should allow cloning of genes that are difficult to isolate in *Plasmodium* and other organisms.

THOMAS F. MCCUTCHAN
JOANNA L. HANSEN
JOHN B. DAME
JUDITH A. MULLINS
Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

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9. The λgt11 library was constructed as follows. *Plasmodium* DNA (5 to 10 μg) was digested with mung bean nuclease as described (*5*) in 30, 35, 40, and 45 percent formamide. The reaction product from each of the four reactions was diluted fourfold with 0.01*M* EDTA, treated with phenol, and precipitated in 2.5 volumes of ethanol. A portion of each DNA was analyzed by the Southern blot technique with a DNA probe homologous to either actin or tubulin. The DNA from the reactions containing "gene sized" fragments homologous to the probe were combined and used as a source of fragments to ligate into λgt11. We combined the 35 and 40 percent reaction products for *P. falciparum* libraries. (In this case the DNA was treated with Klenow fragment, but in the production of other libraries we have eliminated this step.) Eco RI linkers [Bethesda Research Laboratories (BRL)] were blunt-end–ligated to the treated fragments. After digestion with Eco RI, free linkers were separated from larger fragments by using a 1.5 by 20 cm Sepharose 4B column. λgt11 (*18*) was self-ligated and digested with Eco RI. The *P. falciparum* fragments were ligated to the prepared λgt11 DNA overnight at 12°C with T4 DNA ligase (BRL) under the conditions recommended by the supplier. The ligation reaction products were packaged into infectious phage in vitro (Promega Biotec). From an initial 3 μg of starting genomic DNA, 4×10^5 packaging events were scored by detectable interruption of the β-galactosidase gene of λgt11 on RY1090 growing on LB agar supplemented with Xgal and isopropylthiogalactoside (IPTG). A *P. knowlesi* DNA library was prepared in the same way except that we used the mung bean nuclease cleavage products obtained in 40 and 45 percent formamide.
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DNA Cloning of *Plasmodium falciparum* Circumsporozoite Gene: Amino Acid Sequence of Repetitive Epitope

Abstract. *A clone of complementary DNA encoding the circumsporozoite (CS) protein of the human malaria parasite* Plasmodium falciparum *has been isolated by screening an* Escherichia coli *complementary DNA library with a monoclonal antibody to the CS protein. The DNA sequence of the complementary DNA insert encodes a four–amino acid sequence: proline-asparagine-alanine-asparagine, tandemly repeated 23 times. The CS* β*-lactamase fusion protein specifically binds monoclonal antibodies to the CS protein and inhibits the binding of these antibodies to native* Plasmodium falciparum *CS protein. These findings provide a basis for the development of a vaccine against* Plasmodium falciparum *malaria.*

Malaria remains a formidable health problem in large areas of the world, affecting more than 150 million people a year. Of the four plasmodial species that cause malaria in humans, *Plasmodium falciparum* is responsible for most of the severe infections and the highest mortality rates. The spread of drug-resistant *P. falciparum* in many areas and the occurrence of severe epidemic outbreaks of this disease lend particular urgency to recent attempts at developing a malaria vaccine (*1*).

Under normal conditions, a malarial infection is initiated by the introduction of sporozoites into the host's bloodstream through the bite of infected mosquitoes. Hence, inactivation of the sporozoites would block completely the development of this infection. A number of recent findings point to the feasibility of developing an antisporozoite vaccine. Sporozoites are highly immunogenic and, more important, capable of eliciting a protective immune response in several host species, including humans [reviewed in (*2*)]. The immunogenicity of sporozoites resides largely, if not exclusively, in a single antigen, the circumsporozoite (CS) protein (*3*), which covers the entire parasite surface (*4*). In turn, the immunogenicity of the CS protein is restricted almost entirely to a single epitope (*3*) that is identically or quasi-identically repeated several times in tandem (*5, 6*).

Monoclonal antibodies against the CS protein (molecular weight 58,000) of *P. falciparum* sporozoites abolish the infectivity of these parasites (*7*). These monoclonal antibodies bind to a repeated epitope, which is common to different isolates of the parasite obtained from 11 areas in South East Asia, Central and South America, and West and East Africa (*8*). A prerequisite for the develop-

