Estimating Biologically Relevant Parameters under Uncertainty for Within-Host West Nile Virus Infection

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Supplementary Information

Parameter Sampling

We set up a framework to sample parameter values within the biologically plausible parameter space to determine which combinations of parameters could feasibly have generated the observed viremia curves.

Our goal is to determine the plausible range of biological parameters rather than the single set of parameters that give the best fit. Hence we sample the parameter space uniformly at random from the intervals listed in Table 2. Each set of sampled parameters was given as input to a subroutine *refineparameters* implemented in Matlab [1], which we developed to identify sets of model parameters that produce viremia curves consistent with observed data. *refineparameters* repeatedly calls the Matlab local optimization routine *lsqcurvefit* on each parameter set to compare curves generated by those parameters to observed viremia curves. The final output of this computational procedure is an ensemble of model parameters. The computational approach is outlined in Fig. 1. The steps of the method are:

Step 1: Identify values of V_0, β, p and δ that are consistent with the target cell limited model (Eqn. 1-4) and viremia curves from immunocompromised mice. We iterated through specific values of T_0, k and γ within the biological bounds identified above (see Constraints on Model Parameters) in order to determine how those parameters affect the parameters we are attempting to predict. We chose 7 values of T_0 between 2.2×10^4/mL and 2.4×10^7/mL, 3 values of the duration of the eclipse phase ($1/k$) from 6 to 10 hours, and 3 values of the viral clearance rate (γ) between 10/day and

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44.4/day (Table 1).

For each of these 63 combinations, we sampled the parameters (V_0, β, p, δ) uniformly at random within their plausible ranges (Table 2). We did so by dividing the range of these 4 parameters into 10 intervals on the logarithmic scale, and randomly sampled 10 values within those intervals. Each parameter value was then combined with all combinations of the remaining parameters to generate 10^4 samples for the parameters (V_0, β, p, δ) for each value of T_0, k and γ, giving a total of $7 \times 3 \times 3 \times 10^4 = 630,000$ samples. *refineparameters* identified parameter sets (V_0, β, p, δ) that produce viremia curves consistent with observed data. We retained all solutions that yielded a sum of squared residual less than a threshold ($4[log_{10}\text{PFU/mL}]^2$) that was chosen to give visually good fits. This yielded 59,520 sets of values of (V_0, β, p, δ) that lead to viral kinetics consistent with observed viremia data in IgM knockout mice. These values were input to Step 2.

Step 2: Re-estimate V_0 for the immune response model. For each set of model parameter estimates (V_0, β, p, δ) from the target cell limited model in Step 1, the parameter V_0 was estimated again to accommodate the difference in day 2 viremia for wildtype and knockout viremia curves. The subroutine *refineparameters* was given the list of 59,520 solutions (V_0, β, p, δ) from Step 1. For each combination of (β, p, δ), *refineparameters* estimated the value of ($\overline{V_0}$) that best fit the first 4 days of the viremia data for wild type mice. The output from this step is a new set of 59,520 parameter solutions ($\overline{V_0}$, β, p, δ) in which only $\overline{V_0}$ is re-estimated. These solutions are the input to Step 3.

Step 3: Estimate immune response parameters ρ and t_i. Given the estimates from Step 2, the immune response parameters (ρ, t_i) were estimated by a new call to *refineparameters*.

A search grid was created by sampling 25 values of ρ between 1 and 60 $\text{PRNT}_{50}^{-1}\text{day}^{-1}$ and 9 values of t_i between 2 and 4 days. From these $25 \times 9 \times 59,520 = 13,392,000$ solutions, we retained all solutions that yielded a sum of squared residual less than a threshold ($0.1[log_{10}\text{PFU/mL}]^2$) that was chosen to give visually good fits. This threshold is lower than the one for the target cell limited model in Step 1 since there are fewer data points available for the wildtype study. The output of this step is a set of combinations of (V_0, β, p, δ, ρ, t_i) that fit the wild type viremia curve within the threshold. We considered this set of solutions as the feasible parameter values that could have generated the viremia curves for the knockout and wildtype mice (Tables 1 and 2).

Analysis of data on in-vitro infection of dendritic cells

We analyzed data from experiments where immature dendritic cells were infected in-vitro with WNV NY99 (passage 7) [2]. The studies were conducted with an MOI of 5. The supernatant was removed at fixed time intervals and virus titers were determined by plaque assay on Vero E6 cells. We fit a target-cell limited model (Eqs. 1 - 4) to this data (Fig. 1). We reduce the viral concentration by a factor every time the media is changed (the rate of virus clearance, γ, was set to 0). We tried different factors and found a decrease of 10^5 in virus concentration gave the best fit. We varied the duration of eclipse phase ($1/k$) from 3 hours to 12 hours and estimated V_0, β, p, δ. We estimated the productively infected cell lifespan ($1/\delta$) for immature dendritic cells in-vitro to be between 4 and 8 hours.

Analysis of data on in-vitro infection of keratinocytes

We analyzed data from primary human keratinocytes infected in-vitro with an infectious cDNA clone of WNV [3]. At various time points post-infection, the medium was harvested and virus was quantified by plaque assay in Vero cells. The experiment we analyze was conducted with a multiplicity of infection (MOI) of 10. We fit a target cell limited model (Eqs. 1 - 4) to this data (Fig. 2). The rate of virus clearance, γ, was set to 0 since the medium was not changed for the duration of the experiment. We then estimated V_0, β, p, δ while varying the duration of eclipse phase ($1/k$) from 3 hours to 12 hours. We estimated the productively infected cell lifespan ($1/\delta$) for keratinocytes in-vitro to be between 4 to 6 hours.

