum* after NaDodSO$_4$/PAGE separation and electrophoretic transfer to nitrocellulose membranes. The antigens were probed with patients' or immune sera diluted 1:300. Lane 1, donor Kinon; lane 2, ASF; lane 3, IS-6; lane 4, IS-8; lane 5, FG; for further information on these donors, see Table 3. Lane 6 was probed with antibodies from donor Kinon, eluted from monolayers of *P. falciparum*-infected RBCs (2). The numbers are relative molecular weights, shown as $M_r \times 10^{-3}$. Arrows indicate the position of Pf 155. The heavily stained band at M_r 50,000 represents human immunoglobulin heavy chains contaminating the antigen preparation (2).

However, this antigen is easily detected after immunoblotting of NaDodSO$_4$/PAGE-separated parasite extracts (2). It is the major antigen recognized by antibodies responsible for the RBC-surface IF as judged from immunoblots probed with antibodies eluted from monolayers of infected RBCs (Fig. 5, lane 6, and ref. 2). When 10 of the 11 sera were used as probes in this assay at three different concentrations, a very good correlation was seen between their content of anti-Pf 155 antibodies and their titers in the RBC-surface IF assay or in the inhibition assay (Table 2 and Fig. 5, lanes 1–5). No such correlations were seen for any of the other parasite-derived bands appearing in the immunoblotting tests.

DISCUSSION

Using an *in vitro* growth inhibition assay we here show that human antibodies against *P. falciparum* antigens that are transferred from the parasites to the RBC membrane at invasion are strongly inhibitory. Inhibition was mainly, if not entirely, due to prevention of RBC reinvasion. With the total IgG fraction from a hyperimmune Liberian donor (Kinon) complete inhibition was achieved with <50 μg/ml. Although this preparation was the most inhibitory encountered in this investigation, it was not exceptional and similar results have been obtained with several other preparations, including some from patients with acute *P. falciparum* infections (data not shown). The Kinon IgG eluted from infected RBCs was 40 times more efficient. This efficiency is higher than what has been reported previously for either human or monkey antibodies or for mouse monoclonal antibodies against defined *P. falciparum* antigens (14–19).

The total Kinon IgG reacts with ≈25 parasite-derived polypeptides when tested by immunoblotting of parasite extracts separated by NaDodSO$_4$/PAGE. In contrast, in the same test the eluted Kinon IgG reacts only with a few parasite components. The antibodies to Pf 155 were most abundant and appear primarily to be responsible for both RBC-surface IF and reinvasion inhibition. However, the eluted IgG also reacts weakly with 2 or 3 additional parasitic polypeptides (2) and the participation of the corresponding antibodies, present at low concentrations, is not excluded.

The importance of these antibodies for blocking RBC infection was further supported by a detailed investigation of 11 different sera. These had been selected for differences in their capacities to react in the RBC-surface IF. For all 11 sera or their IgG fractions, there was an excellent correlation between their titers in RBC-surface IF and in growth inhibition. Moreover, the IgGs that were strong reactors in RBC-surface IF or in growth inhibition had easily detectable antibodies to Pf 155 as revealed by immunoblotting, whereas the reverse was true for those that were weak reactors or were negative. Negative or low reactivity in these assays was not due to a more general lack of anti-*P. falciparum* antibodies, as apparent from (*i*) their titers in indirect IF for intracellular parasites (Table 3) and (*ii*) immunoprecipitation after NaDodSO$_4$/PAGE of [^{35}S]methionine-labeled parasite extracts.

Other authors have reported previously that serum antibodies or monoclonal antibodies against a variety of *P. falciparum* antigens may inhibit RBC reinvasion or parasite growth *in vitro* (14–19). In view of previous reports, we would have expected to find some inhibition with IgG preparations that lacked demonstrable antibodies to Pf 155 as some of these were from clinically immune donors and were rich in antibodies against other *P. falciparum* antigens. However, this was not the case and further studies of a larger serum material and different *P. falciparum* strains are needed to clarify the relative role of antibodies to different parasite antigens in the invasion process.

