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Immunology

Antigenic variation and the within-host dynamics of parasites

(models/trypanosomes/malaria)

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ABSTRACT **Many parasites exhibit antigenic variation within their hosts. We use mathematical models to investigate the dynamical interaction between an antigenically varying parasite and the host's immune system. The models incorporate antigenic variation in the parasite population and the generation of immune responses directed against (*i*) antigens specific to individual parasite variants and (*ii*) antigens common to all the parasite variants. Analysis of the models allows us to evaluate the relative importance of variant-specific and cross-reactive immune responses in controlling the parasite. Early in the course of infection within the host, when parasite diversity is below a defined threshold value (the value is determined by the biological properties of the parasite and of the host's immune response), the variant-specific immune responses are predominant. Later, when the parasite diversity is high, the cross-reactive immune response is largely responsible for controlling the parasitemia. It is argued that increasing antigenic diversity leads to a switch from variant-specific to cross-reactive immune responses. These simple models mimic various features of observed infections recorded in the experimental literature, including an initial peak in parasitemia, a long and variable duration of infection with fluctuating parasitemia that ends with either the clearance of the parasite or persistent infection.**

As molecular techniques are used more widely in epidemiological studies of infectious diseases, antigenic variability is found in many host–parasite associations (1–3). The production of immunologically novel parasite strains or variants can affect the dynamics of parasite populations at both the between-host and the within-host levels. At the between-host or epidemiological level, the generation of antigenically different strains (which elicit little or limited cross-immunity in the host) will allow the infection of individual hosts with several distinct parasite strains, thus increasing the possible size of the parasite population within the host community (4, 5). The influenza viruses (6), the cholera bacillus *Vibro cholerae* (7), the malaria parasite *Plasmodium falciparum* (8), and *Giardia lamblia*, the protozoan that causes Giardiasis (9), exhibit strain variation in human populations. At the within-host level, the rapid generation of antigenic variants can enhance the likelihood of parasite persistence in the face of a hostile immune response, thereby prolonging the duration of infectiousness and concomitantly increasing the potential for transmission to a new host. The protozoans *Trypanosoma brucei* (10, 11) and *Plasmodium falciparum* (12) as well as viruses such as human immunodeficiency virus (HIV) (13, 14) appear to change their antigenic properties during the course of an infection.

Several studies have used mathematical models to investigate the dynamics of antigenically varying parasites within their hosts (15–19). The studies of Kosinski (15) and Agur *et al.* (16) were directed towards explaining the ordered appearance of antigenic variants in trypanosome infections. The other studies have focused on antigenic variation of the HIV virus (17–19). Models of the dynamics of the interaction between HIV and the human immune system are based on a set of antigenic drift equations to describe a situation in which there is antigenic variation of the virus as well as virus-induced destruction of immune cells. In this paper we construct a set of antigenic drift equations to describe the dynamics of antigenically varying parasites when the variants do not directly impair the host's immune response. Before we describe the model and analyze its properties, we briefly comment on the experimental literature on the dynamics of antigenically varying parasites within their hosts. We do so by focusing on the dynamics of trypanosomes, as their mechanism of antigenic variation is well characterized at the molecular level and there is a substantial literature on their within-host dynamics.

The general pattern of parasitemia of an antigenically varying parasite, reproduced in many textbooks and review articles, is based on the course of parasitemia of *Trypanosoma gambiense* infection in a single patient who received drug treatment. The time course of parasitemia exhibited regular periodical fluctuations (20). In contrast with the studies of parasitic infections of humans, which are frequently complicated by the use of drugs to control parasitemia, studies of infections of other animals offer a rich source of data on the dynamics of parasites in untreated hosts. There is, for example, data from carefully designed studies of *Trypanosoma vivax* infections in a variety of animals including cattle (their natural hosts) as well as goats and mice (21, 22). As can be seen in Fig. 1 the dynamics of *T. vivax* within its natural host (cattle) begins with a rapid rise in parasitemia, which is followed by a long and variable duration of infection and the eventual clearance of the parasite or its control at very low densities. In addition to the complex pattern of parasitemia, we observe a large diversity in the profiles of parasitemia in different individuals. This diversity is observed following the delivery of matched inocula of a given parasite species into different host species as well as in infections of genetically identical hosts with genetically identical (cloned) parasites (21). This observation suggests that some of this variation may be inherent to the interaction between an antigenically varying parasite and the host's immune defenses.

