MOLECULAR CLONING OF *PLASMODIUM FALCIPARUM* BLOOD STAGE ANTIGENS AND APPLICATION OF THE RECOMBINANT PROTEINS IN SERODIAGNOSIS

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Abstract. A *Plasmodium falciparum* genomic DNA library was established in the expression vector lambda gt11, cloned in *Escherichia coli.* The library was screened with human hyperimmune sera by *in situ* hybridization. Twenty clones expressing *P. falciparum* sequences as polypeptides fused to β-galactosidase were identified. One, CD3A/9025/60, reacted with all immune sera and expressed polypeptides that were larger than β-galactosidase as well as reacting with antibodies to β-galactosidase and to *P. falciparum.* When the fusion proteins were used as target antigens to diagnose malaria antibodies, a result was obtained which correlated well with indirect fluorescence assay.

INTRODUCTION

Serologic assay is a major means of measuring a specific immune response that is useful in epidemiological studies of malaria. For serodiagnosis, reliable antigens are important. However, the purification of blood-stage *Plasmodium falciparum* proteins by conventional procedures from parasites grown *in vitro* (Trager and Jensen, 1976) is seriously limited by the requirements for human erythrocytes and serum in the culture system. Using recombinant DNA techniques to express antigens from blood stage of *P. falciparum* in *Escherichia coli* can circumvent these problems. We describe here the construction of a *P. falciparum* genomic DNA library in expression vector lambda gt11, screening of the library with human immune sera and examination of the feasibility of using fusion proteins as target antigens in detection of malaria antibody.

MATERIALS AND METHODS

Construction of a *P. falciparum* genomic DNA library

Genomic DNA of *P. falciparum,* Malayan camp strain parasites, knob-positive, cytoadherence-positive from *Aotus* monkeys, was prepared from the parasites by phenol extraction and ethanol precipitation (Wallach *et al,* 1984), treated with EcoR1 methylase, then digested with DNAse 1 to produce fragments of approximately 1 Kb. After repair and addition of EcoR1 linkers, these DNA fragments were ligated to the EcoR1 site of lambda gt11 (Young and David, 1983). The ligated DNA was packaged *in vitro* (Maniatis *et al,* 1982) and plated onto the R^-M^+ derivative of *E. coli* Y1090.

Screening of the DNA library

The library was screened firstly with antibodies from an *Aotus* monkey (animal 9025) infected previously with the Malayan camp strain. Then the positive clones were plaque-purified and re-screened with human immune serum probes (Kemp *et al,* 1983). Antibodies to *E. coli* in the human sera collected from a *P. falciparum* endemic area of Papua New Guinea (PNG) were removed by incubating serum with lambda gt11 infected *E. coli* Y1090 at the radio of 5:1 for 1 hour at 4°C.

Analysis of fusion proteins by Western blot

The clones reacting with all human sera were picked and recombinant proteins as the β-galactosidase fusion proteins were isolated and fractionated on 7.5% polyacrylamide/SDS gels. The gels were silver stained and proteins from the gels were transferred electrophoretically to nitrocellulose filters and incubated for 1 hour at 4°C in 5%

milk-PBS before reaction for 2 hours with rabbit anti-β-galactosidase or anti-*P. falciparum* antibodies. The filters were then washed, reacted with anti-human or anti-rabbit horseradish peroxidase conjugated IgG, rewashed, and developed by incubation with the substrate 4-chloro-1-naphthol.

Identification of fusion protein antigenic specificity by Dot-ELISA

The supernatant containing the fusion protein of induced recombinant phage was employed to bind nitrocellulose filters using a BIO-DOT apparatus. The procedure of Dot-ELISA was performed as described (Londner *et al,* 1987) to detect malaria antibodies.

RESULTS

Identification of *E. coli* clones that express *P. falciparum* antigens

When the genomic DNA library was screened with serum, the ^{125}I-labeled anti-*P. falciparum* antibodies, from an *Aotus* monkey infected previously with the Malayan camp strain, twenty clones were found to be expressing antigen (Fig 1). The 20 positive clones were plaque-purified, plated and reacted separately with 30 human immune sera from patients with history of *P. falciparum.* Two, CD3A/9025/56, CD3A/9025/60, reacted detectably with all immune sera (Fig 2) but not with normal human sera. The clone CD3A/9025/60 was selected for further study.

