Zika plasma viral dynamics in nonhuman primates provides insights into early infection and antiviral strategies

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Zika virus (ZIKV) is a flavivirus with a ∼10.7-kb positive sense single-stranded RNA genome that is primarily transmitted among humans via a mosquito vector. First identified in Uganda in 1947 (2), it has come to prominence due to an outbreak in 2007 in Micronesia (3) and emergence in Brazil in 2015 (4). Human infection with ZIKV is usually accompanied by a relatively mild, self-limiting fever (3, 5) but can be associated with more severe effects such as Guillain–Barré syndrome (6) and fetal microcephaly (7). Recent reports of non-mosquito-borne transmission (reviewed in ref. 8) including sexual transmission (9, 10) and maternal transmission (11, 12) raise the concern that ZIKV may spread and persist in large parts of the world. Vaccines represent the most effective way to counter the epidemic, with promising results in preclinical studies (13, 14). Despite the fact that human trials have begun, approved vaccines may not become available for years (15). Treatment with antivirals is needed, especially in some populations, such as severely infected patients or individuals for whom viremia remains detectable in the blood or in other compartments for extended periods, as has been observed in pregnancy (16) and in semen (17, 18). So far no antiviral drug has shown a significant effect against ZIKV in vivo. The WHO has highlighted a number of potential drugs that could be relevant against ZIKV (www.who.int/blueprint/priority-diseases/key-action/zika-rd-pipeline.pdf?ua=1) and a high throughput in vitro screen (19) has identified a number of potential small molecules with activity against ZIKV but that will require further testing in animal models.

With that perspective, the establishment of a relevant nonhuman primate (NHP) model (14, 20–22) represents crucial progress toward a better understanding of virus pathogenesis and the development of more efficacious therapeutic strategies. Zika infection in NHPs has recapitulated many of the key clinical findings in human infection, including rapid control of acute viremia, early invasion of the central nervous system, and prolonged viral shedding and fetal pathology in pregnant animals (14, 20, 21, 23, 24). Thus, the study of ZIKV in NHP models can provide insight into viral dynamics as well as providing an essential tool for testing potential antiviral drugs and vaccines (14).

Mathematical modeling of viral dynamics has provided a quantitative understanding of viral dynamics for a number of viruses, including HIV (25, 26), influenza (27), and the flaviviruses hepatitis C virus (28), West Nile virus (WNV) (29), and dengue (30–32). One of the main insights of these models was to provide estimates of parameters that are not easily measurable, such as the half-life ($t_1/2$) of productively infected cells in vivo and the basic reproductive ratio, $R_0$, the number of secondary cells infected by viral production from one infected cell in a population of target cells. In the context of viruses for which antivirals are available, such as HIV and hepatitis C virus, mathematical modeling has been used to optimize and evaluate in silico the impact of antiviral strategies (33–36).

Significance

In light of the recent outbreak of Zika virus (ZIKV), an understanding of the within-host viral dynamics is essential to assess persistence in vivo, transmission risk, and antiviral therapeutics. Using mathematical modeling we find that, in nonhuman primates, the viral dynamics of ZIKV are characterized by a short lifetime of infected cells (<10 h) during which enough viral particles are produced to infect ∼11 other cells. Higher disease burden is associated with changes in natural killer cell subset concentrations and with elevated expression of the cytokine MCP-1, although the mechanisms behind these associations remain unclear. In order for an antiviral treatment to effectively reduce the time to plasma viral clearance therapy should be initiated at the time of infection or given prophylactically.


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Here we use mathematical modeling to characterize ZIKV dynamics in vivo in NHPs, to explore correlations between viral dynamic parameters and the immunologic response, and to simulate the effects of a potential antiviral agent.

Results
Zika Viral Kinetics. After s.c. infection of nine rhesus macaques with 10^6 pfu of a Thai isolate of ZIKV (Materials and Methods), the plasma viral load (VL; data given in SI Appendix, Table S1) was high by day 1 postinfection (>10^4 RNA copies per mL in all monkeys) and peaked at day 2 (Fig. 1). The peak VL was above 10^6 copies per mL for all monkeys and above 10^5 copies per mL for six of the nine monkeys. Following peak viremia, there was a rapid decline in VL, and the VL became undetectable (<200 copies per mL) in eight of the nine monkeys by day 5. The VL in the remaining monkey was undetectable by day 10.

Viral Kinetic Model and Parameter Estimates. Using nonlinear mixed effects modeling we fit eight different models to the plasma VL data, including ones with immune effects via measured IFN-α, natural killer (NK) cell, or monocyte chemoattractant protein 1 (MCP-1) dynamics. Using model selection theory (SI Appendix) we were unable to distinguish models incorporating explicit immune effects from a target cell-limited model, because all models had similar Bayesian information criterion (BIC) values (details are given in SI Appendix).

We chose to use a simple model, a target cell-limited model with an eclipse phase as given by Eq. 4 in Materials and Methods, because it allowed us to test the effects of antiviral therapy. We considered fits of this model with different fixed values of $V(0)$ (effective initial plasma VL concentration), $k$ (rate of transition to productive infection), and $c$ (rate of viral clearance). The model fit with $V(0) = 10^6\text{ mL}$ and $k = 6 \text{ d}^{-1}$ was chosen based on the BIC across all values of $c$ tested (SI Appendix, Figs. S1A and S2A). With these parameter values, the quality of model fit with $c = 5 \text{ d}^{-1}$, $10 \text{ d}^{-1}$, or $15 \text{ d}^{-1}$ was broadly indistinguishable (within ~2 BIC points, SI Appendix, Fig. S1A), and we selected the model with $c = 10 \text{ d}^{-1}$ to analyze further.

We also considered the effect of the initial target cell density, $T(0)$ or $T_0$, selecting $T_0 = 10^6\text{ mL}$ (SI Appendix and SI Appendix, Fig. S1 C and D) and $\omega$, the SD of the random effect term, selecting $\omega = 0.4$ (SI Appendix and SI Appendix, Fig. S1B). With these values, the fitting process provided an estimate of the median basic reproductive ratio $R_0$ of 10.7 (SI Appendix, Fig. S2B) with a relative SE (r.s.e.) of 18% and the productively infected cell death rate, $\delta$, was estimated as 4.5 $\text{d}^{-1}$ (SI Appendix, Fig. S2C, r.s.e. 15%). The estimated value of $p$ is dependent on $T_0$, the initial target cell density, and only the product $pT_0$ can be reliably estimated (38), with the fitting process estimating $pT_0$ as 1.2 $\times 10^5\text{ d}^{-1}\text{ mL}^{-1}$ (SI Appendix, Fig. S2D). The individual parameters derived from this population fit provide estimated VLs for each monkey as shown in Fig. 1, and the predicted target cell dynamics for each individual monkey (SI Appendix, Fig. S1) show that target cells are largely but not completely eliminated by infection. Estimates of the value of $R_0$ for each monkey range between 8.5 and 16.5 (Fig. 1). The goodness of fit and estimated parameters for each combination of $V(0)$ and $k$ are shown in SI Appendix, Fig. S2, and under the model averaging approach (Materials and Methods) the distribution of $R_0$ in the population had a median of 10.5 with range 5.3–19.5 (5th–95th percentiles). We also estimated parameters $R_0$, $\delta$, and $pT_0$ by fitting the model to each individual monkey using a nonlinear least-squares approach (SI Appendix, Fig. S4). This approach gave values of $R_0$ that ranged between 5.3 and 25.9 and that have a very similar median (10.6) to that estimated by population modeling.