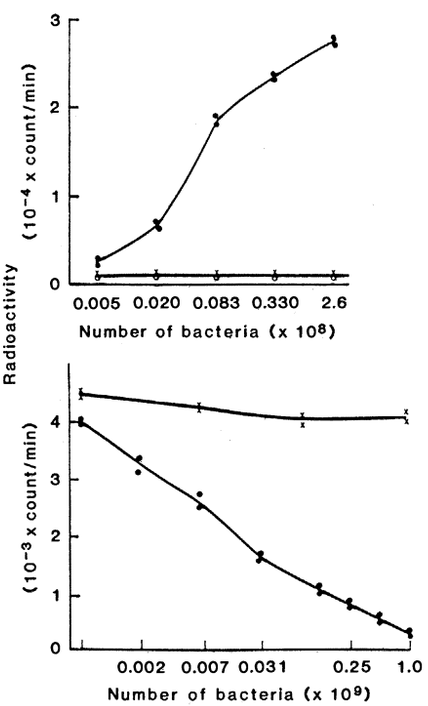

Fig. 1. (A). Two-site immunoradiometric assay. This assay was performed as described elsewhere (*17*). Briefly, wells of flexible microtiter plates (Dynatech) were coated with *P. falciparum* monoclonal antibody 2A10 to *P. falciparum*. After repeated washes with phosphate-buffered saline containing 1 percent bovine serum albumin, the wells were incubated with twofold serial dilutions of lysates of *E. coli* LE392 containing either ● p277-19 or (○) pBR322. After a 2-hour incubation at room temperature, the wells were washed and 30 μl of ^{125}I-labeled monoclonal antibody 2A10 (1×10^5 count/min) were added. After a 1-hour incubation at room temperature, the wells were washed, dried, and counted in a gamma counter. Lysates of *E. coli* LE392 containing plasmid p277-19 were also tested by using plates coated with either monoclonal antibody 2A10 or an unrelated ^{125}I-labeled monoclonal antibody (x). (B). Inhibition assay. Ten microliters (5×10^4 count/min) of ^{125}I-labeled monoclonal antibody 2A10 were incubated with 30 μl of twofold serial dilutions of lysates of *E. coli* LE392 containing either (●) p277-19 or (x) the pBR322 vector. After a 30-minute incubation at room temperature, 30 μl of these mixtures were transferred into microtiter plates that had been coated with an extract of *P. falciparum* sporozoites (*3*). After 1 hour of incubation, the wells were washed, dried, and counted in a gamma counter.

ment of a sporozoite vaccine is the identification of the amino acid sequence of the CS epitopes. This we report in the present communication.

A library of complementary DNA (cDNA) was constructed from polyadenylate [poly(A)]-containing RNA (*9*) extracted from the thoraces of laboratory-bred mosquitoes infected by membrane feeding on *P. falciparum*–infected blood obtained from patients in Thailand. By means of the dC-dG tailing method (*10*), the cDNA was inserted at the site at which the restriction enzyme Pst I cuts pBR322 in order to generate recombinant plasmids that could express the inserts as fusion proteins with the β-lactamase encoded by the vector (*11*). The resulting bacterial clones were screened for the expression of CS antigen by a filter immunoassay in situ (*12*). Approximately 10,000 colonies were screened with a monoclonal antibody (2A10) to the CS protein, and a strongly positive clone, p277-19, was identified.

Escherichia coli, strain LE 392 (*11*), was used as the host bacterium for the plasmid p277-19. Extracts of the host with the vector were then tested in a two-site immunoradiometric assay by using monoclonal antibody 2A10 immobilized in plastic wells, and the same ^{125}I-labeled antibody in the fluid phase (*13*). As shown in Fig. 1A, the recombinant protein expressed by clone p277-19 is able to bind simultaneously both the immobilized and the radioactively labeled antibody. This indicates that the recombinant protein, like the authentic CS protein, contains at least two epitopes that are recognized by monoclonal antibody 2A10. The specificity of this reaction was further verified by the inability of the same extract to react with an unrelated monoclonal antibody, and by the failure of monoclonal antibody 2A10 to bind extracts of bacterial cells containing the pBR322 plasmid without an insert. Bacterial extracts made from LE392 (p277-19) also inhibited the binding of labeled monoclonal antibody to the epitopes of native *P. falciparum* CS proteins (Fig. 1B). Further evidence of the specificity of this reaction was provided by the inability of p277-19 cell extracts to inhibit the binding of a monoclonal antibody to the corresponding CS protein of *P. berghei* (data not shown). All together, these data show that the recombinant protein encoded by p277-19 exhibits the antigenic features of the *P. falciparum* CS protein.

The nucleotide sequence of the p277-19 insert is presented in Fig. 2. The simplicity of the corresponding amino acid sequence is striking. Proline, asparagine, alanine, and asparagine are repeated 23 times in tandem, without variation. However, at the nucleotide level, the repeats present eight variants. Both of the asparagine codons, three of the four proline codons, and two of the four alanine codons are used. This repetitive pattern of four amino acids is by far the shortest of the three known CS protein repeats. In fact, the repeats of *P. knowlesi* and *P. cynomolgi* (Gombak strain), two simian malaria parasites, are 12 and 11 amino acids long, respectively (*5, 6*).

