

## Rabbit and human antibodies to a repeated amino acid sequence of a *Plasmodium falciparum* antigen, Pf 155, react with the native protein and inhibit merozoite invasion

(malaria/synthetic peptide/immunoblotting/immunofluorescence/growth inhibition)

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**ABSTRACT** The *Plasmodium falciparum*-derived antigen of  $M_r$  155,000 designated Pf 155, deposited in the membrane of infected erythrocytes, contains at least two blocks of tandemly repeated amino acid sequences. The peptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala, which corresponds to a subunit of a C-terminally located repeat, was synthesized. Rabbits immunized with the octapeptide conjugated with either keyhole limpet hemocyanine or tetanus toxoid formed antibodies against the octapeptide. These antibodies reacted with Pf 155 as detected by immunoblotting or a modified immunofluorescence assay. Sera from humans exposed to *P. falciparum* also contained antibodies binding to the octapeptide in a dot-blot immunoassay. Their anti-octapeptide titers were correlated with their immunofluorescence titers in the assay detecting Pf 155 and other parasite antigens in the membrane of infected erythrocytes. Human octapeptide-reactive antibodies were isolated on an affinity column with the octapeptide conjugated to bovine serum albumin as ligand. These human antibodies reacted with Pf 155 in immunoblotting and strongly stained the surface of infected erythrocytes in the modified immunofluorescence assay. Approximately 20% of this immunofluorescence activity in a high-titered human serum could be recovered from the octapeptide column, indicating that a significant fraction of these anti-parasite antibodies react with epitopes associated with the octapeptide. Furthermore, the human octapeptide-reactive antibodies very efficiently inhibited merozoite reinvasion into erythrocytes *in vitro*. Similarly purified rabbit antibodies also significantly inhibited reinvasion. Our results suggest that the C-terminal segment of repeated peptides in Pf 155 is a major antigenic region of the molecule and may contain target sites for protective immunity in *P. falciparum* malaria.

In the light of the worldwide increase of *Plasmodium falciparum* malaria, the development of a vaccine against the disease is of high priority. Antigens involved in protective immunity against *P. falciparum* sporozoites (1, 2) or in transmission-blocking immunity against the gametes of this parasite (3) have been identified. Certain antigens from the antigenically complex asexual blood stages are also being assessed for their possible involvement in protective immunity against these stages (4-8). We recently identified one *P. falciparum* antigen of  $M_r$  155,000, designated Pf 155, deposited in the erythrocyte (RBC) membrane during merozoite invasion (9, 10). Human antibodies to this antigen were extremely efficient in inhibiting *P. falciparum* reinvasion in *in vitro* cultures (10, 11). Furthermore, in children living in a holoendemic area of Africa, a correlation was found between high levels of antibodies to Pf 155 and acquired *P. falciparum*

immunity (9, 12). As the antigen was shown to bind to glycoporphin, was heat stable and poor in methionine (9, 13), it is probably identical with the glycoporphin-binding *P. falciparum* protein of  $M_r$  155,000 described by Perkins (14). Pf 155 appears also to be the same as RESA (ring-infected erythrocyte surface antigen), a *P. falciparum* antigen recently described by Coppel *et al.* (15). In its C-terminal part, this antigen was shown to comprise a region of repeating subunits of 8, 4, and 3 amino acids (15). Another block of repeated 10- and 11-amino acid sequences is in the N-terminal part and displays some immunological cross-reactivity with the C-terminal repeat region (16).

In this study we used a synthetic peptide, corresponding to the 8-amino acid repeat of RESA (15), for immunization of rabbits and for isolation by affinity chromatography of human immunoglobulins binding to the peptide. Immunoblotting and immunofluorescence (IF) were used to study the reactivity of the anti-peptide antibodies with *P. falciparum* antigens. The capacity of both rabbit and human antibodies to inhibit merozoite reinvasion was studied in *P. falciparum in vitro* cultures.

### MATERIALS AND METHODS

Parasite material was from *in vitro* cultures of the Tanzanian *P. falciparum* strain F32. For antigen analysis, merozoite-enriched fractions or, alternatively, supernatants from spent culture medium were prepared and processed as described (10).

