

Novel Host-associated Mechanisms to Control Malaria Infection.

Brendan McMorran
Menzies Research Institute
University of Tasmania
Hobart



Malaria

• Plasmodium species affecting humans:

- falciparum
- vivax
- ovale and malariae
- knowlsei

• *P. falciparum*

- 2.37 billion people at risk
- 100's millions clinical cases
- >1 million deaths (mostly children)
- Drug resistance problems

• *P. vivax* ; “milder” form

- widespread in Asia and Americas
- Dormant liver stage and drug resistance

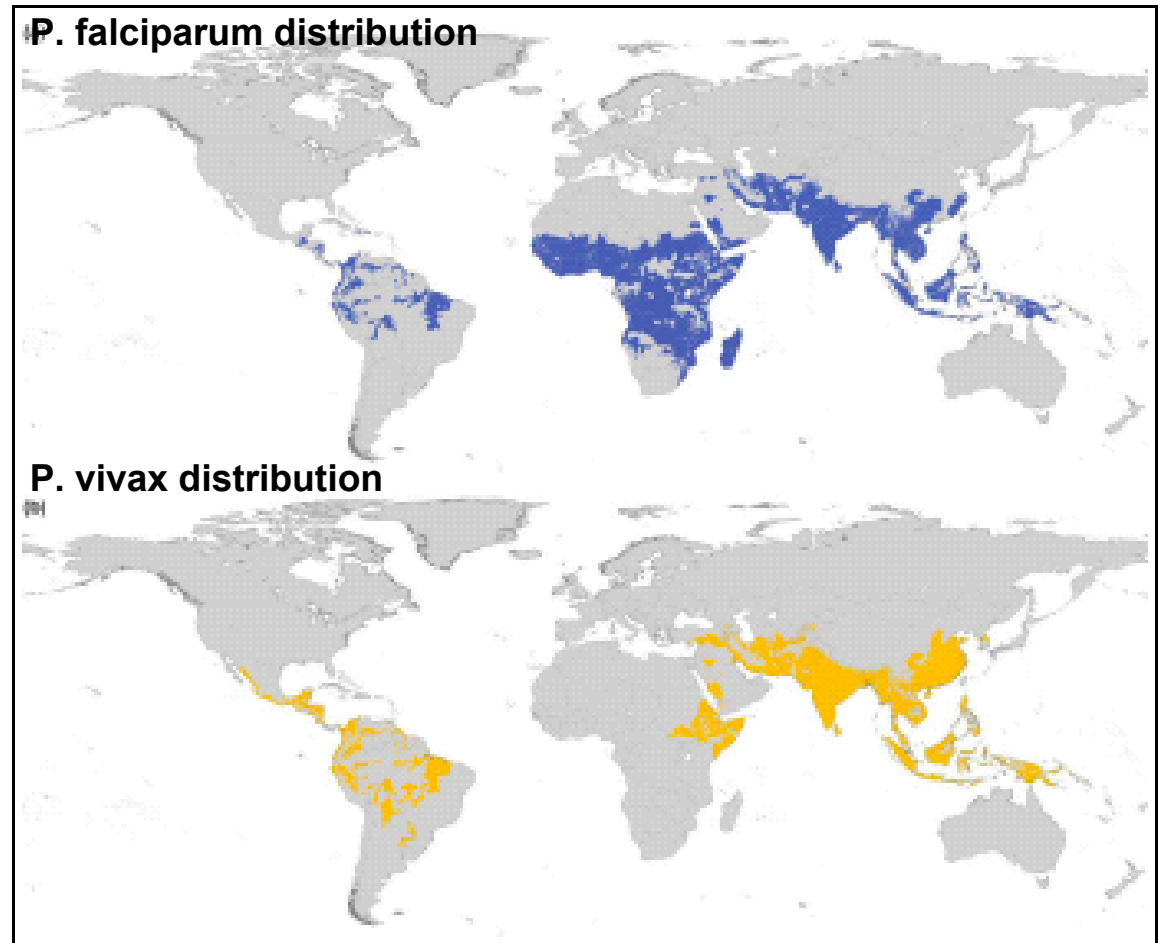
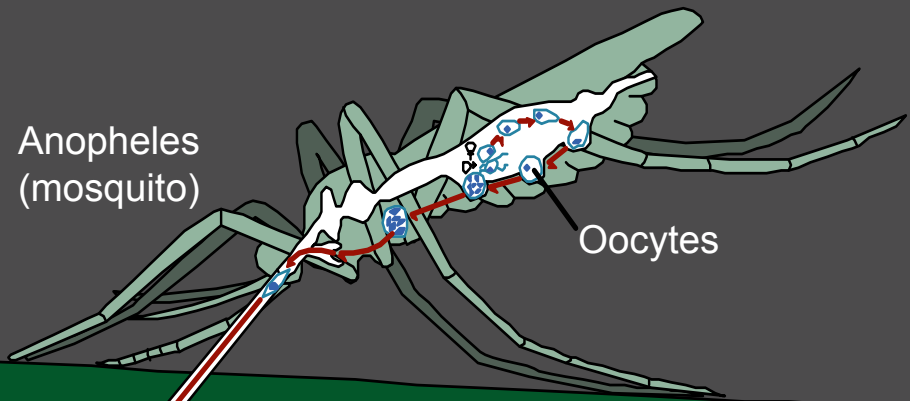


Figure 1. Global distribution of *P. falciparum* and *P. vivax* in 2000. (A) Global distribution of *P. falciparum*. (B) Global distribution of *P. vivax*. Current reservoirs of infection for each species are indicated by shaded areas. The shaded areas represent the current distribution of the species in 2000. The shaded areas represent the current distribution of the species in 2000. The shaded areas represent the current distribution of the species in 2000. The shaded areas represent the current distribution of the species in 2000.

P. falciparum life cycle



Human host

Bloodstream

Schizonts

Liver

Sporozoites

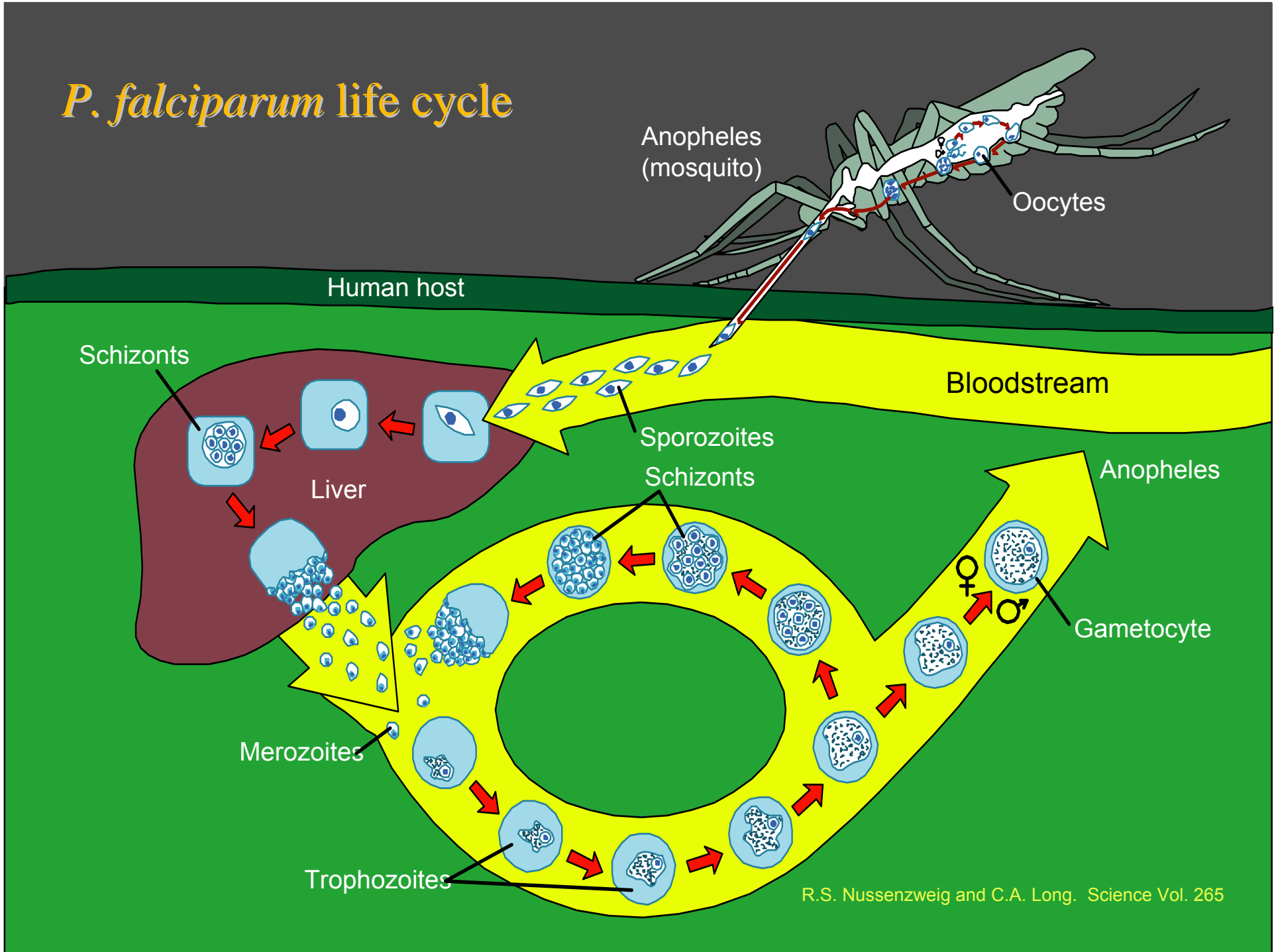
Schizonts

Anopheles

Gametocyte

Merozoites

Trophozoites



Malaria control in the 21st century

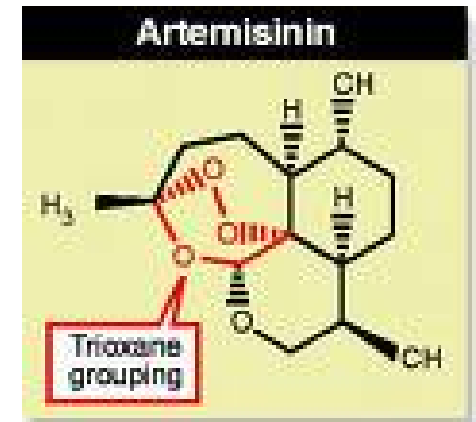
All current anti-malarials suffer from drug resistance

Significant vaccine efforts are underway

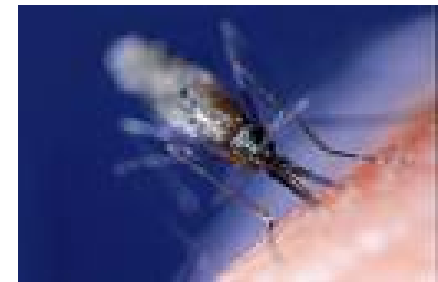
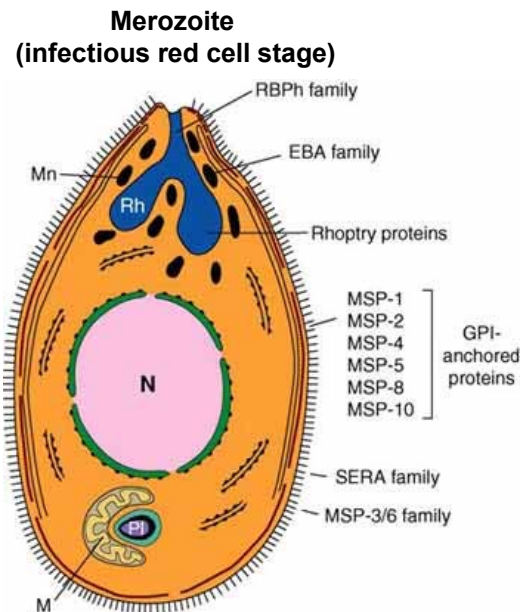
> 50 under development

RTSS in Phase III (30-40% protection)

?But long-term efficacy questionable?

