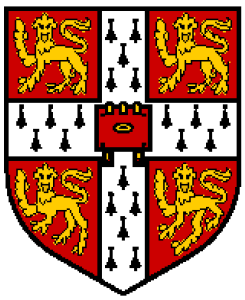
Phenotypic variation in erythrocyte invasion by *Plasmodium falciparum* isolates from Peru



A dissertation submitted for the degree of
Master of Philosophy

Richard Hesketh

Wellcome Trust Sanger Institute

Christ's College

University of Cambridge

Contents

Declaration

This thesis is a summary of research conducted within the Wellcome Trust Sanger Institute between September 2009 and August 2010. This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. None of the research described, in its entirety or in part, has been submitted for any other qualification at any other University or similar institution.

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Abbreviations

A – adenine
bp – base pairs
BSA – bovine serum albumin
C – cytosine
cDNA – complementary DNA
CIDR – cysteine rich interdomain region
CSP – circumsporozoite protein
DBL - Duffy binding like
DDAO-SE – 7-hydroxy-9*H*-(1,3-dichloro-9,9-dimethylacridin-2-one) succinimidyl ester
DDT – dichlorodiphenyltrichloroethane
DMSO –dimethyl sulfoxide
DNA – deoxyribonucleic acid
DNase - deoxyribonuclease
EBA – erythrocyte binding antigen
EBL – erythrocyte binding ligand
EM – electron microscopy
EMP-1 –erythrocyte membrane protein-1
FITC – fluorescein isothiocyanate
FSC – forward scatter
G – guanine
GA – glutaraldehyde
GAG – glycosaminoglycan
GYP – glycophorin
Ht – haematocrit
MSP – merozoite surface protein
NTS – N-terminal segment
PBS – phosphate buffered saline
PCR – polymerase chain reaction
Pf –Plasmodium falciparum
PFA - paraformaldehyde
PMR – parasite multiplication rate
pRBC – parasitised erythrocytes
RBC – erythrocytes
Rh –reticulocyte binding protein homolog
RNA – ribonucleic acid
RNase – ribonuclease
SNP – single nucleotide polymorphism
sRBC – DDAO-SE stained erythrocytes
SSC – side scatter
T – thymine
TRAP – thrombospondin-related adhesive protein
TSP – thrombospondin
UNAP – Universidad Nacional Amazonia de Peruana
VSA – variant surface antigen

Abstract

Plasmodium falciparum invasion of erythrocytes marks the onset of the intra-erythrocytic stage of the parasite life cycle that is responsible for the generation of the symptoms and pathology associated with malaria in humans. The invasion process is complex and incompletely understood. However, specific ligand interactions between the parasite and host erythrocyte must take place to initiate the invasion process. Preventing these interactions inhibits erythrocyte invasion and therefore they have been closely studied as a potential vaccine target.

Plasmodium falciparum erythrocyte invasion is a variable phenotype and parasite isolates can use a number of alternative receptor-ligand interactions to catalyse invasion, resulting in a number of different invasion pathways. The redundancy in pathways allows *P. falciparum* parasites to invade erythrocytes of any age and reduces the possibility of host variation and immune responses having a detrimental effect upon parasite development. Invasion pathways are routinely studied by enzyme treatment of erythrocytes to remove specific receptors and observing the ability of the parasite to invade. Previously assays have been carried out using microscopy to detect invasion events. A two-colour flow cytometry-based assay has been developed at the Sanger Institute to increase the potential throughput of the assay, while also increasing the reproducibility and sensitivity.

Sequence polymorphism and differential expression of parasite ligands can both result in binding to different erythrocyte receptors, but clear genotype-phenotype associations for invasion pathways have not been established. Previous studies have focussed on selected single nucleotide polymorphisms in only a few specific genes. With the development of next generation sequencing technologies, whole genome and transcriptome sequencing of parasite isolates is now possible, allowing the use of genome-wide studies to identify the genotypes that are associated with specific pathways.

This study investigates invasion pathway variation using *P. falciparum* field isolates from Peru. It combines phenotyping, using a newly developed flow cytometry-based assay, with whole genome sequencing and transcriptome analysis to produce an in-depth study of the underlying mechanisms of variation.

Variation in the invasion pathways utilised between Peruvian field isolates was comparable to that in lab strains and field isolates from other countries. Whole genome sequencing and the powerful genotyping analysis tools available were used to identify non-synonymous SNPs in 62 invasion-related genes. Genotyping also revealed the presence of contamination in a number of Peruvian isolates. The project demonstrated that high throughput phenotyping and genotyping can be combined to produce detailed correlations with invasion, and highlights rate limiting steps for such future studies.