

**MOLECULAR INTERACTIONS INVOLVED  
IN ERYTHROCYTE INVASION BY  
MALARIA PARASITE**

Thesis Submitted to the

**Jawaharlal Nehru University**



for the Award of the Degree of  
**DOCTOR OF PHILOSOPHY in MOLECULAR GENETICS**

by

**RICCARDO S. GATTA**



**International Centre for Genetic Engineering  
and Biotechnology**

New Delhi-110067

INDIA

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## INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

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ICGEB Campus, P.O. Box: 10504, Aruna Asaf Ali Marg,  
New Delhi – 110 067, India  
<http://www.icgeb.org>

Tel : 91-11-6189358 / 60 / 61  
Fax: 91-11-6162316

APRIL 14, 2003

### CERTIFICATE

The research work embodied in this thesis entitled '**Molecular Interactions Involved in Erythrocyte Invasion by Malaria Parasite**' has been carried out in the ICGEB, New Delhi. This work is original and has not been submitted so far in part or in full, for the award of any degree or diploma of any university.

***RICCARDO S. GATTA***  
(Candidate)

***Dr. Chetan E. Chitnis***  
(Supervisor)

***Prof. Virander. S. Chauhan***  
(Director)

April 2003  
INTERNATIONAL CENTER FOR GENETIC  
ENGINEERING AND BIOTECHNOLOGY.  
New Delhi-110067  
India.

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*Dante Alighieri,  
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*E quindi uscimmo a riveder le stelle.*

*Inferno, Canto XXXIV, 139*

**RICCARDO S. GATTA**

***Dedicated to***

***my Parents,  
my Grandparents,  
my Family in the old world,  
Tereza and my new Family in Nagaland, and to  
my Friends***

***without whose support this would have remained...***

*"...but a dream within a dream."*

*Edgar Allan Poe*

*Nil sapientiae odiosius acumine nimio.*

*Seneca*

## Abbreviations

|                    |  |
|--------------------|--|
| 6xHis              | Hexa-histidine tag                                       |
| aa                 | amino acid   |
| Ab                 | Antibody   |
| <i>AcNPV</i>       | <i>Autographa californica</i> Nuclear Polyhedrosis Virus |
| AIDS               | Acquired Immune Deficiency Syndrome                      |
| AMA                | Apical Membrane Antigen                                  |
| Amp                | Ampicillin   |
| APS                | Ammonium PerSulfate                                      |
| ATCC               | American Type Cell-culture Collection                    |
| bp                 | base pair  |
| B.C.E.             | Before Christian Era                                     |
| $\beta$ -ME        | $\beta$ -MercaptoEthanol                                 |
| BEVS               | Baculovirus Expression Vector System                     |
| BHC                | Benzene-HexaChloride                                     |
| BSA                | Bovine Serum Albumin                                     |
| BV                 | BaculoVirus  |
| CBB                | Commassie Brilliant Blue                                 |
| C.E.               | Christian Era  |
| cFA/iFA            | Complete/Incomplete Freund's Adjuvant                    |
| CPD                | Citrate Phosphate Dextrose                               |
| CpG                | Cytosine-phosphate-guanosine dinucleotide                |
| CR1                | Complement Receptor 1                                    |
| CSA                | Chondroitin Sulfate A                                    |
| CSP                | CircumSporozoite Protein                                 |
| C'-terminal        | Carboxyl-terminal  |
| DAB                | 3,3'-DiAminoBenzidine                                    |
| DABP               | Duffy Antigen-Binding Protein                            |
| DARC               | Duffy Antigen Receptor for Chemokines                    |
| DBL                | Duffy-Binding Ligand                                     |
| DBP                | Duffy-Binding Protein                                    |
| DBPT               | DiButylPhThalate   |
| °C                 | Degree Celsius   |
| ddH <sub>2</sub> O | double distilled water                                   |