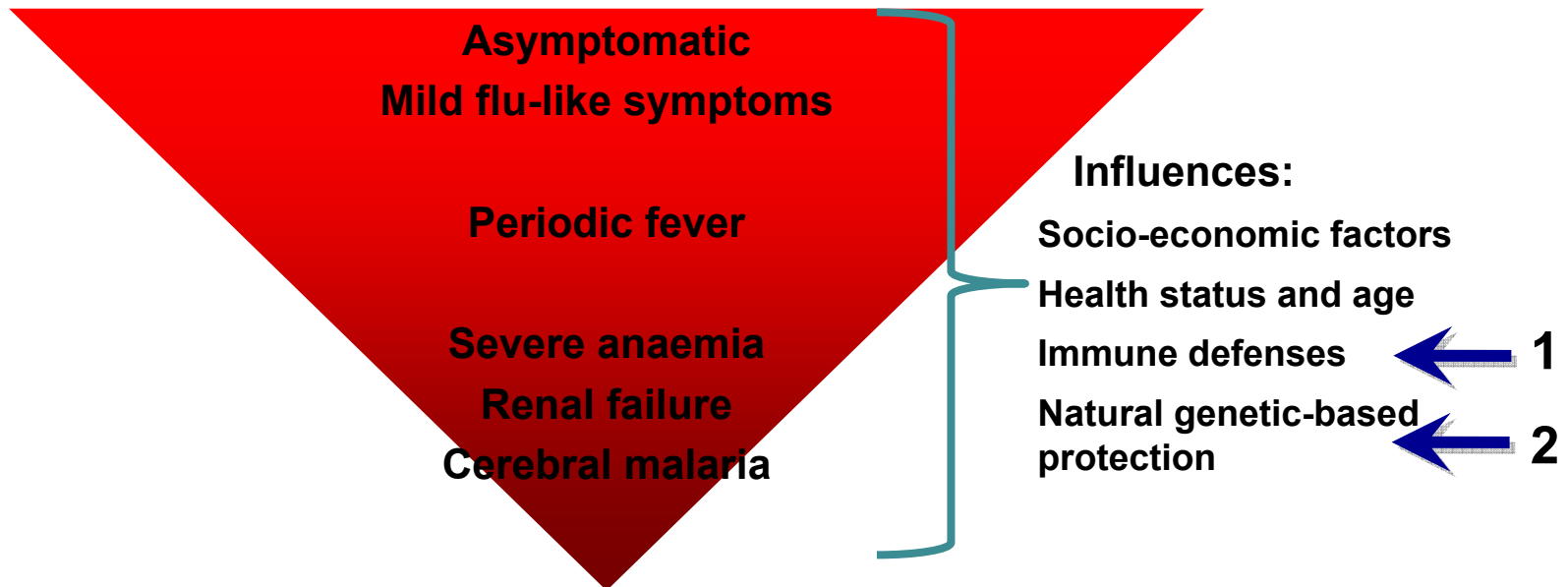


Bed nets and mosquito control remain most effect strategies

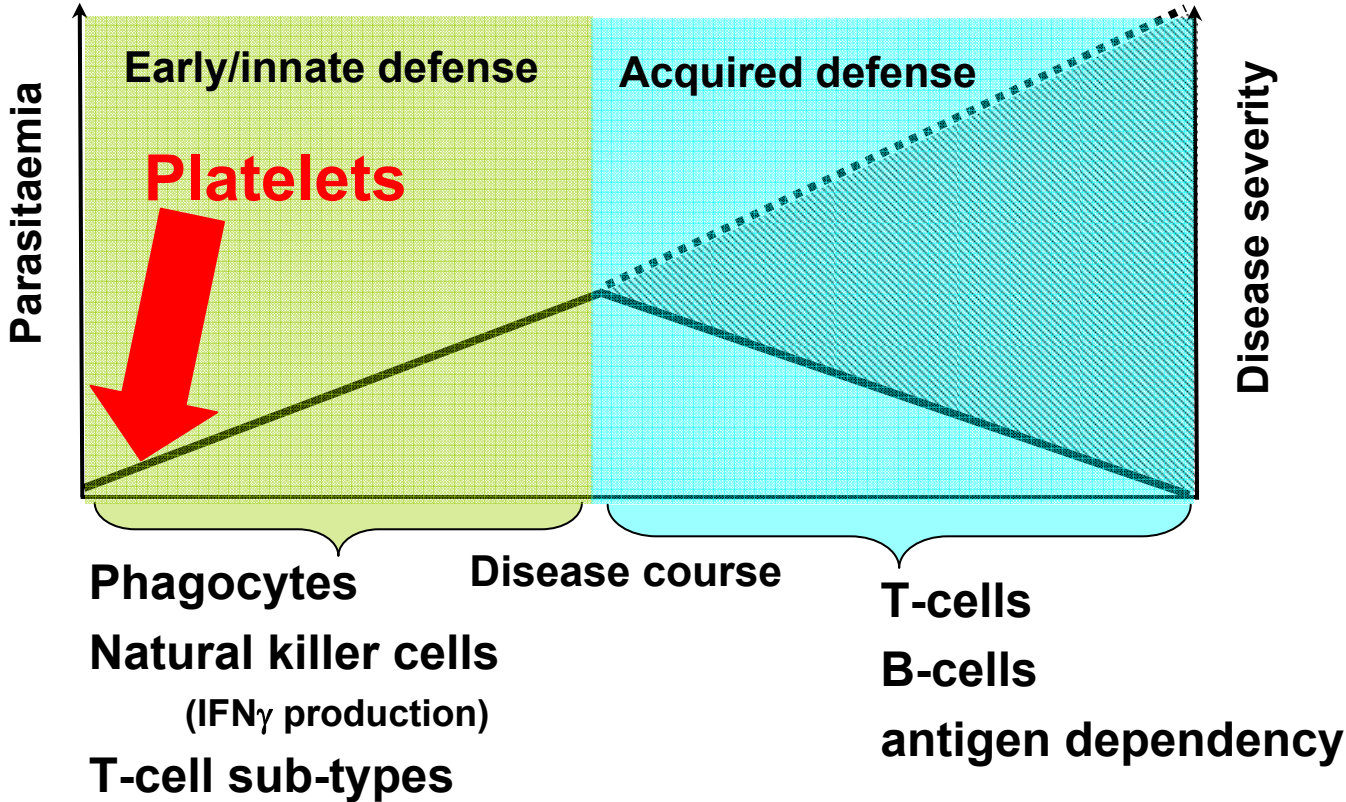


The Importance of Host Defenses

Spectrum of clinical consequences during malaria infection



Immune Defense Against Blood Stage Malaria



Platelets and Host Defense

Archetypical properties of immune cells:

Cytokine and TLR receptors (host or microbe stimulation)

Antibody receptors

Mediate CD154-CD40 costimulation

Oxidative burst and free radical generation

Alpha-granule proteins:

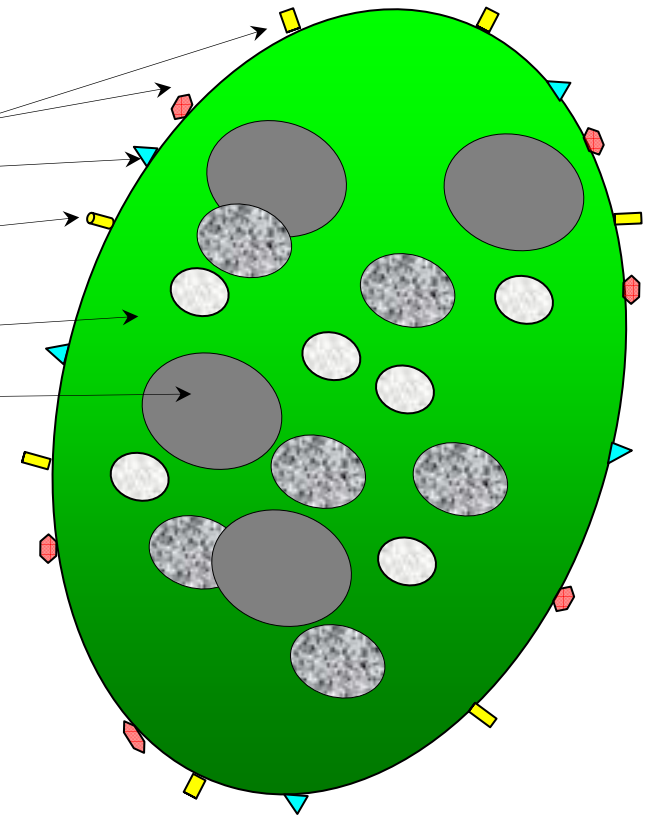
chemokines

antimicrobial peptides

Interact and kill bacteria, fungi and protozoa

Abundant and exquisitely sensitive

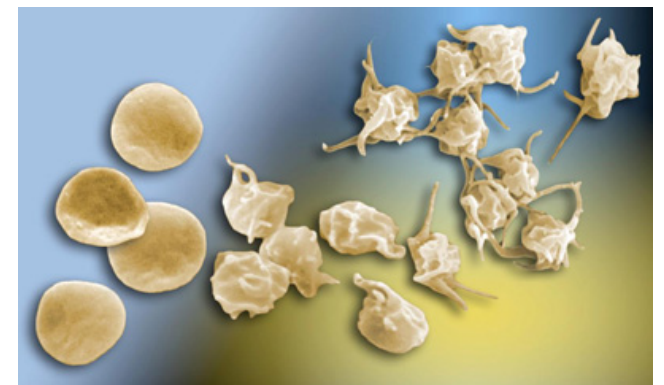
→ Sentinel function



Adapted from Yeaman and Bayer 2007

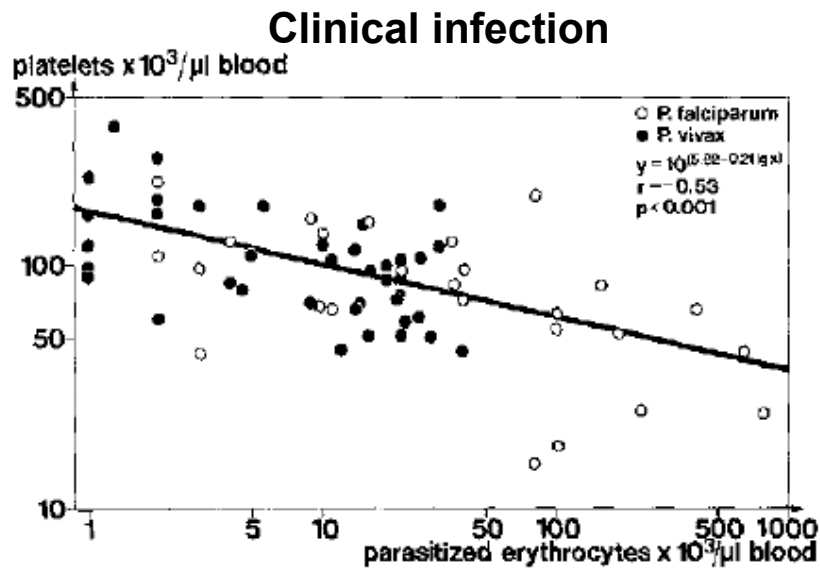
Yeaman (1997) Clin Infect Dis 25: 951-965

von Hundelshausen and Weber (2007) Circ. Res. 5: 27-40



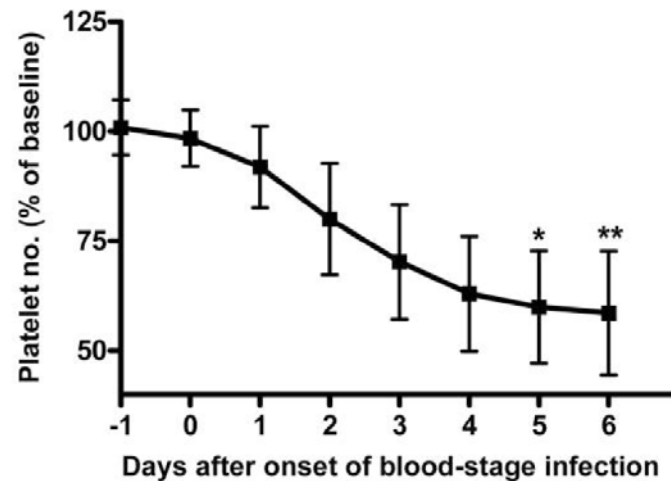
Thrombocytopenia and Malaria

- Common in all malarias (human and mouse)
- Correlates with high parasitaemia and poor outcome
- Develops early, before onset of severe disease
- Cause?