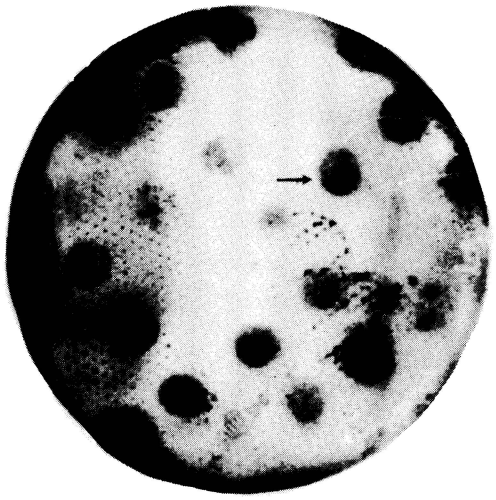

Fig 1—Library screening.
Arrow: positive clones identified by monkey anti-*P. falciparum* serum.

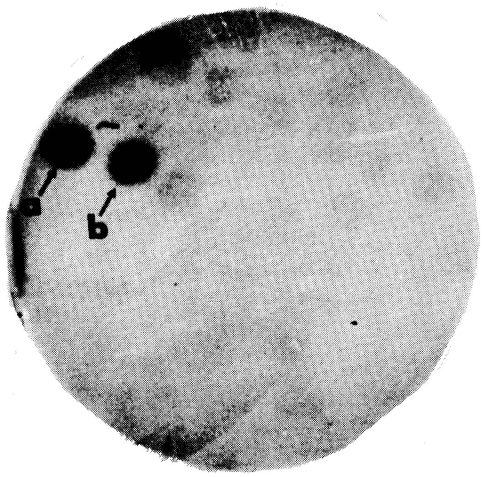

Fig 2—Screening of 20 clones with one of 30 human immune sera.
a) CD3A/9025/56, b) CD3A/9025/60.

***P. falciparum* antigens expressed as fusion protein to β-galactosidase**

We examined proteins from lysates of induced antigen-positive clone CD3A/9025/60 by SDS/PAGE. Staining with silver revealed that the clone produced abundant unique polypeptides larger than β-galactosidase (Fig 3). When the proteins from CD3A/9025/60 were transferred from gel to nitrocellulose filter and probed with anti-β-galactosidase rabbit serum, the unique large polypeptides from clone CD3A/9025/60 reacted strongly with anti-β-galactosidase serum. When equivalent filters were probed with anti-*P. falciparum* human serum, the results were identical to rabbit anti-β-galactosidase serum. As expected, no reaction was observed in wild-type lambda gt11 control (Fig 4).

Feasibility of fusion protein as antigen for serodiagnosis

Dot-ELISA using supernatant containing the recombinant protein as the target antigen was used to detect the presence of antibodies in 48 human serum specimens collected in an area of holoendemic malaria transmission in PNG. Forty-

five out of 48 sera were judged to be positive when the sera were diluted to 1 : 100, a positive rate of 93%. To verify the results, all 48 human sera were tested by IFA which used whole parasites as target antigens. A good correlation was obtained between the two methods (Fig 5, Table 1).

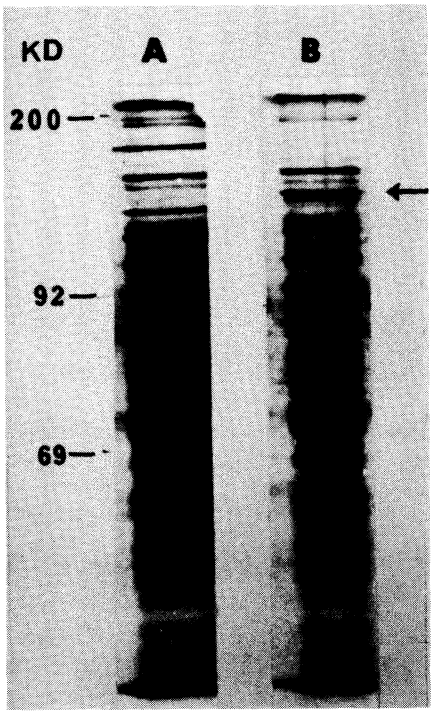

Fig 3—Electrophoretic pattern of the fusion protein.
A: lambda gt11.
B: recombinant phage clone CD3A/9025/60.

