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Synthetic gene construct expressing a repeated and highly immunogenic epitope of the *Plasmodium falciparum* antigen Pf155

(fusion protein/human malaria/reinvasion inhibition)

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ABSTRACT **The *Plasmodium falciparum*-derived antigen Pf155 contains two blocks of tandemly repeated amino acid sequences. A pair of complementary oligonucleotides, encoding the C-terminally located repeat Val-Glu-His-Asp-Ala-Glu-Glu-Asn, were synthesized. The oligonucleotides were polymerized by ligation, and the resulting multimers were cloned into an expression vector. One construct that contained four copies of the repeat was expressed in *Escherichia coli*. The product, a fusion protein, was soluble and produced in high amounts. It reacted in immunoblotting with a monoclonal antibody to a synthetic octapeptide (Glu-Glu-Asn-Val-Glu-His-Asp-Ala). Rabbits immunized with partially purified fusion protein, either with or without adjuvant, formed antibodies against this octapeptide. These antibodies reacted with Pf155 both in parasite extracts and when deposited in the membrane of infected erythrocytes. Furthermore, these antibodies inhibited merozoite reinvasion *in vitro* as efficiently as human antibodies to the octapeptide sequence in Pf155, induced by natural infection. The results suggest that products of synthetic gene constructs may be a suitable basis for an anti-merozoite vaccine.**

In view of the global resurgence of *Plasmodium falciparum* malaria, the most lethal of the malarias affecting man, extensive efforts are being made to define parasite antigens to be used in a malaria vaccine. Unusual approaches to vaccine production, including the use of synthetic peptides coupled to a carrier protein (1–3) or genetic engineering techniques (3, 4), have been used to study the role of the major circumsporozoite protein for inducing protective immunity. However, since the asexual blood stages of the parasite cause the clinical disease, the development of vaccines based on defined antigens from these stages is considered to be of great importance (5). Recently, we have identified a *P. falciparum* antigen of M_r 155,000 (designated Pf155) that is deposited in the erythrocyte (RBC) membrane during merozoite invasion (6, 7). Human antibodies to this merozoite-associated antigen were shown to efficiently inhibit *P. falciparum* reinvasion in *in vitro* cultures (8). In addition, a correlation was found between high levels of antibodies to Pf155 and acquired *P. falciparum* immunity in children living in a holoendemic area of Africa (9).

Pf155 appears to be identical with RESA (ring-infected erythrocyte surface antigen), a *P. falciparum* antigen described by Coppel *et al.* (10). The C-terminal part of RESA has been shown to comprise a region of repeating subunits that are eight, four, or three amino acids long (10, 11). We recently have demonstrated that sera from rabbits immunized with the octapeptide subunit Glu-Glu-Asn-Val-Glu-His-Asp-Ala reacted with Pf155 as revealed by immunoblotting, immunofluorescence (IF), or reinvasion inhibition *in vitro* (12). Moreover, a major fraction of human antibodies to Pf155 also bound to this linear sequence. Affinity-purified human antibodies to this octapeptide were extremely efficient inhibitors of merozoite reinvasion *in vitro*. Taken together, these results suggest that the octapeptide is part of a major antigenic region of Pf155 (13) and, hence, is a possible target structure for the induction of protective immunity against *P. falciparum* merozoites.

In the present study, we prepared synthetic oligonucleotides coding for the octapeptide repeat and ligated these to create a repetitive gene. The latter was cloned in an expression vector, and large amounts of a fusion protein were synthesized in *Escherichia coli*. The bacterial expression products were partially purified and used for immunizing rabbits. The rabbit sera reacted both with the synthetic octapeptide and with *P. falciparum* antigens in ELISA, IF, and immunoblotting. Furthermore, they inhibited *P. falciparum* merozoite reinvasion *in vitro* efficiently.

MATERIALS AND METHODS

Oligonucleotides. Two complementary oligonucleotides, each 24 nucleotides long, were synthesized. When hybridized, the oligonucleotides form a duplex with 5′-protruding complementary ends of three bases (Fig. 1*A*). The oligonucleotides code for the sequence Val-Glu-His-Asp-Ala-Glu-Glu-Asn.