Although neither the DNA nor the protein sequences of these three sets of repeats appear related to one another, they exhibit similarities in their amino acid composition. Alanine and asparagine are present in the repeats of all three CS proteins; proline is present in *P. knowlesi* and *P. falciparum*; and glutamic acid and glycine are present in *P. knowlesi* and *P. cynomolgi* (Gombak).

The results of genomic DNA mapping experiments (data not shown) suggest that, as in the simian malarias (*14*), the CS protein of *P. falciparum* is encoded by a single-copy gene. In a series of comparative studies no hybridization could be detected between p277-19 and the DNA of *P. vivax* (another human malaria), three strains of *P. cynomolgi*, and two species of rodent malaria parasites (*P. berghei* and *P. chabaudi*) (data not shown). This is an agreement with the observations that the repetitive epitopes of the CS proteins are generally not shared by different species (*3*).

	Pro	Asn	Ala	Asn	
G[15]	CCA	AAT	GCA	AAC	
	C	T	A	C	
	A	C	A	C	
	C	T	A	T	
	T	T	A	C	
	C	T	A	T	
	T	T	A	T	
	T	T	C	T	
	A	T	A	T	
	T	T	A	C	
	C	T	A	T	
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	A	C	A	C	
	C	T	A	T	
	T	T	C	T	
	A	T	A	T	
	A	T	A	C	
	A	T	A	C	
	A	T	A	C	
	C	T	A	T	
	CCT	AAT	AAA	AAC	
	AAT	CAA	GCC	CCC	C[18]

Fig. 2. DNA sequence of the cDNA clone p277-19 encoding 23 repeats of the four amino acid sequence Pro-Asn-Ala-Asn. The sequence is aligned as a matrix with the reading frame in register with that of the β-lactamase (*18*). The sequence was derived according to the method of Maxam and Gilbert (*19*) with the Hpa II site 5′ to the Pst I site insert in pBR322 and a Hinf I site 3′ to the insert being used as labeling sites. There has been a 300-base-pair deletion in the pBR322 on the 3′ side of the insert, bringing the Hinf I site to within ten base pairs of the 3′ end of the dC-tailed cDNA insert. It has not been determined whether any of the C residues at the 3′ end of the insert are derived from the mRNA.

Work is in progress to identify precisely the minimal amino acid sequence which binds to the combining site of the monoclonal antibodies to *P. falciparum* CS protein. The shortest polypeptide tested thus far, a synthetic trimer of the tetra-amino acid (Pro-Asn-Ala-Asn), has been found to inhibit the binding of monoclonal antibodies to a sporozoite extract (*15*).

The present findings indicate that the immunodominant epitope of the CS protein of *P. falciparum* consists of a simple sequence of amino acids that does not appear to require further modification to be antigenic. This should facilitate the production of antigenically active protein through recombinant DNA methods in heterologous systems, such as bacteria and yeast. Further, the finding that a small synthetic peptide of the CS antigen of *P. knowlesi* elicits the formation of antibodies that neutralize sporozoite infectivity (*16*), should encourage efforts toward the development of a synthetic vaccine.

VINCENZO ENEA, JOAN ELLIS
FIDEL ZAVALA, DAVID E. ARNOT
Division of Parasitology,
Department of Microbiology,
New York University Medical Center,
New York 10016
ACHARA ASAVANICH
Department of Medical Entomology,
Mahidol University, 42016 Rajvthi
Road, Bangkok 10400, Thailand
AOI MASUDA
Department of Microbiology,
New York University Medical Center,
ISABELLA QUAKYI
Immunology Unit, Noguchi Memorial
Institute for Medical Research,
University of Ghana, Legon, Ghana
RUTH S. NUSSENZWEIG
Division of Parasitology,
Department of Microbiology,
New York University Medical Center

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12. This is an adaptation (*6*) of a published procedure [D. M. Helfman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 31 (1983)]. The bacterial colonies were grown on a nitrocellulose filter; the filter-containing plates were kept inverted over 0.2 ml of chloroform for about 15 minutes. The filters were then peeled off and submerged in a solution containing saline (0.15*M* NaCl, 50 m*M* tris, *p*H 7.5), 3 percent bovine serum albumin (BSA), 1 m*M* $MgCl_2$, 1 μg of deoxyribonuclease and 40 μg of lysozyme per milliliter, and 0.1 m*M* phenylmethylsulfonyl fluoride for 60 minutes at room temperature. The filters were rinsed in saline and then incubated in 3 percent BSA-saline containing ^{125}I-labeled monoclonal antibody 2A10 (5×10^5 cpm/ml; specific activity 2×10^7 cpm per microgram of protein) at room temperature for 3 hours. The unbound radioactivity was washed off in saline plus 0.1 percent NP40 at room temperature, and the filters were mounted for autoradiography. Presumptive positive clones were picked, expanded, recloned, replicated, and retested with monoclonal antibody 2A10 and other monoclonal antibodies of the same isotype but of different specificity. The purification of monoclonal antibodies and the procedures for labeling were as described [see (*3*)].
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Expression of a Retrovirus Encoding Human HPRT in Mice