Mathematical Model and Results

We formulate a model that keeps track of the populations of the parasite variants and the immune responses they elicit within a single host. It consists of a system of ordinary differential equations, whose structure reflects what we hypothesize to be the key features of infections with antigenically varying parasites. These are as follows: (*i*) the parasite produces antigenic variants, (*ii*) parasite antigens unique to indi-

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Abbreviation: HIV, human immunodeficiency virus.

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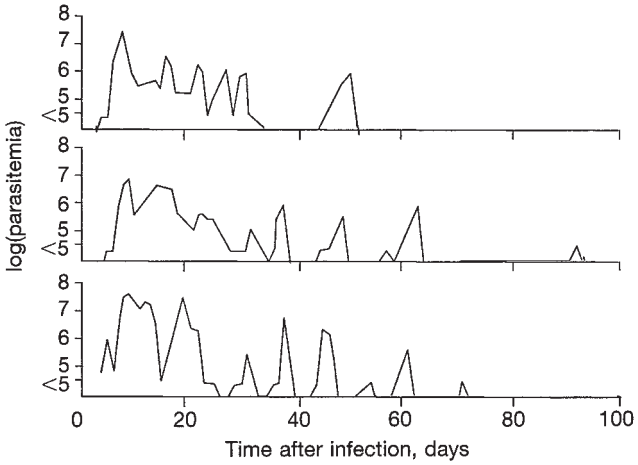

FIG. 1. The dynamics of parasitemia in trypanosome infections. Parasitemia following the delivery of matched inocula of *T. vivax* in individual cattle (from ref. 21). The parasitemia is measured as the number of parasites per milliliter of blood.

vidual variants elicit a "variant-specific" immune response, and (*iii*) parasite antigens shared by all of the variants will elicit a "cross-reactive" immune response that recognizes all variants. We let p_i, x_i, and z represent the populations of parasite variant i, of variant-specific immune cells, and of cross-reactive immune cells, respectively (we have equated the intensity of an immune response directed at a particular variant with the number of immune cells generating the corresponding response). The total parasite population is represented by $P = \Sigma p_i$. The rates of change in the populations of parasite variants and immune responses with respect to time are as follows:

$$\frac{dp_i}{dt} = rp_i\left(1 - \frac{P}{c}\right) - kp_ix_i - k'p_iz \qquad i = 1 \ldots n, \qquad \textbf{[1]}$$

$$\frac{dx_i}{dt} = \rho x_i\left(\frac{p_i}{p_i + \phi}\right) - \mu x_i \qquad i = 1 \ldots n, \qquad \textbf{[2]}$$

$$\frac{dz}{dt} = \rho' z\left(\frac{P}{P + \phi'}\right) - \mu' z. \qquad \textbf{[3]}$$

In Eq. **1** the rate of change in the population of variant i equals the sum of its growth rate (which is assumed to be logistic with maximum rate r and carrying capacity c; this assumption is made in line with conventional wisdom in population ecology and denotes the effect of resource limitation within a host on parasite growth) and the rates at which it is killed by variant-specific and cross-reactive immune responses, which equal kp_ix_i and $k'p_iz$, respectively. In Eqs. **2** and **3**, the rate of change in the population of immune cells equals the sum of the rate at which they proliferate and their death rate. The per capita rate of proliferation of immune cells is assumed to be a simple saturating function of the parasite density. The total number of parasite variants, n, is not constant because new variants can be generated from an existing variant. This introduces a stochastic element, where the probability of generation of a new variant per unit time equals $mP(t)$, where m equals the mutation rate.

