

# Supplement: in-host multi-strain malaria system

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## Model setup

**Target cells** (RBC): susceptible population  $x$ ; homeostatic maintenance, depletion by invading merozoites.

**Parasites:** iRBC  $y$  (circulating and sequestered),  $M = r y$  merozoites,  $r$ -replication factor

**Immune effectors** <sup>1, 2</sup> (specific Ab): anti-erythrocyte  $u$  (binds to EMP, prevents sequestration); anti-merozoite  $v$  (binds to MSP, prevents RBC invasion).

Antigenic variation (AV): each strain consists of  $m$  EMP-variants that stimulate variant-specific effectors  $(u_1; \dots; u_m)$ , and a single (non-specific) MSP – effector  $v$

## Processes

**Invasion:** newly invaded RBC  $Y = (1 - e^{-pM/x})x$ ,  $p$  – probability of invasion, or  $Y_i = (1 - e^{-p_i M_i/x})x$  for multi-strain  $(Y_1; \dots; Y_n)$

## Immune clearing

Innate (febrile) control “switch”  $f(TY) = \frac{1}{1 + (TY / y_F)^p}$  - density-dependent surviving fraction of I (

$TY = \sum_i y_i$  - total parasitemia iRBC),  $y_F$  - febrile threshold

Adaptive effector variables (Ab)  $u$

- Anti-erythrocyte:  $\phi(Ku)Y$  – surviving fraction of newly invaded RBC  $I$  ( $u$ - effector level,  $K$  – affinity). For cross-reacting multi-strain systems  $\phi(\sum_j K_{ij} u_j)Y_i$ . Clearing function  $\phi(Ku)$  is derived in Appendix (equations (1.4)-(1.5))
- Anti-merozoite:  $p(v) = \frac{P_0}{1 + K_v v}$  - reduction in RBC-invasion by  $M$ .

## Single-strain system

**Variables:** (i) normal RBC -  $x$  (homeostatic maintenance, depletion by invading merozoites); (ii) Infected iRBC -  $y$ , free merozoites  $M = r y$  ( $r$ -replication factor); (iii) specific immune effector (Ab)  $u$  ( $^1, ^2$ ); binds to iRBC (prevents sequestration and clears  $y$ )

**Processes:**

1. **RBC Invasion:** newly invaded RBC  $Y = (1 - e^{-pM/x})x$ ,  $p$  – probability of invasion
2. **Immune clearing:**
  - a. Innate (febrile): sigmoid  $f(Y) = \frac{1}{1 + (Y/y_F)^p}$  - density-dependent surviving fraction of  $Y$   
with febrile threshold  $y_F$
  - o Adaptive  $u$ : surviving fraction of newly invaded RBC  $\phi(Ku)Y$ ,  $K$  – affinity. Clearing function  $\phi(Ku)$  is derived in Appendix (equations (1.4)-(1.5))
3. **Immune stimulation:** homeostatic maintenance of baseline  $u$  and its stimulation (growth/proliferation) by  $y$ .

The system is run in discrete time steps  $\Delta t = 48$  h (merozoite replication cycle), as map of current state  $(x, y, u)$  at time  $t$  (the end of cycle) to next state  $(x', y', u')$ , at  $t + \Delta t$

$$\begin{aligned}
 x' &= \underbrace{(1 - \delta)x_0}_{\text{production}} + \underbrace{\delta x}_{\text{loss}} - \underbrace{Y}_{\text{merozoite invasion}} \\
 y' &= \underbrace{\phi(u)f(Y)Y}_{\text{immune survival}} + \underbrace{S}_{\text{source/inoculae}} \\
 u' &= \underbrace{(1 - \delta_u)u_0}_{\text{baseline maintenance}} + \underbrace{\delta_u u}_{\text{loss}} + \underbrace{\sigma(y, u)}_{\text{stimulation}}
 \end{aligned}$$

**Table 1:** Model equations (single-variant, single-strain case)

Compartment	Equations	Auxiliary variables, triggers
RBC	$x' = (1 - \delta)x_0 + \delta x - Y$	RBC invasion: $Y = (1 - e^{-pM/x})x$
iRBC	$y' = f(Y)\phi(u)Y + S$ - source	Free merozoites: $M = r y$ Immune clearing (iRBC survival fraction) Innate: $f(Y) = \frac{1}{1 + (Y/Y_T)^m}$ , $m$ - Hill exponent Specific (Ab): $\phi(u)$
Specific IE	$u' = (1 - \delta_u)u_0 + \delta_u u + \sigma(u, y)$	immune stimulation: $\sigma(y, u) = \frac{\sigma_0 y}{y + y_T} \cdot \frac{u}{1 + u/u_T}$

