The within-host cellular dynamics of bloodstage malaria: theoretical and experimental studies

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SUMMARY

The properties of a mathematical model of bloodstage infection with a single strain of malaria were investigated. Analysing the cell population dynamics in the *absence* of a host immune response we demonstrate a relationship between host and parasite parameters that defines a criterion for the successful invasion and persistence of the parasite. Important parameters are the rates of merozoite production and death and those of erythrocyte production, death and invasion. We present data from experiments designed to evaluate the erythrocyte invasion rate in a rodent malaria system. The model generates patterns of parasitaemia in good qualitative agreement with those seen in *Plasmodium berghei* infections. The sole force behind the rise and fall in parasitaemia in the model without immunity is the density of susceptible erythrocytes, suggesting that resource availability is an important determinant of the initial pattern of infection *in vivo*. When we incorporate a simple immune response into the model we find that immunity against the infected cell is much more effective at suppressing parasite abundance than immunity against the merozoite. Simulations reveal oscillating temporal patterns of parasite abundance similar to *P. c. chabaudi* infection, challenging the concept that antigenic variation is the sole mechanism behind recrudescing patterns of infection.

Key words: dynamics, malaria, erythrocyte, immunity, merozoite, invasion.

INTRODUCTION

The starting point of this study is the mathematical model of bloodstage malaria proposed by Anderson, May & Gupta (1989). Gravenor, McLean & Kwiatkowksi (1995) have recently used this model to investigate the role of competition between parasites for erythrocytes as a mechanism which controls parasitaemia, estimating parameters for *P. falciparum* infection from clinical data. An alternative theoretical framework suggested by Kwiatkowski & Nowak (1991) proposed that the sometimes chaotic nature of *P. falciparum* abundance within the human host is regulated by an interaction between host temperature and the varying temperature sensitivity of different parasite stages. Here we assume few external forces, but consider the cellular interactions as a predator–prey relationship in which host erythrocytes are a resource whose density is governed by birth and death processes and parasite-induced mortality. Initially the parasite–host interaction is viewed in its simplest form, by considering the densities of the principal host and parasite cell populations in the *absence* of immunity and gametocytogenesis. We determine whether a set of 3 simple, non-linear, ordinary differential equations can encapsulate the temporal patterns of infection observed in animal models. The value of formulating the model is in highlighting those biological processes that are most important and, in particular, the criteria that define whether or not the parasite is able to successfully invade and persist in the presence or absence of a defined immune response. Parameter estimation is central to such analysis and we utilize existing data as well as presenting our own experimentally derived estimates of the erythrocyte invasion rate for a strain of rodent malaria.

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MATHEMATICAL MODEL OF BLOODSTAGE MALARIA IN THE ABSENCE OF AN IMMUNE RESPONSE

Formal description

The mathematical framework for the study of bloodstage malaria is summarized in Fig. 1. It considers the changing densities of 3 cell populations: healthy erythrocytes, X; infected erythrocytes, Y; free merozoites, M. In order to produce a tractable model of the biological system there are 2 principal simplifications: we neglect the effects of gametocytogenesis which are considered secondary to other processes in the initial dynamics of infection (see Hellriegel, 1992); and we assume that merozoites invade erythrocytes entirely at random, i.e.

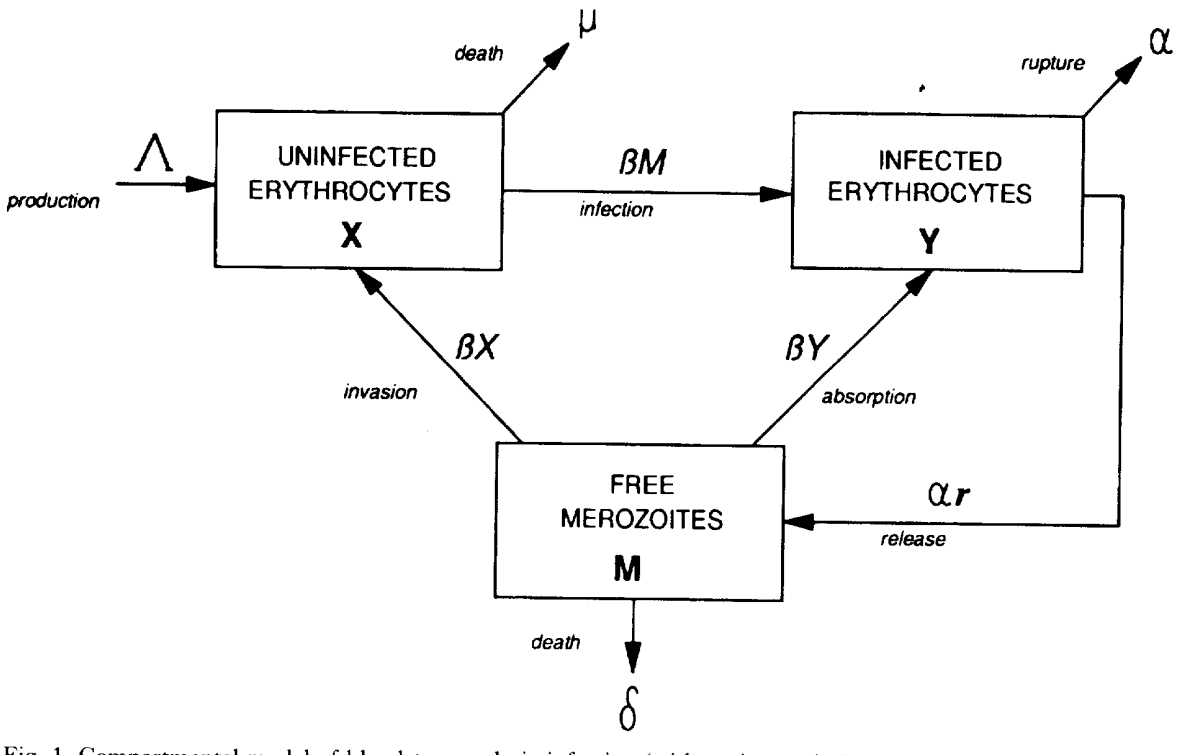


Fig. 1. Compartmental model of bloodstage malaria infection (without immunity).

have no predilection for erythrocytes of a particular age or type. In the first instance, we deliberately consider the system in the absence of any host immune response in order to analyse the underlying dynamics before introducing layers of biological complexity.

The cell population dynamics are regulated by a series of assumptions based on the known biology of the host–parasite system. It is assumed that uninfected erythrocytes are produced at a constant net rate Λ by the bone marrow and die (or are removed) at a *per capita* (i.e. per cell) rate μ. Infection is viewed as a mass action process; uninfected erythrocytes move into the infected class at a rate proportional to their own density multiplied by that of free merozoites. The constant of proportionality β is the *per capita* rate of erythrocyte invasion and comprises the dual probability of contact between an uninfected erythrocyte and a merozoite and of such a contact resulting in productive invasion. Infected cells experience a *per capita* death rate α due to parasite-induced rupture; α is large compared to the natural death rate μ. Each ruptured infected cell produces an average of r merozoites such that the net rate of merozoite production is αrY. Free merozoites invade susceptible erythrocytes at a net rate βXM and die at a *per capita* rate δ. Distinct from the model of Anderson *et al.* (1989), and based on *in vivo* observations of multiple invasions (Garnham, 1966), it is proposed that merozoites are equally likely to be absorbed into already infected erythrocytes, providing an additional loss term of βXY. It is assumed for simplicity that the secondary merozoite perishes without adversely affecting the maturation of the primary merozoite. The additional loss of merozoites, however, is not a major determinant of the system's dynamics.

