Density-Dependent Blood Stage *Plasmodium falciparum* Suppresses Malaria Super-Infection in a Malaria Holoendemic Population

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Abstract. Recent studies of *Plasmodium berghei* malaria in mice show that high blood-stage parasitemia levels inhibit the development of subsequent liver-stage infections. Whether a similar inhibitory effect on liver-stage *Plasmodium falciparum* by blood-stage infection occurs in humans is unknown. We have analyzed data from a treatment-time-to-infection cohort of children < 10 years of age residing in a malaria holoendemic area of Kenya where people experience a new blood-stage infection approximately every 2 weeks. We hypothesized that if high parasitemia blocked the liver stage, then high levels of parasitemia should be followed by a “skipped” peak of parasitemia. Statistical analysis of “natural infection” field data and stochastic simulation of infection dynamics show that the data are consistent with high *P. falciparum* parasitemia inhibiting liver-stage parasite development in humans.

INTRODUCTION

Plasmodium falciparum commences with the bite of a mosquito inoculating sporozoites through the skin, followed by infection of liver cells. Hepatic schizogony progresses over 6.5–10^{1.2} days culminating in merozoite release and erythrocyte invasion. Blood-stage parasitemia is characterized by cycles of merozoite release every 42–48 hours and an increase in parasite biomass that underlies fever and other clinical manifestations of malaria. A high rate of biting by infected mosquitoes and a high proportion of individuals with detectable blood-stage parasitemia in cross-sectional surveys suggests that the liver- and blood-stages may frequently co-exist in the same individual, and this is further supported by genetic studies showing that many individuals are co-infected with multiple strains of malaria.³

The liver and blood stages of *P. falciparum* are thought to proceed independently of one another. However, it has been suggested that in murine models of malaria infection, high blood-stage parasitemia may inhibit the development of subsequent liver-stage infections^{4–6}; this is thought to occur indirectly as a result of stimulation of inflammatory pathways, and possible roles for nitric oxide and hepcidin in inhibiting liver-stage parasite development have been proposed. A possible evolutionary advantage for the parasite is that one parasite strain suppresses the growth of late-arriving competitor strains,⁶ and this observation opens the potential for therapeutic intervention to suppress infection. Whether these interactions between the blood and liver stages seen in murine malaria are also seen in human *P. falciparum* infection is unclear, and difficult to address experimentally. Some have argued specifically against a major role for hepcidin in human infection, pointing out that the emergence of blood-stage parasitemia is reduced in the presence of hepatitis C infection, despite low levels of hepcidin in hepatitis C.⁷ However, it seems that hepcidin is not the only mechanism acting on the liver stage.⁶ Moreover, it is not clear that the same mechanisms observed in mice must also act in human infection. Before embarking on a debate about mechanisms in

humans, it has yet to be shown whether the phenomenon of high blood-stage parasitemia inhibiting the liver stage is even observed in human infections.

Investigating interactions between liver- and blood-stage malaria in humans is problematic because of the obvious inability to induce and monitor high level parasitemias or directly detect and quantify concurrent liver-stage infection. However, natural history cohorts provide some opportunity to investigate this effect, i.e., in a region of high transmission individuals should undergo multiple rounds of liver-stage infection from sequential bites, some of which occur during periods of high blood-stage parasitemia. By analyzing the spacing of sequential “peaks” of parasitemia, we should observe an increased spacing of peaks after high blood-stage parasitemia. We performed a cohort study in western Kenya in 2003 of 201 asymptomatic adults and children who were treated with Coartem, regardless of blood-stage *P. falciparum*, and then followed weekly to understand the dynamics of infection⁸ (Figure 1A). This is a population exposed to an extremely high biting rate by infected mosquitoes,⁹ and the asymptomatic carriage rate among young children was ≈70% at the start of the study. After treatment and clearance of parasitemia, we observed sequential peaks of parasitemia in most of the young children (< 10 years of age). We hypothesized that if high blood-stage parasitemia blocks the liver stage (as seen in murine studies), then high “peak” parasitemias in the patients would be associated with a delay in the next peak of parasitemia in these children. We thus divided the observed peaks of parasitemia according to size and measured the delay to a subsequent peak, and how low the parasitemia dropped between peaks. We used a simulation approach to account for the variability of weekly sampling and randomly occurring mosquito bites, to simulate the patterns of peak spacing we expect in the presence or absence of blood-stage interference with the liver stage. We found that higher blood-stage parasitemias were associated with a delay in detection of the subsequent peak, as predicted by the observations in the murine model.