Human sera were from Liberian donors (Kinon, IS-7, IS-8, and L-30) living in a *P. falciparum* holoendemic area (17) or from South American donors (AM, ASF, MV, JOR, and FG) living in an endemic area of Colombia (for details, see refs. 10 and 11).

**Synthetic Peptide.** The octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala was synthesized by the standard method of Merrifield (18) by Pharmacia (Uppsala, Sweden), and was kindly donated by L.-E. Larsson. The peptide was 62% pure, containing two major peptide contaminants as analyzed by HPLC. Amino acid analysis was performed on a LKB Alpha Plus model 4151 analyzer at the Department of Biochemistry, University of Uppsala, Sweden. The octapeptide was conjugated by means of glutaraldehyde (GA) (19) with either keyhole limpet hemocyanine (KLH; A grade, Calbiochem), bovine serum albumin (BSA; fraction V, Boehringer Mannheim) or tetanus toxoid (TT; State Bacteriological Laboratory, Stockholm, Sweden) at approximate molar ratios 200:1, 20:1, and 20:1, respectively.

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Abbreviations: BSA, bovine serum albumin; GA, glutaraldehyde; RBC, erythrocyte; IF, immunofluorescence; CS, circum sporozoite; KLH, keyhole limpet hemocyanine; TT, tetanus toxoid.

**HPLC.** HPLC was performed with a Varian 5000 system (Varian) and a 150 × 4 mm reversed-phase column (Bio-Sil ODS-55, Bio-Rad). The eluent was a continuous gradient of acetonitrile (8–30%) in 0.1% trifluoroacetic acid, and the absorbance of the eluted material was measured at 215 nm.

**Rabbit Antisera.** New Zealand White rabbits were immunized at biweekly intervals with an amount of conjugate (KLM or TT) corresponding to 50 μg of octapeptide. For injection, the conjugates were emulsified in half their volume of Freund's complete adjuvant. The rabbits were bled 1 week after the last immunization, beginning after two injections. IgG was prepared from the sera by ammonium sulfate precipitation and ion-exchange chromatography on DEAE-Sephadex (Pharmacia).

**Affinity Chromatography.** Octapeptide-BSA was coupled to CNBr-activated Sepharose (Pharmacia) (3 mg of octapeptide per 5 mg of BSA per ml of packed beads) according to the instructions of the manufacturer. For affinity chromatography, 5 ml of a 1:10 dilution of test serum in phosphate-buffered saline (0.15 M NaCl in 0.015 M phosphate buffer, pH 7.4; PBS) was mixed with 2 ml of packed beads and incubated on a roller drum for 1 hr at room temperature and then overnight in the cold. After extensive washing with PBS, the beads were applied to a column and the bound antibodies were eluted with 3 M KSCN. One-half milligram of BSA was added per ml of eluate. The eluates were then dialyzed against PBS, and immunoglobulin concentrations were determined by ELISA.

**Elution of Antibodies from RBC Monolayers.** Antibodies binding to the surface of infected RBC (GA-fixed and air-dried) were eluted with 0.2 M glycine buffer (pH 2.8) as described (10).

**Dot Immunoblotting.** Two μl of octapeptide-BSA conjugate (0.5 mg of octapeptide per ml) were applied onto nitrocellulose strips (Bio-Rad). After being dried, the nitrocellulose was incubated in 3% (wt/vol) BSA in PBS for at least 2 hr and then for 2 hr with test serum diluted in PBS containing 1% BSA, 0.1% Triton X-100, and 0.02% NaN<sub>3</sub> (PBS-T). The nitrocellulose was washed 5 times for 5 min in PBS-T containing 0.2% BSA. It was then incubated for another 2 hr with the proper anti-immunoglobulin antibodies conjugated to alkaline phosphatase (20) diluted in PBS-T containing 1% BSA. After the washing procedure was repeated, the nitrocellulose was stained for alkaline phosphatase (21). For detection of antibodies reacting with protein residues modified by the coupling reagent, BSA treated with GA was used as control antigen.

**Other Procedures.** Immunoblotting of merozoite polypeptides separated by NaDodSO<sub>4</sub>/PAGE (reducing conditions) was performed as described (10, 21).