Our assumptions lead to the following set of coupled non-linear ordinary differential equations:

$$\textit{uninfected cells} \quad \frac{dX}{dt} = \Lambda - \mu X - \beta XM \tag{1}$$

$$\textit{infected cells} \quad \frac{dY}{dt} = \beta XM - \alpha Y \tag{2}$$

$$\textit{merozoites} \quad \frac{dM}{dt} = \alpha rY - \delta M - \beta(X+Y)M \tag{3}$$

Criteria for initial invasion and long-term persistence of the parasite

The system is at equilibrium (or steady state) when the densities of merozoites and uninfected and infected erythrocytes are constant, that is, when the rate of change over time of each cell population is simultaneously zero. The system described by equations 1–3 has 2 equilibrium points. At the first (equation 4), termed the infection-free state, the level of uninfected erythrocytes is as normal (the ratio of their production and death rates) and both parasite stages are absent:

infection-free equilibrium

$$X_{eqm} = \frac{\Lambda}{\mu}, \quad Y_{eqm} = M_{eqm} = 0. \tag{4}$$

Table 1. Value of parameter r for selected *Plasmodium* spp. infecting rodents and man

(Data from Killick-Kendrick & Peters, 1978; Garnham, 1966.)

Plasmodium species	Merozoites/infected cell (r)
P. berghei	6–10 (mice), approx. 16 (rat), up to 18 (natural rodent host)
P. yoelii yoelii	12–18 (rodent)
P. chabaudi chabaudi	4–8 (rodent)
P. vinckei vinckei	8 (mice), 12–14 (natural rodent host)
P. vivax	12–24 (man)
P. ovale	4–10 (man)
P. falciparum	8–32 (man)
P. malariae	8–12 (man)

Theoretically, the infection-free state exists just before infection or following the elimination of an infection.

The second steady state is a long-term endemic equilibrium representing *parasite–host cell coexistence*, or in other words, successful parasite invasion of the host:

$$X_{eqm} = \frac{\beta\Lambda + \alpha\delta}{\beta(\mu + \alpha(r-1))}. \tag{5}$$

$$Y_{eqm} = \frac{\beta\Lambda(r-1) - \mu\delta}{\beta(\mu + \alpha(r-1))} \tag{6}$$

$$M_{eqm} = \frac{\alpha(\beta\Lambda(r-1) - \mu\delta)}{\beta(\alpha\delta + \beta\Lambda)} \tag{7}$$

We consider 2 questions. First, what are the conditions determining whether or not a parasite can initially invade the virgin, infection-free state and produce an epidemic of parasitaemia (i.e. positive parasite growth)? Second, having successfully invaded the blood system, what determines whether the parasite will persist and establish a stable, endemic equilibrium within the host rather than being eliminated? To determine the conditions for successful invasion we employed conventional local stability analysis (May, 1974) to assess the consequence of small perturbations (such as the inoculation of 1 merozoite) to the infection-free state. Analysis indicates that the infection-free state is locally stable and will resist invasion when:

$$\frac{\Lambda}{\mu} < \frac{\delta}{\beta(r-1)}. \tag{8}$$

Note that r must exceed unity for this expression to be meaningful; typical values of r for selected *Plasmodium* species are shown in Table 1.

The conditions governing the long-term persistence of the parasite are deduced by examining equations 6 and 7. The parasite is said to persist if both Y_{eqm} and M_{eqm} are greater than zero, and this will be true for $\alpha > 0$ (always true) and:

$$\frac{\Lambda}{\mu} > \frac{\delta}{\beta(r-1)} \tag{9}$$

The above criterion also defines the resistance of the endemic equilibrium to small perturbations in 1 or more of the cell populations. Thus, the parameter conditions ensuring a stable state of parasite–host coexistence are identical to those which allow parasite persistence in the system, and exclude the possibility of a stable infection-free equilibrium. In biological terms, if the attributes of the parasite are such that a small inoculum is able initially to produce an invasion epidemic in the host erythrocyte population then, in the system without immunity, it will always establish a stable equilibrium with the host. Conversely, if the biological properties of the parasite are insufficient to support the initial invasion, then the system will return to the stable infection-free equilibrium and the parasite will die out.

The model suggests that whether or not the parasite is able to establish within the host is a conflict between opposing forces, namely, the life-expectancy of the merozoite stage, the probability of successful cell invasion, the level of susceptible erythrocytes and the number of merozoites produced per infected cell. The fact that merozoites are expended once they have infected a cell also acts as a disadvantage (i.e. a negative force) to parasite survival. Equation 9 also indicates that the size of the initial inoculum is not important for successful infection in this model, and this is confirmed later by numerical simulation.

Parameter values

Most of the population parameters defined in the bloodstage malaria model have been quantified for several combinations of host–parasite species. In order to validate the output of the model, we make use of data from experimental rodent infections of which there are many published examples of infection time-courses in genetically identical rodent strains, with measurements of parasitaemia and erythrocyte numbers over time in untreated, non-immune animals. Similar data are rarely, if ever, available for human malaria infection.

The parameter values used in the numerical analysis are based upon an infection of *P. berghei* in a rodent host. The biological definition, magnitude and source of each parameter estimate are given in Table 2. The derivation of average *per capita* parameter values from data assumes that rates are constant with time and cell density. The parameter for which little direct information exists is the complex rate β, the *per capita* erythrocyte invasion rate. Our analysis above indicates that β partly

Table 2. Values of parameters and initial variables used in the model of murine bloodstage malaria

(Rates apart from Λ are *per capita* rates, that is, the net loss or gain per unit time is obtained by multiplying the rate by the appropriate cell density.)

	Definition	Value	Source
Parameter			
Λ	Rate of erythrocyte production	*$2{\cdot}5 \times 10^8$ cells ml^{-1} day^{-1}	—
μ	Death rate of uninfected erythrocyte (= 1/life-span)	0·025 day^{-1}	Russell & Burnstein (1966)
β	Rate of merozoite–erythrocyte contact resulting in successful infection	?(cell/ml)$^{-1}$ day^{-1}	—
α	Death rate of infected erythrocyte (= 1/duration of schizogony)	1 day^{-1}	Garnham (1966)
r	No. of merozoites released per infected cell	12	Killick-Kendrick & Peters (1978)
δ	Death rate of merozoite (= 1/life-span)	48 day^{-1}	McAllister (1977)
Variable			
X_o	Initial density of uninfected erythrocytes (= Λ/μ)	10^{10} cells ml^{-1}	Russell & Burnstein (1966)
Y_o	Initial density of infected erythrocytes	0 cells ml^{-1}	—
M_0	Initial density of merozoites	10^6 cells ml^{-1}	Vanderberg (1977)

* To maintain erythrocytes at their normal density, $\Lambda = \mu \times X_o$ cells ml^{-1} day^{-1}.

determines the ability of the parasite to invade and persist within the host, so the magnitude of β is likely to have a significant impact on the potential of the immune system to control an infection. In view of the importance of β, and the difficulty of guessing its magnitude, we carried out experiments to evaluate β within the framework of the model.