METHODS

Field study of *P. falciparum* infection. The detailed description of the cohort study can be found in Reference 8. Briefly,

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FIGURE 1. A field study of malaria infection dynamics. (A) Sample of longitudinal parasite levels for one patient from field study data, illustrating how peak spacing and nadir parasitemia are measured. (B) A “survival curve” of the proportion of children remaining uninfected at different times, divided into groups of children 0–4 (solid circles) and 5–9 (solid diamonds) years of age. (C) Infection curves of groups of patients with given maximal peak parasitemia: circles and dashed line – maximal peak is within 0–8,000 par./μL, diamonds and dashed line—8,000–16,000 par./μL, triangles and dashed line—16,000–24,000 par./μL, turned up triangles and solid line 24,000 + par./μL.

upon entry into the study (Day 0) study participants ($N = 201$) were treated with Coartem, which is expected to eradicate blood-stage infection but is not effective against liver-stage parasites,¹⁰ and blood smears were monitored weekly for 11 weeks by microscopy. Subjects were analyzed in two groups, being < 10 years of age or ≥ 10 years of age. Ethical approval for human investigation was obtained from the Institutional Review Board at Case Western Reserve University, University Hospital of Cleveland and the Ethical Review Committee at the Kenya Medical Research Institute. Adult participants signed a consent form in English or Duhluo (the local language); parents or guardians signed in the case of minors.

Analyzing features of the field data. To understand whether high peaks of parasitemia were more likely to be followed by a “skipped” peak, we first analyzed the proportion of peaks of different sizes that were followed by an undetectable parasitemia reading as follows: 1) We counted a peak as a “peak followed by undetectable parasitemia” if a reading of zero concentration of parasites (the concentration of parasites below detection limit) occurred before the next episode of infection (or the end of the study). 2) We counted a peak as “followed by continuously detectable parasitemia” if the peak was not followed by a zero measurement before the next peak.

Peaks were excluded from the analysis if they were followed by treatment of clinical malaria in both of the previous cases, or if there were missed measurement either before the next peak in case (2) or before the undetectable parasitemia in case (1).

To calculate the distance after the given peak, we simply subtracted the time of the given peak from the time of the following one. We considered the measurement as a nadir after the given peak if it is less than previous measurement and less or equal to the next measurement.

Testing whether biting rate is a confounder. One explanation for high parasitemia being associated with the increased spacing of peaks is that some people get fewer bites on average, and these fewer bites lead to increased spacing of peaks, and to lower immunity and higher parasitemia as a consequence of low immunity. To check the hypothesis that people with higher peaks of parasitemia get less bites, we separated patients into four groups according to their highest values of parasitemia measurements. These groups used the same cutoffs for parasitemia as used elsewhere. Patients with any missed measurements were excluded,

because we could not be sure that the missed measurement may not have been the peak. We built the survival (infection) curves for each group using the Kaplan-Meier method. The comparison of the survival curves was performed by the Log-rank (Mantel-Cox) test (using GraphPad Prism, GraphPad Software, Inc., San Diego, CA) and showed no statistically significant difference among the groups (P value = 0.454) (Figure 1C). We also performed the comparisons of the survival curves that contained the patients with the missed measurements, to include more patients in this study, and found no statistically significant difference as well. Therefore, this does not support the hypothesis that the individuals with the high peaks of parasitemia receive less infective bites.

Modeling of parasite dynamics in the blood. Analysis of the relationship between the size of a peak of parasitemia and subsequent infection dynamics suggested that sampling may have played a role in our observations. In particular, in our patient population we have random (unobserved) biting and random weekly sampling, not directed sampling of the peak. Thus, we may often miss the true peak of parasitemia and sample just before or after it. To examine the effects of this, we developed a simulation approach to investigate how the random occurrence of infective bites, random magnitude of peaks, sequestration of parasites, and discrete sampling affect the relationship between observed peak size and subsequent levels of parasitemia and delays until the next peak. In particular, this model allows us to run two identical simulations, one with an effect of blood-stage parasitemia on the liver, and one without. This allows us to observe which parts of the relationship between peak size and outcome occur simply as a result of sampling, and which parts occur only in the presence of the “blood-liver effect.” Thus, our aim is to plot the relationship between peak size and outcome observed in the two simulations (with and without the blood-liver effect) with random sampling, to see which more closely resembles the field data.