Vector. The bacterial expression vector pATH11 (14) (constructed and donated by T. J. Koerner) was used for cloning and expression of the fusion protein. The fusion protein contained 333 amino acids from the TrpE protein and is under the control of the *trp* promoter.

Enzymes. Restriction endonucleases, ligase, and Klenow polymerase were purchased from New England Biolabs or P-L Biochemicals and used as recommended by the manufacturers. *Bam*HI linkers (12 nucleotides) were obtained from P-L Biochemicals and treated with polynucleotide kinase before use.

Transformation. The recombinant plasmids were transformed into the *E. coli* strain HB101. After colony hybridization, selected plasmids were used to transform *E. coli* C600. Transformation was performed by the method of Maniatis *et al.* (15).

Screening for Recombinant Clones Expressing a Fusion Protein. Induction of the fusion protein and screening for

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Abbreviations: FCA, Freund's complete adjuvant; IF, immunofluorescence; Pf155, *Plasmodium falciparum* antigen of M_r 155,000; RBC, erythrocyte(s); RESA, ring-infected erythrocyte surface antigen.

recombinant clones was performed as described by Spindler *et al.* (14).

DNA Sequence Analysis. Sequence analysis was performed by the method of Maxam and Gilbert (16).

Purification of the Fusion Protein. Induced bacteria were sonicated for 8 min at 20 W, and DNase was added to a final concentration of 2 μg/ml. After centrifugation at 10,000 × *g* for 15 min, the supernatant was fractionated by gel filtration on Sephacryl S200 (Pharmacia). Protein was eluted with phosphate-buffered saline (0.15 M NaCl/0.015 M phosphate buffer, pH 7.4), and the protein concentration was determined by reading the absorbance at 280 nm. Eluted fractions were assayed for the fusion protein in ELISA (17). Recovery of the fusion protein was determined densitometrically (LKB 2202 Ultroscan, LKB) after $NaDodSO_4$/PAGE separation and Coomassie blue staining. Immunoblotting was performed by previously described methods (6, 18) using an octapeptide-specific mouse monoclonal antibody for probing. The latter was produced by immunizing mice with the synthetic octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala coupled to the carrier protein keyhole limpet hemocyanine (13, 19).

Rabbit Antisera. New Zealand White rabbits were immunized intramuscularly with 100 μg of gel-filtrated fusion protein either in Freund's complete adjuvant (FCA) or without adjuvant. A second injection, given 4 weeks later, used Freund's incomplete adjuvant instead of FCA. The rabbits were bled 3 weeks after the first injection and again 1 week after the second. IgG was isolated on a protein A-Sepharose column (Pharmacia).

Parasite Material. *In vitro* cultures of the Tanzanian *P. falciparum* strain F32 were used. For antigen analysis and ELISA, merozoite-enriched fractions were prepared and processed as described (6, 17).

Antibody Titration. Rabbit antibodies binding to the synthetic octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala or to parasite antigens were measured in ELISA (17). Polystyrene microtiter plates (Dynatech, Alexandria, VA) were coated either with synthetic octapeptide conjugated to bovine serum albumin or with a merozoite-enriched parasite fraction. Control plates were coated with bovine serum albumin-conjugated polyasparagine (Sigma) or with normal erythrocyte membranes. The coated plates were incubated for 1 hr at 37°C with various dilutions of rabbit serum. After incubation for 1 hr at 37°C with anti-rabbit IgG conjugated with alkaline phosphatase and for 1 hr at room temperature with *p*-nitrophenyl phosphate, the resulting color was registered at 405 nm.

Indirect IF. Indirect IF of glutaraldehyde-fixed and air-dried monolayers of *P. falciparum*-infected erythrocytes was performed as described by Perlmann *et al.* (6).

Immunoblotting. Parasite extracts were separated by $NaDodSO_4$/PAGE under reducing conditions, electrophoretically transferred to a nitrocellulose membrane, and probed with rabbit serum diluted 1:50 (6, 18).

Affinity Chromatography. Synthetic octapeptide conjugated to bovine serum albumin was coupled to CNBr-activated Sepharose (Pharmacia) (3 mg of octapeptide per 5 mg of albumin per ml of packed beads) according to the instructions of the manufacturer. For affinity chromatography, 1 ml of the IgG fraction isolated from rabbit serum was incubated with 5 ml of packed beads applied to a column. After extensive washing with phosphate-buffered saline, bound antibodies were eluted with 0.2 M glycine buffer followed by elution with 3 M KSCN. The eluates were dialyzed against phosphate-buffered saline, and immunoglobulin concentrations were determined by ELISA.