EXPERIMENTAL ESTIMATION OF THE CELL INVASION RATE, β

The aim of the experimental approach was to evaluate the number of successful invasions resulting from a known number of merozoites. To achieve this, the dynamics of the first round of invasions in a bloodstage infection of *P. berghei* in naive rats were examined. The experiment was designed to ensure consistency between the experimental system and the assumptions underpinning the model, in particular: the model assumes that the bloodstage infection is synchronous and therefore animals were mechanically inoculated with freshly isolated mature schizonts; as the model neglects merozoite loss due to gametocytogenesis, we measured the invasion of an asexual clone of *P. berghei*; to minimize the effects of immunity we measured the invasion of this parasite in young rats which possess little protective immunity (Zuckerman, 1957). Furthermore, as the total duration of infection was no more than 20 h, it is improbable that a significant host immune response was induced (although some innate immunity may have operated). The experimental conditions permit the following statements. (1) Natural erythrocyte death and replacement rates *in vivo* are negligible during the short duration of infection (μ and $\Lambda = 0$). (2) Total erythrocyte levels remain constant as no parasite-induced rupture occurs. Upon invasion, erythrocytes merely pass from the uninfected to the infected class. (3) By 18 h post-inoculation (p.i.), the merozoites contained in the inoculum will either be dead or will have penetrated host cells. Thus, the density of free merozoites will be zero at 18 h p.i. (4) Consequently, by 18 h p.i., all primary invasions will have occurred, so the rate of change of the densities of uninfected and infected erythrocytes will be effectively zero.

These boundary conditions enable us to solve the model at the quasi-steady state when the merozoite population (whose cell dynamics are much more rapid) has reached its equilibrium. We obtain the following expression for β in terms of the merozoite life-span, δ, the initial density of merozoites, M_o, the initial density of susceptible erythrocytes, X_o, and the density of successfully infected cells after one round of invasion, Y_t:

$$\beta = \frac{\delta \ln\left(\frac{X_o}{X_o - Y_t}\right)}{M_o - Y_t} \qquad (10)$$

Experimental materials and methods

Parasites. Clone 233L of *P. berghei* ANKA strain (kindly provided by R. E. Sinden, Imperial College, originally from D. Walliker, University of Edinburgh) produces asexual infections upon mechanical passage. Parasites derived from a third passage were used.

Animals. The invasion experiments were carried out in male Sprague–Dawley (SD) rats weighing 220–275 g, aged 6–8 weeks.

Table 3. Summary of the experimental data used to derive β

Rat no.	M_o ($\times 10^6$ ml^{-1})	X_o ($\times 10^9$ ml^{-1})	P_t at 18 h (%)	Y_t at 18 h* ($\times 10^6$ ml^{-1})
1	122·6	9·46	0·441	41·6
2	110·5	9·98	0·313	30·9
3	52·59	9·81	0·250	24·5
4	39·79	9·75	0·199	19·5
5	19·54	9·83	0·109	10·8
6	19·03	9·96	0·095	9·46
7	4·409	9·62	0·027	2·60
8	3·767	9·80	0·023	2·25

* Assuming that the total erythrocyte density remains constant during the experiment, $Y_t = P_t X_o/100$.

Estimation of parasitaemia. The percentage parasitaemia was evaluated throughout by counting the number of infected cells within at least 5×10^3 erythrocytes in a methanol-fixed, Giemsa-stained bloodfilm, using blood taken from the tail of an infected animal or from *in vitro* culture.

Preparation of schizonts. Large numbers of 233L parasites were generated by i.p. inoculation of SD rats with approximately 10^6 parasitized erythrocytes. The blood from 2 animals with a parasitaemia of not more than 2% was collected by cardiac puncture under deep anaesthesia. A concentrated preparation of schizont stages was obtained according to described methods (Mons *et al.* 1985). Briefly, the blood was mixed with an equal volume of CCM (complete culture medium: RPMI 1640 containing 25 mM HEPES, 0·3 g/l L-glutamine, 2 g/l $NaHCO_3$, 20% foetal calf serum and 50 μg/ml gentamycin) containing 20 i.u./ml heparin and centrifuged at 200 ***g*** for 10 min. The supernatant fraction and upper layer of cells, containing the majority of superinfected erythrocytes and schizonts, were discarded. The remaining erythrocytes, consisting mainly of uninfected cells and immature parasites, were cultured at a density of 2% (v/v, packed cells/medium) in Erlenmeyer flasks (40 ml/200 ml flask). The flasks were filled with a 10% O_2/5% CO_2/85% N_2 gas mix and incubated at 37 °C on an orbital shaker at 60 rpm. After about 16 h incubation, the immature parasites had developed into early schizonts; the shaker was halted and the culture suspension sampled hourly to determine the maturity of the schizonts. Unagitated schizonts of *P. berghei* persist *in vitro* for up to 48 h without releasing their merozoites (Mons *et al.* 1985). When approximately 90% of the schizonts contained 12–16 merozoites, the suspension was concentrated on a 44% Nycodenz density gradient (Nycomed). Purified schizonts were collected from the interface, washed, resuspended in CCM and counted. The parasitaemia and the average number of merozoites per schizont were calculated from a bloodfilm. The average number of *P. berghei* ANKA merozoites per mature schizont was 13·5 (range 6–22, $n = 78$). By determining the erythrocyte density in a haemocytometer, the number of merozoites/ml of schizont preparation was evaluated.

Estimation of the invasion rate. To assess the effect of varying the initial merozoite density upon the invasion rate, the schizont preparation was diluted with CCM to produce 4 suspensions containing densities of merozoites ranging from $1{\cdot}276 \times 10^8$ to $3{\cdot}09 \times 10^9$ merozoites/ml. A known volume of each preparation (300–500 μl) was inoculated into the tail vein of 2 lightly anaesthetized rats. The total blood volume of each animal was estimated from its bodyweight using a conversion factor of 5·5 ml blood/100 g (Russell & Bernstein, 1966). For each rat the initial merozoite density (M_o) was calculated as the ratio of the number of merozoites in the inoculum and the total blood volume (including the inoculum volume). The initial density of susceptible erythrocytes (X_o) in each rat was evaluated by haemocytometry. Due to variations in erythrocyte density, total blood volume and the volume of the inoculum, each animal effectively represented a unique set of initial conditions.

Merozoites of *P. berghei* which successfully invade develop into mature schizonts within roughly 23 h. By 18 h the maturing parasites are visible under the light microscope but have yet to leave the peripheral circulation and sequester in deep tissues (Mons *et al.* 1985). Thus, the fraction of erythrocytes containing late trophozoites/young schizonts was assessed at 18 h p.i. by counting in excess of 5×10^4 erythrocytes in a film of blood taken from the tail of each rat. As the original inoculum contained synchronized mature schizonts, all parasitized cells detected 18 h p.i. were assumed to be the product of the primary invasion.