In the simulation, we assumed that new blood-stage infections occur randomly. The time between new infections has an exponential distribution with the mean waiting time $1/k$ (where k is the rate of initiation of new blood stage infections). Let us denote the time of bite by t_0 . The liver stage after the infective bites lasts for τ days, and then the parasites are released into the blood with an initial concentration p_0 and grow logistically¹¹ with some initial growth

rate r_g , until the concentration reaches the value $p_{\max}$, which is a fraction α of saturation value M . We assume that M is a random variable to produce the peaks of the different magnitudes observed in the experimental data. After the peak, parasites decay logistically with an initial decay rate r_d . A fraction s of parasites during the blood stage sequesters and is not detectable during sampling. Each new infection is assumed to grow in blood independently. The concentration of the parasites derived from one new infection $P(t)$ at time t that were inoculated by bite at the time t_0 , can be defined by

$$P(t) = \begin{cases} \secv(t) \frac{Mp_0 e^{r_g(t-\tau-t_0)}}{M + p_0(e^{r_g(t-\tau-t_0)} - 1)}, & t_0 + \tau \leq t < t_{\max}, \\ \secv(t) \frac{Mp_{\max} e^{-r_d(t-\tau-t_0-t_{\max})}}{M + p_{\max}(e^{-r_d(t-\tau-t_0-t_{\max})} - 1)}, & t_{\max} \leq t < t_z, \\ 0, & t \geq t_z \end{cases} \quad (1)$$

where the function $\secv(t) = 1 - s(1 + \cos\pi(t - t_0 - \tau))/2$ defines the sequestration of parasites, s is the maximal fraction of sequestered parasites. The time

$$t_{\max} = \tau + t_0 + \frac{1}{r_g} \ln \frac{p_{\max}(M - p_0)}{p_0(M - p_{\max})},$$

when parasite reaches the maximal concentration $p_{\max}$ can be found as a solution of equation (2) with respect to t

$$p_{\max} = \frac{Mp_0 e^{r_g(t-\tau-t_0)}}{M + p_0(e^{r_g(t-\tau-t_0)} - 1)}. \quad (2)$$

When parasites of a given strain decay below a threshold concentration z (we also count sequestered parasites) we set the concentration equal to 0. This happens at the time moment

$$t_z = t_{\max} + \tau + t_0 + \frac{1}{r_d} \ln \frac{p_{\max}(M - z)}{z(M - p_{\max})},$$

the solution of the equation for decay (3)

$$z = \frac{Mp_{\max} e^{-r_d(t-\tau-t_0-t_{\max})}}{M + p_{\max}(e^{-r_d(t-\tau-t_0-t_{\max})} - 1)}. \quad (3)$$

The total concentration of parasites in blood can be found simply by summing concentrations of parasites of all concurrent infections.

Presence of the “blood-liver effect” in the model. When we wanted to see the effects of the blood-liver effect, we allowed the liver stage to be aborted in the simulation whenever the blood concentration of parasites reached the “blood-liver” threshold T at any time during the liver stage, or the liver stage occurred < 3 days after this threshold concentration of parasites, consistent with effect reported in mice.⁶ The threshold observed in mice was around 1% parasitemia. Therefore, this threshold was used in most of the simulations.

Parameters of the simulations. To keep our conceptual model simple we estimated the initial growth rate and the rate of initiation of new blood-stage infections from the survival (time-to-initial-infection after treatment) curves fitted to the experimental survival proportions for children

