BS42012: Parasitology

Antigenic variation in malaria parasites

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I – Cytoadherence and pathology

II – Molecular basis of cytoadherence
Introduction

Antigenic variation
➢ Common immune evasion mechanism used by several pathogens.
➢ Immune evasion = subpopulation survives and infection persists.

Cytoadherance
➢ Different cell type tropism prevents infected erythrocytes from encountering the spleen where they will be eliminated.
➢ Clinical manifestation e.g. = cerebral malaria.

Other forms of severe malaria involve the adhesion of infected erythrocytes to endothelium or other erythrocytes (rosetting).

Both the above occur in mature parasitized erythrocytes and can be mediated by PfEMP1.

*PfEMP1 = *Plasmodium falciparum* Erythrocyte Membrane Protein 1.*
The \textit{P. falciparum} life cycle.
Parasitised cells bind to endothelium or placenta.
This binding leads to pathology.

From: Miller et al., 2002 The pathogenic basis of malaria. Nature \textbf{415}, 673.
Adhesion to endothelium in the brain can lead to cerebral malaria.

Infected red blood cells are ‘sticky’

‘Rosetting’
Adhesion to red blood cells can lead to blockage of micro-vessels.

Adhesion to endothelium in the brain can lead to cerebral malaria.
PfEMP1 and PfEMP1 receptors

200-350 kDa proteins.

PfEMP1 is a ligand for adhesion to many distinct receptors. Some examples are listed:

➢ Complement receptor 1 Rosetting with uninfected erythrocytes
➢ CD36 Severe non-cerebral malaria
➢ ICAM1 Cerebral malaria association
➢ Chondroitin sulfate A Tropism for placenta

N.B. adhesion to each of these involves different PfEMP1 proteins. Other proteins (some as yet unknown) may mediate binding to similar or other receptors.

PfEMP1 is localised in ‘knobs’ on the erythrocyte cell surface. Knobs are necessary for efficient cytoadherence as demonstrated by disruption of the KAHRP (knob-associated histidine-rich protein) gene.
Knobs on the erythrocyte cell surface are required for cytoadherence
Knobs on the erythrocyte surface and cytoadherence II
Schematic showing how iRBCs rosette / cytoadhere
PfEMP1 variants, cytoadherence and pathology

From: Miller et al., 2002 The pathogenic basis of malaria. Nature 415, 673.
PfEMP1 variants, cytoadherence and pathology

From: Kraemer & Smith, 2006
A family affair: var genes, PfEMP1 binding, and malaria disease.
Current Opin. Microbiol. 9, 374-380.
II – Molecular basis of cytoadherence

PfEMP1 Protein structure

➢ Transmembrane proteins

➢ Cysteine rich inter-domain regions CIDR (adhesion to CD36)

➢ An intracellular acidic terminal segment anchors PfEMP1 in the knob structure.

➢ 2-4 Duffy-binding like domains in the extracellular portion (adhesion).
  ▪ Also found in EBA-175 and Duffy-binding protein.
  ▪ Erythrocyte binding and invasion - merozoites.
Schematic representation of *PfEMP-1* proteins

**Extracellular**
- DBL - Duffy-binding like domain
- CIDR - Cysteine rich interdomain region

**Transmembrane region**

**Intracellular**
- ATS - Acidic terminal segment
Duffy-binding-like domains are found in other *Plasmodium* proteins

- **Parasite (protein)**
  - *P. vivax*
  - *P. falciparum* (EBA-175)
  - *P. falciparum* (MAEBL)

- **Host receptors**
  - Duffy blood group
  - Glycophorin A
  - Unknown

[Diagram showing the domain structure of different proteins and their interactions with host receptors]
var genes encode *PfEMP1* variants

Southern blots:
Three isolates probed with exon 2

Exon I
Extracellular and transmembrane domains.

Exon II
Intracellular segment.

Intron (~1 kb).

From Su et al., 1995.
Cell, Vol. 82, 89-100
Highly polymorphic both within and between isolates.

- Multiple different \( var \) genes per haploid genome (~60).
- Most \( var \) genes are sub-telomeric.

\textit{var} Gene organisation in \textit{P. falciparum} isolates
var genes in *P. falciparum* 3D7.

Promoter types: UpsA (green), UpsB (red), UspC (blue).
Schematic representation of a typical *P. falciparum* telomere.

Typical arrangement of *var*, *rifin* and *stevor* genes.
Generation of var gene diversity

a  Minimal overlap between var gene repertoires

3D7  HB3  Dd2

var1CSA
var2CSA
type 3 var

b  Nuclear architecture of telomeric and central var genes

ups-type var gene
FISH probes:
- upsB
- upsA (upsD,E)
- upsC

C  Schematic view of telomere cluster

x  Ectopic recombination

Nuclear membrane

Annu. Rev. Microbiol. 62:445–70
var gene expression

➢ Developmentally regulated.

➢ Rate of switching may be as high as 2% per generation.

➢ Switching occurs by transcription activation/inactivation (in-situ switching).
  i.e. There are multiple sites of var gene expression (telomeric and chromosome internal).

➢ Expression appears to be mutually exclusive (one at a time). Produces defined adhesive phenotypes.
Epigenetic factors contributing to antigenic variation

Host immune factors
- Selection of variants
- Switching rate modulation?

Surface expression of var

Monoallelic expression

Histone marks
Nuclear localization

TPE PfSir2

Transcriptional memory
Switching

Additional putative epigenetic factors:
- ncRNA silencing → Silencing
- DNA enhancer → Counting mechanism
- Other histone modifications
- Histone binding proteins
- Chromatin remodellers
- Histone variants

Activation
Silencing
Insulators
Nuclear localization
Transcriptional memory
Do other surface exposed non-PfEMP1 proteins contribute to antigenic variation?

Annu. Rev. Microbiol. 62:445–70
PfEMP1 antigenic and functional heterogeneity (var gene switching) contributes directly to immune evasion and acute malaria pathology = important virulence factors.

PfEMP1 (var) expression or adhesion = possible target for chemotherapy.

PfEMP1 may be involved in naturally acquired immunity = possible vaccine candidate. Specific PfEMP1 antisera can block adhesion to specific receptors.

Other variant genes have also been reported: e.g. rif, STEVOR.

Genome sequencing:
- *P. vivax* = 600-1000 sub-telomeric *vir* genes
- *P. yoelii* = 838 *yir* genes
Some current research

➢ Var gene expression – how is monoallelic expression controlled?

➢ To define the adhesion phenotypes for each PfEMP1.

➢ To develop PfEMP1 as a vaccine for placental malaria.

➢ to define the mechanism of PfEMP1 transport to the cell surface.

➢ To understand Stevor and Rifin function.
SnapShot: var Gene Expression in the Malaria Parasite

A. Surface expression of PEMP1

B. Monoallelic expression

C. Genomic organization of var genes

D. Nuclear architecture

E. Potential role of the intron in a silencing and counting mechanism

F. Genetic and epigenetic control elements