|                |  |
|----------------|--|
| DDT            | 1,1,1-trichloro-2,2-bis(parachlorophenyl) ethane   |
| DMEM           | Dulbecco's Modified Eagles Medium  |
| DNA/RNA        | Deoxyribose/Ribose Nucleic Acid  |
| dNTP           | deoxyNucleotide TriPhosphate   |
| DOPE           | dioleoylphosphatidyl ethanolamine  |
| DOSPA          | 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propaninium trifluoroacetate |
| DTT            | DiThioThreitol   |
| EBA            | Erythrocyte Binding Assay  |
| EBA-175        | Erythrocyte-Binding Antigen-175  |
| EBP            | Erythrocyte Binding Protein  |
| <i>E. coli</i> | <i>Escherichia coli</i>  |
| EDTA           | Ethylene Diamine Tetra Acetic acid   |
| EGF            | Epidermal Growth Factor  |
| ELISA          | Enzyme-Linked Immuno-Sorbant Assay   |
| EMP            | Erythrocyte Membrane Protein   |
| FITC           | Fluorescein IsoThioCyanate   |
| FT             | Flow-Through   |
| g              | gram   |
| GAG            | GlycoAmino-glycan  |
| GFP/BFP/YFP    | Green/ Blue/ Yellow Fluorescent Protein tag  |
| GPI            | GlycosylPhosphatidyl-Inositol  |
| GST            | Glutathione-S-Transferase tag  |
| G-TS           | Giemsa Thick-Smear   |
| h              | hour   |
| HA             | Hyaluronic Acid  |
| HC             | HematoCrit   |
| HPLC           | High Performance Liquid Chromatography   |
| HRP            | Histidine Rich Protein   |
| <i>HSVgD</i>   | <i>Herpes Simplex Virus</i> glycoprotein D   |
| IAA            | IodoAcetAmide  |
| IC             | Immuno-Chromatographic   |
| ICAM-1         | IntraCellular Adhesion Molecule  |
| ICT            | Immuno-Chromatographic Test  |
| Ig             | Immunoglobulin   |
| IL             | Interleukin  |
| INF            | Interferon   |
| IPTG           | IsoPropyl $\beta$ -D-ThioGalactopyranoside   |
| kb             | kilo base  |

|                          |  |
|--------------------------|--|
| kD                       | kilo Dalton                                      |
| LAPDAP                   | Combination drug with chlorproguanil and dapsone |
| LB                       | Luria Broth                                      |
| μ                        | micron   |
| μg                       | micro gram                                       |
| μl                       | micro litre                                      |
| μM                       | micro Molar                                      |
| M                        | Molarity   |
| mAb                      | monoclonal Antibody                              |
| MCS                      | Multi-Cloning Site                               |
| ME                       | Multiple T cell Epitopes                         |
| MGSA                     | Melanoma Growth Stimulatory Activity             |
| MHC                      | Major Histocompatibility Complex                 |
| min                      | minute   |
| ml                       | milli litre                                      |
| mM                       | milli Molar                                      |
| MOI                      | Multiplicity Of Infection                        |
| MSP                      | Merozoite Surface Protein                        |
| N                        | Normality  |
| ng                       | nano gram  |
| NIH                      | National Institute of Health (USA)               |
| Ni-NTA                   | Nickle-NitriloTriacetic Acid                     |
| nM                       | nano Molar                                       |
| N <sup>7</sup> -terminal | Amino-terminal                                   |
| O/N                      | OverNight  |
| OD <sub>x nm</sub>       | Optical density (taken at x nm)                  |
| OPD                      | o-Phenylene diamine dihydrochloride              |
| <i>P.</i>                | <i>Plasmodium</i>                                |
| PAGE                     | PolyAcrylamide Gel Electrophoresis               |
| PBS                      | Phosphate Buffered Saline                        |
| PCR                      | Polymerase Chain Reaction                        |
| PCV                      | Packed Cell Volume                               |
| %                        | Percentage                                       |
| pfu                      | plaque forming unit                              |
| pi                       | post-infection                                   |
| ppt                      | precipitate                                      |
| PRBC                     | Parasitized Red Blood Cell                       |
| PVDF                     | PolyVinilidenDiFluoride                          |