Correlation Between Plasma VL and VL in Other Compartments. There was a statistically significant positive correlation, after Bonferroni correction for multiple testing, between the area under the log10 VL curve (AUC) between days 0 and 14 in plasma calculated by the model and in saliva (SI Appendix, Fig. S5A, Spearman correlation, $P = 0.05$). There were no other statistically significant correlations at the $P = 0.05$ level between modeled AUC in plasma and AUC in other compartments (SI Appendix, Fig. SS B–E) or between peak log10 VL in plasma as predicted by the model and peak log10 VL in other compartments (SI Appendix, Fig. S6).

Immunological Markers During ZIKV Infection. We tested for correlations between immune cell subset or cytokine concentrations during ZIKV infection and the modeled plasma VL. None of the Spearman correlations were statistically significant after correction for multiple testing, but we considered those cell subsets and cytokines with the strongest correlations with VL to look for suggestions of an immune response to ZIKV infection that we could further examine via model fitting.

Of the cell subsets, NK CD16+ CD69+ cells showed the strongest positive correlation ($r = 0.83$ between subset concentration at day 0 and peak log10 VL, SI Appendix, Fig. S7 A and B), and NK CD16− CD56− cells (SI Appendix, Table S4) show the strongest negative correlation ($r = −0.83$ between subset concentration at day 2 and log10 VL AUC between days 0 and 14, SI Appendix, Fig. S7 C and D). Biologically we might expect NK cells to aid in clearance of infected cells, but model fitting incorporating the NK CD16− CD56− subset did not provide strong support for or against these cells controlling ZIKV VL (Model G, SI Appendix, Table S5).

Of the cytokines, MCP-1 (SI Appendix, Table S2) showed the strongest positive correlation ($r = 0.88$ between MCP-1 concentration at day 1 and estimated infected cell half-life, SI Appendix, Fig. S7 E and F). A mechanism by which MCP-1 recruits target cells might explain the observed positive correlation, but model fitting (Model H, SI Appendix, Table S5) does not provide strong evidence for or against this hypothesis. Additionally, we hypothesized that IFN-α (SI Appendix, Fig. S8 and Table S3) might be involved in immune control of VL, despite the absence of a correlation with VL (SI Appendix, Fig. S9). Models incorporating an IFN-α response (Models D–F, SI Appendix, Table S5) were all indistinguishable from our basic model, Eq. I, using model selection theory.

Evaluating the Effect of an Antiviral Treatment. For an antiviral that reduces the viral production rate, the effect on the VL dynamics can be assessed using a target cell-limited model if the $EC_50$
against ZIKV and the free drug concentration are known. Because we do not know how lowered VL due to antiviral therapy will influence immune responses, models incorporating the measured immune markers in these data are not suitable for studying the effect of an antiviral. The model, Eq. 1, predicts that when the drug concentration $C$ is low relative to its EC$_{50}$, $R_0$ declines almost linearly with increasing drug concentration (Fig. 2A). However, once the drug concentration is well above the EC$_{50}$ further increases in $C$ have little effect. To reduce the median value of $R_0$ to below 1, meaning no establishment of infection, a constant free drug concentration 9.7 times the EC$_{50}$ (i.e., 9.7 EC$_{50}$) is required. Similarly, the peak VL decreases monotonically with increasing $C$ (Fig. 2B).

Despite the monotonic reduction in $R_0$ with increasing $C$, the time until VL becomes undetectable initially increases with $C$ before declining at higher ratios of $C$ to EC$_{50}$ (Fig. 2C). This initial increase at low drug concentrations is due to the effect of the drug in reducing the number of viral particles produced and therefore the rate at which target cells become productively infected. Consequently, the time until the target cell population is substantially depleted is increased in the model, leading to a prolonged viral infection, albeit at lower levels than without treatment. The median time until the VL becomes undetectable is maximal, at 16.1 d, when $C$ is 7.6 EC$_{50}$ and becomes lower than the time with no drug (4.7 d) when $C$ is 12.8 EC$_{50}$, reaching 3 d when $C$ is $\sim 15$ EC$_{50}$. The VL AUC exhibits similar behavior as constant $C$ is increased (Fig. 2D). Whether infection will be prolonged at low drug concentrations in reality is unclear because an adaptive immune response most likely will arise and limit the infection (23).

To model the effects of an antiviral drug with activity against ZIKV under more realistic conditions of changing drug concentration we examined favipiravir, an antiviral drug, approved for human use, with activity against many RNA viruses (39) and with known pharmacokinetics (PK) in monkeys (40). We used a previously developed PK model that shows differences between male and female monkeys (SI Appendix), assumed a 150 mg/kg twice daily dosing regime initiated at the time of infection, and chose PK (for male monkeys) and viral kinetic parameters randomly from their estimated distributions to simulate VL dynamics for 5,000 monkeys. The peak median VL was substantially reduced by $\sim 3$ logs for male monkeys and the time to first undetectable median VL was shortened by $\sim 2$ d (Fig. 3A, red). Initiating treatment 2 d after infection had essentially no effect on peak log$_{10}$ VL and only shortened the time to undetectable VL by $\sim 0.5$ d (Fig. 3A, blue). Assessing the reduction in VL burden by the AUC calculated between 0 and 14 d postinfection (dpi) (Fig. 3B) shows a reduction in median log$_{10}$ VL AUC from 14.5 d copies per mL with no drug (Fig. 3B, black) to 4.7 d copies per mL (with large variation between simulations) when treatment is initiated at the time of infection (Fig. 3B, red). When treatment is initiated 2 d after infection, the effect on median log$_{10}$ VL AUC in less substantial (Fig. 3B, blue). The equivalent analysis for female monkeys shows a less substantial effect on VL (SI Appendix, Fig. S10).

**Discussion**

This study presents an analysis of the within host dynamics of ZIKV in NHPs. After s.c. infection with 10$^6$ pfu of a Thai isolate, ZIKV is found at high concentrations in the plasma at day 1 and peaks at day 2 in these animals, followed by a rapid decline. These rapid viral kinetics resemble those observed in influenza A infections in humans, where VL tends to peak 2–3 dpi and declines to undetectable levels by 6–7 dpi in many individuals (27). This similarity motivated the use of a target cell-limited viral infection model developed by Baccam et al. (27) for influenza to fit the ZIKV plasma VL data, and the use of similar methods of analysis as in ref. 41.