Indirect IF of GA-fixed and air-dried monolayers of *P. falciparum*-infected erythrocytes was performed as described by Perlmann *et al.* (10). Inhibition of the IF was assayed as described (10) by using either native octapeptide or octapeptide-BSA at concentrations corresponding to 2–200 μg of octapeptide per ml. When octapeptide-BSA was used, GA-treated BSA was the control antigen.

Inhibition of merozoite reinvasion in *P. falciparum* *in vitro* cultures was performed with serum or isolated IgG fractions as described (11).

## RESULTS

The octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala was synthesized and analyzed by HPLC (Fig. 1). One main peak containing 62% of the material and two minor peaks containing 3% and 23% of the material were obtained. Amino acid analysis of these peaks showed that they had the expected composition of the octapeptide (Table 1); however, the composition of the two minor peaks deviated slightly.

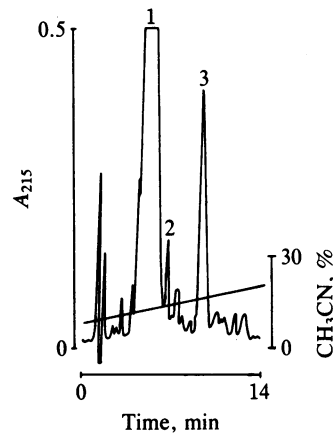


Fig. 1. HPLC separation of the octapeptide preparation by reversed-phase chromatography. Elution was performed with a gradient of acetonitrile (8–30%) in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. The absorbance at 215 nm was measured, and the peaks designated 1, 2, and 3 were analyzed further (see text).

Antigenic aspects of these three peaks will be dealt with below. The whole unseparated octapeptide preparation was used in the following experiments if not otherwise mentioned.

Rabbits were immunized with the octapeptide conjugated with either KLH or TT, and the sera were then tested for octapeptide-reactive antibodies in a dot-immunoblotting assay. Both sera reacted with an octapeptide-BSA conjugate, while the preimmune sera were negative. When titrated, the anti-octapeptide-KLH antiserum reacted with octapeptide-BSA up to a dilution of 1:6,400, whereas the end-point titers of anti-octapeptide-TT was 1:12,800. The anti-octapeptide-KLH reacted weakly with GA-treated BSA (1:100), indicating that it contained only low amounts of antibodies reactive with protein residues modified by the coupling reagent (22). In contrast, the antiserum to octapeptide-TT reacted significantly with GA-treated BSA, although at lower titers than with octapeptide-BSA.

The antisera were tested for reactivity with *P. falciparum* polypeptides by means of immunoblotting after NaDodSO<sub>4</sub>/PAGE of merozoite extracts. When probed with human immune serum, at least 20 bands, representing parasite antigens, appear on the blots (10, 11). In contrast, when probed with the rabbit anti-octapeptide antisera, the only strongly stained band was Pf 155. In addition, a few polypeptides of lower molecular weight were weakly stained (Fig. 2). The same *M<sub>r</sub>* 155,000 polypeptide was the major stained band when merozoite extracts or soluble parasite components from culture supernatants were used as antigen (10, 13). The weakly stained bands included primarily polypeptides of *M<sub>r</sub>*s 135,000 and 120,000, previously shown to be antigenically related to Pf 155 (13). The anti-octapeptide-KLH and anti-octapeptide-TT antisera gave identical results in this assay (not shown).

Table 1. Amino acid composition of the HPLC-separated octapeptide preparation

Amino acid	Percentage composition			
	Expected*	Peak 1	Peak 2	Peak 3
Glu	37.5	38.0	38.3	35.6
Asx	25.0	24.6	23.2	23.3
Ala	12.5	12.4	11.9	11.3
Val	12.5	12.2	11.2	16.9
His	12.5	11.9	10.9	10.7
Gly		0.9	2.1	1.3
Ser			1.6	0.7
Lys			0.8	
Phe				0.2

\*Expected values for octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala.