0–4 years of age and 5–9 years of age. We included only children in these age groups and excluded older children and adults, because the latter did not have any parasitemia peaks that exceeded 24,000 parasites/ μ L, and the murine model suggested that the blood-liver effect was only seen at high parasitemia. At the beginning of the parasitemia (before the detection threshold of 40 parasite/ μ L), the exponential growth will not differ significantly from logistic. Assuming the initial exponential growth of parasite in blood as described by the formula (4):

$$P(t) = p_0 e^{r_g(t-\tau)}, \quad (4)$$

the survival (time-to-initial-infection) function that was fitted to the experimental infection proportions is defined by formula (5):

$$S(t) = \begin{cases} e^{-k(t - \ln(T/p_0)/r_g - \tau)}, & t > \ln(T/p_0)/r_g + \tau, \\ 1, & t \leq \ln(T/p_0)/r_g + \tau. \end{cases} \quad (5)$$

where T is the detection threshold of 40 parasites/ μ L (the minimal concentration in the analyzed data table, which completely conforms to the range of 20–50 parasites/ μ L reported in References 12 and 13), p_0 is the initial concentration of parasites in the blood after a single bite equals ~ 0.05 parasites/ μ L,¹³ the duration of the liver stage $\tau = 7$ days.¹³ After fitting the function (5), we found that the growth rate of blood-stage parasitemia for children 0–4 years of age is 0.77 per day and for 5–9 years of age is 0.5 per day. The rate of initiation of new blood-stage infections were respectively 0.08 infections/day and 0.066 infections/day. In our simulation we used the mean of these values, $r_g \approx 0.64$ per day and $k \approx 0.073$ infections/day. Similar values of growth rates and infection rates can be found in published data.^{14–19} We assumed that the initial decay rate is equal to the initial growth rate because we could not obtain the better information from the data $r_d \approx 0.64$ per day. The distribution for M was found experimentally by multiple simulation with the different distributions so that the resulting distribution of the observed magnitude of peaks after the weekly sampling would be similar to the observed in the field study data. The chosen distribution was the Lognormal with parameters 21 and 5 truncated at 5×10^4 parasite/ μ L, the maximal value observed in the data (resulting in a mean peak size of 1,517 parasites/ μ L, with a standard deviation of 5,953 parasites/ μ L). Sequestration fraction s was taken as 0.7 parameter $z = 0.05$ parasite/ μ L, $\alpha = 0.98$.

We simulated 60,000 days of exposure with sequential infection and parasite growth (equivalent to $\approx 1,000$ patients for the study period), and analyzed the relationship between peak height and subsequent parasitemia. The first 300 days of simulation are shown in (Figure 2). We then applied a “blood-liver effect” by introducing different threshold levels of parasitemia levels at which any intercurrent liver stage was blocked, and observed the resulting relationship between observed peak of parasitemia and subsequent dynamics. When the threshold level of parasitemia that blocked the liver stage was set to 45,000 parasites/ μ L, ($\sim 1\%$ parasitemia, as reported in the murine studies⁶), we found the relationship between peak height and subsequent parasitemia was similar to the experimental relationship.

FIGURE 2. Example of the simulated blood stage parasite dynamic with and without “blood-liver effect.” (A and B) Simulation without the blood-liver effect, continuous (A) and weekly sampling (B). (C and D) Simulation with the blood-liver effect, continuous (C) and weekly sampling (D). Triangles over the x axis are infective bites that “passed” the liver stage; crossed out triangles over the x axis are infective bites that were “blocked” because of the “blood-liver effect.” Solid circles on the parasite density line are observed peaks and minima after the weekly sampling.

To test the robustness of the simulation we also varied the growth rates, infection rates, and fraction of sequestered parasites (by running simulations in which individual parameters we doubled or halved). In addition, we replaced logistic growth and decay with exponential growth and decay. In these simulations, we compared the expected relationship between observed peak parasitemia and subsequent nadir level of parasitemia and distance to next peak—in the presence and absence of an effect of blood stage on liver stage—and found similar patterns to those shown in (Figure 3). Thus, the features are not highly dependent on the particular parameters or assumptions of the model.

RESULTS

Higher peaks are more likely to be followed by undetectable parasitemia. Our cohort included very young children up to adults. However, we initially limited our analysis to children < 10 years of age, where parasitemias were highest and we usually observed multiple sequential “peaks” of parasitemia over the course of the study. An important feature of these young children is the high rate of blood-stage infection. We first estimated the rate of initiation of new blood-stage infections, by monitoring the rate of initiation of new blood-stage infections after treatment. This showed an infection dynamic consistent with a new blood-stage infection arising every 10–14 days (Figure 1B). Thus, individuals received on average an infective bite every 2 weeks,

therefore a high peak of parasitemia has a good chance of blocking the liver stage of the next infectious bite.