## Multi-strain system

Parasite types labeled by  $i = 1, 2, \dots$

- **Table 2:** Dynamic variables and functions used in multi-variant

Variable	Notation
Uninfected red blood cells [ $\mu\text{L}^{-1}$ ]	$x$
Merozoites	$M_1, M_2, \dots, M_n$
Newly invaded RBC [ $\mu\text{L}^{-1}$ ]	$Y_1, Y_2, \dots, Y_n$
Sequestered iRBC [ $\mu\text{L}^{-1}$ ]	$y_1, y_2, \dots, y_n$
Anti-EMP (VSA) effectors	$u_1, u_2, \dots, u_n$
Anti-MSP effector	$v$

- **Table 3:** Model equations (single-strain case)

Compartment	Equations	Auxiliary variables; immune triggers
RBC	$x' = (1 - \delta)x_0 + \delta x - Y$	$M_i = \sum_j a_{ij} r_j y_j$ ; total $M = \sum_{i=1}^n M_i$
Sequestered iRBC	$y_i' = f(Y)\phi(u_i)Y_i$	$Y_i = \frac{M_i}{M} Y$ ; total $Y = (1 - e^{-pM/x})x$
u- effector	$u_i' = (1 - \delta_u)u_0 + \delta_u u_i + \frac{\sigma_u Y_i}{Y_i + y_T} \cdot \frac{u_i}{1 + u/u_T}$	$Y_i = \sum_m K_{im} [\alpha Y_m + (1 - \alpha)y_m]$ $u = \sum_i u_i$

**Table 4:** Parameter names and values/ranges used in the model

Parameter Definition	Notation	Value range
Normal red blood cell level [ $\mu\text{L}^{-1}$ ]	$x_0$	$5 \times 10^6$
Red blood cell loss rate (survival fraction) <sup>1</sup>	$\delta$	0.98
Parasite clearing cut off [ $\mu\text{L}^{-1}$ ]	$y_c$	$10^{-6}$
Parasite replication rate [per cycle 1-2 days]	$r_i$	6 -12
Immune stimulation threshold	$y_T$	0.001 - 0.003
Baseline immune level (dimensionless)	$u_0$	.05-.1
	$v_0$	.01-.05
Immune efficiency (affinity)	$K_{ij}$	0-1
Saturation threshold for immune growth	$u_T, v_T$	20-50
Maximal proliferation rates	$\sigma_u$	2-4
	$\sigma_v$	1.2-1.8
Immune memory (loss rate/cycle) <sup>2</sup>	$\delta_u$	0.9 - 0.95
	$\delta_v$	.9-.99

## Genetically structured parasite

Parasite makeup: collection of var-genes

Locus	1 (growth)	2 (anti-erythrocyte)
1	$r_1$	$u_1$
2	$r_2$	$u_2$
...	...	...

### AV-switching

Different patterns of mutation matrix:  $A = (a_{ij})$ ,  $\sum_{i=1}^n a_{ij} = 1$ .

- 1) Uniform  $a_{ij} = \varepsilon$  ( $i \neq j$ ), with rate  $\varepsilon = 10^{-9} - 10^{-4}$   $a_{ii} = 1 - \sum_{j \neq i} a_{ji}$
- 2) Sequential band-diagonal  $A = (a_{ij})$ ,  $a_{ij} = \varepsilon$  ( $|j-i|=1$ ) or 0 ( $|j-i|>1$ ).
- 3) Structured mutability based on network (graph) with links  $\{i \rightarrow j\}$

<sup>1</sup> Based on 100-day life-span of RBC

<sup>2</sup> Based on immune (Ab) duration of 40-100 days

4) Structured mutability based on genotypes (alleles on different loci control basic phenotypes growth, antigenic types, etc.),

So clone  $(i, j, k)$  has growth -  $r_i$ , anti-RBC effector -  $u_j$ , anti-merozoite effector -  $v_k$ . Such structured quasi-species can be divided into cross-reacting groups.

### Input parameters

Strain AV-diversity:  $n = \#$  distinct AV types. Effective parasite replication  $r = (r_1; r_2; \dots)$  ;

switching/mutability  $\varepsilon$ . Max immune stimulation proliferation  $(\sigma_u; \sigma_v)$ , immune thresholds  $y_T; M_T$  ;

memory loss  $\delta_u; \delta_v$ , memory rates (survival fractions)  $\delta_u; \delta_v$  .

### Estimated outputs

Individual histories and “host ensembles” with various inputs, including (i) random AV-switching rate and pattern  $A = A(\varepsilon)$  ; (ii) combinations of uncertain in-host parameters.