From: Horstmann et al. 1981

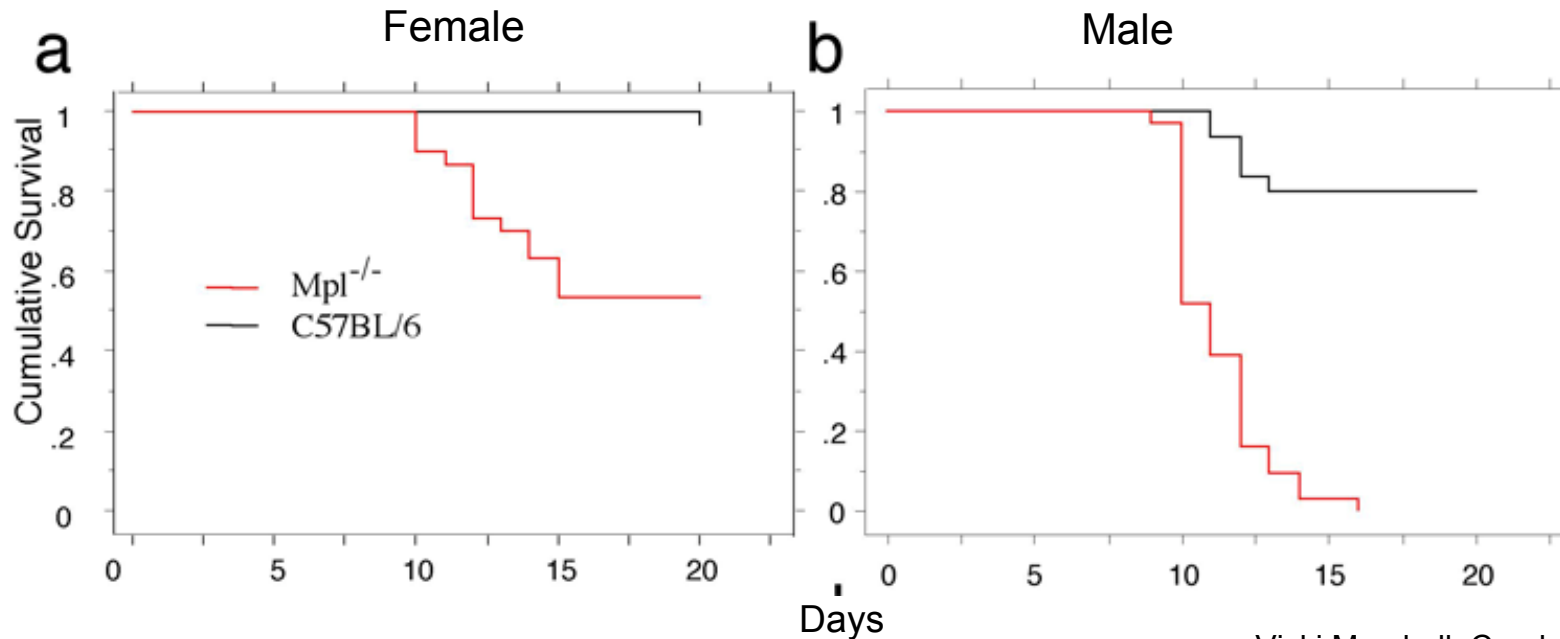
Experimental human infection



From: de Mast et al. 2007 JID

Platelet-Deficient Mice Are Susceptible to *P. chabaudi* Malaria

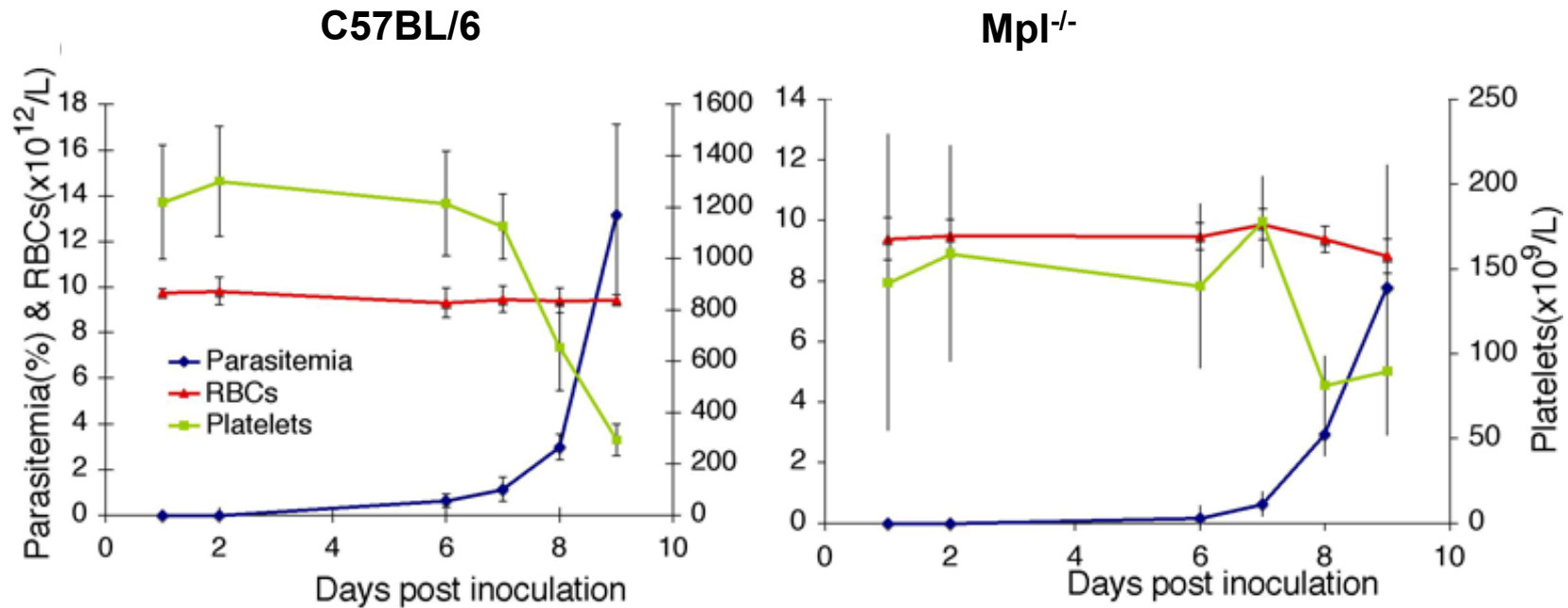
- Mpl encodes the thrombopoietin receptor
- Mpl^{-/-} mice have 10% normal platelet levels, on a C57BL/6 background
- *P. chabaudi* infections result in acute parasitaemia and anaemia.
- **Aspirin-treated mice are more susceptible to *P. chabaudi* infection.**



p<0.0001 (Mantel-Cox log rank test)

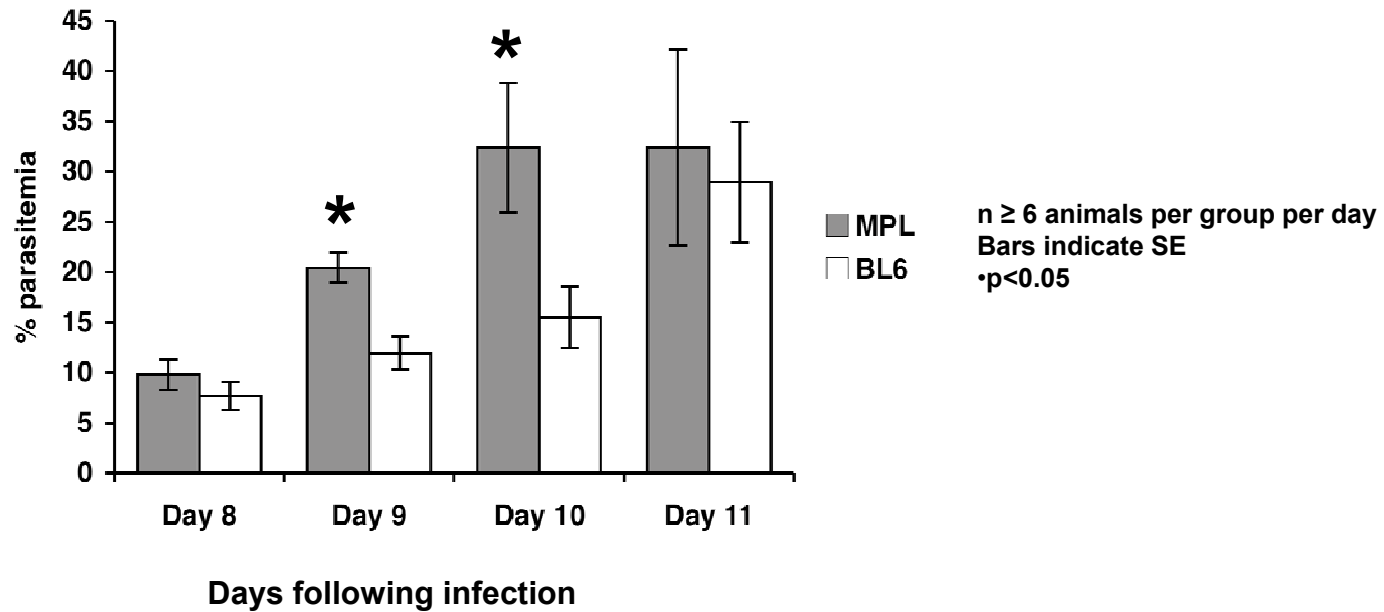
Vicki Marshall, Carolyn de Graaf and
Simon Foote, WEHI

Parasitaemia and Haematological Parameters



Vicki Marshall, Carolyn de Graaf and
Simon Foote, WEHI

Platelets directly control parasite growth?



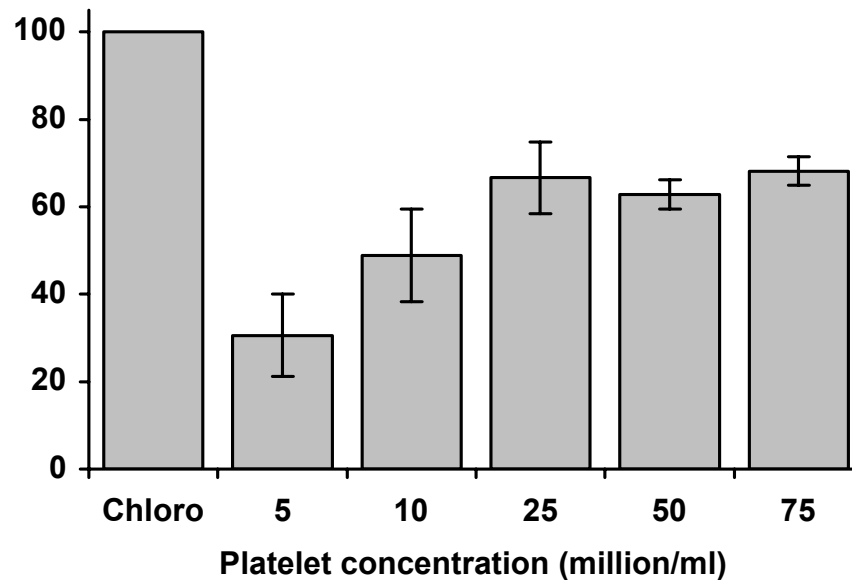
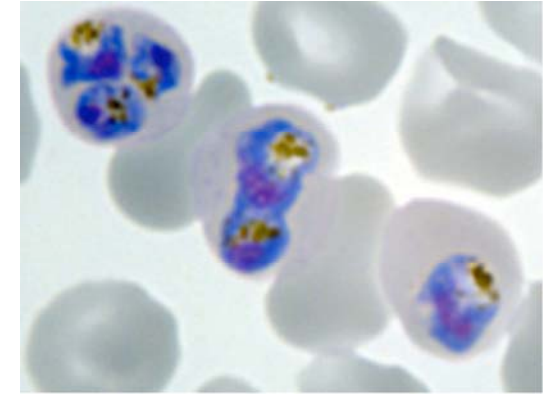
No differences in:

- Rates of erythropoiesis
 - Immunological responses (lymphocytes and serum cytokines)
-