Elution of Antibodies from RBC Monolayers. Antibodies binding to the surface of infected RBC (glutaraldehyde-fixed and air-dried monolayers) were eluted with 0.2 M glycine buffer (pH 2.8) (6).

***In Vitro* Inhibition of *P. falciparum* Merozoite Reinvasion.** The assay was performed in *in vitro* cultures with serum or isolated IgG fractions as described (8).

RESULTS

Construction of a Multimeric Gene. Two complementary synthetic oligonucleotides, 24 bases long, which encode one octapeptide repeat from the 3′ region of RESA (Pf155) (10, 11) were constructed and hybridized (Fig. 1*A*). The resulting duplexes had complementary ends. Thus, it was possible to ligate the duplexes to each other, creating a multimeric gene encoding octapeptide repeats (Fig. 1*B*). Because of the structure of the duplex, there were three extra nucleotides at the very end of each polymeric fragment coding for a valine residue. A flow chart of the construction is shown in Fig. 1*C*. The oligonucleotides were ligated under limiting conditions (1 hr at 15°C) to preserve most of the material as small multimers. The 5′-protruding ends of the multimers were filled in using Klenow polymerase, creating blunt ends. Twelve-nucleotide-long *Bam*HI linkers were then ligated onto the multimers. After cleavage with *Bam*HI, the different multimeric fragments were separated in a 10% polyacrylamide gel and eluted. Individual fragments were finally ligated into the *Bam*HI site of the pATH11 vector, and the recombinant plasmids were transformed into *E. coli* HB101. Plasmid DNA was prepared from clones that were positive by colony hybridization and transformed into *E. coli* C600. The latter was found to be a more suitable host for the production of fusion protein as previously reported (14).

Expression of the Fusion Protein in *E. coli*. The recombinant clones were screened for expression of the fusion protein after induction with indoleacrylic acid, and one recombinant clone that expressed the fusion protein at a high level was chosen for further characterization. It contained four repeats and was designated 4A:1. The nucleotide sequence of the construct is shown in Fig. 2. In addition to the repeats, the fusion protein consisted of 333 amino acids at the N-terminal end derived from the TrpE protein and 24 amino acids at the C-terminal end encoded from the polylinker. Immunoblotting of unfractionated bacterial lysates revealed two strong bands when probed with a mouse monoclonal antibody specific for the octapeptide repeat (Fig. 3*A*). The upper and lower bands were estimated to have M_r values of 49,000 and 43,000, respectively. The theoretical size of the fusion protein was M_r 43,000 as deduced from the amino acid sequence. No similar proteins were seen in *E. coli* C600 lysates or lysates of *E. coli* C600 containing pATH11 plasmids without inserts.

Purification and Characterization of the Fusion Protein. The fusion protein was soluble, which facilitated further processing. Thus, after lysis and centrifugation of the recombinant bacteria, almost all of the fusion protein was found in the supernatant. The latter was fractionated by gel filtration, and each fraction was assayed for fusion protein by using an ELISA with the octapeptide-specific mouse monoclonal antibody (13, 19). Fractions 42–50 reacted strongly with the antibody (Fig. 3*B*). Other protein-containing fractions, including the void volume, did not react with this antibody.

Immunoblotting with the monoclonal antibody revealed the same two polypeptides in the ELISA-positive fractions as were seen in the unpurified lysates (not shown). Fractions 42–50 were pooled, and the recovery of the fusion protein in the pool was estimated to be ≈50%. By using this procedure, ≈125 mg of gel-filtered fusion protein was prepared from 0.5 liter of bacterial culture.