The early peak VL (2 dpi) makes it unlikely that an adaptive immune response has been mounted and is responsible for the postpeak viral decline. However, innate immune responses may play a role, as has been suggested for dengue (31), and cytokines such as type I IFN could place some uninfected cells into an antiviral state, protecting them from infection. Fitting models that include IFN-α, using its measured dynamics, did not improve the fit to the data sufficiently to justify including these innate immune responses in the model, consistent with the observation that IFN-α is not correlated with VL (SI Appendix, Fig. S9) and that ZIKV disrupts IFN signaling via STAT2 degradation (42–44). Similarly, the modeling did not strongly support or reject including an NK cell-mediated innate immune response. It may be that these data do not contain enough information to allow inference about the presence of an innate immune response, and additional VL measurements at earlier time points or after infection with lower doses may allow more detailed assessment of the contribution of the innate immune system to control of ZIKV in these animals. It may be that inclusion of immune data could reduce the unexplained individual variability of the parameters, but these data do not contain enough information to allow us to estimate this. Given the positive relationship between

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**Fig. 2.** The predicted effect of a constant concentration, $C$, of an antiviral drug initiated at the time of infection that reduces viral production rate $p$ from infected cells by a factor $1 - e^{-C}$ (where $e = C/(EC_{50} + C)$ is the drug effectiveness) on the (A) basic reproductive ratio, $R_0$ peak VL, (C) time until the VL becomes undetectable (below 200 RNA copies per mL), and (D) log$_{10}$ VL AUC. At each value on the x axis, 5,000 viral dynamic parameter sets were sampled from the estimated parameter distributions as determined by mixed effects model fitting. Black lines represent the median value across simulations and gray lines represent the 5th and 95th percentiles. In A, the horizontal black dashed line represents $R_0 = 1$. **Fig. 3.** The predicted effect in male macaques of a 150 mg/kg twice daily regime of favipiravir, initiated at time of infection (red) or 2 d after infection (blue) on (A) median VL and (B) distribution of log$_{10}$ VL AUCs. In A, the horizontal black dashed line represents the limit of detection of the experimental assay, 200 RNA copies per mL. In B, median AUC values are 14.5 (no drug), 11.8 (2 dpi), and 4.7 (0 dpi). Data shown are from 5,000 repeated simulations, each including independent simulations of the PK profile and VL dynamics determined by parameters $R_0$, $\delta$, and $p$ selected at random from the population distribution predicted by mixed effects model fitting.
the expression of MCP-1, a cytokine implicated in recruitment of cells to the site of infection (45), and the predicted infected cell half-life (SI Appendix, Fig. S7F), we also considered a viral dynamics model in which the target cell population was increased in an MCP-1-dependent manner (model H, SI Appendix, Table S5). In fitting this model to the VL data from each monkey, the model BIC suggested that the available data cannot support or reject the hypothesis that MCP-1 affects the VL via this mechanism.

We highlight the point that these data do not contain enough information to allow for either acceptance or rejection of the hypothesis that an immune response is responsible for control of plasma ZIKV in these animals. Our modeling approach is to select the simplest model from those that provide equivalently good fits to the data, and as such we have performed analysis without explicit inclusion of an immune effect.

The initial viral infection is characterized by a basic reproductive ratio ($R_0$) of 10.7, suggesting that early after infection each infected cell on average infects ~11 other cells. To attain rapid spread, we estimate the mean eclipse phase for ZIKV (i.e., the time between infection and viral production) is ~4 h. This estimated eclipse phase length is shorter than that for WNV in mice (29) and that estimated for influenza A in humans (27). In vitro ZIKV viral production from infected human skin cells was detected at 6 h, the earliest time examined (46). We also estimate that once cells begin producing virus they live an average of 5.3 h to yield a total lifespan, which includes the eclipse phase, of slightly less than 10 h.

Using the model, Eq. 1, we can only estimate the product $pT_0$ (38), where $T_0$ is the initial target cell density and $p$ is the rate of viral production from a productively infected cell. Because the target cells for ZIKV are not currently fully characterized, we have no a priori estimate of $T_0$. However, we can constrain $p$ because the amount of infectious virus made over an infected cell’s productive lifetime of 5.3 h needs to be greater than $R_0$. For a French Polynesian isolate of ZIKV it has been estimated that 500–1,000 virions correspond to 1 pfu (20). Thus, with $R_0$ ~11, an infected cell needs to produce at a minimum 500 $\times$ 11 = 5,500 virions over 5.3 h. This implies a daily production rate $p \geq 25,000$ virions d$^{-1}$. From our model fitting, we estimated that $pT_0 = 1.2 \times 10^4$ d$^{-1}$ mL$^{-1}$, so that with $p \geq 25,000$ virions d$^{-1}$ we estimate $T_0 \leq 4.8 \times 10^4$ mL. Our finding of a low initial target cell density combined with the broad range of cell types that ZIKV has been shown to be able to infect, because the amount of infectious virus made over an infected cell on average infects ~11 other cells. To attain rapid spread, we estimate the mean eclipse phase for ZIKV (i.e., the time between infection and viral production) is ~4 h. This estimated eclipse phase length is shorter than that for WNV in mice (29) and that estimated for influenza A in humans (27). In vitro ZIKV viral production from infected human skin cells was detected at 6 h, the earliest time examined (46). We also estimate that once cells begin producing virus they live an average of 5.3 h to yield a total lifespan, which includes the eclipse phase, of slightly less than 10 h.

The early peak viremia and rapid viral decay in this NHP model, along with the limited longitudinal sampling, means that the chosen value of $V(0)$ and the estimate of $R_0$ should be taken cautiously. Plasma samples taken during the first few hours postinfection would allow for a more accurate assessment of the likely value of $V(0)$ and would give insight into how rapidly a s.c. infection is trafficked to the blood. Also, studies with different inoculum sizes would help in determining a relationship between dose and $V(0)$. The number of data points available also limits our ability to estimate the distribution of parameters found in the population, because these data are unable to support fitting the variance of the estimated parameter distributions. Additional animals as well as more frequent sampling would improve the estimates of these distributions. The lack of VL AUC correlation between the plasma and semen compartments (SI Appendix, Fig. S5D) might be due to the small number of data points available in this study, or it might indicate that different mechanisms affect the VL in the semen, which would restrict our ability to assess sexual transmission risk from plasma samples. Overall, the persistence of virus in saliva and semen (17) and the prolonged presence in the plasma of pregnant macaques and humans (16, 20) as well as in a child born to ZIKV-infected parents (51) remains to be explained.

In VL dynamics from a study with different ZIKV strains and with lower inoculum doses (25) it was seen that peak VL tended to be later than in these data, and a model incorporating immune control from IFN-α (reducing viral production) and antibodies (reducing viral infectivity) was able to improve the fit of the model. The estimated value of $R_0$ was found to be very similar to this study, whereas the estimated value of $R_0$ was much lower (~3). This discrepancy between the estimated values of $R_0$ can be attributed to a later peak with lower inoculum doses as well as a higher estimate of $V(0)$ with equivalent inoculum doses, highlighting the need for early samples to assess the true initial plasma viral concentration.

Because antiviral drugs against ZIKV are being tested in rhesus macaques we investigated the effect of an antiviral drug at concentration C that reduces the production of virus by a factor of $1 - C/(EC_{50} + C)$. Increasing the drug concentration reduces $R_0$, but we estimate that a drug concentration of at least 128 EC$_{50}$ is required to reduce the time to undetectable VL (Fig. 2), where these results are based on our target cell limitation model assumptions. This approach can allow for rapid screening of potential repurposed drug candidates: if a drug is unable to reach this concentration with a safe dose, it is unlikely to have a substantial positive impact of the VL dynamics, although both differences between EC$_{50}$ in vitro and in vivo and differences between the VL dynamics in the NHP model and in humans need to be accounted for. We identified favipiravir, a small-molecule antiviral, as capable of reducing the median viral burden in NHP infections in simulations and found that substantial clinical benefit can be achieved for the majority, but not all, of simulations when treatment is started at the time of infection. The PK of favipiravir is highly variable in NHPs (40), resulting in the broad distribution of VL AUCs seen in our simulations (Fig. 3B), although the sustained infections seen in our simulations when the PK is unfavorable, giving low drug concentrations, would likely be mitigated by later immune responses. However, if an antiviral strategy is to have any substantial effect on peak VL, time to undetectable viremia or VL AUC it needs to be initiated before peak viremia. Because peak plasma VL occurs very early in this NHP model, this effectively means that treatment needs to be given prophylactically. In humans where lower viremia levels are often observed (e.g., refs. 18, 53, and 54), as well as when lower inoculum doses are used in NHPs (14) the viral dynamics will be altered and the peak of plasma viremia will likely be delayed, affecting the assessment of antiviral impact and potentially allowing for later antiviral treatment initiation.