In this study area, the entomologic inoculation rate (EIR)⁹ and the rate of initiation of new blood-stage infections was so high that many parasitemia peaks may have coalesced. Moreover, because our sampling was weekly, we may have regularly missed the true “peak” of a given infection. However, if a high peak of parasitemia leads to “blocking” of an intercurrent liver-stage infection, we expect to see a period of undetectable parasitemia after the high peak. Accordingly, we sorted peaks according to size, and analyzed what proportion of peaks had an undetectable parasitemia (zero reading) in the subsequent weeks before the next peak. Comparing the highest peaks of parasitemia (> 24,000 parasite/μL, $N = 8$) with those slightly lower (16,000–24,000 parasite/μL, $N = 30$) we observed a higher proportion of zero readings after high peaks, suggesting “skipped peaks” after high parasitemia. However, our results indicate there is an underlying pattern of a decrease in the proportion of zero readings with increasing peak height, up to the level of ~24,000 parasite/μL. However, peaks with > 24,000 parasite/μL were followed by a higher proportion of zero readings than peaks of 8,000–24,000 parasite/μL, although this was not significant (P value = 0.2, Mann-Whitney test), suggesting a “blocking threshold” exerted by higher peaks.

Simulating liver- and blood-stage infection. The unusual shape of the graph in (Figure 3A) suggests that some feature other than simply suppression of the liver stage is determining the pattern of blood-stage infection. Therefore,

FIGURE 3. The relationship between parasite density (i.e., peak size) and subsequent parasitemia. The data for the field study (top panels, A–C) and the simulation (bottom panels, D–F) are shown. The simulations without any effect on liver (left panels, D–F) and with an effect of blood stage on liver stage (right panels, D–F) are shown (simulation without blood-liver effect, striped bars; simulation with blood-liver effect, checked bars). Individual peaks of parasitemia are sorted according to size (x axis) and then the post-peak dynamics are compared for peaks of different size. The fraction of peaks followed by undetectable parasitemia (A and D), the median distance to the next peak (B and E), and the median of concentration of parasites in the nadir after peaks (C and F) are compared. The field data (top) follow the patterns seen in the simulation when high parasitemias were able to block the liver stage of infection (right-hand panels, D–F).

we developed a stochastic stimulation of inoculation and infection events to understand how peak parasitemia might affect subsequent parasitemia in the presence and absence of an effect of the blood stage on the liver. In this model, we assumed infective bites arrived randomly (at the experimentally observed rate of initiation of blood-stage infection), with logistic growth and peak size drawn from a log-normal distribution with a maximum 50,000 parasite/ μ L. We simulated 60,000 days of exposure first in the absence of any effect on the liver stage, and then assumed that parasitemia above the threshold level (45,000 parasite/ μ L $\approx$ 1% parasitemia) aborted any intercurrent liver-stage infection, or any new liver stages arising in the following 3 days (as observed in the murine studies).⁶

The results of this simulation showed that in the absence of any effect on the liver stage, the proportion of peaks followed by undetectable parasitemia after peak declined with increasing peak size—consistent with the pattern we observed at lower parasitemias. However, when we allowed high parasitemia to inhibit liver-stage growth in the simula-

tion, there was an increase in the proportion of undetectable parasitemias following high blood level parasitemias (Figure 3D). Comparing the pattern observed in our data versus that seen in the simulation with and without a blood-liver effect, the pattern in the data clearly resembled the simulation with the blood-liver effect.

High peak parasitemias are followed by low nadir levels of parasitemia and a delay until subsequent peaks. Our simulation of infection with and without a blood-liver effect allows us to predict and examine other features of infection that we might expect in the presence of high blood-stage parasitemia blocking the liver stage. First, our simulation showed that we would expect no association between peak size and delay between peaks in the absence of a liver effect (Figure 3E). However, in the presence of a blood-liver effect we would see high peaks of parasitemia followed by an increased delay in the subsequent peaks. Consistent with a blood-liver effect, a significant delay after high parasitemia was observed in the experimental data (Figure 3B and E). Second, the simulation suggested that the minimal parasite

level between peaks should increase with peak size in the absence of an effect on liver, but should be reduced if high parasitemia affects liver-stage development. Again, the data showed a pattern consistent with an effect on the liver stage (Figure 3C and F).