Immunization of Rabbits with Fusion Protein. Rabbits were immunized twice with fusion protein in FCA or without adjuvant, and the sera were tested in ELISA for antibodies binding either to *P. falciparum* merozoite extracts or to the synthetic octapeptide (12, 19). Table 1 shows that the rabbit

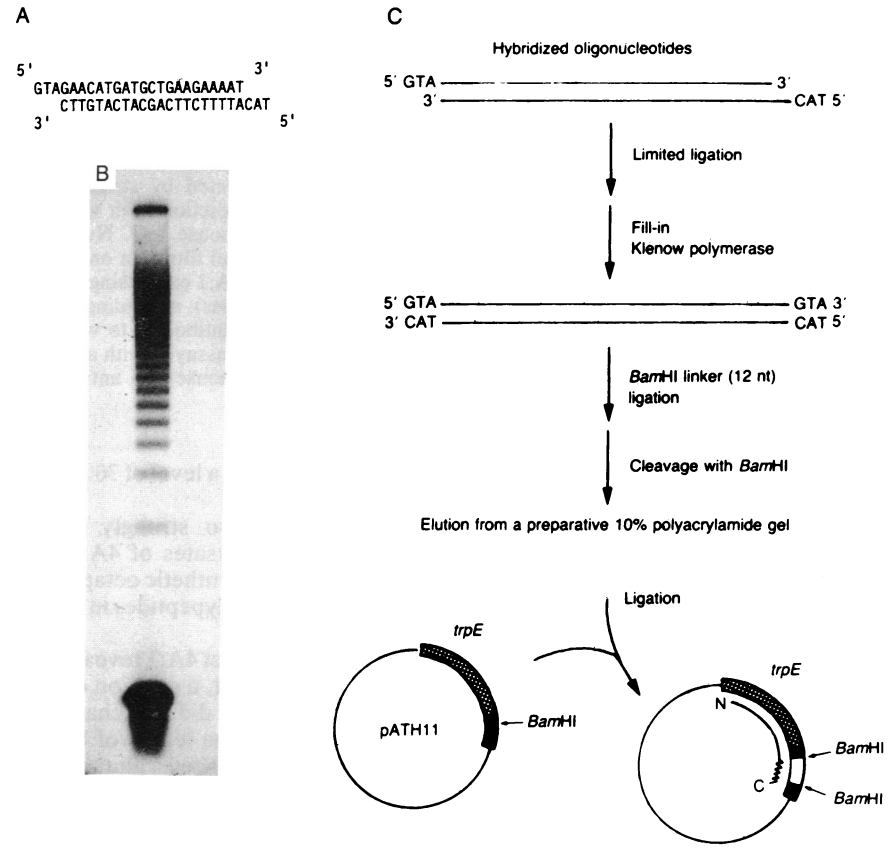

FIG. 1. (*A*) Structure of the two complementary synthetic oligonucleotides encoding one octapeptide repeat from the 3′ region of RESA (= Pf155). (*B*) The hybridized and ligated oligonucleotides were separated on a 10% polyacrylamide gel. The oligonucleotides were radioactively labeled by [γ-^{32}P]ATP. (*C*) A flow chart of the gene construction.

immunized with the fusion protein in FCA reacted both with parasitized erythrocytes and the synthetic octapeptide (end-point titers were 1:1000 and 1:100, respectively). Serum from the rabbit immunized with the fusion protein without adjuvant reacted strongly with the octapeptide (1:5000), while the reaction with parasites was weaker (1:100).

The rabbit antisera were also tested in indirect IF on glutaraldehyde-fixed and air-dried monolayers of *P. falciparum*-infected RBCs (Table 1). This assay mainly reveals Pf155 deposited in the membrane of infected RBCs (6). Serum from the rabbits immunized twice with fusion protein with or without FCA stained the surface of infected RBCs strongly, giving end-point titers of 1:1250 and 1:625, respectively.

To further identify the parasite components reacting with the antibodies, rabbit sera were tested for reactivity with *P. falciparum* polypeptides in immunoblotting of merozoite extracts. The only stained band was Pf155, which reacted strongly with antisera from both rabbits (Fig. 4).