The mathematical modeling approach in this study describes the characteristics of the viral dynamics of ZIKV as observed in the plasma of an NHP model and provides a tool by which to assess, in silico, the effect of candidate antivirals.

Materials and Methods

ZIKV-Infected Rhesus Macaques. Ten Indian-origin rhesus macaques (five male and five female) were infected s.c. with 1$^{10^5}$ pfu of a Thai isolate of ZIKV as described in ref. 21. Samples were collected from blood (plasma), urine, saliva, cerebrospinal fluid, semen, and vaginal secretions for 4 wk postinfection. Samples were taken regularly, and viral RNA levels in samples were assessed using real-time PCR, with a limit of detection of 200 copies per mL. Additionally, peripheral blood mononuclear cells (PBMC) were analyzed from the same blood samples. PBMC samples were stained with fluorescent antibody mixes and flow cytometry used to quantify the concentration of T-cell, B-cell, and NK subsets. The set of immune cells measured includes total lymphocytes, eight T-cell subsets, three B-cell subsets, and four NK cell subsets, and both total concentration and activated (as indicated by expression of CD69) concentration were measured (21). Cytokine expression in plasma samples was also measured by multiplex bead immunoassays at days 0, 1, 3, and 7. All animals were housed at Bioqual Inc. or the Wisconsin National Primate Research Center (WNPRC). The described studies were approved by the Institutional Animal Care and Use Committee of Bioqual Inc. or WNPRC, respectively.

Viral Dynamic Model. Plasma viral dynamics in nine of the 10 animals were characterized using a standard target cell-limited model with an eclipse phase originally developed to analyze influenza infection kinetics (27). One animal in which there were only two plasma VLs above the detection limit of the assay was excluded from this analysis. The model with an eclipse phase was
used in preference to a model without an eclipse phase (SI Appendix). The model is given by the following system of ordinary differential equations:

\[
\begin{align*}
\frac{dT}{dt} &= -\beta VT, \\
\frac{dI_1}{dt} &= \beta VT - k_i I_1, \\
\frac{dI_2}{dt} &= k_i I_1 - k_d I_2, \\
\frac{dV}{dt} &= -p_I V - cV.
\end{align*}
\]  
[1]

In this model, target cells (T) are infected by free virus (V) at a rate proportional to both virus and target cell concentrations, as given by the law of mass action term \(\beta VT\). After infection, the cells enter an eclipse phase, denoted \(I_1\), where it is infected but does not produce virus. It then transitions to a productively infected state \(I_2\) at rate \(k_i\), that is, \(1/k_i\) is the average duration of the eclipse phase. Productively infected cells are cleared at per capita rate \(k_d\), incorporating the observed cytopathic effects of ZIKV (55, 56), and produce virus at rate \(p_I\) per cell. Free virus is cleared at rate \(c\). Because of the short-term nature of the infection as measured in blood with a peak VL at day 2 in all nine animals, target cell replenishment was ignored in the model.

The model predicts an initial decline of virus until the end of the eclipse phase of the first infected cells, followed by an exponential increase until target cells become limiting. At the end of this expansion phase, the target cells are largely but not completely depleted (SI Appendix, Fig. S3), the number of new infections is low, and the loss of infected cells largely outnumber the number of new cell infections. Consequently, the number of infected cells, and hence the total viral production, declines. Mathematical analysis shows that this decline is exponential with a rate proportional to the infected cell death rate, \(\delta\), when it is much smaller than \(c\) and \(k\) (57). In this model all parameters were assumed to be constant, but in principle they could vary over time. Thus, for instance, an adaptive immune response, if present, could be associated with an increasing value of \(\delta\). The model derives from the basic reproductive number, \(R_0\), representing the number of secondary infected cells that would be created by a single infected cell introduced into a population of \(T_0\) target cells, given by \(R_0 = \beta k_i T_0 c / \delta\). The model was reparameterized as a function of \(R_0\), which we constrained to be larger than 1 to ensure productive infection. More details on viral dynamics models during acute infection can be found in refs. 57–59. We additionally tested a number of other potential models, including a model without eclipse and another model incorporating immune parameters (SI Appendix). Model selection was based on BIC, with more complex models only being accepted if an improvement in BIC of >2 points was observed (60).

Fixed Parameters. Because not all model parameters can be estimated using only VL data (38) a number of parameters had to be fixed (details are given in SI Appendix). First the number of nonproductively and productively infected cells at time of infection is assumed to be \(0(|I_1| = |I_2| = 0)\), and the number of target cells at the time of infection, \(T(0)\), is assumed to be \(10^5\) per mL. As shown in ref. 38, for a standard viral dynamic model the viral production rate \(\beta\) and the initial target cell density \(T(0)\) are dependent, and with measurements of VL only one is able to be estimated. Here the assumed value of \(T(0)\) was chosen to give biologically reasonable estimates of \(\beta\) (SI Appendix). Note that although the value of \(T(0)\) was chosen to be plausible it may affect the specific model conclusions. The findings regarding the basic reproductive number, \(R_0\), the half-life of infected cells, and the effect of antiviral treatment. Additionally, we do not assume that all target cells and infection events are in blood, but model the effective impact of target cell infection on the plasma VL as has been done, for example, in models of hepatitis viruses (28), whose target cells are mainly or exclusively in the liver. After initial model fitting to determine the effect of the rate of clearance of free virus, \(c\), on the model fit (details are given in SI Appendix) we set \(c\) to a fixed value of 10⁻³.

Estimated Parameters. The macaques were inoculated s.c. with \(10^5\) pfu ZIKV. Assuming that 1 pfu represents between 500 and 1,000 copies of viral RNA (20) and that a typical macaque plasma volume is about 300 mL, the inoculum would at most generate between \(1.7 \times 10^5\) and \(3.3 \times 10^5\) viral RNA copies per mL of plasma. Because it is likely that much of the early infection occurs in the skin and that only a small proportion of the inoculum is transported to the blood immediately after infection, we used values of \(V(0)\) between \(10^3\) and \(10^5\) viral RNA copies per mL in our model fitting. Here \(V(0)\) should be interpreted as an effective initial viral concentration.