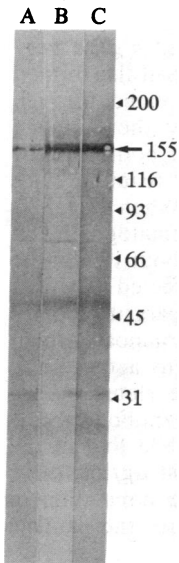


FIG. 2. Immunoblotting analysis of merozoite polypeptides separated by NaDodSO<sub>4</sub>/PAGE and probing with serum (1:100 dilution) of a rabbit immunized with octapeptide-KLH conjugate (lane A), human antibodies affinity-isolated on monolayers with *P. falciparum*-infected erythrocytes (lane B), or human antibodies affinity-isolated on octapeptide-BSA-Sepharose (lane C). Bound antibodies were detected by staining for alkaline phosphatase after reaction with either sheep anti-rabbit Ig (lane A) or rabbit anti-human Ig (lanes B and C) conjugated with alkaline phosphatase. The broad stained band at  $M_r$  50,000 represents human Ig heavy chains contaminating the antigen preparation (10). Numbers indicate the approximate molecular weights  $\times 10^{-3}$ .

The reactivity of the anti-peptide antisera with parasite antigens was further established by indirect IF on GA-fixed and air-dried monolayers of *P. falciparum*-infected RBC. This IF assay mainly lights up Pf 155 but also some other *P. falciparum* antigens deposited in the membrane of the infected RBCs (10). Both antisera gave a distinct surface IF of infected RBCs at dilutions of 1:25 to 1:125. This IF of both rabbit antisera was inhibited in a dose-dependent manner by addition of either native octapeptide (10–200  $\mu\text{g}/\text{ml}$ ) or octapeptide-BSA but was unaffected by corresponding concentrations of GA-BSA (not shown).

To investigate the occurrence of octapeptide reactive antibodies in donors exposed to *P. falciparum* malaria, such human sera were analyzed by dot-immunoblotting with octapeptide-BSA as antigen. Most sera containing antibodies to Pf 155 as detected by IF or immunoblotting also contained anti-octapeptide antibodies. The anti-octapeptide titers were well correlated with the titers in the RBC surface IF assay (Table 2). Ten normal human sera so treated did not react with octapeptide-BSA at dilutions of  $>1:100$  (not shown).

The octapeptide-reactive antibodies of the serum from a *P. falciparum* immune Liberian (Kinon) were isolated by affin-

Table 2. Serum titers in dot-immunoblotting with octapeptide-BSA as antigen or in surface IF of infected RBC

Donor*	Titers <sup>†</sup>	
	Dot-blot <sup>‡</sup>	IF <sup>§</sup>
Kinon <sup>¶</sup>	1600	15,000
AM <sup>  </sup>	800	12,500
IS-7 <sup>¶</sup>	800	625
L-30 <sup>¶</sup>	800	625
ASF <sup>  </sup>	400	250
MV <sup>  </sup>	200	625
JOR <sup>  </sup>	100	125
FG <sup>  </sup>	100	5
IS-8 <sup>¶</sup>	<100	25
NS	<100	<5

\*Sera from nine donors exposed to *P. falciparum* and one normal Swedish donor (NS).

<sup>†</sup>Reciprocal of serum dilution, end-point titers.

<sup>‡</sup>Lowest serum dilution tested was 1:100; <100 = negative.

<sup>§</sup>Lowest serum dilution tested was 1:5; <5 = negative.

<sup>¶</sup>Clinically immune.

<sup>||</sup>Acute infection.

ity chromatography with octapeptide-BSA as ligand on Sepharose 4B beads. Approximately 0.15% of the serum IgG applied to the column could be eluted from the adsorbent with 3 M KSCN. When calculated on the basis of the IF titers, the total serum volume added to the octapeptide column, and the volume of the eluates, the anti-octapeptide activity recovered amounted to  $\approx 20\%$  of the antibody activity giving rise to surface IF of infected RBC (13%, 20%, and 25%, respectively; three experiments). As  $\approx 60\%$  of this activity passed through the octapeptide columns, the total recovery of IF activity ranged from 70% to 85%. Similar experiments with the rabbit anti-octapeptide-KLH antiserum indicated that  $\approx 10\%$  of the IF activity could be eluted from the octapeptide column. However, in this case the fraction passing the column had no antibody activity. Addition of an excess of octapeptide or octapeptide-BSA (200  $\mu\text{g}$  of octapeptide per ml) in the IF assay had no inhibitory effect on the staining with the human antibodies eluted from the octapeptide column. In contrast, the surface IF of infected RBC obtained with rabbit anti-octapeptide antibodies, similarly isolated by affinity chromatography, was readily inhibited at low concentrations of native octapeptide or octapeptide-BSA (2  $\mu\text{g}$  of octapeptide per ml).