Human Platelets Inhibit Growth of Cultured *P. falciparum*

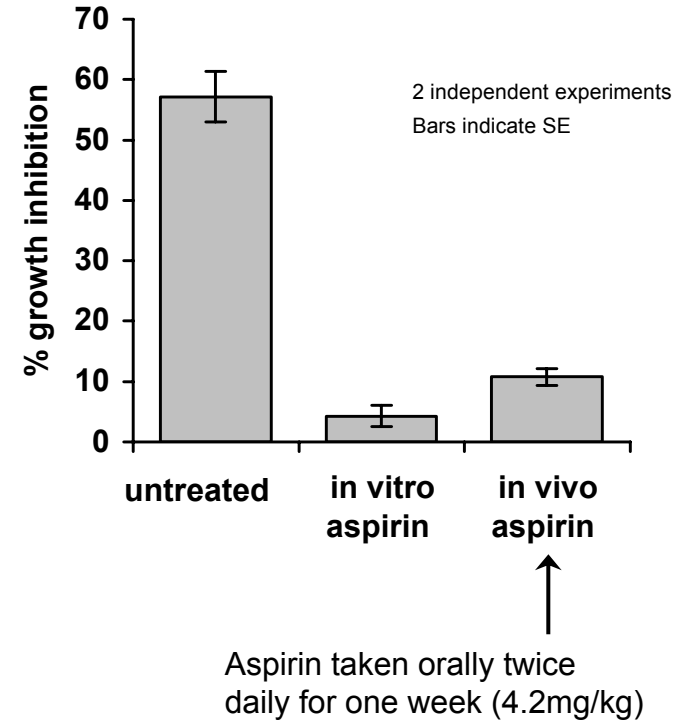
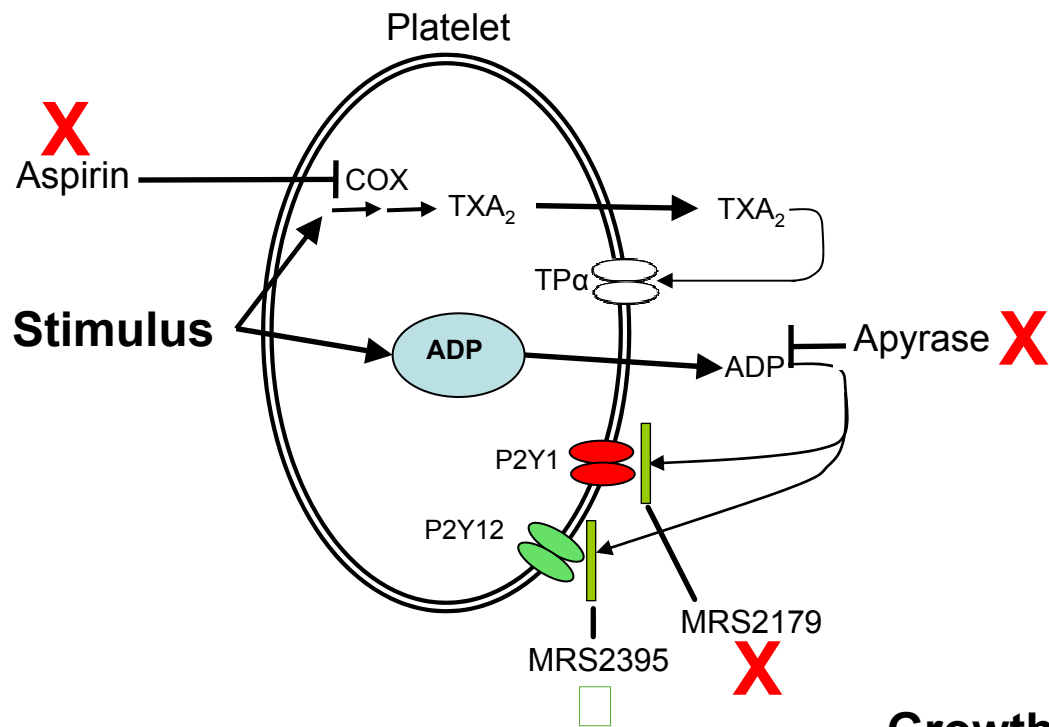
Methods

- Continuous culture of *P. falciparum* 3D7 in human RBCs (Trager and Jensen 1976)
- Freshly purified human platelets (by centrifugation and washing)
- Co-incubate for 48 h (synchronised at trophozoite stage) \approx 1 cycle of growth
- Compare to platelet buffer treated cultures



≥ 3 independent experiments
Bars indicate SE

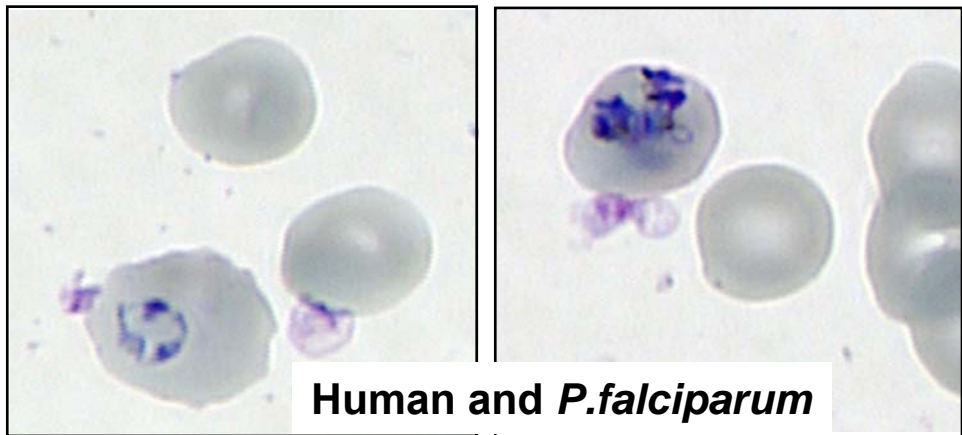
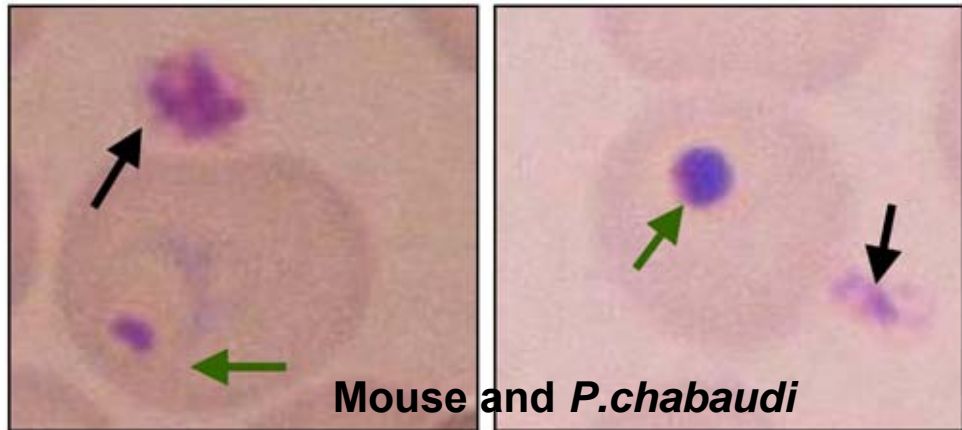
Platelet Function Antagonists Block Growth Inhibition



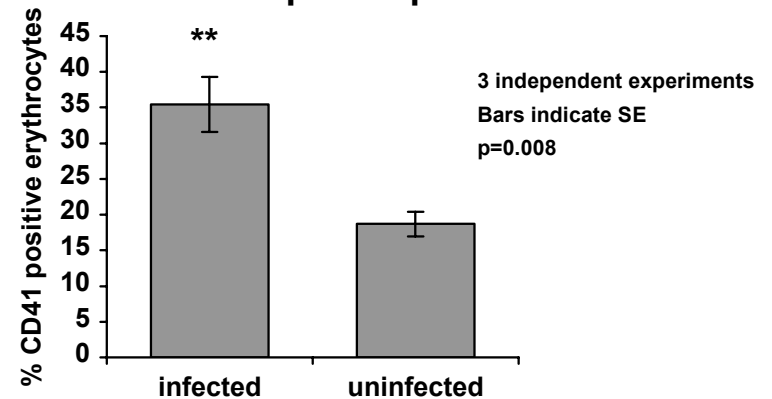
Growth inhibition also blocked by:

- PGE₁ (cAMP)
- NO (cGMP)

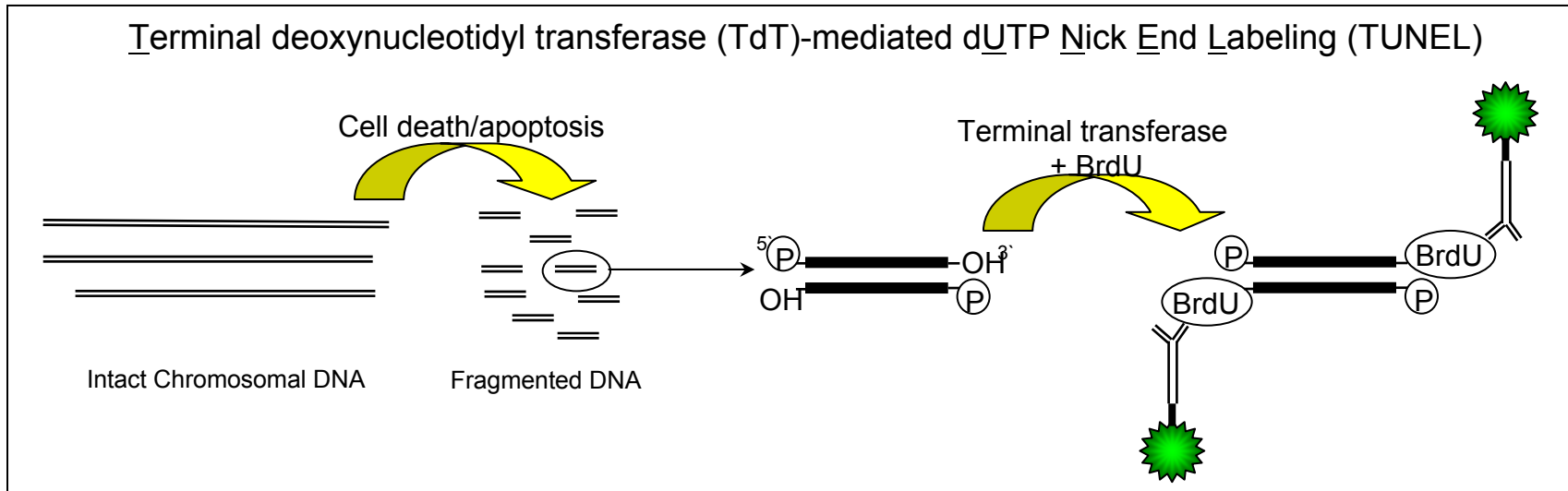
Platelets Preferentially Bind Infected Red Blood Cells



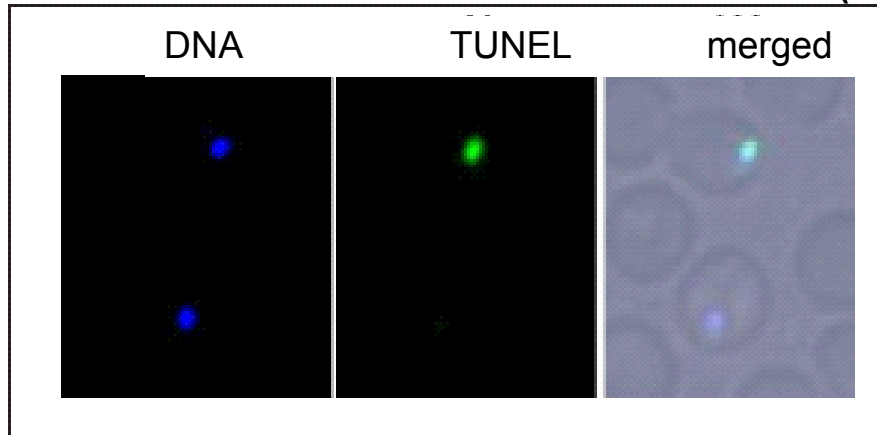
Flow cytometry: preferential association between mouse platelets (CD41) and falciparum parasites



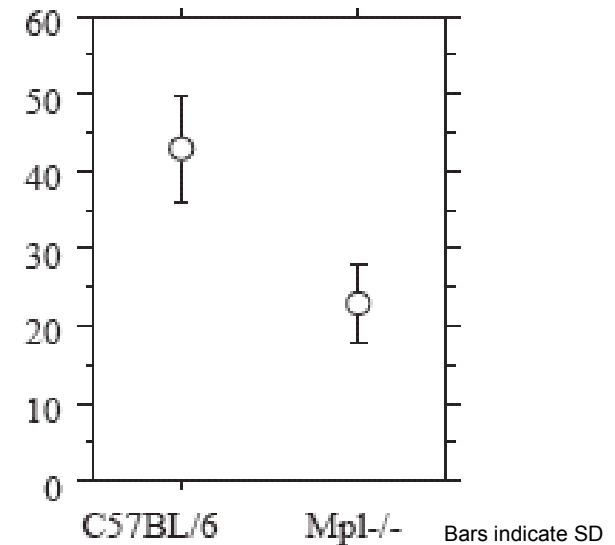
Lower Proportions of 'Dead' Parasites in Mpl Mice



Blood smear from *P. chabaudi*-infected mouse (7d)