Before we proceed to examine the properties of these equations in detail, we consider the relative magnitudes of the various parameters (23). We assume that on introduction of each variant its population size equals unity. We scale the populations of specific-immune cells so that on introduction of a new parasite variant, the population of variant-specific immune cells equals unity, and we similarly set the population of cross-immune cells to unity at $t = 0$. Immune cells and the parasite are assumed to be capable of dividing about once per day (i.e., ρ and r are in the range of 0.5 to 2.0 per day), and in the absence of antigen, immune cells are assumed to have a half-life of a few weeks (i.e., $\mu \approx 0.1$ per day) (24). We expect that the parasite density (ϕ) at which the immune response grows at half its maximum rate is much greater than the initial parasite density but much smaller than the carrying capacity (c). Additionally, since parasite-specific immune cells must attain a high density to control the parasite, we expect that the rate constant for immune-mediated clearance of the parasite (k) is much less than the initial intensity of immunity (unity in our scaled equations). Finally, since the cells generating the variant-specific and cross-reactive immune responses are similar we assume that they have comparable growth and death rates but that the parasite density at which these cells grow at half their maximal rates can be very different (i.e., antigens that generate cross-reactive responses are likely to have a lower density on the parasite surface and consequently have a higher ϕ than those that generate specific responses).

$$k < \mu(\approx 0.1) < r,\ \rho(\approx 1.0) \ll \phi \ll c. \qquad \textbf{[4]}$$

The main properties of the above model can be understood from analytical and numerical studies of the set of equations described above. We first examine the special case where there is only variant-specific immunity and then add the cross-reactive immune response.

Model with Variant-Specific Immunity. By setting the cross-reactive immunity to zero (i.e., $z = 0$), we examine the effect of having only variant-specific immunity. We consider how the total parasitemia and immune response change with the number of distinct antigenic variants, n, within the host (n will also be referred to as the measure of parasite diversity). Let P and X denote the total parasitemia and immunity at equilibrium—i.e., $P = \Sigma p_i = np$ and $X = \Sigma x_i = nx$. There is a "trivial" steady state when n equals 0, corresponding to an uninfected host—i.e., $P = 0$ and $X = 0$. For $P > 0$ the outcome depends on whether the parasite diversity (i.e., the number of variants) n exceeds a critical value, n_s, given by

$$n_s = \frac{c(\rho - \mu)}{\mu\phi}. \qquad \textbf{[5]}$$

When the diversity is less than n_s, the equilibrium parasitemia is maintained at less than the carrying capacity and increases linearly with the number of parasite variants. The parasitemia reaches the carrying capacity when the number of variants is equal to n_s, and the parasitemia is maintained at the carrying capacity when the number of variants is greater than n_s. Immunity obtains a maximum value when the number of variants equals half the diversity threshold n_s and declines for both lower and higher n, vanishing when $n = 0$, or is greater than or equal to n_s.

$$\text{If } n < n_s\text{:} \quad P = \frac{n\mu\phi}{(\rho - \mu)} \quad \text{and} \quad X = \frac{nr}{k}\left(1 - \frac{P}{c}\right). \qquad \textbf{[6]}$$

$$\text{If } n > n_s\text{:} \quad P = c \quad \text{and} \quad X = 0. \qquad \textbf{[7]}$$

Initially, when a single parasite variant is present at steady state, it will coexist with a low level of immunity. As the number of variants increases, each variant being antigenically distinct, their total density increases linearly with the number of variants and is limited only by the carrying capacity. The diversity threshold n_s corresponds to the number of parasite variants present when the equilibrium parasite density just attains the carrying capacity. When the number of variants exceeds the diversity threshold, the carrying capacity restricts the density of each variant p_i to such a low level that the rate at which each variant induces the proliferation of the specific-immune cells is less than the death rate for immune cells. We

thus observe that when the number of variants exceeds the threshold n_s, immunity to all parasite variants falls to zero, and resource limitation, not the immunological defenses of the host, constrains parasite population growth. The magnitude of the diversity threshold n_s can be determined if we know the relative magnitude of the parameters in Eq. **5**. Through use of the approximations in Eq. **4**, and if the parasite density ϕ for stimulation of the immune response is about 3 logarithms less than the maximum parasitemia, then n_s will be $\approx 10^4$.