Experimental results

The experimental data used to calculate the invasion rate in 8 rats are summarized in Table 3. It is clear

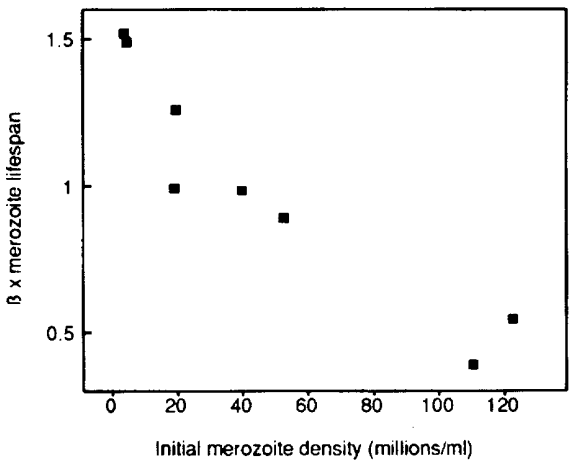


Fig. 2. Experimental estimates of β multiplied by the merozoite life-span ($1/\delta$), ($\times 10^{-10}$ (cell/ml)$^{-1}$) plotted against the density of the initial merozoite inoculum.

from equation 10 that β is linearly dependent upon δ and, as the exact value of δ is not known, it is convenient to rearrange equation 10 and to express our results in terms of β/δ. Fig. 2 plots our estimates of β/δ (where $1/\delta$ = merozoite life-span) for each initial merozoite inoculum. As we did not observe multiple infection of erythrocytes, the influence of superinfection was neglected for the purposes of this calculation. Moreover, at the low parasitaemias recorded, accounting for superinfection has a minor impact upon the results. Our estimates of β/δ lie in the range $0{\cdot}39$–$1{\cdot}54 \times 10^{-10}$ (cell/ml)$^{-1}$. In earlier experiments we similarly estimated β/δ for *P. berghei* in Theiler's Original mice ($n = 6$) to be $1{\cdot}375 \times 10^{-10}$ (cell/ml)$^{-1}$, consistent with the present findings.

NUMERICAL ANALYSIS OF THE MODEL OF BLOODSTAGE MALARIA WITHOUT IMMUNITY

The equations of the model can be solved numerically for a given set of parameters to provide a picture of the within-host cell dynamics of malaria infection over time. This allows us to investigate the effect of varying parameters and initial conditions upon, for example, the peak level of parasitaemia or whether the infection takes off at all. *In vivo* data are used to provide a comparison between the early dynamics of infection in the model and temporal patterns of infection *in vivo*.

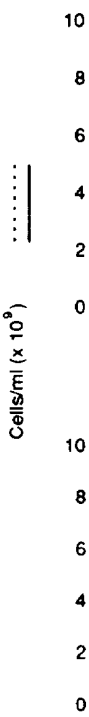
Numerical analysis of the equations was carried out using 4th-order Runge-Kutta numerical methods (Solver v4.01, APIC, Department of Statistics and Modelling Science, Strathclyde University, Glasgow) and the *P. berghei*–mouse parameters given in Table 2. Based on our experimental findings in rodents, a value for β of 2×10^{-9}/(cell/ml)$^{-1}$ day^{-1} was used in the simulations unless otherwise stated. Our observations suggest that the initial invasion rate of *P. berghei* is of a similar magnitude in naive mice and rats. We have based parameters on the *P. berghei*–mouse interaction principally because more detailed time-course data exist for this host–parasite combination. As indicated in Table 2, at the beginning of the simulation the density of uninfected cells, X_0, is the natural equilibrium between production and removal (Λ/μ) while Y_0 is zero. The initial merozoite inoculum M_0 is calculated from Vanderberg (1977), who found 5·6–266 liver schizonts after mosquito feeding. If each releases an average of 20000 merozoites (Garnham, 1966) then a natural range for M_0 is in the region $0{\cdot}05$–3×10^6/ml.

Results of numerical simulation and comparison with in vivo *data*

A typical example of the numerical patterns of the model is presented in Fig. 3, where it is compared with time-course data from studies of individual mice infected with *P. berghei* (Eling, van Zon & Jerusalem, 1977; Roth & Herman, 1979; Dearsley, Sinden & Self, 1990). These data sets are useful as the mouse has little immune protection against infection by *P. berghei* (Jayawardena *et al.* 1975) and because both erythrocyte and parasite densities have been recorded. Measurements of the parasitaemia alone can be more difficult to interpret as fluctuations may be due to a change in either infected or uninfected cell levels. The experimental data sets vary in the timing and the value of the peak parasitaemia and explanations for this include differences in the genetic backgrounds and age of the mice, in the virulence of the *berghei* isolate and in the size of the initial inoculum. Thus, rather than fitting the model to each time-course, we focus upon the qualitative properties of the model's output.

The infection dynamics of the simple system fall into 2 qualitatively distinct categories: rapid elimination of the parasite or the establishment of a persistent infection. The parasite is unable to initiate an increasing parasitaemia (a within-host epidemic) if parameter conditions are such that the infection-free state is locally stable (equation 8). Conversely, in the absence of immunity, merozoites entering the blood system will produce an initial epidemic of parasitaemia if this state is unstable to small perturbations (equation 9). Moreover, in the absence of immunity, the successful parasite will always persist at some stable, non-zero level within the host erythrocyte population. Whether or not an infection takes off at all is entirely dependent upon the relationship between the host/parasite-derived parameters given by equation 8.

In the model simulation (Fig. 3A), the level of uninfected erythrocytes initially falls while parasitized erythrocyte numbers rise as long as the erythrocyte production rate is exceeded by the high death rate of infected cells. However, parasite levels



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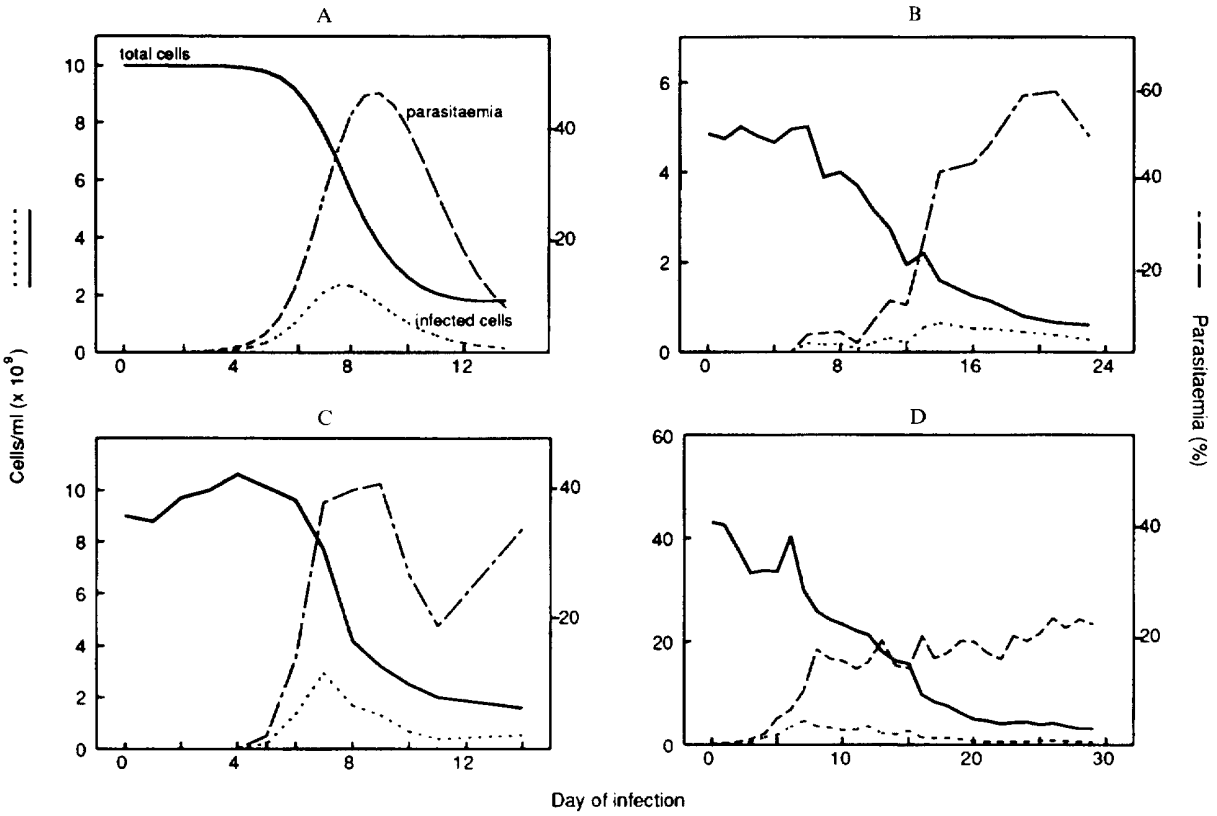