Summary of analysis of data from in-vitro infection experiments and in-vivo replicon experiments

In order to get independent estimates of productively infected cell lifespans ($1/\delta$) and compare them to our estimates in mice, we analyzed data from different sources. Analysis of in-vitro infection data suggests that productively infected lifespan of dendritic cells in-vitro is between approximately 4 and 8 hours (Fig. 1). Analysis of in-vitro infection data in keratinocytes produce estimates of productively infected cell lifespans between 4 to 6 hours (Fig. 2). This compares favorably and is consistent with our estimated range for productively infected cell lifespans of 1 to 14 hours in mice (Table 2).

References

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3. Lim, P.-Y., Behr, M. J., Chadwick, C. M., Shi, P.-Y. & Bernard, K. A., 2011 Keratinocytes are cell targets of West Nile virus in vivo. *J Virol* **85**, 5197–201. ISSN 1098-5514. (doi:10.1128/JVI.02692-10).

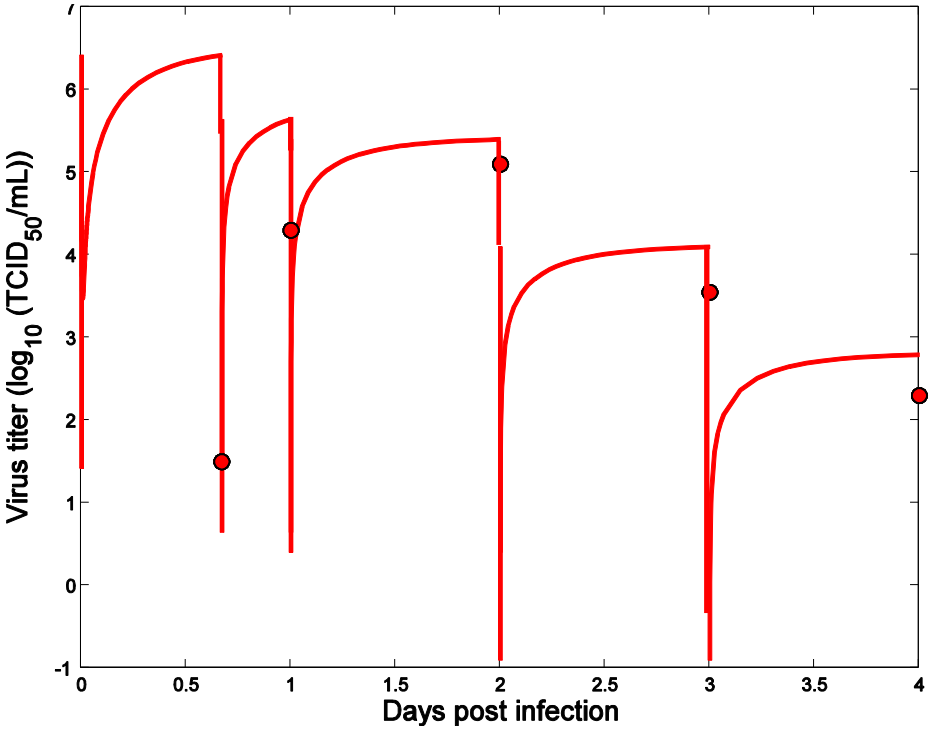

Figure 1. Fig. S1. Target cell limited model (Eqs. 1-4) fit to data on in-vitro infection of immature dendritic cells (data from [2]).

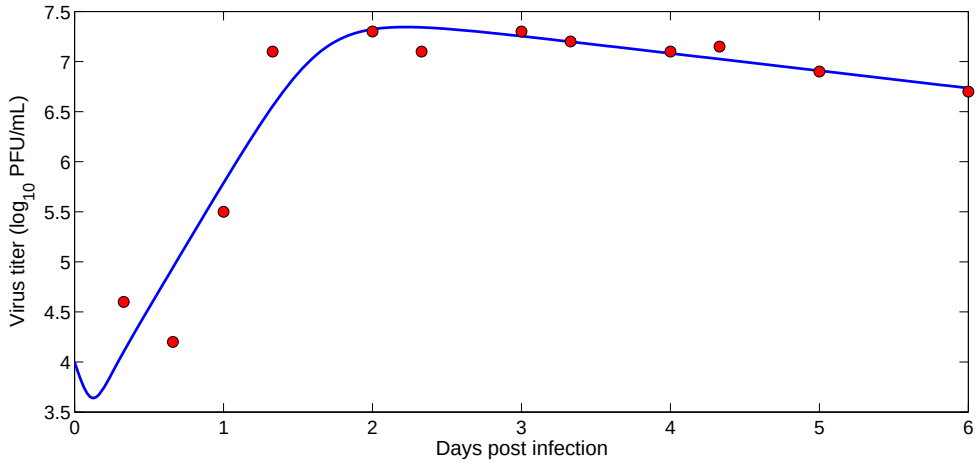

Figure 2. Fig. S2. Target cell limited model (Eqs. 1-4) fit to data on in-vitro infection of keratinocytes (data from [3]).