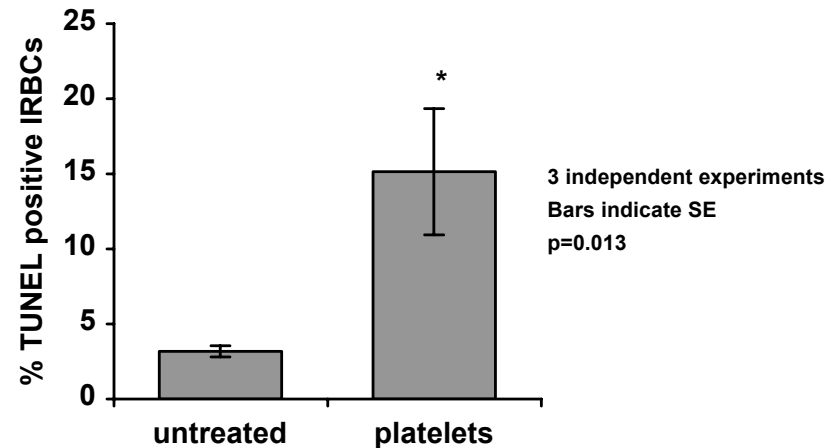


% TUNEL positive *P. chabaudi*

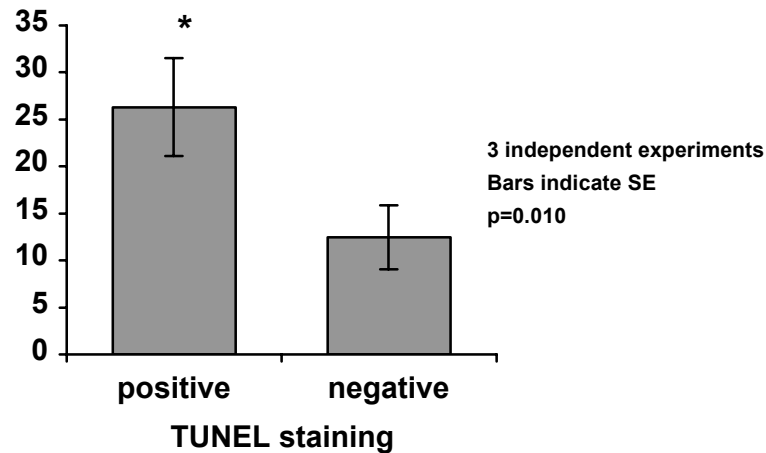


Direct Association Between Platelets and Dead Parasites

Human platelet treatment increases *P. falciparum* death

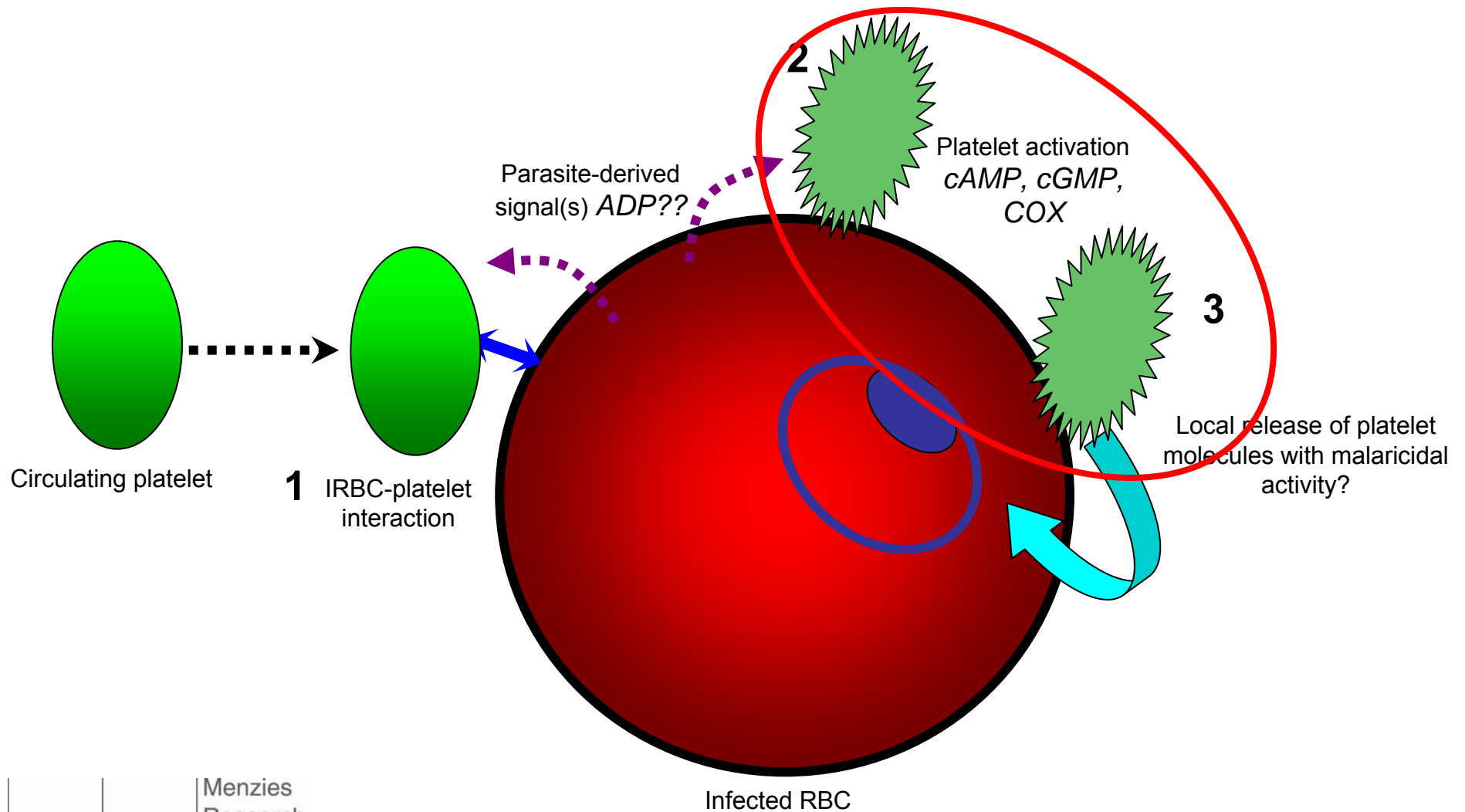


Human platelets associate with dead/dying parasites



Proposed Model

McMorran *et al.* 2009 Science 323: 797-800



Platelet contact with infected red cells triggers activation?

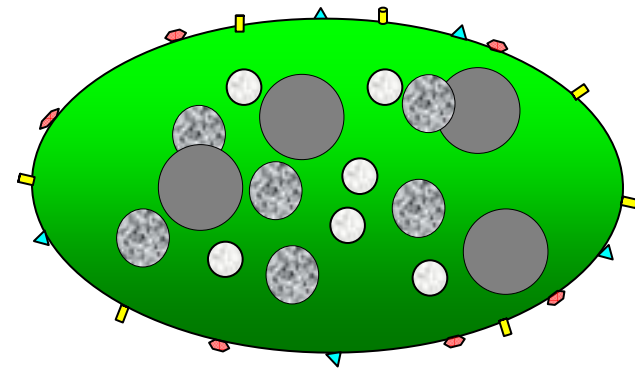
Platelet Factor 4 (PF4, CXCL4)

Abundant platelet-specific protein (~25%)

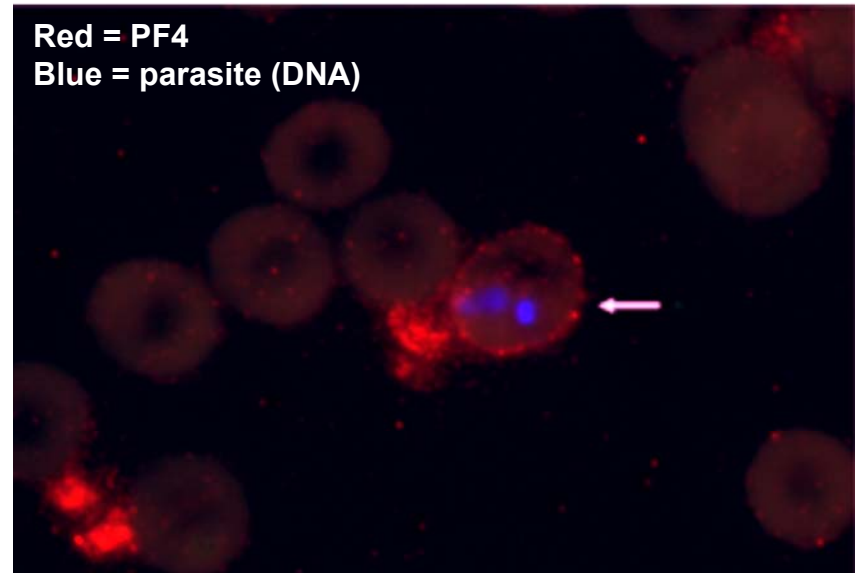
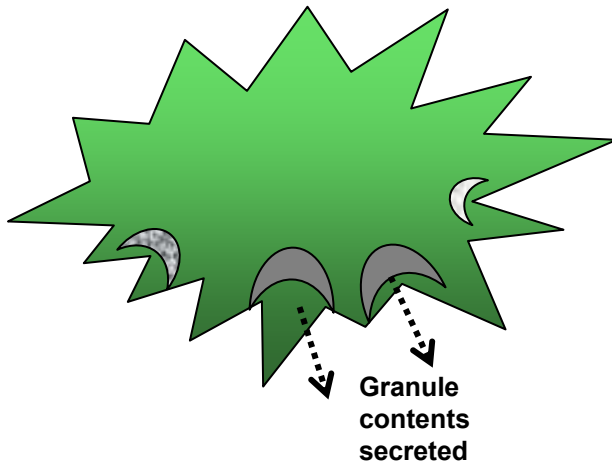
Located in alpha-granules

Released upon platelet activation (≈ activation marker)

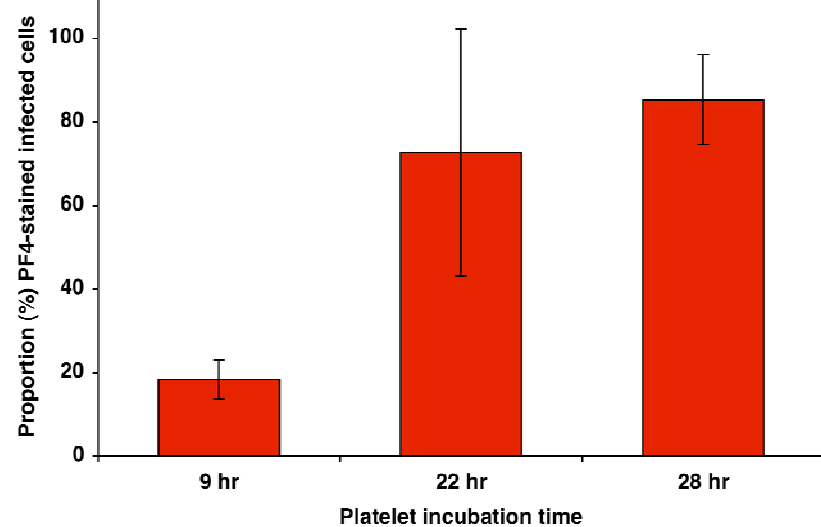
Chemokine/cell growth regulator



activation



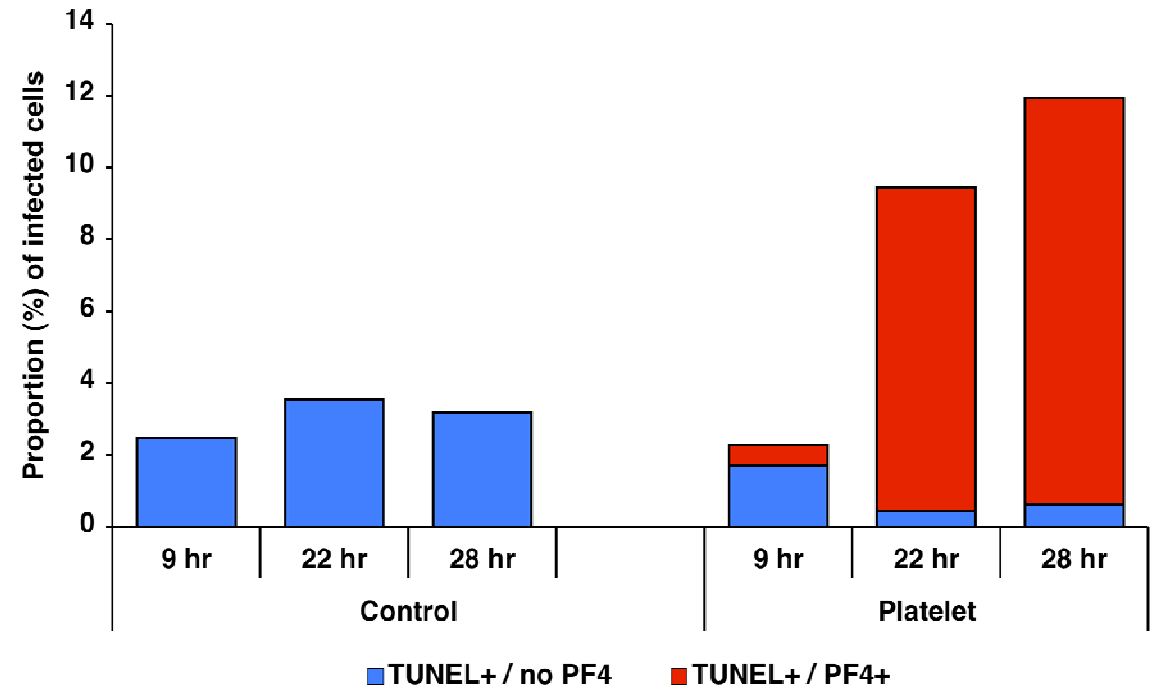
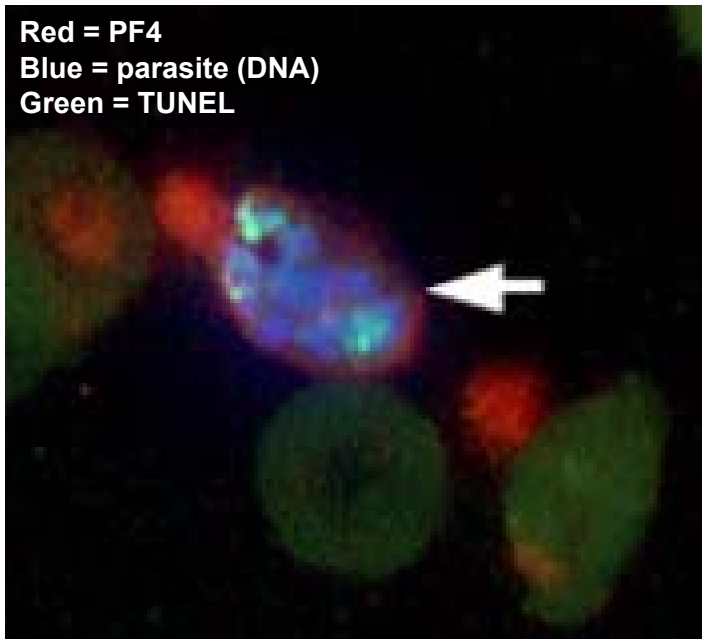
Quantification of PF4-stained infected red cells