In immunoblotting, the eluted human antibodies gave a strong staining of Pf 155 and weak staining of a few components of lower molecular weight (including the  $M_r$  135,000 and  $M_r$  120,000 polypeptides), similar to that obtained with antibodies from the same individual but isolated by elution from monolayers of *P. falciparum*-infected RBC (10) or with rabbit anti-octapeptide antibodies (Fig. 2). The amount of immunoglobulin (human or rabbit) eluted from a BSA-Sepharose column was at most 1/10th of that eluted from the octapeptide-BSA columns, and the eluted immunoglobulins were not reactive in either the IF or the immunoblotting assay (not shown).

To investigate if the major peptide components in the octapeptide preparation displayed any major antigenic differences, antibodies from the human serum used above were adsorbed to Sepharose beads bearing BSA conjugates of either of the two major octapeptide fractions obtained by HPLC (HPLC peaks 1 and 3, respectively, in Fig. 1). The antibodies eluted from these adsorbents were used for probing in dot-immunoblotting with BSA conjugates of the peptides from each of the three different HPLC peaks. Both antibody preparations reacted at similar titers with all three peptide conjugates.

The IgG fraction of the Liberian serum Kinon efficiently inhibits reinvasion of RBC by *P. falciparum* merozoites in *in vitro* cultures. This inhibitory activity can be highly enriched by prior elution of the antibodies from monolayers of infected RBCs (11). As shown by the results of a representative experiment given in Table 3, the octapeptide-reactive anti-

Table 3. *P. falciparum*-reinvasion inhibition *in vitro* and surface IF of *P. falciparum*-infected RBC by human antibodies

Source of Ig	Ig, $\mu\text{g}/\text{ml}$	
	Reinvasion inhibition*	IF <sup>†</sup>
Ig eluted from monolayers of infected RBC	0.7	0.1
Ig eluted from octapeptide-Sepharose	3.0	$\leq 0.1$
Anti-octapeptide-depleted Ig, eluted from infected RBC	11.0	0.4

\*Ig concentration needed for 50% inhibition.

<sup>†</sup>Lowest Ig concentration giving positive IF.

bodies eluted from the octapeptide-BSA column also inhibited reinvasion completely, with a 50% inhibition titer of 3  $\mu\text{g}/\text{ml}$  (Table 3). Immunoglobulin eluted from BSA control columns had no inhibitory effects (not shown). Passing of the Kinon serum three times over octapeptide-BSA columns depleted it of octapeptide-reactive antibodies as detected by dot-immunoblotting. Thereafter, the depleted serum was incubated on monolayers of *P. falciparum*-infected RBC, and the bound antibodies were eluted with glycine buffer. These eluted antibodies had a reduced capacity by more than a factor of 10 to inhibit parasite reinvasion as compared with monolayer eluted antibodies tested before passage over the octapeptide column. The fraction depleted of anti-octapeptide reactive antibodies was also the least reactive one in the surface IF assay (Table 3). Similar results were obtained with another Liberian serum (L-30, Table 2). The reinvasion inhibitory capacity of antibodies reactive with the octapeptide sequence of Pf 155 was also demonstrated with affinity-purified anti-octapeptide antibodies from the two rabbit antisera (Fig. 3). These antibodies inhibited reinvasion to 100%, and their 50% inhibition titers were 65 or 75  $\mu\text{g}$  of IgG per ml, respectively. In contrast, the total immunoglobulin fraction of these sera gave a maximum of 30% inhibition at about 2–3 mg/ml. As for the human antibodies, a correlation was found between the reinvasion inhibition titers and the lowest concentration of IgG giving positive surface immunofluorescence. Thus, for the anti-octapeptide-TT antiserum, the end-point titers in the IF assay were 60  $\mu\text{g}/\text{ml}$  for the antibodies eluted from the octapeptide-BSA column and 600  $\mu\text{g}/\text{ml}$  for the total IgG fraction.