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Gly Ser Gly Val Asp Asp Ala Glu Glu Asn
GGA TCC GGG GTA GAT GAT GCT GAA GAA AAT

Val Glu His Asp Ala Glu Glu Asn
GTA GAA CAT GAT GCT GAA GAA AAT

Val Glu His Asp Thr Glu Glu Asn
GTA GAA CAT GAT ACT GAA GAA AAT

Val Glu His Asp Ala Glu Glu Asn Val Pro Gly
GTA GAA CAT GAT GCT GAA GAA AAT GTA CCC GGA
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FIG. 2. The nucleotide sequence of the recombinant clone 4A:1 comprising a tetramer of the octapeptide repeat. The boxes indicate the *Bam*HI linker. In the first repeat, a deletion of three bases (AAC) had occurred (indicated by a black triangle), which replaced a glutamic acid and a histidine residue with an aspartic acid residue. Furthermore, a base substitution (indicated by a black dot) changed a guanosine to an adenosine resulting in a change of an alanine residue to a threonine in the third repeat.

To determine the relative amount of antibodies reacting in the octapeptide ELISA or giving rise to the surface IF of infected RBCs, the IgG fraction from the serum of a rabbit immunized with fusion protein without adjuvant was affinity-purified on synthetic octapeptide-bovine serum albumin conjugate coupled to Sepharose 4B. Of the serum IgG applied to the column, ≈0.1% was eluted with 0.2 M glycine buffer. Calculations based on the ELISA titers indicated that the anti-octapeptide activity recovered amounted to ≈45% of the antibody activity present before fractionation. The corresponding recovery of activity giving rise to surface IF of infected RBCs was 10%. Subsequent elution with 3 M KSCN resulted in recovery of an additional 0.3% of the serum IgG applied to the column. Since no detectable antibody activity was recovered in the flow-through fraction, antibodies representing higher affinities for the octapeptide would be expected in the KSCN-eluted fraction. However, the KSCN-eluted fraction completely lacked activity in the octapeptide ELISA and failed to stain the surface of infected RBCs, presumably due to denaturation of the antibodies.

***In Vitro* Inhibition of *P. falciparum* Merozoite Reinvasion.** Octapeptide-reactive antibodies from human immune sera or from rabbits immunized with synthetic peptides have been shown to inhibit *P. falciparum* merozoite reinvasion *in vitro* (12). This inhibitory activity could be further enriched by prior elution of the antibodies from monolayers of infected RBCs (12). Antibodies produced after immunization of rabbits with fusion protein also inhibited reinvasion (Table 2). The 50% inhibition titers for the IgG fractions from the rabbits immunized with fusion protein with or without FCA were 1.3 and 2.5 mg/ml, respectively. Enrichment for parasite-specific antibodies by affinity purification of the IgG fractions on monolayers of *P. falciparum*-infected RBCs resulted in 1000-fold increase of these titers. The end-point IF titers of the affinity-purified antibodies were increased ≈150-fold as compared to the total IgG fractions (Table 2).

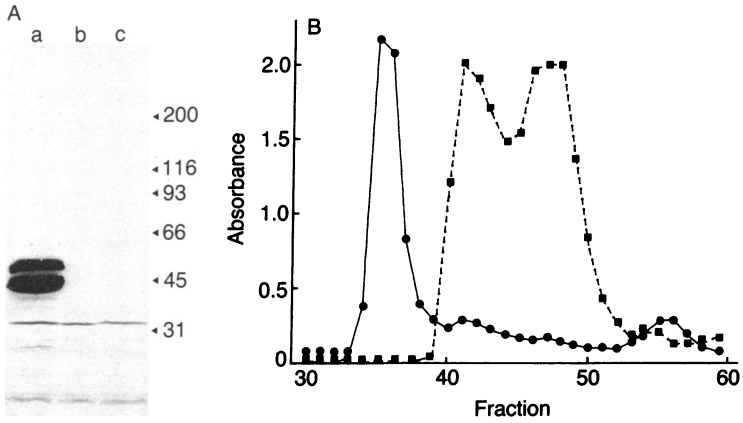

FIG. 3. (*A*) Immunoblotting analysis of the fusion protein with a mouse monoclonal antibody to the synthetic octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala. Lanes: a, a lysate of recombinant clone 4A:1; b, a lysate of *E. coli* strain C600; c, a lysate of *E. coli* C600 containing the pATH11 vector. Bound antibodies were detected by staining for alkaline phosphatase activity after reaction with alkaline phosphatase-conjugated rabbit anti-mouse IgG. Numbers are approximate $M_r \times 10^{-3}$. (*B*) ●, Gel filtration on Sephacryl S-200 of lysate from the clone 4A:1 expressing fusion protein (absorbance at 280 nm of eluates); ■, binding of the mouse monoclonal anti-octapeptide antibodies to wells of ELISA plates coated with eluate and assayed with alkaline phosphatase-conjugated rabbit anti-mouse IgG antibodies (absorbance at 405 nm).

