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PROGRESS REPORT NO. 1

DNA PROBE FOR THE DIAGNOSIS OF VIVAX MALARIA

A RESEARCH PROJECT

U.S. - ISRAEL COOPERATIVE DEVELOPMENT

RESEARCH PROGRAM

GRANT NO. 936-5544-G-00-8047-00

SUBMITTED BY

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PROJECT PROFILE

Country : Thailand

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CONTENTS

1. Background/Introduction

Malaria is still an important public health problem plaguing the economic development of countries of the third world (1). Vivax malaria, which is caused by the parasitic protozoan *Plasmodium vivax*, is second in occurrence to falciparum malaria which is the most frequency worldwide disease caused by *P. falciparum*. Although vivax malaria does not cause as much mortality as falciparum malaria, nevertheless, it causes incapacitation by preventing people from work and thus undermines productivity of the nation. In addition, *P. vivax* is the major species responsible for relapsing malaria (2).

For more than a century, microscopic examination of blood smears has been used for diagnosis of malaria. However, this technique is laborious, requiring a well-trained personnel, difficult to reproduce quantitatively and not suitable for surveillance or for a large scale epidemiological studies. Very recently, DNA probes have been developed and used for rapid diagnosis of infections caused by many infectious agents including viruses, bacteria and protozoa (3,4,5,6,7,8). The DNA probes specific for *P. falciparum* have been reported but none to date for *P. vivax* (9,10,11,12,13).

2. Objectives : The objectives of the present study are the followings :

1. To construct genomic DNA library of *P. vivax* in appropriate vectors of *Escherichia coli*.

2. To identify and isolate the appropriate vectors containing P. vivax DNA inserts for use as probes for detection of P. vivax in clinical specimens.

3. To apply the selected DNA probes as a tool for specific diagnosis of P. vivax infection in the field.

3. Methods and Results

3.1 Purchase of equipment

Equipment purchased

3.1.1 Microwave oven

3.1.2 Vaccuum pump and accessory

3.1.3 Agarose electrophoresis system

Equipment being ordered

3.1.4 Microcentrifuge

3.1.5 UV Transluminator

3.1.6 Polaroid camera and accessories

3.1.7 Shaking water bath

3.2 Collection of P. vivax infected cells

P. vivax infected red cells were obtained from patients with vivax malaria admitted to the Bangkok Hospital for Tropical Diseases of the Faculty of Tropical Medicine. The parasitemia varied from 0.1% to 0.7%. They were enriched using the method previously described (14). Briefly, the infected blood was collected in heparinized tube (15 IU heparin/ml). The specimens were either processed immediately or kept overnight at 4°C. The infected blood was centrifuged at 500xg for 10 minutes

and the plasma was collected. The cell sediments were resuspended to the original volume with phosphate buffered saline pH 7.5 (PBS) and a one ml aliquot was passed through a column consisting of an equal volume of sulfoethyl cellulose and Sephadex G-25 previously equilibrated with PBS. The columns were washed with 40 ml of PBS, the eluates were centrifuged and the supernates were discarded. The cell sediments were pooled and reconstituted to a 50% hematocrit. Each two ml of the cell suspension was layered on top of discontinuous gradient consisting of successive layers of two ml each of 75%, 60%, 50% and 40% of Percoll (Pharmacia-LKB) in the same buffer. After centrifugation at 2,600 rpm for 10 minutes at 4°C, three bands of cells were observed. The first two bands localized in 50% and 60% Percoll consisted of 30%-99% of red cells infected of mature forms of P. vivax (Figure 1) while the third band at the interface between 60% and 75% Percoll consisted of 20%-99% of red cells infected with ring stages of the parasites (Figure 2).

It was found in the present study that the enrichment process gave high efficiency when fresh blood from the patients were used. Eighty-five blood samples were processed and approximately 1.5×10^9 P. vivax infected cells were obtained. All samples were kept at -70°C for further use in the recombinant DNA work.

Furthermore, approximately 50-100 µl of P. vivax infected blood were collected during August to September 1988 in capillary tubes from 150 patients with vivax malaria in Amphur Mad Sod, Tak Province and kept at -20°C for DNA probing.

3.3 Collection of antisera

One hundred and fifteen plasma samples were obtained from patients with vivax malaria admitted to the Bangkok Hospital for Tropical Diseases. The plasma were kept in small aliquots at -20°C for further screening for specific antibodies against P. vivax.