## DISCUSSION

Several tandem repeats of the octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala, followed by tandem repeats of half of this sequence (Glu-Glu-Asn-Val), constitute the main components of a C-terminally located block of conserved amino acid sequences in a  $M_r$  155,000 *P. falciparum* antigen (15, 16). In this study we demonstrate that antibodies made in rabbits against the octapeptide conjugated with carrier proteins react with native *P. falciparum* antigens in a variety of test systems. In addition, our results suggest that a significant portion of the anti-Pf 155 antibodies found in humans exposed to *P. falciparum* react with epitopes associated with the same octapeptide unit as was used to immunize the rabbits.

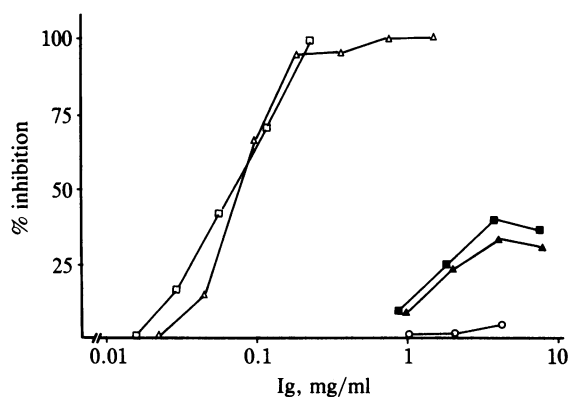


FIG. 3. Reinvasion inhibition *in vitro* either by (i) total IgG from rabbit anti-octapeptide-KLH antiserum (■), rabbit anti-octapeptide-TT antiserum (▲), or preimmune serum from rabbit immunized with octapeptide-TT (○) or by (ii) IgG fractions affinity-purified on octapeptide-BSA Sepharose from rabbit anti-octapeptide-KLH antiserum (△) or from rabbit anti-octapeptide-TT antiserum (□). The percentage inhibition was determined after 20 hr of incubation as described (11).

The antibodies raised in rabbits with the octapeptide conjugated with two different carrier molecules gave very similar reactions. Although one of the rabbits had also formed antibodies directed against determinants created through modification of carrier protein residues by the coupling reagent GA (22), the other had not, and for both sera the octapeptide specificity of the antibodies reacting with plasmodial antigens was clearly established. When the rabbit antisera were further purified by affinity chromatography on octapeptide-BSA immunoadsorbents, only about 10% of the antibody activity responsible for the IF of infected RBC was recovered in the eluates. There was no anti-parasite activity detectable in the fraction passing the immunoadsorbent column, indicating that a large portion of this activity was either lost by denaturation or could not be eluted by the methods used. As the IF of the eluted antibodies was inhibited by the octapeptide at 2  $\mu\text{g}/\text{ml}$  while that of the unfractionated antiserum required up to 200  $\mu\text{g}/\text{ml}$ , these antibodies probably represented a selected population of molecules with comparatively low affinity for the parasite antigen.

Antibodies reactive with the octapeptide-BSA conjugate in the dot-blot assay were also present in human malarial sera, with titers well correlated to those of the modified IF assay for Pf 155 and other parasite antigens deposited in the RBC membrane. Direct evidence for the IF activity and Pf 155 specificity of the human octapeptide-reactive antibodies was obtained by affinity chromatography. For the one human serum studied in detail,  $\approx 20\%$  of the antibody activity responsible for the IF of the surface of infected erythrocytes could be eluted from octapeptide-BSA immunoadsorbent. In contrast to what was found with the rabbit antisera, a significant IF activity was also present in the antibody fraction depleted of octapeptide-reactive antibodies, indicating the involvement of other epitopes (and/or antigens) in this activity. Nevertheless, the results suggest that octapeptide-associated epitopes are immunodominant in naturally occurring immunization by infection. This is in line with the results of Cowman *et al.* (16).