Fig. 3. (A) Simulation of malaria infection in the absence of immunity using the parameters in Table 2. Results are plotted as parasitaemia (–·–), density of infected cells (····) and total cell density (infected plus uninfected erythrocytes, ——). Shown for comparison are time-course data for *Plasmodium berghei* infections in individual mice redrawn from (B) Roth & Herman (1979), (C) Eling *et al.* (1977) and (D) Dearsley *et al.* (1990).

fall once there are no longer sufficient uninfected erythrocytes available for invasion. Analogous to a typical host–pathogen interaction, the subsequent net increase in the level of uninfected cells due to new recruits in turn eventually provides adequate resources for renewed parasite population growth. If followed for a sufficient length of time (e.g. 20–40 days) the host–parasite cell populations undergo increasingly damped oscillations towards a stable equilibrium. However, this equilibrium is a longer-term dynamic property of the model which may not be applicable to the *in vivo* situation when the effect of an immune response becomes significant. As long as the conditions for primary invasion are satisfied, varying the parameter values merely affects the kinetics and severity of the ensuing infection. For example, increasing β/δ or r leads to a more rapid and increased peak in parasitaemia and a more rapid achievement of the endemic equilibrium. Varying the initial number of infected cells or merozoites has no effect on the final equilibrium level attained as predicted earlier, while the initial density of erythrocytes is an important determinant of successful invasion.

In vivo (Fig. 3 B–D), we also observe an initial rise and fall in infected cell numbers accompanied by a dramatic decrease in the density of uninfected cells. The fall is intuitively predicted even in the model without immunity, and is because levels of susceptible cells fall below the threshold necessary to sustain positive parasite growth. However, despite strong qualitative agreement, there are minor differences between the pattern of infection predicted by the model and the *in vivo* picture. For example, the susceptible erythrocyte density remains lower and the parasitaemia generally remains higher than in the simulation. Such discrepancies can provide important information and are discussed later.

MATHEMATICAL MODEL OF BLOODSTAGE MALARIA WITH A SIMPLE IMMUNE RESPONSE

We extended the model to incorporate a simple immune response directed against the bloodstages of a single strain of malaria parasite. Though the response elicited by the parasite is highly complex it is probable that relatively few components are truly important in the regulation of parasite population growth. For example, a wide body of evidence indicates that protective anti-bloodstage immunity is dependent upon $CD4^+$ T cells (e.g. Suss *et al.* 1988). We therefore consider effective immunity as a population of activated T cells and assume that the resulting immune response is a direct function of

their density. We define a population of activated parasite-specific immune cells, T, which are recruited when their resting precursors contact free merozoites and infected cells at net rates $\gamma_m M$ and $\gamma_y Y$ respectively (γ_m and γ_y are constants). For simplicity it is assumed that activation is proportional to the density of these parasite stages and that the precursors are not limiting. The immune cells expand at a net rate p, which encapsulates the positive feedback upon the immune system (including T cell proliferation, macrophage activation, and help for B cell proliferation and antibody production). It is further assumed that a regulatory negative feedback force, such as the effects of cell density, inhibitory cytokines or natural apoptosis, operates to suppress immune population growth at a net rate proportional to the square of its density (bT^2). This is not an entirely arbitrary function as we frequently observe convex or plateauing relationships between the rate of T cell proliferation and antigen concentration (Lamb *et al.* 1983; Matis *et al.* 1983), implying a regulation of the response at high antigen concentrations. Merozoites and infected cells are destroyed at a rate proportional to their own density multiplied by that of the immune population at net rates ωMT and κYT respectively.

The amended set of equations is given by:

$$\frac{dX}{dt} = \Lambda - \mu X - \beta XM \tag{11}$$

$$\frac{dY}{dt} = \beta XM - \alpha Y - \kappa YT \tag{12}$$

$$\frac{dM}{dt} = \alpha r Y - \delta M - \beta(X+Y)M - \omega MT \tag{13}$$

$$\frac{dT}{dt} = \gamma_m M + \gamma_y Y + pT - bT^2 \tag{14}$$

where parameters Λ, μ, β, α, r and δ are as before.

Criteria for invasion and persistence in the system with an immune response

The determination of equilibria in the immune model is significantly more difficult, but inspection reveals that 3 categories of equilibrium exist: the first is the infection-free, naive equilibrium where $X = \Lambda/\mu$ and $Y = M = T = 0$. When $dT/dt = 0$ there is a second equilibrium point where $X = \Lambda\Lambda/\mu$ and $Y = M = 0$, but $T = p/b$. At this steady state, the immune population has been triggered but the parasite has been eliminated and the system has returned to an infection-free state with a degree of residual immunity (an uninfected immune state). The third type of equilibrium represents host–parasite coexistence and is characterized by non-zero levels of Y and M, $X < \Lambda/\mu$ and $T > p/b$.

Again, it is useful to consider the local stability of these equilibria to disturbance by the parasite. In the absence of infection, the immune population (and therefore the naive equilibrium) is inherently unstable and will not return to zero once it has been triggered by the presence of the parasite. Nevertheless, as in the system without immunity, the parasite is only capable of initially invading the blood system if the criterion in equation 8 is satisfied. It is intuitively obvious that this condition should still hold as the initial level of immune cells is zero even in the system with potential immunity.

The infection-free, immune equilibrium, where $T = p/b$, is stable if:

$$\frac{\beta\frac{\Lambda}{\mu}\left(\alpha(r-1)-\kappa\frac{p}{b}\right)}{\left(\alpha+\kappa\frac{p}{b}\right)\left(\delta+\omega\frac{p}{b}\right)} < 1 \tag{15}$$

This biologically important criterion determines how a parasite, following successful invasion, will eventually be controlled by the immune response: if the criterion is not satisfied, the parasite will not be eliminated but will persist at an equilibrium density within the host. Alternatively, in a host who has undergone successful anti-malarial therapy, whose immune cells have settled at their equilibrium level, the criterion determines whether a parasite strain with similar attributes is able to overcome the stability of this equilibrium and reinfect.