3.4 Construction of P. vivax genomic DNA library

A preliminary work on construction of P. vivax genomic DNA library was done through the assistance of Dr. Chev Kidson at the Queensland Institute of Medical Research, Australia. Enriched P. vivax infected cells from 4 vivax malaria patients were lysed by saponin and digested by proteinase K and the DNA was extracted (15). The purified DNA was partially cleaved with Sau3A and ligated to the Bam H1 site of pUC13 (Pharmacia-LKB) then transformed into Escherichia coli JM109 host cells. Transformed colinies were transfered to nitrocellulose filters and screened with ^{32}P-labeled nick-translated human, P. falciparum or P. vivax DNA. A clone containing P. vivax DNA insert was found and is now being characterized.

4. Conclusion/Remark

5. Workplan for the Next Period

5.1 Construction of P. vivax genomic DNA library using lambda phage vector EMBL and Escherichia coli (to be done at the Hebrew University of Jerusalem, Israel).

5.2 Screening of the phage vector containing P. vivax DNA inserts (to be done at the Hebrew University of Jerusalem, Israel and Mahidol University).

5.3 Field blood collection. Arrangement will be made with either with the Malaria Division, Ministry of Public Health or other institution to collect blood specimens from individuals living in malaria endemic areas.

5.4 Characterization of P. vivax antigens using immune plasma from patients with vivax malaria (to be done at Mahidol University).

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7. Figures

Figure 1. Erythrocytes infected with mature forms of *P. vivax* enriched by Percoll discontinuous gradient centrifugation A:trophozoites and B:schizonts

A

B

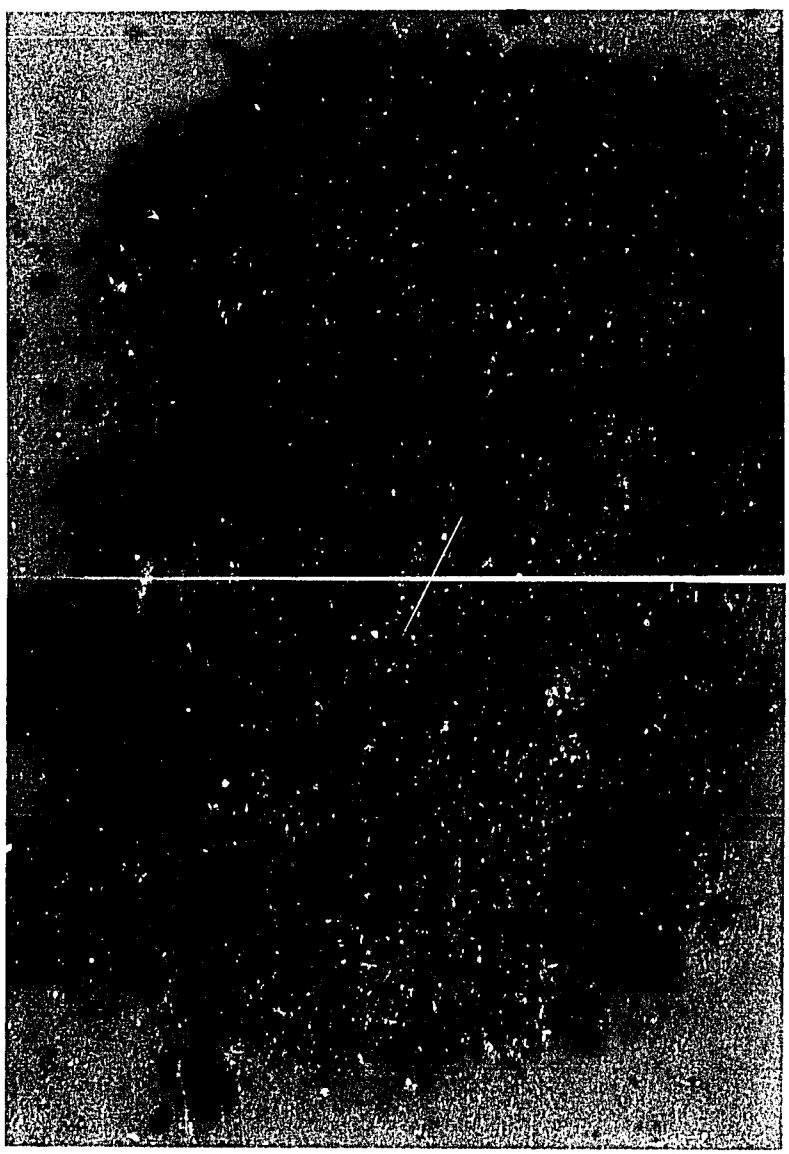

Figure 2. Erythrocytes infected with ring forms of *P. falciparum* enriched by Percoll discontinuous gradient centrifugation