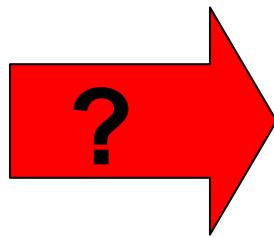
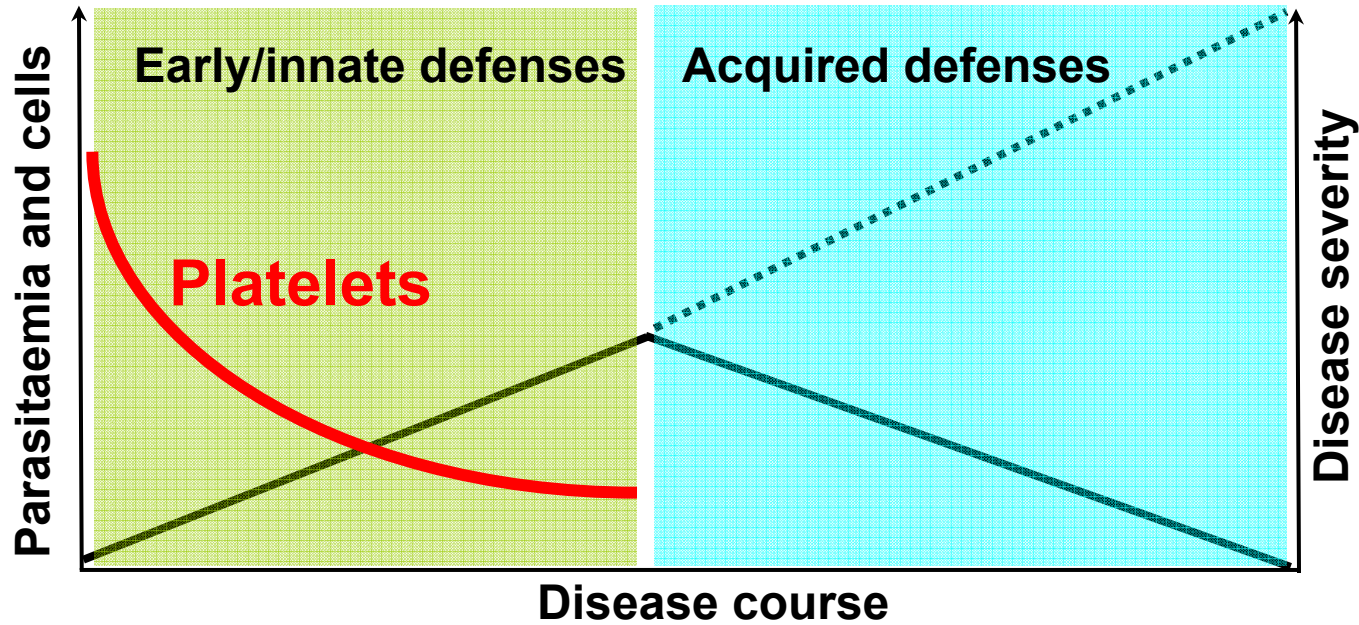
Association between PF4 and TUNEL staining

Microscopy Analysis

(3x independent expts, ~100 cells counted/expt)



A Role for Platelets in Malaria Immune Defense



Aspirin usage and malaria
Platelet-parasite interaction during CM
Platelet-IRBC binding and thrombocytopenia

Human Genetics and Natural Protection from Malaria

High selective pressure exerted by malaria during evolutionary history

Polymorphisms/mutations arisen that protect against infection or disease symptoms

1. Parasite entry
Duffy chemokine receptor

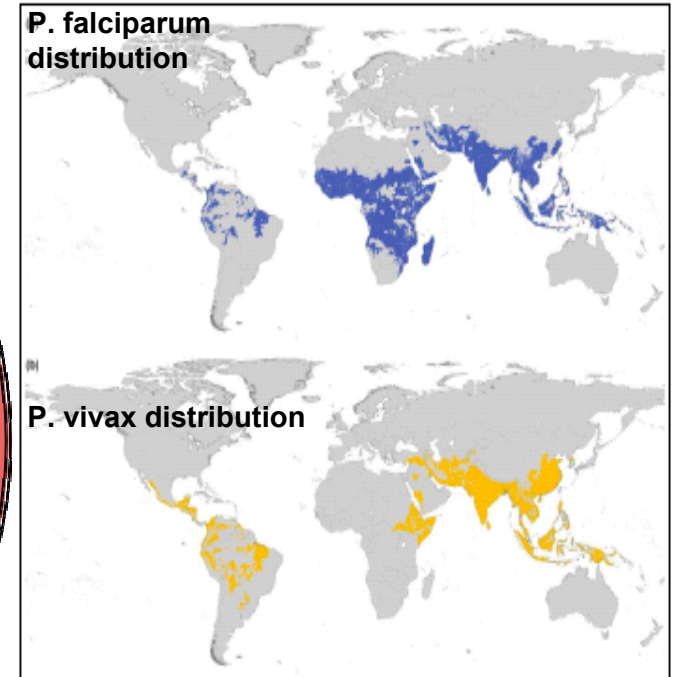
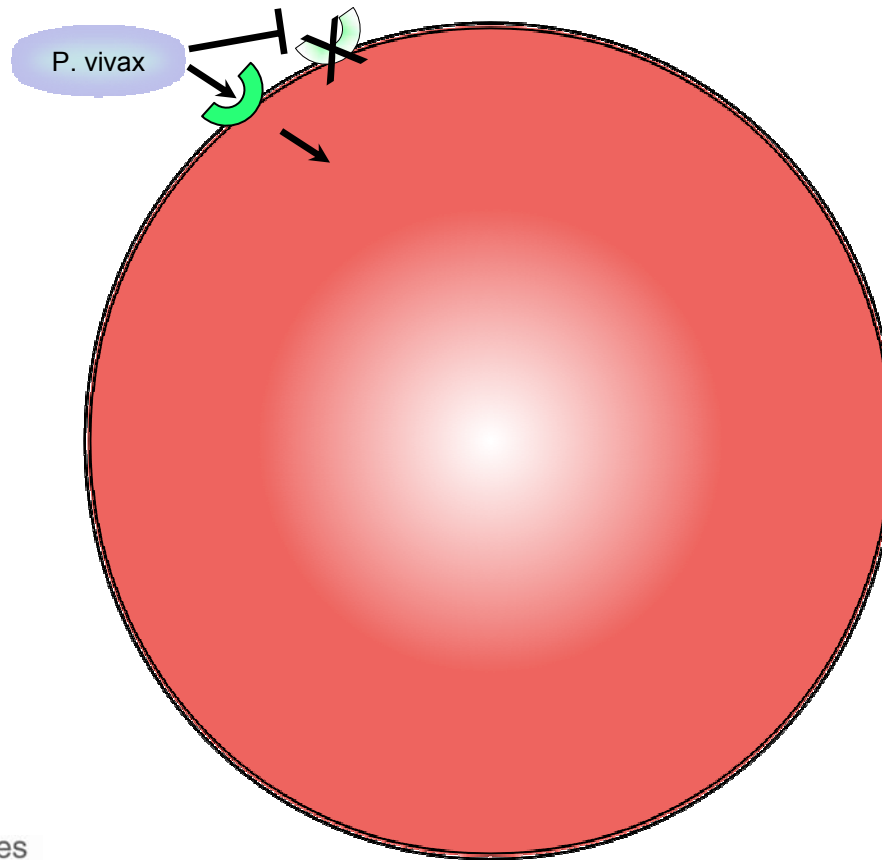


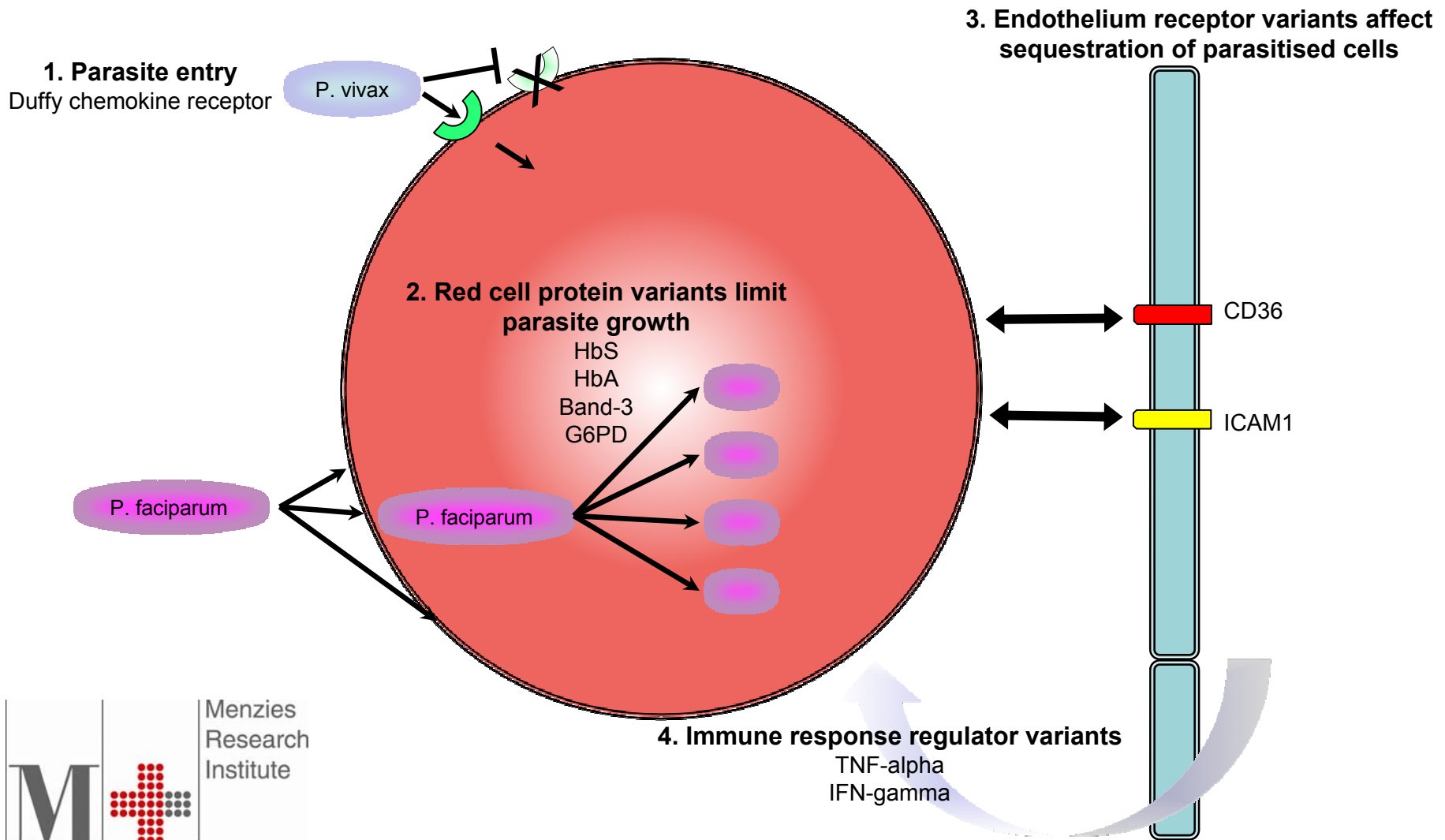
Figure 1. Global distribution of *P. falciparum* and *P. vivax* in 2010. (A) Distribution of *P. falciparum*. (B) Distribution of *P. vivax*. Several sources of information on malaria risk include ITN use, malaria drug resistance, climate data for mosquito breeding, and human population density. These data have been combined in a geographic information system to generate these maps. See the step-by-step guide in the sidebar for a full description of the methods used to create these maps.