The form of equation 15 illustrates that the inclusion of a minimal immune response generates complex criteria for the persistence or elimination of infectious agents. As before, the outcome of infection depends upon the balance between parasite and host attributes. As the negative forces acting against the parasite, namely the merozoite and infected cell death rates (δ, α) and the rates of parasite clearance (ω, κ), increase, the immune equilibrium becomes more stable and the host is able to eliminate the parasite more easily. Similarly, the higher the equilibrium level of immunity p/b, the greater the parasite invasion rate β (and/or r) must be to achieve reinfection. It is interesting to note that in this system the stability of the immune equilibrium does not depend upon the rates of T cell activation by the parasite (γ_m and γ_y). However, the speed at which the system moves towards equilibrium, and the equilibrium levels of immune cells and parasites, will be influenced by the magnitude of these rates.

The importance of the target of the immune response

We can use the model to examine the impact of immunity upon the bloodstage malaria system (compared to no immunity) as well as to compare the effect of directing immunity against the merozoite or against the infected cell. As before, the equations of the model were solved over time using numerical methods. Parameters associated with the malaria

Table 4. Baseline values of immune parameters used in model simulations

Parameter	Definition	Value
κ	Rate of immune killing of infected cells	1×10^{-8} (cell/ml)$^{-1}$ day^{-1}
ω	Rate of immune killing of merozoites	1×10^{-8} (cell/ml)$^{-1}$ day^{-1}
γ_y	Rate of immune cell activation by infected cells	0·1 day^{-1}
γ_m	Rate of immune cell activation by merozoites	0·1 day^{-1}
p	Rate of proliferation of immune cells	0·1 day^{-1}
b	Density-dependent rate of immune cell suppression	1×10^{-9} (cell/ml)$^{-1}$ day^{-1}

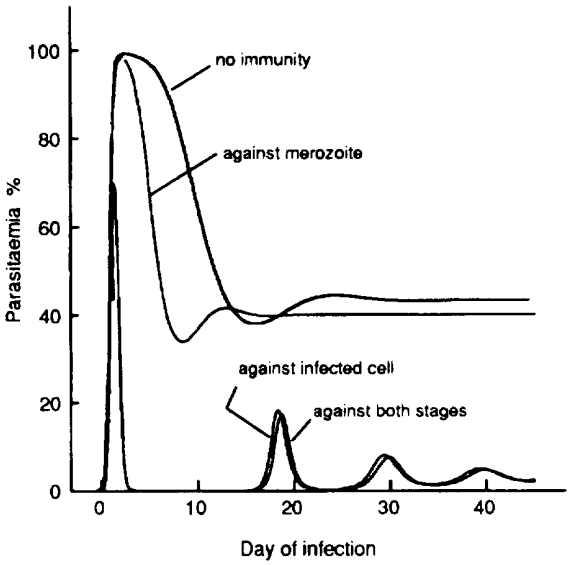

Fig. 4. The target of the immune response strongly influences the outcome of infection in the malaria model with immunity. Parameters are given in Tables 2 and 4.

parasite and non-immune host processes were assigned the values in Table 2, and a value of 2×10^{-8} (cell/ml)$^{-1}$ day^{-1} was used for β. Due to the simplistic representation of the immune response, the estimation of rates such as immune cell activation, proliferation and parasite clearance is not straightforward. Therefore, we assigned relative rates to immune activation by and immune killing of each parasite stage in order to compare the contribution of these to the system's dynamics. The numerical values of immune parameters are summarized in Table 4, and we verified that they generate patterns of infection in agreement with observed data (not shown). Initial levels of X, Y and M were as in Table 2 and the initial density of activated immune cells was assumed to be zero.

Fig. 4 summarizes the output assuming an immune response directed against both stages (rates equally weighted), against the infected cell only (ω and γ_m set to zero), against the merozoite only (κ and γ_y set to zero) or against neither stage (all immune parameter values zero). The results, which are typical, demonstrate that an immune response against the merozoite alone has very little impact upon the course of infection compared to an equal response against the infected cell, assuming identical rates of immune cell stimulation by each stage. Biologically reasonable estimates of parasite-associated parameters and non-immune host parameters were used. Because the death rate of the merozoite (δ) is much greater than that of the infected cell (α), the condition for a stable immune equilibrium (parasite elimination) is easier to satisfy for a given level of anti-cell immunity in the absence of anti-merozoite immunity, rather than vice versa. It can also be seen from equation 15 that the term representing the net rate of killing of immune cells ($\kappa p/b$) doubly promotes the stability of the immune state as, apart from its direct effect, the death of an infected cell also results in the loss of potential merozoites.

Comparison with in vivo *data*

In a study by Finley, Mackey & Lambert (1982), comparing *P. berghei* infection in intact and athymic mice, the latter are not able to control the parasite as well as normal mice (Fig. 5) although the course of infection retains the characteristic peaked property demonstrated by the model simulations. The peak of infected cells is the same in both groups, supporting the concept that erythrocyte availability rather than immunity may regulate the initial severity of infection (though T cell-independent immunity cannot be ruled out). In contrast to the simulations in Figs 3 and 4, the density of infected cells in the T cell-deficient mice remains at a relatively high level after its initial peak, perhaps due to an increased rate of erythrocyte replacement induced by anaemia. However, *in vivo* patterns can be very variable and differences in mouse genetic background, parasite virulence and the rate of erythrocyte replacement by the anaemic host make comparison between experimental data and the output of the simple model difficult. In particular, the striking predilection for reticulocytes exhibited by the *berghei*-type parasites is likely to result in highly heterogeneous mixing patterns that cannot be captured by a simple mass-action model. It may be more appropriate to compare the output with an infection of *P. c. chabaudi*, a species that appears to invade randomly (Jarra &

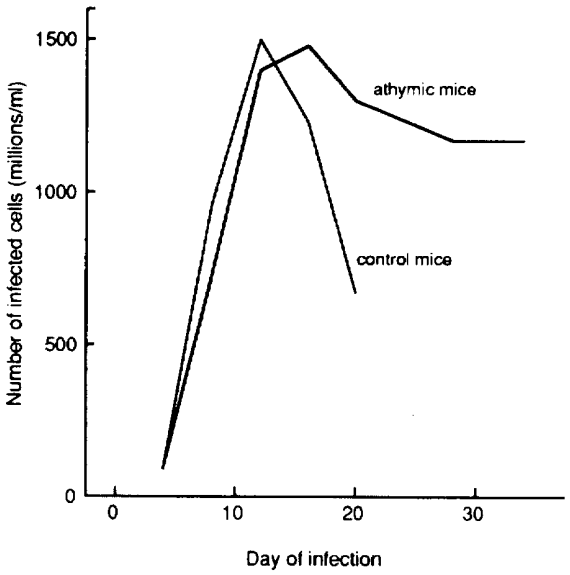

Fig. 5. Time-course of infection in normal and athymic BALB/c mice inoculated with 10^4 *Plasmodium berghei*-infected cells. Redrawn from Finley *et al.* (1982).