Descriptive ensemble statistics include:

1. Observed history duration
2. Max parasitemia, # peaks
3. Fast and slow clearing slopes
4. Virulence (=max RBC loss), cumulative RBC-loss/host mortality
5. Transmissibility based on parasitemia-duration patterns

## Immune clearing and stimulation

Anti-malaria immunity involves different types of antigens and immune effectors (Abs), most important are anti-merozoite MSP-effectors ( $v_1; v_2; \dots$ ), and anti-erythrocyte EMP effectors ( $u_1; u_2; \dots$ ). Both effector types bind to respective antigens:  $u_i$  - to iRBC  $y_i$  (and cross-reacting  $y_j$ ), while  $v_i$  binds to free merozoites  $M_i, M_j$ . We want to estimate surviving fraction  $\phi(u)$  of newly invaded iRBC  $Y$ , as function of immune effector  $u$ . The derivation employs continuous Ab-binding process (DE). Two possible approaches are (i) binding-dissociation kinetics, and transition 'free' to 'opsonized' iRBC,  $y \rightleftharpoons z$ ; (ii) dynamics of opsonization fraction of iRBC,  $0 < s(t) < 1$ , and the resulting survival probability  $\phi(s)$

**Binding- dissociation kinetics.** We use standard reaction kinetic for free and bound iRBC populations

$y(t) \quad z(t)$



with binding rate  $k u$ , proportional to concentration  $u(t)$ , and fixed dissociation  $k_-$ . Opsonized population  $z$  is cleared (spleen) at a rate  $\nu$ , and is removed by the end of the cycle (no merozoite release). So only free (unbound)  $y$  are left to release merozoites for the next cycle.

We want to estimate the surviving fraction of initial population  $Y$  by the end of the cycle  $0 < t < T$  (= 48 hrs). The DE system for reaction kinetics (1.1),

$$\begin{aligned} \frac{dy}{dt} &= -k u y + k_- z; & y(0) &= Y \\ \frac{dz}{dt} &= k u y - (k_- + \nu) z; & z(0) &= 0 \end{aligned} \quad (0.2)$$

initialized at values  $y(0) = Y = (1 - e^{-pm/x})x$ ,  $z(0) = 0$ , all newly invaded iRBC unbound by  $u$ . After

*rescaling* over dissociation rate  $k_-$ ,  $k \rightarrow K = k / k_-$  - affinity,  $\nu \rightarrow \nu / k_-$ , assuming near constant

(mean) effector level  $u$  over the cycle, we write solution of (1.2) via eigenvalues of matrix

$$A = \begin{bmatrix} -Ku & 1 \\ Ku & -(1+\nu) \end{bmatrix}$$

$$\lambda_{1,2}(Ku, \nu) = -\frac{1+Ku+\nu}{2} \pm \sqrt{\left(\frac{1+Ku+\nu}{2}\right)^2 - \nu Ku} \quad (0.3)$$

The survival fraction is given by

$$\frac{y(t)}{Y} = \phi(Ku) = \frac{(Ku + \lambda_2)e^{\lambda_1} - (Ku + \lambda_1)e^{\lambda_2}}{\lambda_2 - \lambda_1} \quad (0.4)$$

Function  $\phi(Ku)$  predicts (approximately) surviving fraction of iRBC by the end of cycle in terms of dimensionless  $Ku$ , and relative clearing rate  $\nu = \nu / k_-$ .

For multi-strain systems with immune effectors  $\{u_1; \dots; u_n\}$  and cross-reactive matrix  $K = (K_{ij})$  we use approximate clearing function (1.3)-(1.4) applied to each strain  $y_i$ , so

$$\frac{y_i}{Y_i} = \phi\left(\sum_{j=1}^n K_{ij}u_j; \nu; T\right) = \phi\left(\sum_{j=1}^n K_{ij}u_j\right) \quad (0.5)$$

**Opsonisation process:** the dynamic variable here is mean coverage level  $s(t)$  of iRBC bound by Ab  $u$ .

The binding-dissociation kinetics gives DE

$$\frac{ds}{dt} = ku(1-s) - k_-s \quad (0.6)$$

Its equilibrium level  $s^* = \frac{Ku}{1+Ku}$ , determines mean survival probability as sigmoid function

$$\phi(s) = \frac{1-s^m}{1+s^m} \text{ (Figure 1).}$$

The resulting survival probabilities in both systems, binding-dissociation (i) and opsonisation (ii) are shown in Figure 2. They are qualitatively similar for both systems.