Model with Variant-Specific and Cross-Reactive Immunity. Next we examine the outcomes of the model when there is a cross-reactive response z that is elicited by all of the parasite variants and is assumed to be equally effective in controlling all variants. The effect of cross-immunity is to limit the total parasitemia to $P = \mu'\phi'/(\rho' - \mu')$ (see Eq. **3**).

If this value of P is greater than the carrying capacity c, then the addition of cross-immunity does not alter the steady-state outcome that pertained in the presence of variant-specific immunity alone. If this value of P is less than the carrying capacity, then the outcome depends on whether the number of parasite variants exceeds the diversity threshold which is now given by n_c:

$$n_c = \left(\frac{\mu'\phi'}{\rho' - \mu'}\right) \Big/ \left(\frac{\mu\phi}{\rho - \mu}\right). \qquad [8]$$

When the number of variants is less than n_c, the parasite is controlled by variant-specific immunity alone and the cross-reactive immune response decays to zero. In this regime the total parasite density and variant-specific immunity are the same as in the absence of cross-immunity (i.e., Eq. **9** is identical to Eq. **6**). If, however, the number of variants exceeds this value n_c, then the parasitemia is controlled by cross-reactive immunity alone and variant-specific immunity tends to zero, as shown in Fig. 2.

$$\text{If } n < n_c: \quad P = \frac{n\mu\phi}{(\rho - \mu)},\ X = \frac{nr}{k}\left(1 - \frac{P}{c}\right),\ z = 0. \qquad [9]$$

$$\text{If } n > n_c: \quad P = \frac{\mu'\phi'}{(\rho' - \mu')},\ X = 0, z = \frac{r}{k'}\left(1 - \frac{P}{c}\right). \qquad [10]$$

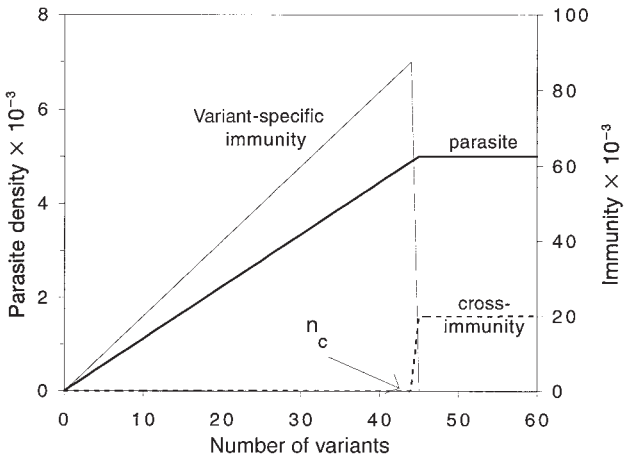

FIG. 2. Steady-state outcome obtained from a model with both variant-specific and cross-reactive immunity. We plot the steady-state density of the parasite and of variant-specific and cross-reactive immune responses as a function of the number of parasite variants (parasite diversity). There is a sudden shift from variant-specific to cross-reactive immune responses when the number of variants exceeds the diversity threshold n_c. Parameters are: $r = 2.0$; $k = 10^{-3}$; $\rho = 1.0$; $\phi = 10^3$; $\mu = 0.1$; $c = 10^6$; $k' = 10^{-4}$; $\rho' = 0.5$; $\phi' = 2 \times 10^4$; $\mu' = 0.1$.

The model therefore suggests that when the number of variants is below the diversity threshold n_c, variant-specific immunity controls the density of each variant, and the combined density of all the variants is not sufficiently large to elicit a cross-reactive immune response. When the number of variants exceeds n_c, then the cross-reactive immune response holds the (steady-state) density of each variant below the level required to elicit a variant-specific immune response, and consequently specific immunity steadily declines. Note that we observe this behavior even though we do not intrinsically assume any competition between variant-specific and cross-reactive immune responses. It is just a question of whether sufficient parasite antigens are present to stimulate a given response.