Seven of the 11 IgGs were positive in the inhibition assay but only 1 of them gave complete inhibition. After adsorption of low-titered and partially inhibitory preparations to monolayers of infected RBCs and subsequent elution, the eluted antibodies from 2 of these donors were as active in both the inhibition assay and the RBC-surface IF as the eluted Kinon IgG. The results suggested that these IgGs contained less antibody that binds to parasite antigens in the RBC membrane. However, the antibodies that were present appeared to be similar with regard to affinity or specificity (or both) for the antigens deposited in the RBC surface. For a third donor, more IgG was eluted from the RBCs but the eluted antibodies were less inhibitory and less active in RBC-surface IF, probably because of the presence of IgG of unrelated specificities (anti-Ig?). In all instances the eluted IgGs contained antibodies to Pf 155 (data not shown). Why only partial reinvasion inhibition was obtained with most of the IgGs before adsorption and elution is not clear. It may reflect a nonspecific stimulation of parasite growth by IgG at high concentrations. Alternatively, these IgG preparations may contain additional (anti-idiotypic?) antibodies interfer-

ing with reinvasion inhibition when present at concentrations above certain critical levels.

In some *P. falciparum* systems, growth inhibition *in vitro* has been reported to be strain specific (20–22). Thus far, no strain specificity has been seen in the RBC-surface IF, using both sera and fresh parasite isolates from different parts of the world (unpublished data). No evidence for antigenic variability has come forth in the present investigation. However, the parasites were all from the same African strain, whereas the sera were of different geographic origin. Studies of *P. falciparum* clones of defined serotype are required to establish the possible existence of variant structures in the present test system.

Antibodies have been reported to inhibit RBC reinvasion by agglutinating merozoites (23, 24). In the present investigation, agglutination of free merozoites released from bursting schizonts was not observed. Alternatively, the inhibitory antibodies may block receptor structures required for efficient parasite–RBC interaction (25–27). The invasion of RBCs is a multi-step process (28, 29) and several parasite polypeptides believed to facilitate merozoite attachment to RBCs have been described (30). With regard to their apparent molecular weights, these carbohydrate binding polypeptides are distinct from those recognized by the antibodies eluted from the surface of infected RBCs. However, Perkins (3) has recently described a *P. falciparum* protein of M_r 155,000 binding to some peptide structures of glycophorin A. This protein is soluble, heat stable, and poor in methionine. It appears to be located on the merozoite surface. This protein seems to be the same as the Pf 155 investigated by us and may thus have adhesive functions that are susceptible to antibody-mediated inhibition.

Although it has frequently been stated that reinvasion inhibition *in vitro* reflects antibody-mediated protection against infection *in vivo*, the evidence for this is circumstantial and has been questioned (31). From our results, no conclusions can be drawn as to the protective significance of the humoral immune response to the parasite antigens deposited in the RBC surface during invasion. Its importance for protection is supported by the distribution of these antibodies in sera from different donor categories (2). In an investigation of 40 children living in a holoendemic area of Liberia, we have found a striking correlation between the age-dependent appearance of these antibodies and the development of clinical immunity (unpublished data). However, it should be noted that the present study included 3 Liberian sera, which, although derived from clinically immune donors, were negative in the inhibition assay and contained no or very little anti-Pf 155 antibodies. This does not contradict our previous results as these sera were specially selected for negativity in RBC-surface IF and negative sera are only rarely found among residents of this Liberian area. Nevertheless, these findings underline the fact—trivial as it may be—that immunity to malaria is building up and maintained by a variety of mechanisms. They certainly do not rule out that antibodies against these parasite antigens may be of great significance for the clearance of acute infections and that inhibition of RBC reinvasion may be an important mechanism in this context. Be this as it may, available evidence is sufficient to consider Pf 155 as a good candidate for a vaccine against the asexual blood stages of *P. falciparum*.

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