DISCUSSION

We have synthesized a set of complementary oligonucleotides that encode an immunodominant octapeptide repeat sequence of the Pf155 antigen from *P. falciparum* merozoites (10, 11). Multimers of the synthetic oligonucleotides were cloned into the expression vector pATH11, giving rise to constructs encoding a fusion protein. The fusion protein that contained 333 amino acids from the TrpE protein was expressed under the control of the inducible *trp* promoter. One construct (4A:1) comprising four copies of the octapeptide repeat of Pf155 was expressed in *E. coli*. In immunoblotting, the fusion protein reacted strongly with a mouse monoclonal antibody against the synthetic octapeptide sequence Glu-Glu-Asn-Val-Glu-His-Asp-Ala. Rabbits immunized with partially purified fusion protein produced antibodies that reacted both with the synthetic octapeptide and with parasite antigens in several test systems. Furthermore, these antibodies efficiently inhibited *P. falciparum* merozoite reinvasion *in vitro*.

A useful property of the fusion protein was its water solubility, which eliminated the need for detergents during purification. The amount of fusion protein produced varied between different experiments, attaining a level of 30% of the protein in a soluble bacterial lysate.

In immunoblotting experiments, two strongly stained bands were consistently found when lysates of 4A:1 were probed with antibodies specific for the synthetic octapeptide. The structural difference between the polypeptides in the two bands remains to be determined.

DNA sequence analysis of the construct 4A:1 revealed two alterations in the sequence (Fig. 2). First, a deletion of three base pairs (AAC) had occurred, which did not change the reading frame; however, it decreased the length of the first repeat unit to seven amino acids and replaced a glutamic acid and a histidine residue with an aspartic acid residue. Second, a base-pair substitution had occurred in the third repeat, changing an alanine residue to a threonine (Fig. 2). These alterations could be due to cloning artifacts or heterogeneity of the oligonucleotides. However, in spite of these alterations, an immunologically highly reactive fusion protein was obtained.

Rabbits immunized twice with the fusion protein either with or without FCA produced antibodies that reacted both with synthetic octapeptide and parasite antigens in ELISA. The rabbit immunized with fusion protein in FCA gave a positive anti-parasite response in ELISA, IF, and immunoblotting only 3 weeks after the first injection (data not shown), while the other rabbit did not respond significantly until after a booster injection. The strong response of the animal immunized with the fusion protein without adjuvant is a notable finding. However, since the fusion protein was only partially purified, it cannot be excluded that its immunogenicity was enhanced by bacterial components.

The specificity of the antibodies was also studied by testing the sera in ELISA for reactivity with a synthetic dimer or tetramer of the sequence Glu-Glu-Asn-Val constituting half

Table 1. Antibody activity in sera from rabbits immunized with fusion protein

Antisera	Dilution‡	ELISA* Merozoites§	ELISA* Octapeptide¶	IF†
Preimmune	100	0	0.03	
	1000	0	0.01	<5
	5000	0.03	0.02	
Immunized	100	0.68	0.94	
with FCA	1000	0.13	0.05	1250
	5000	0.07	0.02	
Preimmune	100	0	0.02	
	1000	0.03	0.01	<5
	5000	0.06	0.03	
Immunized	100	0.30	2	
without	1000	0.05	0.76	625
adjuvant	5000	0.04	0.21	

*Absorbance values at 405 nm; >0.1 is positive.
†Indirect IF of merozoite antigens in the surface of infected RBCs (Pf155): end-point titers. The lowest serum dilution tested was 1:5;<5 were negative.
‡Reciprocal serum dilution.
§Merozoites as the antigen. Values were reduced by subtracting the reactivity of the sera with normal erythrocyte membranes (OD, 0.09–0.22).
¶Bovine serum albumin-conjugated synthetic octapeptide (Glu-Glu-Asn-Val-Glu-His-Asp-Ala) as antigen. Values were reduced by subtracting the reactivity of the sera with bovine serum albumin-conjugated polyasparagine (OD, 0.03–0.10).