The magnitude of the diversity threshold can be estimated if we recall that the specific and cross-reactive immune responses differ principally in the parasite densities (ϕ and ϕ') required to stimulate half-maximal proliferation. This allows us to simplify Eq. **8** to get $n_c \approx \phi'/\phi$—i.e., that its magnitude equals the ratio of the parasite density at which the rate of growth of cross-reactive immune cells is half-maximal to that at which the rate of growth of variant-specific immune cells is half-maximal. If, to a first approximation, the immunogenicity of the antigens that elicit the specific and cross-reactive immune responses is the same, then we might expect that the ratio would be approximately equal to the ratio of the densities of the variable and the conserved antigens per parasite. In trypanosomes the invariant surface molecules are 100 times less abundant than the variable surface glycoproteins that cover most of the parasite surface (25), suggesting that the magnitude of the diversity threshold for cross-immunity will be ≈ 100. We note that this is much less than the diversity threshold for specific immunity.

Dynamics of the Response. The above analysis gives us the steady-state population densities of parasite and immunity for a given number of parasite variants, n. When the rate of production of novel antigenic variants is sufficiently small, the parasite population reaches equilibrium within the host prior to the appearance of the next variant. The parasitemia will thus increase in a stepwise manner, and since the rate of production of new variants in this case will be proportional to the steady-state parasite density P, successive variants will appear more and more rapidly. When the critical number of variants (n_c or n_s) is reached, then the total parasitemia will remain constant.

When the rate of generation of antigenically novel forms is high [as in the case for trypanosome and malaria infections (12, 26)], there will not be sufficient time for the equilibrium to be attained before a new variant is generated. In this case there is initially a high but fluctuating parasitemia. When there is only variant-specific immunity, then with the passage of time the total parasitemia saturates at the carrying capacity; but when cross-reactive immunity is present, the total parasitemia is regulated at a much lower level by the cross-reactive immune response (Fig. 3).

Incorporating Differences in Growth Rates and the Extinction of Variants. The models described above assume that the parasite variants have identical growth properties and can persist at an infinitesimally small density. In this section we examine the consequences of introducing the biological realities that parasite variants are likely to differ in their growth properties and that they will be driven to extinction if their density is very low. We limit the discussion to representative numerical simulations.

We introduce the extinction of parasite variants when their density is low by incorporating a threshold density. When the parasite density falls below this threshold, then it is assumed to go extinct, and in the numerical simulations its density is set to zero. For simplicity, differences in the growth properties of individual variants were introduced by choosing the growth