DISCUSSION

The blood stages of *P. falciparum* contain a plethora of natural immunogens, but which of these many immunogens are important in naturally acquired host-protective immunity is not clear. The identification of immunogenic proteins not only offers avenues for vaccine production but allows development of immunoassays that will detect specific antibodies in human or animal sera. In order to obtain clinically important malarial antigens, we used immune sera from human containing many anti-*P. falciparum* specifities to isolate genomic DNA clones expressing different *P. falciparum* antigens. Clone CD3A/9025/60 produced

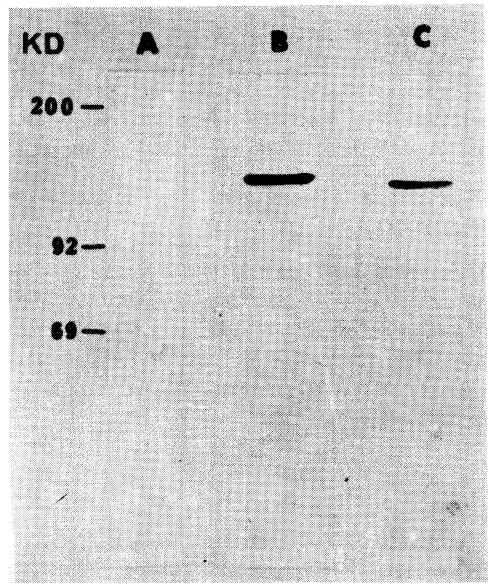

Fig 4—The result of Western blot.
A: lambda gt11.
B: CD3A/9025/60 clone reacted antibody of β-galactosidase.
C: CD3A/9025/60 clone reacted with human serum with anti-*P. falciparum* antibody.

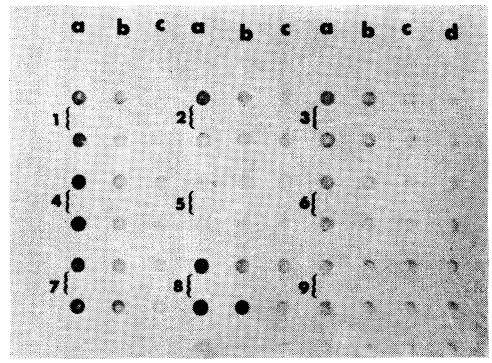

Fig 5—The result of DOT-ELISA.
a,b,c : dilution of serum: 1 : 100, 1 : 1,000, 1 : 10,000.
1,2,3,4,7,8: positive sera in both DOT-ELISA and IFA.
5,6,9: DOT-ELISA negative, IFA positive.
(10 μl supernatant antigen was used to coat nitrocellulose).

Table 1

Comparison between Dot-ELISA and IFA*.

Method	No. tested	Positive	Rate(%)
DOT-ELISA	48	45	93
IFA	48	48	100

* Dilution of serum: 1 : 100.

β-galactosidase fusion proteins of about 150 kDa molecular weight. The finding that the selected fusion protein reacted strongly with both rabbit and human antisera indicates that CD3A/9025/60 apparently expresses an antigenic peptide sequence of *P. falciparum.*

A large antigenic protein, MESA, with the molecular weight of about 300 kDa, accumulates under the plasma membrane when red blood cells are infected with mature asexual stages of *P. falciparum.* Further study showed that the fusion protein from CD3A/9025/60 reacted strongly with polyclonal antibodies from rabbit antiserum raised by immunization with affinity purified MESA, and antibodies reacting with the recombinant protein expressed by CD3A/9025/60 also reacted with MESA of the parasite (data not shown). So we can tentatively conclude that the recombinant protein expressed by CD3A/9025/60 may be a fragment of MESA.

We used crude extracts of fusion proteins which contained a number of *E. coli* proteins to detect *P. falciparum* antibodies, but no non-specific reaction was found between the fusion protein and human serum. It showed that the fusion protein had a high specificity and strong affinity, and the antigenicity of the *P. falciparum* antigen was retained but *E. coli* proteins were not detected when the amount of antigen supernatant used to coat the nitrocellulose paper was very small (10μl supernatant in this study). The Western blot gave only one band of fusion protein also supported this conclusion.

ACKNOWLEDGEMENTS

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