

CONTENTS

ABBREVIATIONS

i - iv

CHAPTER I. General introduction on the disease, rationale and objectives of the study.

1- 16

- Introduction and brief history
- Life cycle of the malaria parasite
- Pathogenesis of the disease in human
- Host genetics and malaria
- Host immunity and malaria
- Malaria control and pitfalls
- Rationale and objectives of the present study

CHAPTER II. Review of literature on cross-reactive, conserved vaccine candidates in different life cycle stages of malarial parasite and drug resistance.

17-28

CHAPTER III. Identification and isolation of gene encoding cross-reactive antigen of *Plasmodium falciparum*.

29- 44

Brief Introduction.

Experimental Methodology.

I: Parasite materials.

- a. *In-vitro* culture of *P. falciparum*
- b. Synchronization of *P. falciparum*
- c. *In-vivo* maintenance of *P. yoelii* in mouse
- d. Removal of white blood cells from infected RBC

II. Construction of *P. falciparum* Genomic expression library in λ gt 11.

- a. Isolation of *P. falciparum* DNA
- b. Insert DNA preparation
- c. Ligation of insert in to the λ gt11 vector
- d. *In-vitro* packaging
- e. Titration of and amplification of phage library

III. Immunoscreening of the λ gt11- *P. falciparum* genomic expression library.

- a. Preparation of convalescent phase mouse anti- *P. yoelii* sera
- b. Human sera from malaria endemic area
- c. Identification of immuno-reactive clone

Results.

Discussion.

CHAPTER IV. Insert size determination and sequence analysis. 45- 67

Introduction.

Experimental Methodology.

I. Insert size determination of the seropositive clone.

- a. Preparation of recombinant phage DNA
- b. Restriction digestion of Phage DNA
- c. PCR amplification of the insert

II. Insert DNA purification from the gel.

III. Human DNA and other malaria parasite DNA.

IV. Southern hybridization.

- a. Preparation of probe
- b. Restriction digestion and gel electrophoresis of DNA
- c. Capillary blotting of DNA to nitrocellulose membrane

V. Dot blot hybridization.

VI. Sub-cloning of insert in pBKS plasmid.

- a. Ligation of insert to pBKS vector
- b. Competent cell preparation
- c. Transformation
- d. Screening of transformants
- e. Large scale DNA preparation of the recombinant plasmid

VII. Sequencing of the insert and sequencing analysis.

Introduction.

Experimental Methodology.

I. Sub-cloning of insert into the pGEX-4T1 expression vector.

- a. Preparation of expression vector arm
- b. Ligation of insert in frame into pGEX- 4T1

II. Small scale expression of recombinant clones.

- a. SDS- PAGE analysis of the recombinant protein
- b. Immuno-blotting to identify expressed recombinant protein

III. Purification of recombinant fusion protein.

IV. Immunization of mice with recombinant protein to raise antibodies.

V. Identification of native protein in the parasite lysate.

VI. Intra erythrocytic *P. falciparum* growth inhibition effect of the antibodies.

VII. Immunofluorescent antibody test.

Results.

Discussion.

CHAPTER VI. Stage specific expression of the cloned protein, full-length characterization and comparative sequence analysis.

Introduction.

Experimental Methodology.

I. RNA isolation.

II. Northern blotting.

- a. Agarose/Formaldehyde gel electrophoresis of RNA
- b. Transfer of RNA to nitrocellulose membrane
- c. Hybridization analysis.

III. RT- PCR assay.

- a. 1st strand cDNA synthesis
- b. PCR amplification

IV. Isolation of full length gene of karyopherin beta of *P. falciparum*.

A. Rapid amplification of cDNA ends

- a. 3' Rapid Amplification of cDNA end (3'RACE)
- b. 5' Rapid Amplification of cDNA end (5'RACE)

B. Use of genome data bases to isolate full-length karyopherin gene of *P. falciparum*

V. Characterization of full-length karyopherin gene of *P. yoelii*.

VI. Comparative sequence analysis.

Results.

Discussion.

REFERENCES