|                  |  |
|------------------|--|
| PVM              | Parasitophorous Vacuole Membrane                               |
| RAP              | Rhoptry-Associated Protein                                     |
| RATES            | Regulated upon Activation normal T-cell Expressed and Secreted |
| RBC              | Red Blood Cell   |
| RESA             | Ring-stage Erythrocyte Surface Antigen                         |
| RIMA             | Ring-Infected Membrane Antigen                                 |
| RP-HPLC          | Reverse Phase High Performance Chromatography                  |
| rpm              | Revolutions Per Minute   |
| RPMI             | Roswell Park Memorial Institute culture medium                 |
| RT               | Room Temperature   |
| <i>S.</i>        | <i>Spodoptera</i>  |
| SAP              | Shrimp Alkaline Phosphatase                                    |
| SD               | Standard Deviation   |
| SDS              | Sodium Dodecyl Sulphate  |
| SDS-PAGE         | Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis     |
| <i>Sf</i>        | <i>Spodoptera frugiperda</i>                                   |
| SP               | combination of Sulfadoxime and Pyrimethamine                   |
| SUB              | SUBtilisin-like Proteins                                       |
| TBV <sub>s</sub> | Transmission Blocking Vaccines                                 |
| TE               | Tris-EDTA  |
| TEMED            | N,N,N',N'-Tetramethyl Ethylene Diamine                         |
| TFA              | TriFluoroacetic Acid   |
| TNM-FH           | <i>Tricopulsia ni</i> Medium-Formulation Hank                  |
| TNF              | Tumour Necrosis Factor   |
| TRAP             | Trombospondin Related Apical Protein                           |
| Tris             | Tris (hydroxy methyl) amino methane                            |
| U                | Unit   |
| UV               | Ultraviolet  |
| VLP              | Virus-Like particles   |
| WHO              | World Health Organization                                      |
| wt               | wild type  |



## TABLE OF CONTENTS

|   | <b>Page</b> |
|---|-------------|
| Frontispiece  | i           |
| Certificate   | ii          |
| Acknowledgements  | iii         |
| Abbreviations   | v           |
| Table of Contents   | ix          |
| <br>  |             |
| <b>1 INTRODUCTION AND REVIEW OF LITERATURE</b>  | <b>1</b>    |
| <br>  |             |
| <b>1.1 General Introduction to Malaria</b>  | <b>1</b>    |
| 1.1.1 The causative agent of malaria  | 2           |
| 1.1.2 The burden of malaria   | 3           |
| <br>  |             |
| <b>1.2 The Biology of the Malaria Parasite</b>  | <b>5</b>    |
| 1.2.1 Life cycle of the malaria parasite  | 5           |
| 1.2.2 Morphology and characteristics of <i>Plasmodium</i> spp.  | 9           |
| 1.2.3 Special characteristics of <i>P. vivax</i>  | 11          |
| <br>  |             |
| <b>1.3 Morphology of Erythrocyte Invasion</b>   | <b>13</b>   |
| 1.3.1 Invasion of erythrocytes by malaria parasites   | 13          |
| 1.3.2 Erythrocyte receptors   | 14          |
| 1.3.3 Parasite ligands  | 16          |
| <br>  |             |
| <b>1.4 Vaccine Development</b>  | <b>21</b>   |
| 1.4.1 Concept and approach for the development of malaria vaccines  | 21          |
| 1.4.2 Status of vaccine and development efforts   | 23          |
| 1.4.3 Resistance to malaria: genetic, natural and acquired immunity   | 27          |
| <br>  |             |
| <b>1.5 Recombinant Protein Expression Methods</b>   | <b>28</b>   |
| 1.5.1 Review of methods available for expressing malaria proteins   | 28          |
| 1.5.2 Baculovirus expression vector system  | 29          |
| <br>  |             |
| <b>1.6 Summary of Thesis: Studies on Recombinant PvRII, the Binding Domain of <i>P. vivax</i> Duffy Binding Protein</b> | <b>31</b>   |