Patterns of parasitemia in adults are consistent with simulation results. Our initial analysis of the field data was restricted to children < 10 years of age, who experienced higher parasitemias than older children or adults. Indeed, none of the older group experienced any peaks > 24,000 parasite/ μ L, and thus we would not expect to observe the blood-liver effect in this group. However, our simulation predicted an association between peak size and subsequent parasitemia even in the absence of this effect. Therefore, we also repeated our analysis on individuals from the same cohort aged > 10 years, in which there were no peaks > 24,000 parasites/ μ L. In this older population the relationship between peak size and the proportion of zero readings after the peak, the delay between peaks, and the nadir level of parasites between peaks was all consistent with the expectations from the model with or without a blood-liver effect (Figure 4).

Variable infection rate is not a cause of delayed peaks. The observed high proportion of undetectable parasites and delayed peaks in parasitemia could have arisen simply because some children experienced a lower EIR than others, and indeed one would expect some variability in this rate. This could be a significant confounder in our study, because children with a lower EIR might be expected to both have weaker immunity (leading to higher parasitemia)²⁰ and a greater delay between peaks. Thus, we compared the rate of initiation of new blood-stage infections (estimated from the initial infection curve after treatment) for the patients with different peaks of parasitemia (see Figure 1C and Methods section). We found no evidence that individuals with a higher peak parasitemia had a lower rate of infection, suggesting the lower nadir parasitemias and delayed peaks were temporally associated with the high peaks themselves and not a general feature of specific patients.

FIGURE 4. The relationship between peak size and subsequent parasitemia in individuals > 10 years of age. Individual peaks of parasitemia are sorted according to size (x axis) and then the post-peak dynamics are compared for peaks of a different size. The fraction of peaks followed by undetectable parasitemia (A), the median distance to the next peak (B), and the median of density of parasites in the nadir after peaks (C). * Only one patient measurement available.

DISCUSSION

Studies of murine malaria infection suggest that high levels of blood-stage parasitemia may inhibit the liver stage of the parasite⁴⁻⁶; this occurs presumably because high levels of parasitemia induce either a direct anti-parasitic effect, or stimulate host responses that drive this. Similar studies are not able to be performed in humans, and it is not known whether similar effects are observed. We analyzed the data from a field study of treatment-infection by *P. falciparum* to measure the effect of high parasitemia on subsequent infection dynamics. Our results using this natural history of infection study in a malaria holoendemic region suggest that after a high peak of parasitemia, an increased delay until the next peak of parasitemia is observed, and a decrease in the parasitemia between these peaks. We used a simulation approach to determine the expected dynamics of infection in the presence and absence of a blood-liver effect, and found the data consistent with the effects predicted by the presence of a blood-liver effect. This is not caused by confounding factors such as an association between a lower infection rate and higher peak, and suggests that high peak parasitemia may have a direct effect on suppressing liver-stage development of *P. falciparum* in humans.

It is important to note that our study does not specifically address the mechanisms driving the associations observed in the field data, i.e., although the observed delay in peak and decrease in parasitemia between peaks is consistent with the conceptual model that high parasitemia may block the liver stage of infection, it is also possible that a variety of other mechanisms may drive this association. For example, high parasitemia may drive a high level of anti-merozoite immunity, which could either block the growth of blood stage infection as it emerges from the liver, or slow the growth of infection. Either of these mechanisms may be expected to cause the features observed in the clinical data. Similarly, high levels of parasitemia could alter the balance of pro-inflammatory versus inhibitory cytokine responses,²¹ or possibly drive other forms of competition between parasite strains, again suppressing new infections. These mechanisms are also not exclusive, and could also act in conjunction with suppression of the liver stage. Our model of inhibition of the liver stage of infection is not intended to prove that this is the mechanism, but instead to illustrate the data is compatible with such an explanation. Thus, the studies by Portugal and others⁶ raise the major question "does the phenomenon of high blood-stage parasitemia inhibiting the liver stage that was seen in mice also occur in humans." To address this we studied infection dynamics in humans. If we had found no association between peak size and subsequent parasitemia, we would have then arrived at the answer "the behavior of *P. falciparum* infection is not compatible with suppression of the liver stage." Conversely, the fact that the infection dynamics are compatible with suppression of the liver stage does not prove this is the mechanism. However, our study is the first to show that high parasitemia alters the dynamics of subsequent infection with *P. falciparum*, and provides an exciting challenge to further explore what drives this phenomenon in natural infection.

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