The eclipse phase duration for ZIKV is unknown. We let \(k\) be 4, 6, or 8 \(d\)⁻¹ (representing a mean eclipse phase of 6, 4, or 3 \(h\)). We restricted our range of possible eclipse phase lengths to short times because the Zika plasma VL can be as high as \(10^5\) RNA copies per mL by 1 d postinfection, suggesting rapid onset of viremia. Additionally, the model is only a basic reproductive number \(R_0\), peak VL time to undetectable VL, and VL AUC for different (constant) values of \(C_{E_{10}}\).
The EC₅₀ values of favipiravir against ZIKV strains was assessed as detailed in SI Appendix and provided values between 2.7 µg/mL and 6.6 µg/mL. Because the PK of favipiravir varies in NHP, while maintaining a trough concentration at day 7 greater than the EC₅₀ against ZIKV (40). Different treatment initiation times were tested, with dosing starting either at the time of infection or 2 dpi (median time of viral peak). For each scenario, 5,000 PK and viral kinetic parameters were drawn from their estimated distributions, giving simulated trajectories of response. Because the PK in male and female animals differed, this process was done for males and females separately. To summarize the simulations, the median of the VLs at each time point and the distribution of the AUC of log₁₀ VL between 0 and 14 dpi over the 5,000 trajectories were reported for males and females separately.

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Viral dynamic models

Below we compare a number of different viral dynamic models that were used to fit the ZIKV plasma viral load (VL) measured in 9 infected rhesus macaques. The plasma VL data is provided in Table S1. Fitting of the data from all 9 monkeys was done simultaneously using Monolix (http://lixoft.com/products/monolix). Bayesian information criterion (BIC) values as reported by Monolix are given for each model and used as a basis for model comparison. Table S5 summarizes our model comparisons. The lower the BIC the better the model, although absolute differences of BIC that are less than 2 are not sufficient to distinguish between models (1).

Mathematical models considered

Model A: Viral dynamic model without an eclipse phase

We assessed the ability of a standard viral dynamics model (Model A) without an eclipse phase to describe the observed plasma viral load data. The model is described by the following set of ordinary differential equations (ODEs):
\[
\begin{align*}
\frac{dT}{dt} &= -\beta VT \\
\frac{dI}{dt} &= \beta VT - \delta I \\
\frac{dV}{dt} &= pI - cV \\
\end{align*}
\]  
(S1)

This model is not realistic in that cells that become infected immediately start to produce viral particles, but nonetheless such models are sometimes useful to summarize viral dynamics data, as shown in (2).

**Model B: Viral dynamic model with an eclipse phase**

In order to assess whether the inclusion of an eclipse phase, during which cells are infected but not producing viral particles, improves the model fit, we compared the fit of Model A to the fit of the model with an eclipse phase (Model B) that is presented in the main text and given below.

\[
\begin{align*}
\frac{dT}{dt} &= -\beta TV \\
\frac{dI_1}{dt} &= \beta TV - kI_1 \\
\frac{dI_2}{dt} &= kI_1 - \delta I_2 \\
\frac{dV}{dt} &= pI_2 - cV \\
\end{align*}
\]  
(S2)
Model C: Infection leads to loss of a viral particle

In a slight extension to the standard viral dynamics model with eclipse phase (Model B), as used to model West Nile Virus (3), we tested whether inclusion of a viral loss term due to infection events improves the fit of the model to the data. This model is described by the following system of ODEs:

\[
\begin{align*}
\frac{dT}{dt} &= -\beta VT \\
\frac{dI_1}{dt} &= \beta VT - kI_1 \\
\frac{dI_2}{dt} &= kI_1 - \delta I_2 \\
\frac{dV}{dt} &= pI_2 - cV - \beta VT
\end{align*}
\]

As in Model B, we estimate the population distributions of \(R_0, \delta\) and \(p\), where for this model \(R_0=p\beta T_0/\delta(c+\beta T_0)\).

Viral dynamic models incorporating immune response

We incorporated an immune effect into our viral dynamic model in a number of different ways and tested whether these extended models provide a better fit to the plasma viral load data than Model B. It is possible to fit models of this type that include an ODE for the assumed dynamics of the immune effect, but we opted instead to make use of the immunological data available from these monkeys. For each of these models, relevant measured immune cell populations or cytokines are incorporated into the modeling via the regressor option in Monolix, using linear interpolation to estimate
values between measurements. We compare model fits on the basis of BIC, accepting a more complex model only if it provides an improvement in BIC of > 2 points (1).

*Model D: IFN acts to reduce viral production rate*

IFNα levels were measured in each of the monkeys at days 0, 1, 2, 3, 5 and 7 after infection (Fig. S8) and some of the monkeys produced a robust IFNα response while others did not. There was no correlation between IFNα response and viral load (Fig. S9). We assumed that IFNα acts to reduce the viral production rate in a model described by the following ODEs:

\[
\begin{align*}
\frac{dT}{dt} &= -\beta VT \\
\frac{dI_1}{dt} &= \beta VT - kI_1 \\
\frac{dI_2}{dt} &= kI_1 - \delta I_2 \\
\frac{dV}{dt} &= \frac{p}{1 + \varepsilon X(t)} I_2 - cV
\end{align*}
\]

where \(X(t)\) is the measured IFNα level. Since IFNα is below the limit of detection in all monkeys at time of infection, we assume that \(X(0) = 0\) and as such \(R_0\) remains the same in Model D (and other IFN models described below) as in Model B. We estimate population distributions of \(R_0\), \(\delta\), \(p\) and \(\varepsilon\).
Model E: IFN acts to reduce the infection rate

As in Baccam et al. (4), we also examined a model where IFNα acts to reduce the infection rate, yielding a model described by the following ODEs:

\[
\frac{dT}{dt} = -\frac{\beta}{1 + \epsilon X(t)} V T \\
\frac{dI_1}{dt} = \frac{\beta}{1 + \epsilon X(t)} VT - k I_1 \\
\frac{dI_2}{dt} = k I_1 - \delta I_2 \\
\frac{dV}{dt} = p I_2 - c V
\]

where \(X(t)\) represents the measured IFNα level.

Model F: IFN protects target cells

We assumed that IFN acts to place target cells in a protected anti-viral state, either permanently or temporarily, in the same way as modeled in Pawelek et al. (5). While cells are in an anti-viral state they are unable to be infected. The model is described by the following system of ODEs:

\[
\frac{dT}{dt} = -\beta V T - \varphi X(t) T + \psi P \\
\frac{dP}{dt} = \varphi X(t) T - \psi P \\
\frac{dI_1}{dt} = \beta V T - k I_1 \\
\frac{dI_2}{dt} = k I_1 - \delta I_2 \\
\frac{dV}{dt} = p I_2 - c V
\]

(S6)
where $P$ denotes cells that are protected from infection by being in an anti-viral state and $X(t)$ represents the measured IFNα level. If the protection induced by IFN is permanent (at least over the timescale of our data) we set $\psi = 0$ and refer to this as model $F(a)$. Alternatively, we fit $\psi > 0$ and call this model $F(b)$. When fitting this model we increased the initial target cell population above that used for other models (setting $T(0) = 10^6$/mL) to allow for protection of a fraction of these cells while maintaining a sufficient target cell population to sustain viremia.