Brown, 1989), and Fig. 6 shows the characteristic pattern of infection (Langhorne, Simon-Haarhaus & Meding, 1990). $CD4^+$ T cell depletion (comparable to our model run without immunity) makes no difference to the height of the initial parasitaemia but allows the parasite to remain at a high level rather than be eliminated.

DISCUSSION

We began by exploring the dynamics and the analytical properties of a mathematical model of bloodstage malaria in which no immune response was represented. The model generated patterns of within-host infection which are non-linear waves, typical of ecological systems whose dynamics are strongly regulated by immigration-death processes and simple mass-action contact. Here, these are the rates of susceptible erythrocyte production (Λ) and loss, due either to natural death or parasite invasion. Non-linear patterns are often seen in rodent malaria infections. The qualitative agreement between the simulations of the model and these *in vivo* observations implies that the early dynamics of many experimental malaria systems are largely driven by cell infection processes and that, initially at least, immunity plays a subordinate role. The immune clearance of parasitized cells or merozoites is not required to explain the primary fall in infected cell density observed *in vivo*: erythrocyte scarcity may be an important biological mechanism regulating early bloodstage infection prior to the induction of an effective immune response. Although not considered here, spatial effects within the host may accentuate local restriction on the availability of susceptible host cells.

Analytical examination of the model revealed an important association between certain rate parameters. We may define this relationship as the within-host reproductive potential (R_0) of the parasite, and it is comparable to the basic reproductive number defined for the population level transmission of directly transmitted diseases such as measles (see Anderson & May, 1992). Here R_0 is given by:

$$R_0 = \frac{\beta}{\delta} X_0 (r-1) \qquad (16)$$

where X_0 is the initial density of susceptible erythrocytes (Λ/μ). More precisely, R_0 is the average number of infected cells generated by 1 infected cell introduced into a totally susceptible cell population. By definition, a requirement for primary invasion is that R_0 must exceed unity. R_0 is directly related to stability: the parasite population is capable of a primary invasion if it is able to overcome the stability of the infection-free equilibrium (the initial state). The expression for R_0 is not affected by superinfection as it describes the potential of the parasite in a totally susceptible erythrocyte population and is therefore identical to the R_0 derived by Anderson *et al.* (1989).

R_0 provides a valuable insight into the antagonistic relationship between certain biological processes of the host and parasite. Before encountering the host immune response, the critical properties of the parasite are the life-expectancy of the merozoite stage ($1/\delta$), the probability of successful cell invasion (β) and the number of merozoites produced per infected cell (r). While the magnitude of the parasite inoculum does not appear to be important, our analysis implies that there is a critical threshold density of susceptible erythrocytes required for the successful establishment of infection in the host. Thus, low-grade anaemia (a reduction in Λ) could potentially render the host refractory to malaria. Similarly, R_0 would be reduced if host factors interfered with the developmental success of the intracellular parasite (a decrease in β or r). Indeed, iron-deficient anaemia and β-thalassaemia can confer protection against malaria (Weatherall, 1988).

The results from the experimental *P. berghei* – rat system provide a value of R_0 between 4·8 and 18·6 (assuming mean $r = 13{\cdot}5$, X_0 as in Table 2), though of course the actual value cannot exceed 13·5. Any value of R_0 obtained from a laboratory model will be an approximation of that in the natural host, which may possess more effective non-specific immune mechanisms and to whose erythrocytes the parasite may be better adapted. Furthermore, we may have overestimated the value of β by assuming that all trophozoites detected at 18 h survive until schi-

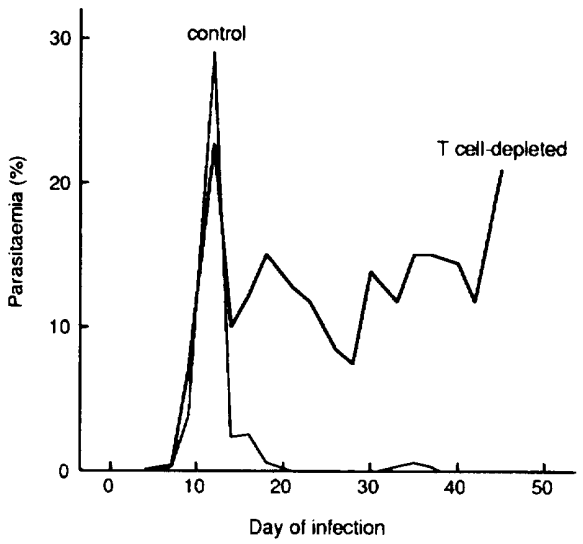

Fig. 6. Time-course of *Plasmodium chabaudi* AS infection in normal C57BL/6 mice and those depleted of $CD4^+$ T cells on day 7. Redrawn from Langhorne *et al.* (1990).

zogony. Lastly, although it may not be a significant factor during initial invasion, loss of asexual reproductive potential through gametocytogenesis will act to lower R_0 in a natural infection.

The data in Fig. 2 indicate that as the density of the merozoite inoculum increases, the estimate of β/δ decreases. In terms of obtaining an order-of-magnitude estimate for β this is of little consequence, but it is an interesting phenomenon with several possible causes. One explanation may be that the merozoite death rate increases with the density of the schizont inoculum due to crowding or mechanical damage. Otherwise, our finding implies a density-dependent trend in β itself. Sampling biases leading to a proportional overestimate of the parasitaemia at lower parasitaemias may have occurred but the consistent and linear decrease in β makes this doubtful. Also unlikely is the existence of an early non-specific anti-merozoite immune response whose magnitude proportionately increases with the size of the inoculum. More plausible explanations are connected with the availability of susceptible cells and spatial mixing effects. Parasites inoculated into the tail vein may not mix randomly with erythrocytes as assumed in the differential equations. The merozoite has limited energy stores and a finite time in which to penetrate the host cell: therefore the probability of a merozoite encountering a susceptible erythrocyte before its death would decrease as the density of merozoites increased. A related explanation is based on evidence that merozoites of *P. berghei* exhibit a strong predilection for immature erythrocytes (reticulocytes) (Garnham, 1966) which normally comprise about 2% of rat blood cells. If invasion requires the immotile merozoites to contact a suitable cell then as merozoite densities increase, the supply of susceptible reticulocytes could become increasingly limited. While these last two explanations seem plausible, they both predict increasing superinfection of erythrocytes or reticulocytes with increasing merozoite density (we assume that no mechanism inhibits the invasion/development of secondary merozoites). No superinfection was observed in the current experiment although a problem in the detection of low numbers of multiply infected cells cannot be excluded. The observed density-dependence may be a result of the combined factors of the fast death rate of the infective stage and the restriction of invasion to cells of a particular age. Merozoites capable of infecting only reticulocytes would experience a significantly reduced host resource, resulting in the death of a greater proportion of the inoculated merozoites which fail to contact a suitable cell before they expire. Support for this comes from many experiments in which *P. berghei* parasitaemia is significantly higher in rodents whose reticulocyte levels are artificially elevated (by chemicals or bleeding) than in their normal counterparts (e.g. Roth & Herman, 1979).