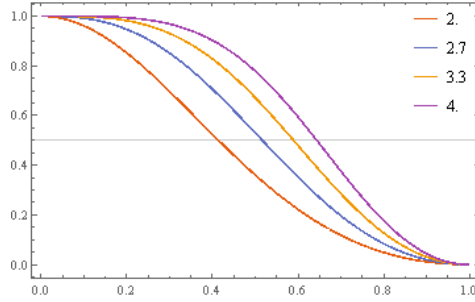


Figure 1: Survival functions  $\phi(s)$  for serial values  $2 \leq m \leq 4$

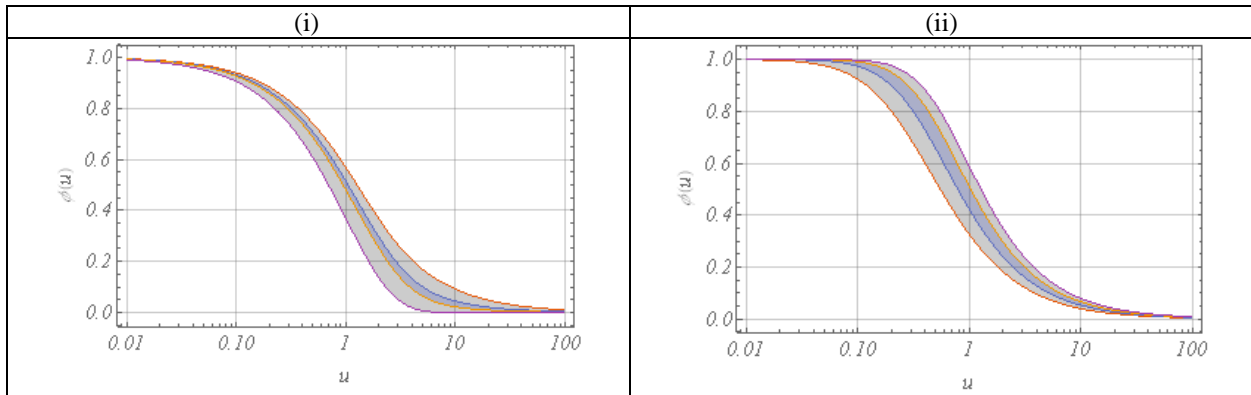


Figure 2: Survival fraction  $\phi(u)$  in 2 cases: system (i) for clearing range  $0 < \nu < \infty$  (top-to-bottom) – shaded; system (ii) for range  $2 \leq m \leq 4$

**Anti-merozoite Abs**  $\nu = (\nu_i)$  have short time to invade RBC, so the clearing is less important ( $\nu = 0$ ), but only unbound fraction  $m$  of freshly released merozoite  $M = r y$  can invade RBC efficiently. We estimate this fraction from equilibrium M- kinetics

$$\frac{dm}{dt} = -k \nu m + k_- (M - m)$$

Hence,  $m^* = \frac{M}{1 + K \nu}$ , with affinity  $K = K_\nu = \frac{k}{k_-}$ , or  $\frac{M_i}{1 + \sum_j K_{ij} \nu_j}$  - for cross-reacting suite  $(\nu_j)$ .

**Immune stimulation/growth** for  $u_i$  (or  $\nu_i$ ) is determined by antigenic load  $Y_i (M_i)$ , or cross-reacting

$$\sum_j K_{ij} Y_j .$$



Variable  $Y$  stimulates proliferation of immune B-cells, their conversion to secreting (plasma) cells and release of antibodies (effector  $u$ ). We lump all these processes into a single immune stimulation function

$$\sigma(Y) = \frac{\sigma_M Y}{Y + y_T}$$

with maximal proliferation rate  $\sigma_M$ , and sensitivity threshold  $y_T$ . So  $\sigma(Y)$  changes from a ‘decay mode’ ( $\sigma(Y) < 1$  at low  $Y$ ) to the ‘growth mode’ ( $\sigma(Y) > 1$ ), as  $Y$  passes a critical threshold.

The exponential growth of  $u$  driven by factor  $\sigma(Y) > 1$  cannot proceed indefinitely, but it should slow down when B-cell populations and effector  $u$  reach sufficiently high levels. While multiple factors and mechanisms control proliferation/growth of immune effectors, in our scheme we implement it through a simple density threshold effect, so it exponentially for low  $u < u_T$  (threshold), and saturates at high levels

by density factor  $\frac{u}{1 + u / u_T}$ . The combined immune stimulation function takes the form

$$\sigma(Y, u) = \frac{\sigma_M Y}{Y + y_T} \cdot \frac{u}{1 + u / u_T} \quad (7)$$

We also allow for slow waning of immune effector  $u$  at a certain rate  $0 < \delta_u < 1$ , due to Ab degradation and memory loss.

1. Struik SS, Riley EM. Does malaria suffer from lack of memory? *Immunol Rev.* 2004;201:268-290.
2. Eckhoff P. P. falciparum infection durations and infectiousness are shaped by antigenic variation and innate and adaptive host immunity in a mathematical model. *PloS one.* 2012;7(9):e44950.