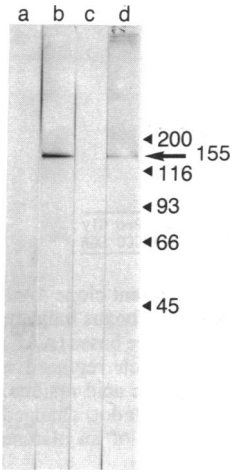

FIG. 4. Immunoblotting of merozoite polypeptides separated by $NaDodSO_4$/PAGE and probed with rabbit preimmune serum (lane a), serum from the same rabbit after two immunizations with fusion protein in FCA (lane b), preimmune serum from a rabbit immunized with fusion protein without adjuvant (lane c), and serum from the same rabbit after two immunizations (lane d). Serum was diluted 1:50 in all cases. Bound antibodies were detected by staining for alkaline phosphatase activity after reaction with alkaline phosphatase-conjugated sheep anti-rabbit IgG. Numbers are approximate $M_r \times 10^{-3}$.

Table 2. Reinvasion inhibition *in vitro* and surface IF of infected erythrocytes by antibodies from rabbits immunized with the fusion protein

Immunization	IgG IC_{50} in reinvasion inhibition,* μg/ml	Minimal IgG in IF,† μg/ml
With FCA	1300	12
	2.3‡	0.1‡
Without adjuvant	2500	45
	2.5‡	0.3‡

*IgG concentration needed for 50% inhibition.
†Lowest IgG concentration giving positive IF.
‡IgG eluted from monolayers of infected RBCs.

of the octapeptide Glu-Glu-Asn-Val-Glu-His-Asp-Ala and repeated >30 times in Pf155 (10, 11). Although a weak binding of the antibodies to these peptides was observed, it was significantly less than that to the octapeptide. Furthermore, when tested with a synthetic dimer of the octapeptide, both sera gave a binding identical to that obtained with the monomer (data not shown). This suggests that the parasite-related response of the rabbits was primarily directed to the Glu-Glu-Asn-Val-Glu-His-Asp-Ala repeats of Pf155.

The results of both the parasite and the octapeptide ELISA paralleled the ability of the rabbit antisera to stain the surface of infected RBCs in indirect IF. The latter assay recognizes Pf155 as the major antigen (6). In line with this, a polypeptide of M_r 155,000 was the only parasite-derived antigen detected when $NaDodSO_4$/PAGE-separated merozoite extracts were probed with the rabbit antisera in immunoblotting. Thus, immunization with the fusion protein gave rise to antibodies that are highly specific for Pf155. As the major part of these antibodies recognize linear sequences in the C-terminal octapeptide repeat of the molecule, the results also imply that these repeats are responsible for an important part of the surface IF of infected erythrocytes. This corroborates previously published results (12, 13).

The present experiments show that immunization of rabbits with the fusion protein gave rise to antibodies that inhibited merozoite reinvasion of RBCs *in vitro* efficiently. The inhibitory titer of the total IgG fractions from these sera was low, but after addition of high amounts of the IgG fraction, a complete reinvasion inhibition was obtained (data not shown). From this observation and from the shape of the titration curves, it could be concluded that the IgG fractions contained no material that interfered with reinvasion inhibition as previously seen with some other inhibitory sera (8, 13). Thus, the strong increase (≈1000-fold) of the inhibitory titer of the IgG obtained by affinity purification on the infected RBCs reflects an enrichment of specific antibodies, the concentration of which was low in the total IgG fraction (0.1–0.3% of total IgG). The specific inhibitory activity of the affinity-purified antibodies (1–3 μg of IgG antibody per ml for 50% inhibition) was high and comparable to that obtained with human anti-octapeptide antibodies (12).

Synthetic gene constructs similar to the one described above might be useful for future production of merozoite-directed vaccines. Recently Young *et al.* (4) described the use of polymerized restriction fragments, derived from the gene for the major circumsporozoite protein, for production of immunogens. However, the use of synthetic oligonucleotides offers a more flexible design. The method might provide a useful alternative to the use of synthetic peptides conjugated to carrier protein as immunogens. With this approach even large polypeptides can be manufactured with good precision and in high amounts at a reasonable cost.