**Model G: NK cells act to enhance clearance of productively infected cells.**

We assumed that NK cells act to increase the death rate of productively infected cells in a mass-action like manner, described by the following system of ODEs:

\[
\begin{align*}
\frac{dT}{dt} &= -\beta VT \\
\frac{dI_1}{dt} &= \beta VT - kI_1 \\
\frac{dI_2}{dt} &= kI_1 - \delta(1+\gamma X(t))I_2 \\
\frac{dV}{dt} &= pI_2 - cV
\end{align*}
\]

(S7)

where $X(t)$ now represents the measured concentration of NK cells. We performed model fitting using the measured concentrations of NK CD16$^-$ CD56$^+$ cells, found to be the cell subset most strongly negatively correlated with viral load burden (Fig. S7d). Since NK CD16$^-$ CD56$^+$ cells are not at concentration 0 at the time of infection, we use the expression $R_0 = \beta T_0 p/(1 + \gamma X(0))\delta c$ to reparametrize this model, estimating population distributions of $R_0$, $\delta$, $p$ and $X$.  


**Model H: MCP-1 recruits target cells**

The observed positive relationship between the expression of MCP-1, a cytokine implicated in recruitment of cells to the site of infection, and disease burden as assessed by log$_{10}$ viral load AUC (Fig. S7f) was one of the strongest correlations in these data, so we considered a viral dynamic model in which the target cell population was increased in an MCP-1 dependent manner. We assumed that target cells are recruited to become available for ZIKV infection at a rate proportional to the MCP-1 expression level. This model is described by the following system of ODEs:

\[
\begin{align*}
\frac{dT}{dt} &= -\beta VT + \phi X(t) \\
\frac{dI_1}{dt} &= \beta VT - kI_1 \\
\frac{dI_2}{dt} &= kI_1 - \delta I_2 \\
\frac{dV}{dt} &= pI_2 - cV
\end{align*}
\] (S8)

where \(X(t)\) represents the observed MCP-1 concentration (Fig S7e). We estimate the population distributions for \(R_0, \delta, p\) and \(\phi\).

**Comparison of model fits**

A summary of the model fit results is shown in Table S5.

*Model with eclipse phase is preferred to model without eclipse phase*

With initial conditions \(T(0) = 10^5 / \text{mL}, I_1(0) = 0\) and \(I_2(0) = 0\) and using different fixed values of \(c\) and \(V(0)\), we fitted parameters \(R_0 = \beta T_0 p / \delta c\), \(\delta\) and \(p\) of Model A (eqn.
The best fit of Model A to observed viral load data provided a BIC of 113.6. Model B (eqn. S2) uses one additional parameter, \( k \), and the model fit as presented in the main text provides a BIC of 109.5 as provided by Monolix. However, since the additional parameter in Model B, \( k \), could only take on one of three biologically realistic values we also re-fitted Model B, allowing \( k \) to be freely fitted. We found that the best fit of Model B was provided when \( k \) is assumed to have no intra-individual variation (being a parameter of the virus rather than of the host), and we obtained an estimate of \( k = 5.6 \text{ d}^{-1} \), giving a BIC of 111.7, which is a 1.9 point improvement over Model A. With \( k = 5.6 \text{ d}^{-1} \) the length of the eclipse phase is approximately 4 hours, and so we decided to retain the estimate of fixed \( k = 6 \text{ d}^{-1} \), found to provide the best fit of Model B, in further modeling.

Additionally, Model B is known to be a more biologically realistic model, since there is a delay between a cell being infected by virus and producing new virus particles. Therefore, we use Model B for further analysis. Model C, where infection events cause loss of one viral particle, provides very similar fits to Model B and could be used in its place.

**Selection of fixed parameters, initial conditions and standard deviation of the random effects term for Model B**

We tested the ability of the viral dynamic model with an eclipse phase (Model B) to fit the observed plasma viral load data for a range of fixed values of \( c \), the viral clearance rate. For fixed \( T(0) = T_0 = 10^5 \text{ cells/mL} \), we fit the data with \( c = 2, 5, 10, 15 \) or \( 25 \text{ d}^{-1} \), with \( k = 4, 6, \) or \( 8 \text{ d}^{-1} \) and with \( V(0) = V_0 = 10^3, 10^4 \) or \( 10^5 \text{ copies/mL} \) (Fig. S1a). We found that the BIC was systematically minimized with \( k = 6 \text{ d}^{-1} \) and \( V_0 = 10^4 \text{ /mL} \), so
we used these fixed values. The model fits with \( c = 5, 10 \) or 15 d\(^{-1} \) were found to be equivalently good (BICs within approximately 2 points), and we selected \( c = 10 \) d\(^{-1} \) for further analysis. We then performed model fitting with fixed \( k = 6 \) d\(^{-1} \), \( V_0 = 10^4 \) /mL and \( c = 10 \) d\(^{-1} \) with different values of \( T_0 \) and as expected from mathematical analysis of the model (6) we found the model fit (Fig. S1c) and parameters except for \( p \) remain broadly constant. Since only the product \( pT_0 \) is identifiable in this model, the fitted value of \( p \) is directly proportional to the fixed value of \( T_0 \), and the burst size \( (p/\delta) \) and the ratio of burst size to \( R_0 \) are similarly related to \( T_0 \). The measured ratio of viral RNA copies to infectious particles is in the range 500 – 1000 (7), and therefore the ratio of burst size to \( R_0 \) (Fig S1d) should be in approximately the same range. We used \( T_0 = 10^5 \) /mL in further analyses unless specified.

We tested model fits of Model B to the observed data with fixed \( c = 10 \) d\(^{-1} \), \( k = 6 \) d\(^{-1} \), \( V_0 = 10^4 \) /mL and \( T_0 = 10^5 \) /mL with different fixed values for the standard deviation of the random effects term, \( \omega \). The model fits with \( \omega = 0.2, 0.3 \) or 0.4 were all found to be equivalently good (within 2 BIC points), while fits with higher or lower \( \omega \) are significantly worse (Fig S1b).

Models incorporating immune response are not accepted

For each of Models D – H described above we compared best model fits obtained to the fit of model B. For each of these model fits, the random effect on each of the fitted parameters \( (R_0, \delta, p \text{ and } \varepsilon, \varphi, \psi \text{ or } \gamma \text{ as appropriate}) \) was fixed at \( \omega = 0.4 \), as for the fit of Model B. Model fits with fixed values of \( c = 5, 10, 15 \) or 25 d\(^{-1} \) and \( V(0) = 10^3, 10^4 \) or 10\(^5 \)
mL\(^{-1}\) were tested, while \(k = 6 \text{ d}^{-1}\) and \(T(0) = 10^5 \text{ mL}^{-1}\) remained fixed. BICs as reported by Monolix are presented in Table S5. None of the models incorporating immune response effects examined here can be viewed as being better than Model B, since none provided a BIC improvement > 2 points. Although Models B, C, D, E, F(a), G and H were found to be equally good based on BIC (within 2 points) we prefer Model B because it is the simplest and can be used to evaluate the effects of antiviral therapy. The model that is closest to being accepted over Model B is Model G, where NK CD16\(^{-}\)CD56\(^{+}\) cells act to increase the rate of death of productively infected cells. This model provides a 0.7 point improvement in BIC but as discussed in Table S5 footnote is unlikely to have biological support.

We note that, while IFN\(\alpha\) is observed in a number of these monkeys, it is perhaps not surprising that the modeling does not suggest a significant effect of IFN\(\alpha\) on viral dynamics, since ZIKV is known to disrupt IFN signaling, for example by degrading STAT2 (8–11), and as such the presence of IFN\(\alpha\) does not necessarily imply any downstream antiviral effect. Further there was no correlation between peak VL and peak IFN\(\alpha\) nor between IFN\(\alpha\) AUC and \(\log_{10}\) VL AUC (Fig S9).