Abstract. *Transmissible retroviruses encoding human hypoxanthine phosphoribosyltransferase (HPRT) were used to infect mouse bone marrow cells in vitro, and the infected cells were transplanted into mice. Both active human HPRT-protein and chronic HPRT-virus production were detected in hematopoietic tissue of the mice, showing transfer of the gene. These results indicate the possible use of retroviruses for somatic cell therapy.*

The unique structure and mode of propagation of retroviruses make them ideally suited for gene transfer. These features include efficient transmission to recipient cells, integration into host chromosomal DNA, plasticity of the viral genome for accommodation of foreign DNA, and a wide cell type and host range. We have described the construction of an infectious retrovirus that contains the coding sequences for human hypoxanthine phosphoribosyltransferase (HPRT; E.C. 2.4.2.8) as a model system (*1*). In this vector (pLPL), a complementary DNA (cDNA) copy of the human gene coding for HPRT is expressed under the transcriptional control of viral long terminal repeats (LTR's). Several investigators have used similar vectors to transfer other genes into cultured cells (*2*). We now show that both enzymatically active human HPRT-protein and production of HPRT-virus can be detected in hematopoietic tissue of mice receiving transplants of bone marrow cells infected with HPRT-virus.

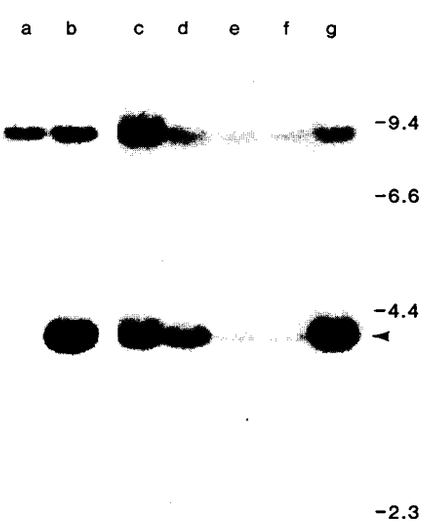

Fig. 1. Analysis of DNA from cultured cells converted to HPRT$^+$ by exposure to tissues from infected mice. HRPT$^+$ colonies induced by exposure to tissues of infected mice were propagated in bulk, and the DNA was extracted and analyzed by Southern transfer (*12*) with the use of an isotopically labeled probe made from a Pst I–Hind III fragment of the HPRT cDNA. (Lane a) Uninfected HPRT$^-$ cells; (lanes b and g) cultured cells infected with the virus used to infect the mice (c7c1); (lane c) DNA from HPRT$^+$ cells induced by exposure to spleen cells from a mouse 41 days after it was injected with bone marrow cells; (lane d) DNA from HPRT$^+$ cells induced by bone marrow cells from the same mouse; and (lanes e and f) DNA from HPRT$^+$ cells induced by spleen cells from two different mice 133 days after they were injected with bone marrow cells.

Mouse bone marrow cells were infected with HPRT-virus either by cocultivation with virus-producing cells or by direct infection with high-titer virus. Helper virus [Moloney strain (MoMLV)] was present in all infections so that rescue of replication-defective HPRT-virus could be monitored in recipient animals. We chose to infect bone marrow cells because pluripotent, self-renewing hematopoietic stem cells in bone marrow are probably among those cells that can be infected (*3*) and because bone marrow cells are readily accessible and have a high proliferation rate after reintroduction into mice. The infected bone marrow cells were then injected into irradiated mice of the same strain as the donor mouse. The radiation dose was chosen so that transplantation of fresh bone marrow was a requirement for survival of the mice. Thus, certain hematopoietic cellular compartments (bone marrow and spleen) depleted by irradiation were repopulated by the virus-infected bone marrow cells.

Spleen and bone marrow cells from mice receiving infected bone marrow were analyzed for production of HPRT-virus. It was expected that some hematopoietic cells from the mice receiving infected bone marrow would be infected with both the HPRT-virus and helper virus and thus would produce HPRT-virus. Spleen and bone marrow cells from mice receiving bone marrow transplants were removed and overlaid on adherent HPRT$^-$, ouabain-resistant cultured mouse cells (*4*). One day later the dishes were washed thoroughly to remove unattached cells and the remaining attached cells were exposed to selective medium containing HAT (see legend to Table 1) and ouabain. This selection ensures that only cultured cells converted to HPRT$^+$ by virus will survive because ouabain kills the donor mouse cells and HAT selects against HRPT$^-$ cells. The development of colonies of cells surviving HAT selection is thus dependent on production of HPRT-virus by the donor