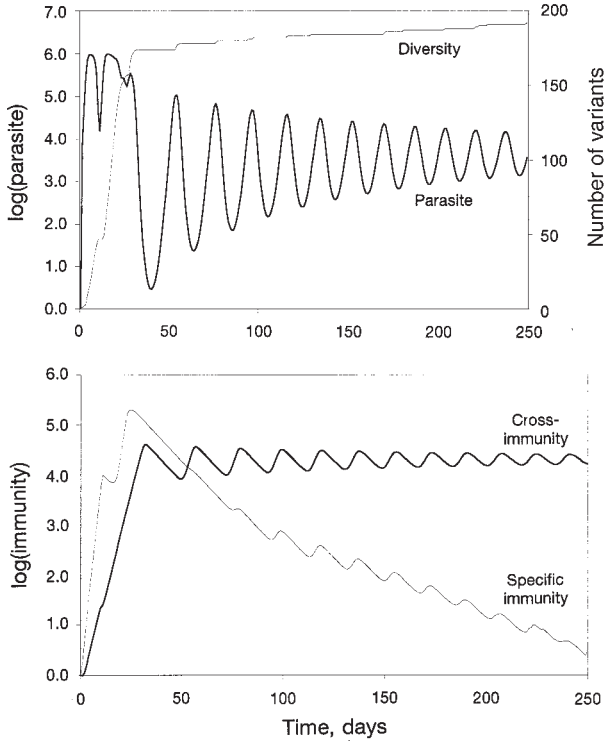

FIG. 3. Dynamics obtained from a model with both variant-specific and cross-reactive immunity. (*Upper*) Total parasite density and parasite diversity (number of different variants). (*Lower*) Total variant-specific and cross-reactive immunity. The dynamics was obtained by numerical simulation of Eqs. **1–3**, with the introduction of variants being stochastic with probability mP. Stochasticity in the precise time of introduction of new parasite variants does not result in much variation between different simulations. Parameters are as in Fig. 2, and $m = 10^{-5}$.

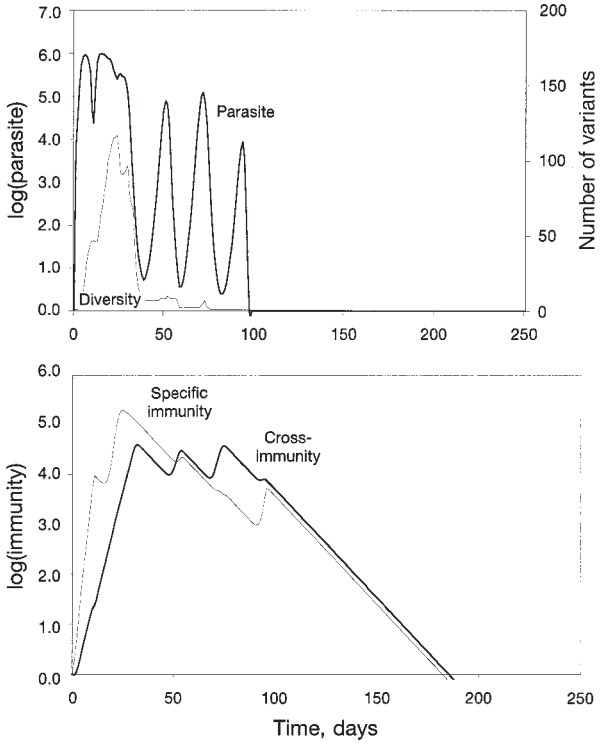

FIG. 4. Dynamics of parasitemia and immunity obtained when we include differences in the growth rate of parasite variants, and variants go extinct if their densities are very low. (*Upper*) Total parasite density and parasite diversity (number of different variants). (*Lower*) Total variant-specific immunity and the cross-reactive immunity. The dynamics is obtained by numerical simulation of the model with both variant-specific and cross-reactive immunity (Eqs. **1–3**) as in Fig. 3, with the growth rate of individual variants chosen from a uniform distribution in the range between 1.75 and 2.25, and extinction of variants when their density falls below 0.5.

rates of individual variants from a uniform distribution with the same average as before. In Figs. 4 and 5 we see that the introduction of these features into the model does not change the dynamics observed shortly after infection. In the longer term, however, we note (*i*) that the parasite can be driven to extinction, (*ii*) the coexistence of both variant-specific and cross-immunity continues while the parasite persists, and (*iii*) heterogeneity in the profiles of parasitemia is observed in the different simulations (Fig. 5).

Discussion

The model emphasizes the role played by cross-reactive immunity in the control of infections by a parasite that can display antigenic variation. This result differs from that of previous mathematical studies of the dynamics of interaction between trypanosomes and the host immune system, as these studies have not considered cross-reactive immunity (15, 16). While most of the experimental research has concentrated on the enormous potential of trypanosomes to generate variable surface molecules, there are several invariant surface proteins at densities about 1/100th of the densities of the variable surface glycoproteins (25). Antibody responses to common or invariant antigens have been detected following infection of cattle with *Trypanosoma congolense* (27, 28). These studies indicate that antibodies to invariant antigens are higher in resistant *N'Dama* than in susceptible *Boran* cattle, suggesting that they may be associated with a capacity to control the disease. Our model can explain these findings and further predicts that the relative abundance of cross-reactive to variant-specific antibodies (and of cross-reactive to specific T cells) will increase as the infection progresses.