The IF of the human antibodies eluted from the octapeptide-BSA immunoadsorbent could not be inhibited by addition of large amounts of octapeptide or octapeptide-BSA. This was different from what was found for the affinity-purified rabbit antibodies, which were easily inhibited by small amounts. This suggests that the human antibodies have a higher affinity for parasite antigen than for the octapeptide. Several not-mutually-exclusive explanations may account for this. (i) The conformation of the natural epitopes of the parasite antigens is more favorable for antibody binding than that of the synthetic octapeptide or its conjugate. (ii) The correct epitopes for the human antibodies may only partially be contained within the octapeptide sequence. Rather, it may comprise sequences shared between neighbor repeat subunits—e.g., the multiple repeated sequence Glu-Glu-Asn-Val or with the block of 5'-repeats present in Pf 155 (15, 16). Other synthetic peptides as well as monoclonal antibodies will be needed for a more detailed analysis of the epitopes involved. (iii) Although there are good reasons to believe that Pf 155 is the major immunogen eliciting the octapeptide-reactive antibodies, the involvement of genetically distinct immunogens displaying certain homologies with the sequences included in the Pf 155 repeats (ref. 23; unpublished data) is not excluded.

Several plasmodial antigens have been shown to contain tandemly repeated amino acid sequences that seem to define major antigenic regions of the molecules (15, 16, 23–28). Synthetic peptides corresponding to sequential units of the major surface protein of the sporozoites [the CS (circum sporozoite) antigen] of *P. knowlesi* bind mouse monoclonal anti-CS antibodies, and for three of four of these, the major

epitope comprised the same sequence of 8 amino acids located within the 12-amino acid repeat unit of this molecule (29). Similarly, rabbit antibodies to synthetic peptides corresponding to two or four repeats of the 4-amino acid subunit, repeated 41 times in the CS-antigen of *P. falciparum*, reacted efficiently with the native protein in several assay systems (30). Hence, both in the case of the CS antigen and Pf 155, the antibodies to repetitive epitopes seem to recognize continuous amino acid sequences in the molecules.

Pf 155 has been shown to bind to glycophorin (9, 13, 14) and is assumed to play a role in the merozoite-RBC interactions resulting in RBC invasion (10). This role is supported by the strong reinvasion inhibitory activity *in vitro* of human anti-Pf 155 antibodies (11, 13). The present experiments show that the octapeptide-reactive fraction of these antibodies is very efficient in inhibiting reinvasion. In fact, these antibodies appear to be more efficient inhibitors than the antibodies eluted from the surface of infected RBC but depleted of the octapeptide reactive fraction. The importance of octapeptide-associated antigenic sites for the function of Pf 155 and related molecules in RBC invasion is also supported by the inhibitory effects of rabbit anti-octapeptide antibodies enriched by elution from the octapeptide-BSA immunoadsorbent. As was to be expected, the rabbit antibodies were less-efficient inhibitors than the corresponding human antibodies. Nevertheless, our results are similar to those obtained in the sporozoite system, where antibodies to synthetic peptides corresponding to the repeat regions of the CS antigen have been shown to inhibit the interaction of the sporozoites with their target cells *in vitro* and to neutralize sporozoite infectivity (30–32). However, the inhibitory effect of the octapeptide-reactive antibodies on RBC infection *in vivo* remains to be established. As these antibodies occur in relatively large amounts in certain human sera and can be isolated by affinity chromatography, their possible protective efficiency *in vivo* can be assessed by passive immunization.

In conclusion, we have demonstrated that the C-terminal repeats of Pf 155 constitute an immunodominant region that might include target sites for a protective humoral immune response to asexual blood stages of *P. falciparum*. It is of importance to note that this region seems to be conserved, as no major antigenic diversity between different isolates or strains of *P. falciparum* has thus far been detected (refs. 10, 11, 13, 15, and 16; H.P., unpublished data). Hence, the antigen stands out as a suitable vaccine candidate, and synthetic peptides corresponding to the major epitopes of this molecule may be proper components of such a vaccine. Gene constructs coding for multiple repeat subunits of the sequences studied here may provide an alternative source of this material (33).

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