In Fig. 3 we compare a typical example of the output of the simple model with *P. berghei* time-course data and the similarities early in the infection are striking. Nevertheless, there are important differences between the output of the model and experimental data. First, during *in vivo P. berghei* infection, the parasitaemia does not fall in the way predicted by the model but remains relatively high, while uninfected cell levels are lower than expected. Second, even by extensively varying parameters such as r, β and δ (which may differ between species), it is difficult to generate simulations which resemble the data in terms of the timing and the value of peak parasitaemia for a given inoculum size. In general, to achieve a similar timing of peak infection, we found that the parameter combination used in the model gave a higher parasitaemia. Parameter estimation may be responsible for quantitative differences, while the over-simplifications in the design of the model may lead to qualitative differences. Our intention was not to fit the model exactly to time-course data by optimizing parameter values, though we thoroughly explored the qualitative patterns generated by the model over a range of values for each parameter. It is likely that minor discrepancies are due to differences between the real and our assumed parameter values, while more important discrepancies are due to the simplicity of the model's assumptions, which we discuss below.

One of the more important over-simplifications is that mixing between merozoites and erythrocytes is assumed to be totally homogeneous. *In vivo*, spatial compartmentalization might reduce contact between merozoites and uninfected cells, while a mechanism such as cytoadherence of uninfected erythrocytes to an infected erythrocyte (described *in vitro* by

Carlsson *et al.* (1990)) would increase the success of invasion (β). Another form of invasion heterogeneity is exhibited by parasite species with predilections for invading erythrocytes of a particular age. The human parasites *P. vivax* and *P. malariae* are notable examples, preferring young and old erythrocytes respectively (Garnham, 1966). A restriction in the number of susceptible cells available to the parasite would lower its reproductive potential and reduce the severity of infection. To address this important question we are currently designing a more complex framework in which β may be varied as a function of host cell age.

In the models, all rates are assumed to be constant and independent of time or cell density. However, during infection, competition for the erythrocyte pool is likely to select for parasites that produce more merozoites per cell or with more efficient invasion. A further example of a non-constant rate is the observation that erythrocyte production by the bone-marrow may be upregulated in an anaemic host, thus supplying more susceptible erythrocytes to the parasite (Roth & Herman, 1979). This might explain why the parasitaemia in the experimental data remains unexpectedly high when total erythrocyte levels are relatively low. There is also evidence in experimental and human malaria that uninfected erythrocytes experience shortened survival even after parasite clearance (see Weatherall, 1988). This may contribute to the observation that erythrocyte levels *in vivo* are significantly lower than those predicted by the model.

Ostensibly, the major drawback of the basic model is the absence of a specific or non-specific immune response, and so we attempted to rectify this in a model with an immune population. Despite the necessarily simplistic representation of the immune response, the framework produces patterns of infection in reasonable qualitative accordance with observed data (Figs 4–6). Looking at the time-course of *P. chabaudi*, it is interesting to note that the model generates the recrudescence of infection seen *in vivo* after the primary wave. Research has suggested that such recrudescences are associated with a decline in the effector arm of the immune response and antigenic variation by the parasite resulting in the evasion of the specific immune response (McLean, MacDougall & Phillips, 1990). In the single-strain model, the small delayed peaks are due to slight recoveries in erythrocyte levels (facilitated by immune control of the parasite) which enable parasite growth to begin again, but at a rate slower than that seen immediately post-inoculation. This suggests that the supply of susceptible erythrocytes remains an important driving force in the system with immunity, and that antigenic variation need not be the only mechanism of recrudescence. Importantly, the model supports the finding of the model by Anderson *et al.* (1989) that immunity directed against the infected cell is several orders of magnitude more effective at reducing parasite levels (which would in turn reduce gametocyte levels and morbidity) than an equivalent level of immunity against the merozoite (for example, invasion-blocking mechanisms). This property of the model arises because the death rate of the merozoite is so much greater than that of the infected cell. The conclusion is not merely an artifact of the form of the immune response, as our model differs from the earlier one in this aspect. Furthermore, a similar result is seen in a model of chemotherapy (i.e. a non-replicating anti-malarial force) directed against either parasite stage (C. Hetzel, unpublished work). Together, these results have implications for the development of stage-specific therapeutic agents.

As before, the simplicity of the immune model leads to inevitable discrepancies between simulations and observed data, and extensive numerical simulations were unable to mimic completely all aspects of *in vivo* patterns. For example, *in vivo*, the height of the initial wave of parasitaemia is often similar in both intact and immunodeficient mice, while in the model, even a low degree of immunity acts to significantly lower the height of the peak. In some cases, the strong reticulocyte preference of the parasite species may explain the disparity: cell availability limitations may be more important than the immune response in regulating the height of the initial wave of parasitaemia. It would be interesting to look at the combined effects of age-dependent invasion and host immune response. In the case of a parasite with no strong invasion preference, such as *P. c. chabaudi*, non-specific immunity may be equal to, or more important than, cell availability in regulating the height of the primary wave under conditions or in some host species. It has been demonstrated that monocytes play a role in controlling the peak parasitaemia and the elimination, though not the early rise, of *P. chabaudi* in C57BL/6 mice (Stevenson *et al.* 1989). The speed of induction of the immune response appears to be faster in the model than in reality, and may explain why an immediate effect on parasitaemia is seen in the simulations. This could be remedied by introducing a time-delay or a degree of non-linearity in the terms describing T cell activation or by accounting for the slower dynamics of naive T cells.

Amongst the most significant aspects of the biological system not accounted for by a simple model is the phenomenon of antigenic variation, which has been observed in many malaria parasite species (McLean *et al.* 1990; Roberts *et al.* 1992). The generation of variants during the course of malaria infection can be likened to that seen during infection with the human immunodeficiency virus or African trypanosome for which simple but effective models have been constructed (Agur, Abiri & van der Ploeg, 1989; Nowak, May & Anderson, 1990). However, it

is valuable to study the dynamics of a single strain as they undoubtedly represent the building blocks of the more complex situation with antigenic variation.

There is also great scope to model the dynamics of the immune response against the parasite more accurately, both in terms of the speed and strength of immune activation and immune killing, and the number of different cell types and their cytokine-mediated interactions. In the case of *P. chabaudi*, there is accumulating evidence that complete parasite clearance requires an activated macrophage ($CD4^+$ Th1-mediated) response during the primary wave of parasitaemia followed by a B cell (Th2-mediated) response during the second infection phase, which is characterized by low recrudescing parasitaemia (Meding & Langhorne, 1991). The possibility that different parasites may differentially activate the T cell population and thereby determine the virulence of infection has also been suggested (Jayawardena *et al.* 1975). However, in order to build more detailed models of within-host malaria infection, quantitative information concerning the interaction between the malaria parasite and the host immune response is needed. For example, the rate of activation of immune cells as a function of parasite density is assumed to be linear here, but is likely to be a more complicated function. Non-linear processes within the system (including immunosuppression and immunoregulation), as well as factors such as limiting precursor immune cell numbers, are capable of generating a wide spectrum of outcomes, which highlights the need for quantitative experimental studies.

The complexities discussed above require consideration in a systematic manner for which the mathematical model is used as a framework for the analysis of each process individually and in combination. In this task the dynamical properties of the two models described here provide an important template. Even these samples generate patterns that are in qualitative agreement with the observation. Furthermore, they provide complex multi-parameter criteria which distinguish between different dynamical outcomes. The within-host dynamics of parasites and their interaction with the host's immune system are rich areas for the study of non-linear dynamics and their use in the interpretation of observed biological patterns.

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