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1. Ballou, W. R., Rothbard, J., Wirtz, R. A., Gordon, D. M., Williams, J. S., Gore, R. W., Schneider, I., Hollingdale, M. R., Beaudoin, R. L., Maloy, W. L., Miller, L. H. & Hockmeyer, W. T. (1985) *Science* **228**, 996–999.
2. Zavala, F., Tam, J. P., Hollingdale, M. R., Cochrane, A. H., Quakyi, I., Nussenzweig, R. S. & Nussenzweig, V. (1985) *Science* **228**, 1436–1440.
3. Mazier, D., Mellouk, S., Beaudoin, R. L., Texier, B., Druilhe, P., Hockmeyer, W., Trosper, J., Paul, C., Charoenvit, Y., Young, J., Miltgen, F., Chedid, L., Chigot, J. P., Galley, B., Brandicourt, O. & Gentilini, M. (1986) *Science* **231**, 156–159.
4. Young, J. F., Hockmeyer, W. T., Gross, M., Ballou, W. R., Wirtz, R. A., Trosper, J. H., Beaudoin, R. L., Hollingdale, M. R., Miller, L. H., Diggs, C. L. & Rosenberg, M. (1985) *Science* **228**, 958–962.
5. Miller, L. H., David, P. H. & Hadley, T. J. (1984) *Philos. Trans. R. Soc. London Ser. B* **307**, 99–115.
6. Perlmann, H., Berzins, K., Wahlgren, M., Carlsson, J., Björkman, A., Patarroyo, M. E. & Perlmann, P. (1984) *J. Exp. Med.* **159**, 1686–1704.
7. Wahlgren, M., Berzins, K., Perlmann, H., Wåhlin, B., Carlsson, J., Björkman, A., Perlmann, P., McNicol, L. A., Dame, J. B. & McCutchan, T. F. (1985) in *Vaccines 85*, eds. Lerner, R. A., Chanock, R. M. & Brown, F. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 51–56.
8. Wåhlin, B., Wahlgren, M., Perlmann, H., Berzins, K., Björkman, A., Patarroyo, M. E. & Perlmann, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7912–7916.
9. Wahlgren, M., Björkman, A., Perlmann, H., Berzins, K. & Perlmann, P. (1986) *Am. J. Trop. Med. Hyg.* **35**, 22–29.
10. Coppel, R. L., Cowman, A. F., Anders, R. F., Bianco, A. E., Saint, R. B., Lingelbach, K. R., Kemp, D. J. & Brown, G. V. (1984) *Nature (London)* **310**, 789–792.
11. Cowman, A. F., Coppel, R. L., Saint, R. B., Favaloro, J., Crewther, P. E., Stahl, H. D., Bianco, A. E., Brown, G. V., Anders, R. F. & Kemp, D. J. (1985) *Mol. Biol. Med.* **2**, 207–221.
12. Berzins, K., Perlmann, H., Wåhlin, B., Carlsson, J., Wahlgren, M., Udomsangpetch, R., Björkman, A., Patarroyo, M. E. & Perlmann, P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1065–1069.
13. Berzins, K., Perlmann, H., Wåhlin, B., Ruangjirachuporn, W., Högh, B., Petersen, E., Björkman, A. & Perlmann, P. (1986) *Mem. Inst. Oswaldo Cruz*, in press.
14. Spindler, K. R., Rosser, D. S. E. & Berk, A. J. (1984) *J. Virol.* **49**, 132–141.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
17. Wahlgren, M., Berzins, K., Perlmann, P. & Björkman, A. (1983) *Clin. Exp. Immunol.* **53**, 127–134.
18. Berzins, K., Wahlgren, M. & Perlmann, P. (1983) *Clin. Exp. Immunol.* **54**, 313–318.
19. Perlmann, P., Berzins, K., Björkman, A., Carlsson, J., Patarroyo, M. E., Perlmann, H., Sjöberg, K., Troye-Blomberg, M., Udomsangpetch, R., Wahlgren, M., Wåhlin, B. & Wigzell, H. (1986) in *Vaccines 86*, eds. Lerner, R. A., Chanock, R. M. & Brown, F. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 149–155.