How sensitive are our conclusions to the particular way in which we have formulated the model? The functional form for the term describing the proliferation of the immune cells in response to the parasite, $\rho x(p/\phi + p)$, is consistent with the clonal expansion of immune cells at a rate that increases with increasing parasite density, saturating as the maximum rate of growth of immune cells is approached at high parasite densities. We find that our basic qualitative result (that there is a diversity threshold for cross-immunity, which divides the outcomes into a regime in which variant-specific immunity is dominant from one in which cross-reactive immunity is dominant) is maintained even when we modify the proliferation term for immune cells in a variety of ways—including (*i*) changing the term to ρp as in the case of published models of HIV dynamics (18); (*ii*) removing the saturation in the term for the generation of immune responses (i.e., changing the term to the form $\rho p_i x_i$); (*iii*) adding to the proliferation term a constant (small) input of naive immune cells from the thymus (in this case neither cross- nor specific-immunity tend to zero before and after diversity threshold is breached, but rather they fall to low levels); and (*iv*) introducing competition between the various immune responses by having a carrying capacity that limits the total immune response—then we can observe suppression of all immune responses when the parasite density is high. This last modification could provide a simple explanation for the generalized immunosuppression reported in the literature (29, 30) and may also give rise to an increase in the duration of infection.

The model also provides a convenient framework to ask questions and undertake further investigations. For example, to what extent does the eventual decline in parasitemia and

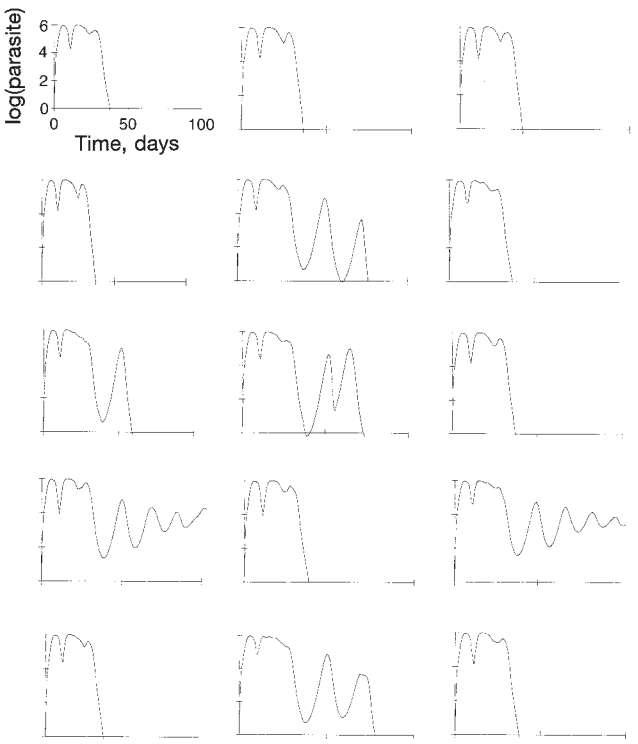

FIG. 5. The diversity of outcomes possible is illustrated by repeated simulations such as the one in Fig. 4. The stochasticity in the precise time of appearance of variants and their growth rate as described in Fig. 4 (and see text) is responsible for the differences between simulations.

possible clearance of the parasite depend on the generation of cross-immunity as opposed to the parasite simply running out of antigenic variants (22)? What can generate an ordered appearance of antigenic variants, which has been suggested for trypanosomes (15, 16). The present model could be modified to describe the within-host dynamics of the antigenically variable merozoite stage of the malaria parasite. For malaria it would be interesting to examine the extent to which the initial oscillation in parasitemia is due to either antigenic variation or the dynamics of erythrocyte infection and death (31–33)?

Finally, we note an obvious applied conclusion resulting from the analysis of the model. If cross-reactive immune responses are important for controlling the parasite, then invariant antigens that elicit cross-immunity even if less dominant than variant-specific antigens could be of use both for the treatment of infected hosts as well as for the generation of vaccines.

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