**Immune marker correlation analysis**

For each immune marker (31 cell subsets and 23 cytokines with concentration measured in the monkeys during infection), we calculate a number of different correlations with viral load. For each of the immune markers we consider four characteristics:

(i) value of the marker at baseline (day 0)
(ii) value of the marker at or near the time of peak viral load: day 1 (for cytokines)
or day 2 (for cell subsets)

(iii) peak value of the marker

(iv) AUC of the marker between days 0 and 14

We also consider three characteristics of the viral load, as predicted by the modeling:

(i) peak log_{10} plasma VL

(ii) log_{10} plasma VL AUC between days 0 and 14

(iii) estimated half-life of infected cells, t_{1/2} = \ln(2)/\delta

Then for each marker we calculate the Spearman correlation coefficient of each marker characteristic against each VL characteristic, giving 12 tests for each marker. We use the Bonferroni correction for multiple testing, with a total of 31 x 12 = 372 tests performed for cell subset correlations and 23 x 12 = 276 tests performed for cytokine correlations. Given this large correction factor, none of the correlations were statistically significant after Bonferroni testing. As such, to look for immune markers that might be involved in the immune response to ZIKV infection we considered those cell subsets and cytokines that showed the strongest correlations in any of their characteristics to one of the viral load characteristics and suggested a feasible biological mechanism influencing the VL. These selected immune markers were then incorporated into viral dynamic models. Additionally we tested models incorporating IFNα data.
Assessment of EC\textsubscript{50} of favipiravir against ZIKV

Three ZIKV strains belonging to both the African lineage (strain Uganda MR766, isolated in Uganda from a Rhesus monkey in 1947 and strain Dakar AR D 41662, isolated in Senegal from \textit{Aedes taylori} in 1984) (12) and the epidemic Asian lineage of the virus (strain H/PF/2013, isolated in French Polynesia from a Zika patient in 2013) (13) were tested. Briefly, the EC\textsubscript{50} determination was based on a virus yield reduction assay \textit{in vitro}. Favipiravir dilutions were added in triplicate to Vero E6 cells grown in a 96-well format, 15 minutes before ZIKV infection. Cells were then incubated at 37°C with 5% CO\textsubscript{2} and supernatants were collected on the third day (in the log phase of virus replication). Virus RNA was extracted (Cador Pathogen 96 kit and Qiacube HT automate, Qiagen) and quantified by qRT-PCR (GoTaq Probe one step qRT-PCR kit, Promega) with reference to quantified T7-generated RNA calibration standards. Inhibition values obtained for each drug concentration tested were used to determine the best sigmoidal curve fitting mean values (Kaleidagraph, Synergy Software) and establish EC\textsubscript{50} values. EC\textsubscript{50} values were 5.0 µg/mL and 6.6 µg/mL for the Uganda MR766 and Dakar AR D 41662 African lineage strains, respectively, and 2.7 µg/mL for the H/PF/2013 Asian lineage strain.

Pharmacokinetic (PK) model of favipiravir in cynomolgus macaques

The pharmacokinetic model used in this study is taken from (14). Briefly, a model including both an enzyme-dependent and an enzyme-independent rate of clearance of drug from the central compartment was used to characterize the drug pharmacokinetics. The enzyme-dependent clearance rate was found to increase over time and differed
between male and female animals. The increase resulted in reduced drug concentration on repeated dosing. The model is described by the following equations:

\[
\begin{align*}
\frac{dA_c}{dt} &= -k_A c - V_{\text{max}} A_e A_c \\
\frac{dA_e}{dt} &= R_{\text{in}} - k_{\text{out}} (1 + C_{\text{el}} k_{\text{el}} e^{-\lambda t}) A_e \\
R_{\text{in}} &= k_{\text{out}} A_{e0} \\
C_{c} &= \frac{A_c}{V}
\end{align*}
\]  

(S9)

In this model, \( A_c \) represents the amount of favipiravir in the central compartment, \( A_e \) represents the activity level of the enzyme and \( C_c \) represents the plasma concentration of favipiravir, which is calculated as the amount in the central compartment divided by its volume, \( V \). Favipiravir is removed from the central compartment at the first order elimination rate \( k \) and additionally by an enzyme dependent elimination rate \( V_{\text{max}} \). Enzyme is synthesized at a constant rate \( R_{\text{in}} \) and degraded at rate \( k_{\text{out}} \). In the presence of favipiravir, the enzyme is additionally degraded in a drug- and time-dependent manner, described by parameters \( k_{\text{el}} \) and \( \lambda \).

Parameter values are selected in (14) to fit the model to observed data, and are found to differ between male and female Mauritian cynomolgus macaques in the estimate of \( k_{\text{out}} \). In this study we use the parameter estimates from (14) for male and female monkeys in our simulations of the effect of favipiravir on the Zika plasma VL. Additionally, we assumed conservatively that only free drug concentrations were active and a value of 50% was used for the plasma protein binding fraction. Likewise, we conservatively used the larger reported value for the EC\(_{50} \), i.e., 6.6 µg/mL, in our simulations.
References

**Supporting Tables**

**Table S1. Plasma VL data**

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The measured log_{10} plasma VL per ml observed in each monkey (animal ID given in left-most column). The limit of detection in this assay is 200 RNA copies /mL (i.e., a VL of 2.301 log_{10}), and when the viral load is undetectable it is shown at this value. Monkey 5829 has only 2 detectable viral loads and was not used in these analyses.
Table S2. Plasma MCP-1 concentrations

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The MCP-1 concentrations in pg/mL in each monkey (animal ID given in the left-most column).
Table S3. Plasma IFNα concentrations

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<th>Days post infection</th>
<th>0</th>
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<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
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</table>

The IFNα concentrations in pg/mL in each monkey (animal ID given in the left-most column). When IFNα is undetectable it is shown in the table at the limit of detection, 0.099 pg/mL.
Table S4. NK CD16⁻ CD56⁺ concentrations

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Days post infection</th>
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</thead>
<tbody>
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The concentrations (in cells /µL) of NK CD16⁻ CD56⁺ cells in each of the monkeys (animal ID given in the left-most column)
### Table S5. Models tested

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
<th>Freely fitted parameters</th>
<th>BIC</th>
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<tr>
<td>A (*)</td>
<td>Model without eclipse phase</td>
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<td>113.6</td>
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<tr>
<td>B</td>
<td>Model with eclipse phase</td>
<td>3</td>
<td>109.5</td>
</tr>
<tr>
<td>C</td>
<td>Model B plus infection leading to loss of a viral particle.</td>
<td>3</td>
<td>109.6</td>
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<tr>
<td>D</td>
<td>IFNα reduces viral production rate</td>
<td>4</td>
<td>111.0</td>
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<tr>
<td>E</td>
<td>IFNα reduces infection rate</td>
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</tr>
<tr>
<td>F(a)</td>
<td>IFNα permanently protects targets</td>
<td>4</td>
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</tr>
<tr>
<td>F(b)</td>
<td>IFNα temporarily protects targets</td>
<td>5</td>
<td>113.3</td>
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<tr>
<td>G</td>
<td>NK CD16+ CD56+ cells enhance clearance</td>
<td>4</td>
<td>108.8</td>
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<tr>
<td>H</td>
<td>MCP-1 recruits target cells</td>
<td>4</td>
<td>111.0</td>
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</table>

The quality of the population level model fit evaluated by the Bayesian Information Criterion (BIC, as provided by Monolix) for each of the models tested. Eight different models were used to fit the data and were then evaluated based on the model fit and number of parameters fitted using model selection theory, via the BIC. The lower the BIC the better the model, and a BIC improvement of 2 points or more provides support for acceptance of a model. By BIC, all the models incorporating immune response, except model F(b), are considered to provide an equivalently good fit as Model B (within 2 BIC points), but since there is not strong support to accept any of these models the simpler model (Model B) is used in the main text for further analysis. Also, Models D-H that incorporate immune responses cannot be used for evaluating the effects of antiviral therapy as the change in the immune response, if any, due to lowered VLs in unknown.