Human Genetics and Natural Protection from Malaria

High selective pressure exerted by malaria during evolutionary history

Polymorphisms/mutations arisen that protect against infection or disease symptoms

Successful! No parasite resistance to these protective effects



Novel anti-malarial: mimic host protective polymorphisms

All current anti-malarials suffer from parasite resistance

Mutation of drug target (DHFR and pyrimethamine)

Upregulation of efflux pumps (chloroquine)

Why? Anti-malarials target parasite proteins/processes

Host protective polymorphisms avoid parasite resistance

Why? Anti-malarial host factors lie outside parasite genetic control

Can we exploit this to develop novel resistance-proof anti-malarials?

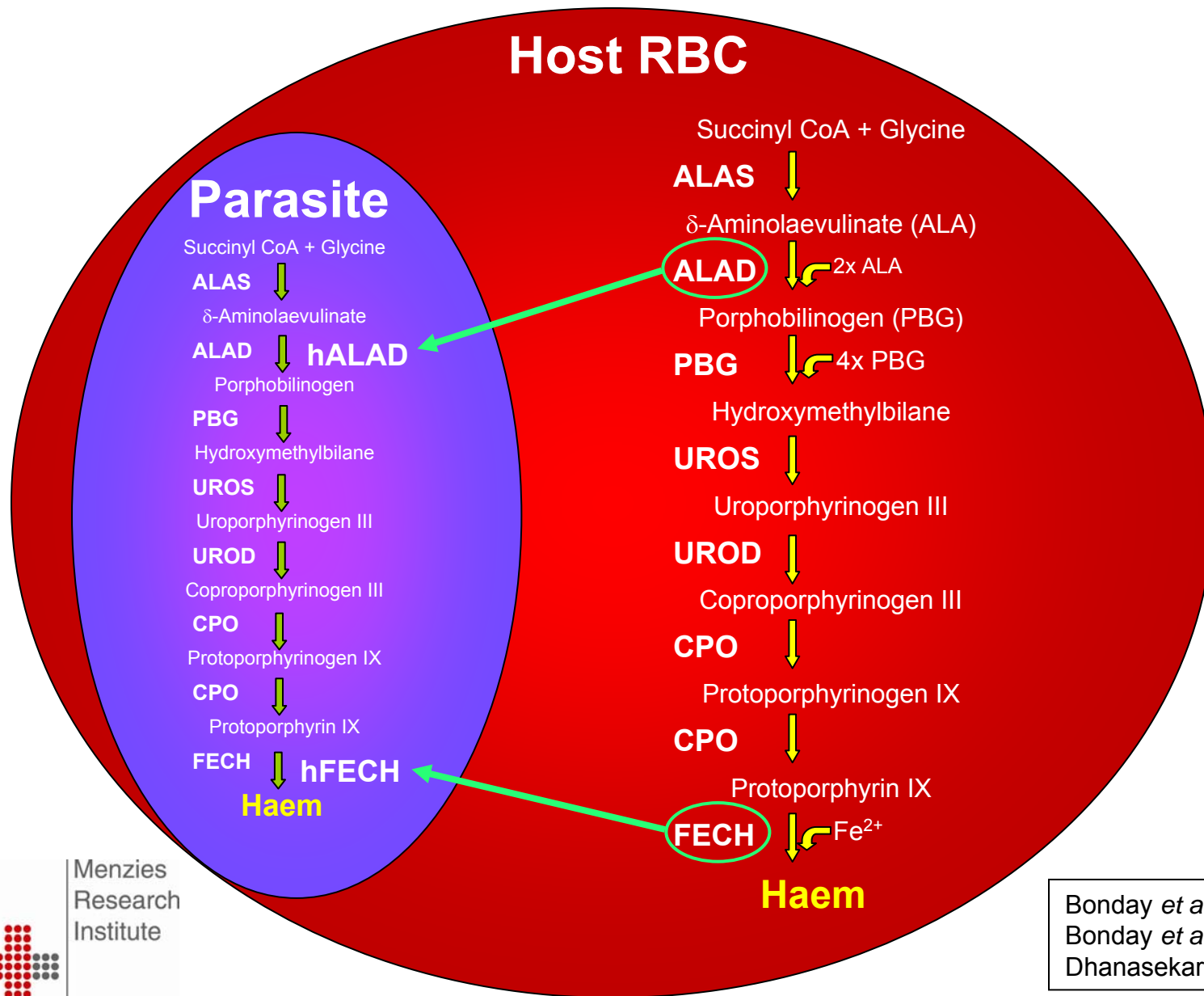
- 1. Target known host enzymes required for parasite growth**
- 2. Identify novel protective host genes amenable to drug targeting**

Host Directed Therapy

(Clare Smith, Hons/PhD student)

- **Aim:** Identify host targets that inhibit/protect from infection
- **Motivation:** Potential to avoid parasite drug resistance
(\approx protective polymorphisms)
- **An ideal HDT; an enzyme:**
 - Abundant in red cell
 - (Non-essential for red cell function)
 - Imported/scavenged by the parasite
 - Utilised by parasite in essential metabolic pathway
 - (Parasite genome lacks the equivalent gene)
- **Parasites scavenge red cell constituents**
 - Haemoglobin
 - Enzymes of the haem biosynthetic pathway

Haem biosynthetic pathway: an ideal HDT target?



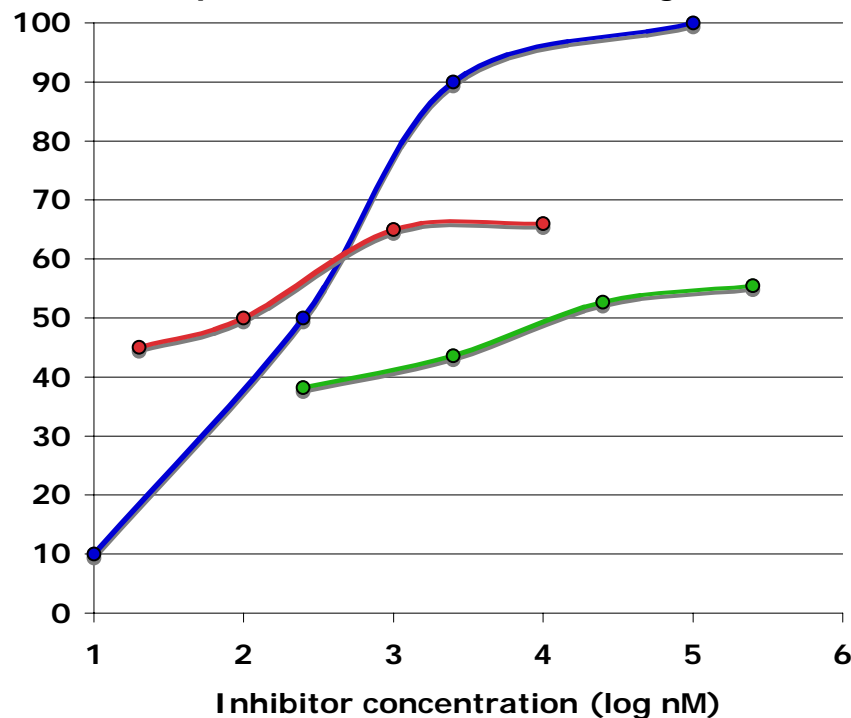
Inhibition of FECH or ALAD prevent parasite growth

FECH = Ferrochelatase, ALAD = δ -Aminolaevulinic acid dehydratase

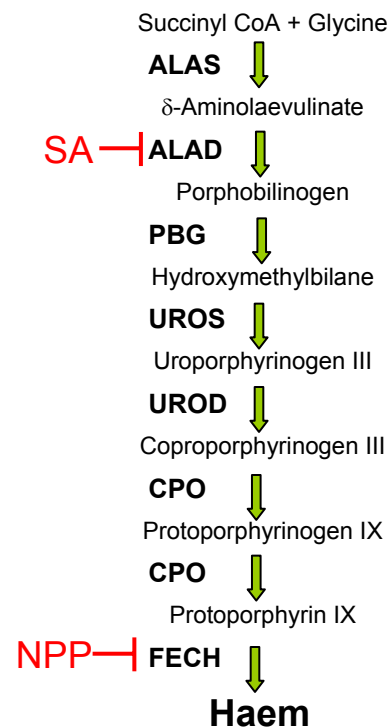
Clare Smith 2007

Parasite growth inhibition assays

P. falciparum 3D7 incubated with drug for 48 hr



- Chloroquine
- N-methylprotoporphyrin (NPP)
- Succinylacetone (SA)



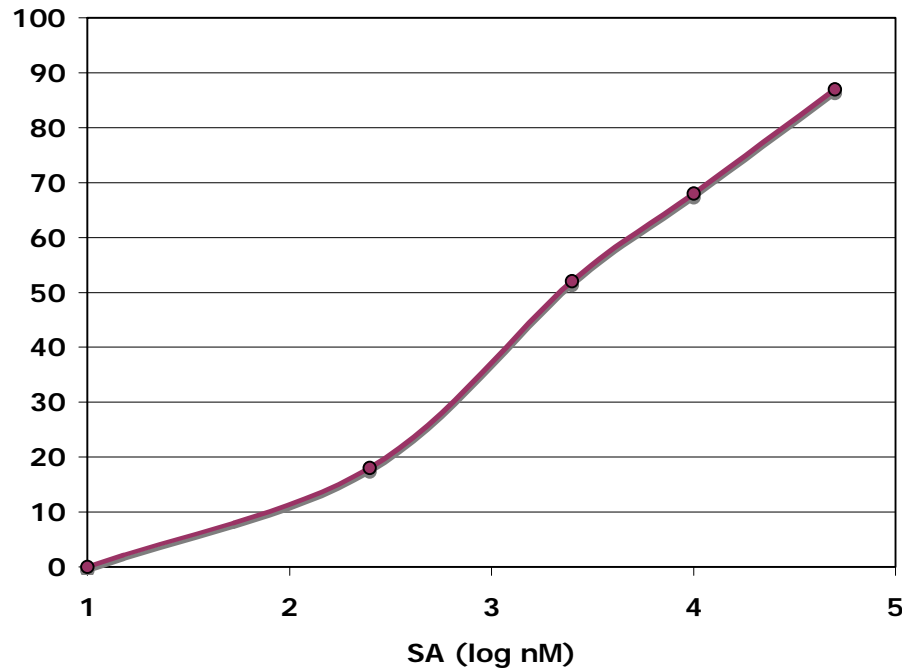
Drug	IC ₅₀ (μM)
Cq	~ 0.2
NPP	0.125
SA	10