|            |  |           |
|------------|--|-----------|
| <b>2</b>   | <b>MATERIALS AND METHODS</b>   | <b>33</b> |
| <b>2.1</b> | <b>Plasmid Construct for Recombinant PvRII Expression in Insect Cells</b>        | <b>33</b> |
| 2.1.1      | Preparation of plasmid DNA for use in insect cells                               | 34        |
| <b>2.2</b> | <b>Generation of Recombinant Baculovirus for Expression of PvRII</b>             | <b>35</b> |
| 2.2.1      | Insect cell line used for generation of recombinant virus and protein expression | 35        |
| 2.2.2      | Generation of recombinant baculovirus by co-transfection                         | 35        |
| 2.2.3      | Plaque assay   | 36        |
| 2.2.4      | End point dilution assay   | 37        |
| <b>2.3</b> | <b>Expression and Characterization of Recombinant PvRII from Insect Cells</b>    | <b>38</b> |
| 2.3.1      | Preliminary screening for best PvRII producing virus                             | 38        |
| 2.3.2      | Screening recombinant virus and production of PvRII                              | 38        |
| 2.3.3      | Purification of recombinant PvRII using metal affinity chromatography            | 39        |
| 2.3.4      | Western blot analysis of PvRII   | 40        |
| 2.3.5      | Mobility of native and reduced PvRII on SDS-PAGE                                 | 40        |
| 2.3.6      | Analysis of purified PvRII by reverse phase chromatography                       | 41        |
| 2.3.7      | Chymotrypsin treatment of erythrocytes   | 41        |
| 2.3.8      | Erythrocyte binding assay on soluble recombinant PvRII                           | 41        |
| <b>2.4</b> | <b>Immunization of Mice with Recombinant PvRII</b>                               | <b>42</b> |
| 2.4.1      | Determination of antibody titer of anti-PvRII mouse sera by ELISA                | 42        |
| <b>2.5</b> | <b>Inhibition of Erythrocyte Binding</b>   | <b>43</b> |
| 2.5.1      | Transfection of mammalian cells with recombinant PvRII                           | 43        |
| 2.5.2      | Detection of PvRII on COS7 cells by immunofluorescence assay                     | 44        |
| 2.5.3      | Erythrocyte binding assay on COS7 cells expressing PvRII                         | 44        |
| 2.5.4      | Detection of PvRII on 293T cells by immunofluorescence assay                     | 45        |
| 2.5.5      | Inhibition of erythrocyte binding by anti-PVRII sera in EBA on plated 293T cells | 45        |

|            |  |    |
|------------|--|----|
| <b>3</b>   | <b>RESULTS</b>   | 47 |
| <b>3.1</b> | <b>Expression, Purification, and Characterization of Recombinant PvRII</b>           | 47 |
| 3.1.1      | Construct for expression of recombinant PvRII  | 47 |
| 3.1.2      | Expression and purification of recombinant PvRII using metal affinity chromatography | 47 |
| 3.1.3      | Silver stain   | 48 |
| 3.1.4      | Mobility shift   | 48 |
| 3.1.5      | Reverse phase liquid chromatography  | 48 |
| 3.1.6      | Erythrocyte-binding assay  | 49 |
| <b>3.2</b> | <b>Immunogenicity of Recombinant PvRII</b>   | 49 |
| 3.2.1      | Immunization of mice with recombinant PvRII  | 49 |
| 3.2.2      | End point titers of anti-PvRII antibodies for all bleeds                             | 50 |
| <b>3.3</b> | <b>Inhibition of Erythrocyte Binding Using Anti-PvRII Antibodies</b>                 | 50 |
| 3.3.1      | Erythrocyte binding assay with COS7 cells expressing PvRII                           | 50 |
| 3.3.2      | Transfection efficiencies using 293T cells   | 51 |
| 3.3.3      | Inhibition of erythrocyte binding to PvRII with antibodies raised against PvRII      | 51 |
| <b>4</b>   | <b>DISCUSSION</b>  | 53 |
| <b>4.1</b> | <b>Conclusion</b>  | 57 |
|            | <b>REFERENCES</b>  | 58 |