(*) In order to fairly compare Model A (BIC of 113.6), the eclipse phase parameter was allowed to be freely fitted in Model B, providing a BIC of 111.7 and an estimate of $k =$
5.6 d⁻¹. Model B with fitted $k$ represents a 1.9 point improvement in BIC above Model A and is more biologically realistic, so we use Model B (with fixed $k$) for further analysis.

(***) The values shown for Model C are from a fit with fixed $c = 10$ d⁻¹, to allow for fair comparison with model B. When $c = 5$ d⁻¹ is used, Model B provides a BIC of 108.8 while Model C provides a BIC of 109.0. Despite this slight improvement of fit with $c = 5$ d⁻¹ we prefer $c = 10$ d⁻¹ since it gives a more biologically realistic estimate of $R_0$.

(****) Even though Model G has the lowest BIC we used Model B in the main text as it allows one to evaluate the effects of antiviral therapy. Also, the biological support for Model G is weak as these NK CD16⁻ CD56⁺ cells were not activated, i.e. were CD69⁻. Further, CD16 is the FcγRIII receptor that binds IgG and allows NK cells to mediate antibody-dependent cellular cytotoxicity. Thus, these CD16⁻ NK cells are not thought to be very cytotoxic. Given the lack of significance after Bonferroni correction of the Spearman correlation between the concentration of these cells at day 2 and the log₁₀ VL AUC, the observed correlation may have occurred by chance.
Supporting Figures

**Figure S1.** The Bayesian Information Criterion (BIC) values from a population level mixed effects model fitting for (a) the indicated fixed values of $V(0)$, $k$ and $c$ with fixed $T(0) = 10^5$/mL and fixed $\omega = 0.4$, (b) the indicated fixed values of $\omega$ with fixed $T(0) = 10^5$/mL, $V(0) = 10^4$/mL, $k = 6$ d$^{-1}$ and $c = 10$ d$^{-1}$, (c) the indicated fixed values of $T(0)$, with fixed $c = 10$ d$^{-1}$, $k = 6$ d$^{-1}$, $V(0) = 10^4$/mL and $\omega = 0.4$. (d) The estimated burst size ($p/\delta$) divided by estimated $R_0$ for different values of $T(0)$, with other fixed parameters as in (c). The grey shaded section indicates values between 500 and 1000, the estimated ratio of RNA copies to PFU (7).
Figure S2. With the indicated fixed values of $V(0) = V_0$ and $k$, the (a) BIC values and estimated population median values for (b) $R_0$, (c) $\delta$ and (d) $pT_0$ derived from fitting a population level mixed effects model to observed data using a standard viral dynamic model with an eclipse phase (Model B). Population model fitting is performed with fixed $T(0) = 10^5$ cells/mL and fixed $c = 10$ d$^{-1}$. Population distributions of $R_0$, $\delta$ and $p$ are assumed to be lognormal, with standard deviation of the mixed effects error term, $\omega$, fixed at 0.4. Colors indicate BIC or parameter values, with dark red indicating lower BIC and dark blue indicating larger estimated parameter values. The model fit with $V(0) = 10^4$ copies/mL and $k = 6$ d$^{-1}$ is presented in Figure 1 in the main text.
Figure S3. For the individual VL fits (black, and shown in Fig. 1) derived from population level mixed effects model fitting, we show the predicted uninfected target (T, blue), infected but not productive (I₁, yellow) and productively infected (I₂, red) cell concentration dynamics.
Figure S4. Individual viral load dynamics (black lines), derived from fitting parameters of the standard viral dynamic model with an eclipse phase (Model B) to data from each individual monkey to minimize the sum of squared errors. Black circles: observed plasma viral loads. Where virus is not detectable in a sample is it plotted at a value equal to the limit of detection, 200 RNA copies/mL. Model fitting was performed with using the values below the limit of detection as censored data with fixed parameters $T(0) = 10^5$ cells/mL, $c = 10$ d$^{-1}$, $V(0) = 10^4$ copies/mL and $k = 6$ d$^{-1}$.
**Figure S5.** The correlation between the log$_{10}$ viral load area-under-the-curve (AUC) between 0 and 14 dpi in each of the additional compartments observed in the study and the modeled plasma viral load. (a) saliva, (b) urine, (c) cerebral spinal fluid (CSF), (d) semen and (e) vaginal secretions (CVS). The correlation coefficient, $r$, and the $p$-value, after Bonferroni correction for multiple testing, from a Spearman correlation are shown. Colors indicate individual monkeys.
Figure S6. The correlation between the peak observed VL in (a) saliva, (b) urine, (c) CSF, (d) semen or (e) CVS and the modeled peak VL in plasma. The correlation coefficient, $r$, and the p-value, after Bonferroni correction for multiple testing from a Spearman correlation are shown. Colors indicate individual monkeys.
Figure S7. (a) The time course in each monkey (indicated by color) of the NK CD16⁺ CD69⁺ cell subset concentration in plasma and (b) the correlation of this marker at day 0 with the peak VL.

(c) The time course in each monkey of the NK CD16⁻ CD56⁺ cell subset concentration and (d) the correlation between the concentration of this marker at day 2 and the log₁₀ plasma VL AUC.

(e) The time course in each monkey of the plasma concentration of the cytokine MCP-1 and (f) the correlation between MCP-1 at day 1 and the estimated productively infected cell half-life.

The correlation coefficient, $r$, and the $p$-value, after Bonferroni correction for multiple testing, from a Spearman correlation are shown in panels b, d and f.
Figure S8. The measured IFNα concentration (green squares, right axis) in each monkey, displayed with the plasma viral load (black circles, left axis)
Figure S9. Correlations between (a) the peak IFNα concentration and the peak viral load in each monkey and (b) the day 0-7 IFNα AUC and the day 0-7 log_{10} plasma VL concentration in each monkey. Neither correlation is statistically significant.
Figure S10. The predicted effect in female macaques of a 150 mg/kg twice daily regime of favipiravir, initiated at time of infection (red) or 2 days after infection (blue) on (a) median VL and (b) distribution of log_{10} VL AUCs. In (a), the horizontal black dashed line represents the limit of detection of the experimental assay, 200 RNA copies/mL. In (b), medians are 14.5 (no drug), 12.3 (2 dpi) and 9.8 (0 dpi). Data shown is from 5,000 repeated simulations, each including independent simulations of the PK profile and VL dynamics determined by parameters $R_0$, $\delta$ and $p$ selected at random from the population distribution predicted by mixed effect model fitting.