Inhibition of host ALAD prevents parasite growth

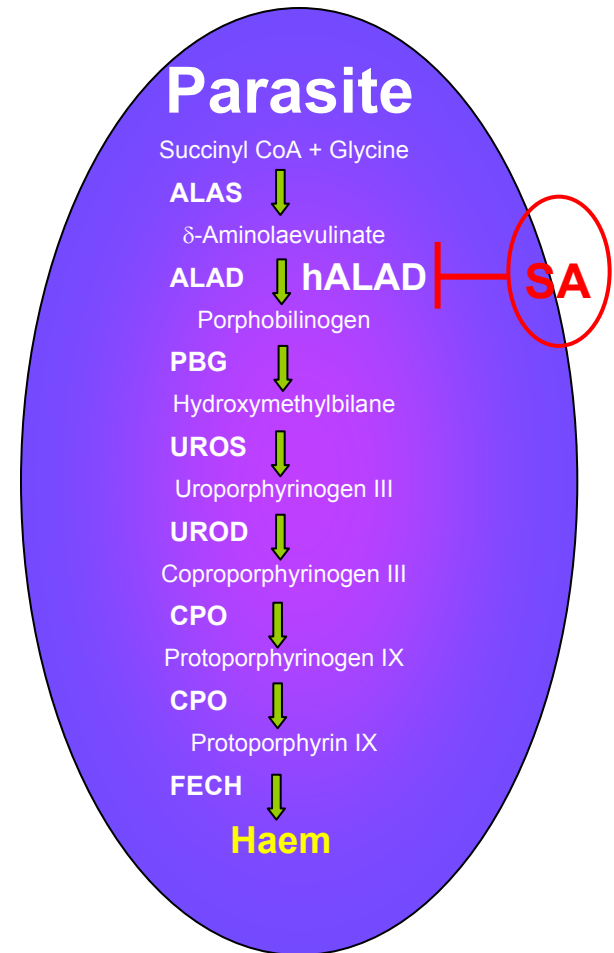
Clare Smith 2007

SA preincubation and washout

Uninfected RBC loaded with SA, washed, then exposed to *P. falciparum*



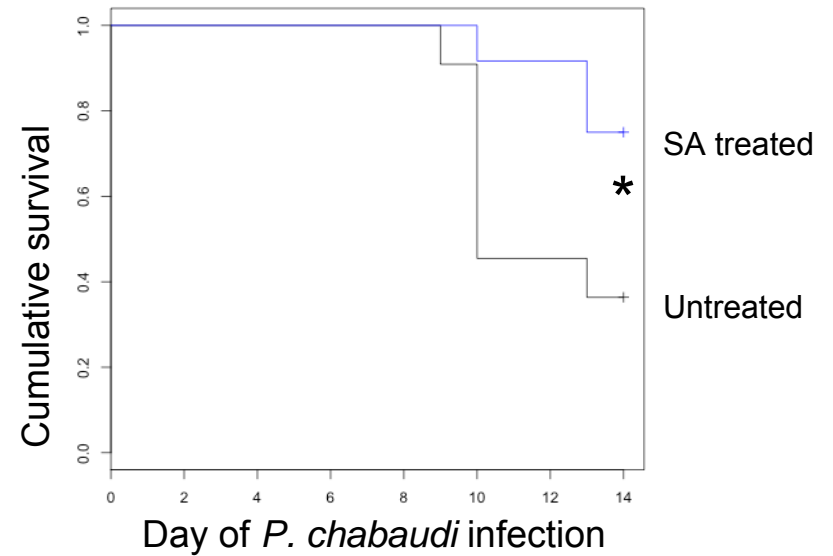
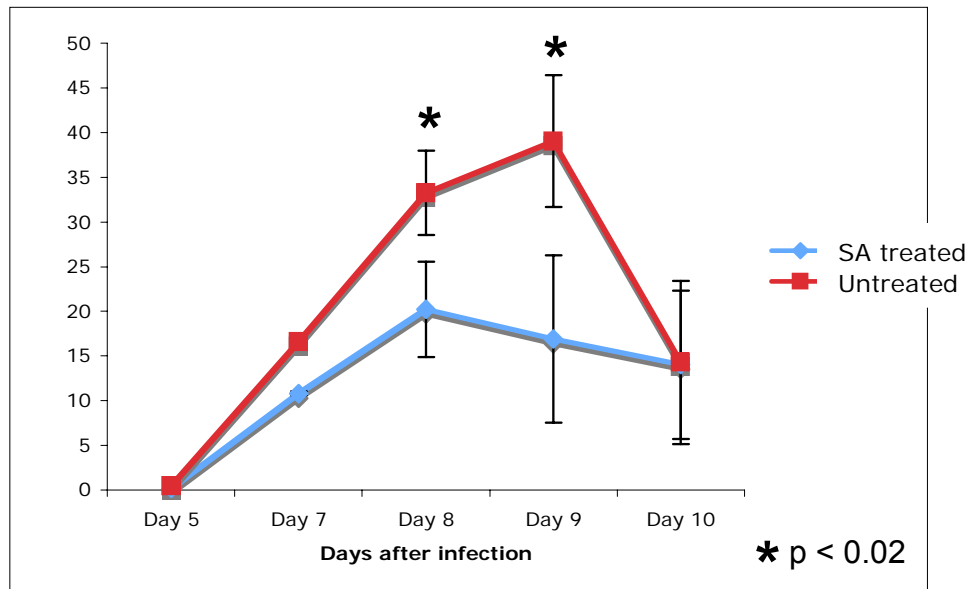
$IC_{50} = 2.5 \mu M$



Anti-malarial efficacy of SA in *P. chabaudi*-infected mice

Clare Smith 2008

SA treatment significantly reduced peak parasitaemia and improved survival



* P= 0.03 (Mantel-Cox log rank test)

Advantages of a haem enzyme HDT

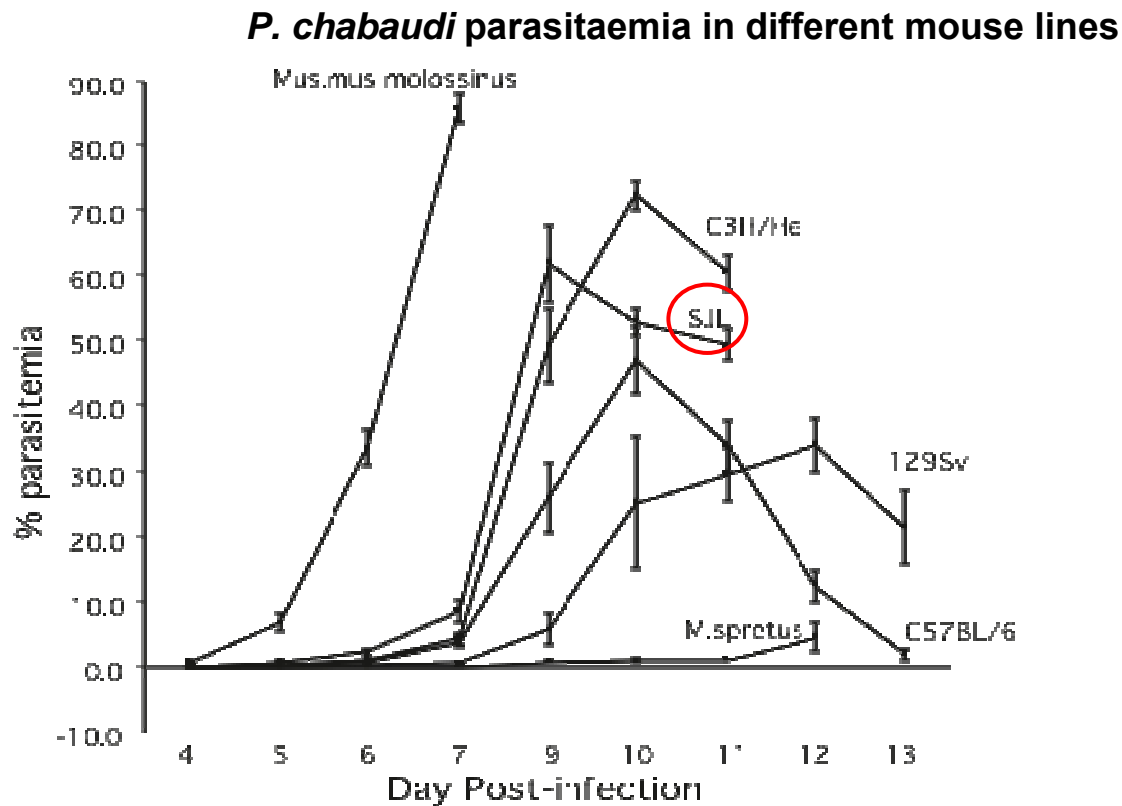
- Haem synthesis is essential for parasite growth.
- Host-derived ALAD is essential for parasite growth
- Some efficacy observed with SA *in vivo*.

- Haem enzymes are abundant in mature RBC, but not necessary for function.
- RBC do not synthesise new proteins.
- RBC life-span ~ 120 days.
- Therefore, an irreversible drug (like SA):
 - allows intermittent treatment, renders red cells impervious to parasite growth for their entire life
 - would act outside parasite, thereby avoiding drug efflux by the parasite

- Caveat: Side effects ≈ porphyria?

Towards new HDT targets: Mouse Genetic Screening

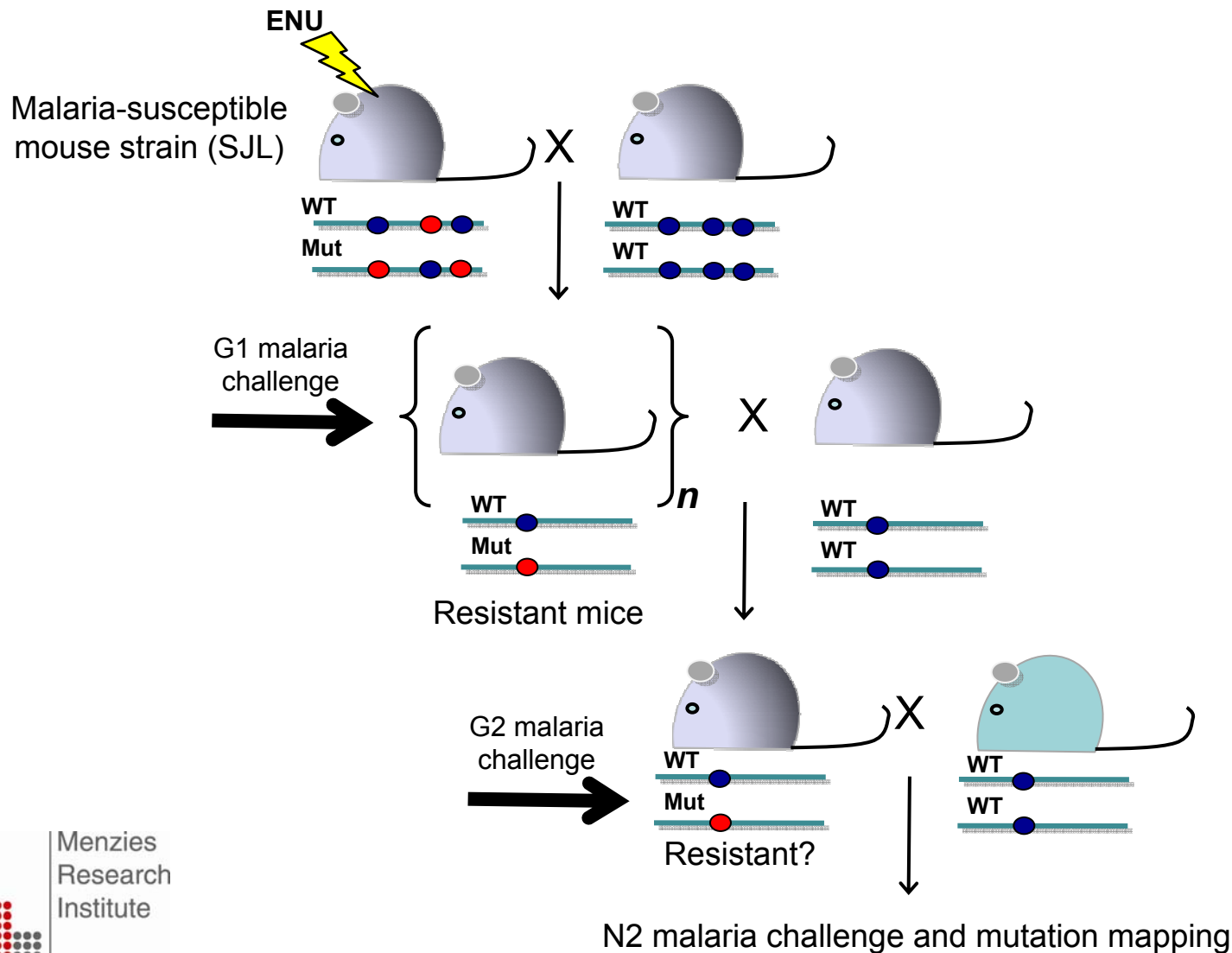
- Human polymorphisms (diseases) provide protection
- Resistance is also genetically determined in mouse models of malaria
- Several resistance loci mapped for *P. chabaudi* and *P. berghei*



Genetic (ENU) screen for protective mutations

(Dr Gaetan Burgio)

ENU (N-ethyl-N-nitrosourea): induces random point mutations = pool of novel genetic variants



Genetic (ENU) screen for protective mutations

(Dr Gaetan Burgio)

Anticipated outcome:

- Identification of novel genes that when disrupted, increase survival to infection

Advantages:

- ENU-induced mutations are simple to map (gross loci mapping and next-gen sequencing)
- Typical mutation disrupts gene function; \approx druggable effect?
- *In vivo* toxicity pre-validated during ENU screen
- High likelihood of discovering new biology

Challenges/Caveats:

- Elucidation of underlying resistance/survival mechanism
- Translation of mouse models to human malaria.

Conclusions

- **Limited life-span for current anti-malarials**
- **Immune response and host genetics important protective mechanisms**
- **Role for platelets in protection; implications for use of COX inhibitors**
- **Polymorphisms protect against infection; resistance-proof**
- **Strategies to identify host-directed targets:**
 - **Bioinformatic: haem pathway**
 - **ENU genetic screening: novel targets?**

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