Supporting Information

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SI Text

Empirical Determination of Model Parameters. The parameters of the model can be grouped into two categories: those parameters related to the virus and those parameters depending on the therapeutic treatment. Most of them can be directly estimated by means of simple experiments that are described here. As a case example, we use data obtained with foot-and-mouth disease virus under the experimental conditions relevant for the cases analyzed in the accompanying paper.

Viral parameters. Several parameters of the model describe the intrinsic characteristics of the virus in the cells that it is infecting. These are the number of replication cycles inside the cell (G), the number of copies per viral genome and replication cycle (m), the rate of production of nonviable mutants (w_0), and the ratio (k) between resistance conferring mutations and deleterious ones.

The experimental measure of the viral productivity $P_0 = m^G(1 - w_0)^G$ in the absence of drugs is easy to perform. If there is no limitation on resources, the population grows exponentially at a rate equal to the viral productivity. As a result, if the population size along successive passages is plotted in a semilogarithmic graph, we obtain a straight line with a slope that is equal to the logarithm of the productivity. Viral productivity is, thus, obtained by quantifying population size in several successive passages. It is measured in units of pfu per milliliter and per passage.

When modeling the dynamics of ssRNA viruses, it should be noticed that each replication cycle requires the synthesis of a complementary antisense RNA that is used as a template for the synthesis of new positive-sense genomes. For this reason, the number of copies per replication cycle is equal to the mean number of genomes produced from a single antisense RNA. Thus, parameter m could be estimated as the ratio between sense and antisense genomes inside the cell. The few available estimations of that ratio indicate a large excess of positive strands from 50 to 1,000 per negative strand (1).

Our parameter w_0 comprises those genomes that carry lethal mutations and all defective genomes that are unable to replicate by themselves, because the former genomes do not play any additional role in the dynamics and the latter genomes are cleared up from the population at each passage when multiplicity of infection is sufficiently low, which is in the case studied. The explicit consideration of the defective type does not modify the quantitative predictions reached. This natural mutation rate can be estimated from a number of experiments that have determined the effect of mutations on viral fitness and the ratio of defective forms to WT genomes. Lethal mutations affect from 20% to 55% of total genomes produced under replication (2, 3), and therefore, a reasonable, although still quite rough, estimate for w_0 would be between 0.4 and 0.9, assuming one-half of the mutations are lethal and one-half of the mutations cause defects that prevent completion of a viral infectious cycle.

Regarding the mutation ratio k, biological knowledge about the virus is also necessary to estimate its value. In any case, because it can be hypothesized that only one (or a few) mutations at specific sites of the genome generate resistance to the inhibitor, the ratio k should be of the order of the inverse of the genome size.

Parameters related to experimental conditions. The treatment is described by two experimental parameters: the inhibition factor i and the increased mutation rate $w \geq w_0$.

In a particular realization of the therapy, both parameters can be experimentally obtained as the decrease in viral productivity in the presence of the inhibitor or mutagen separately. In the presence of a mutagen, viral productivity is $P_w = m^G(1 - w)^G$. This value can be experimentally measured in the same way as before by plotting the evolution of the population size in a semilogarithmic graph and taking the slope of the resulting line. If the productivity with mutagen is lower than one, that slope will be negative, which means that the population is decreasing (Fig. S1). After the productivity has been determined, the parameter w can be calculated by using the previously estimated values for m and G. The case for the inhibitor is analogous. Now, the expression of the productivity becomes $P_i = (im(1 - w_0))^G$, and parameter i can be determined after the productivity has been experimentally measured. In this case, some care must be taken when calculating productivity, because the slope changes as resistant mutants appear after a few passages.

Predicted parameter values for foot-and-mouth disease virus. Comparison of the titers experimentally obtained with those titers predicted by the model allows us to fix the values of all model parameters. We now use the mathematical expressions obtained for the combination (Y_T^C) or sequential (Y_T^S) treatment. First, we select a pair of values m and w_0 within the estimated interval and by using the basal productivity P_0, obtain the corresponding value of G, which was explained above. Second, use of P_w and P_i immediately yields the corresponding values of parameters w and i. Third, we represent the experimentally obtained titer as a function of the calculated i. Because all parameters are now fixed, the titer predicted by the model with varying i is obtained by direct substitution into the expressions for Y_T^C and Y_T^S. Finally, we evaluate the error produced by this set of parameters by calculating the sum of the squared distance between data and titer estimated through the model. The steps above and the evaluation of the error are repeated for all compatible pairs of m and w_0. The combination yielding the smaller error is accepted as optimal given the experimental data.

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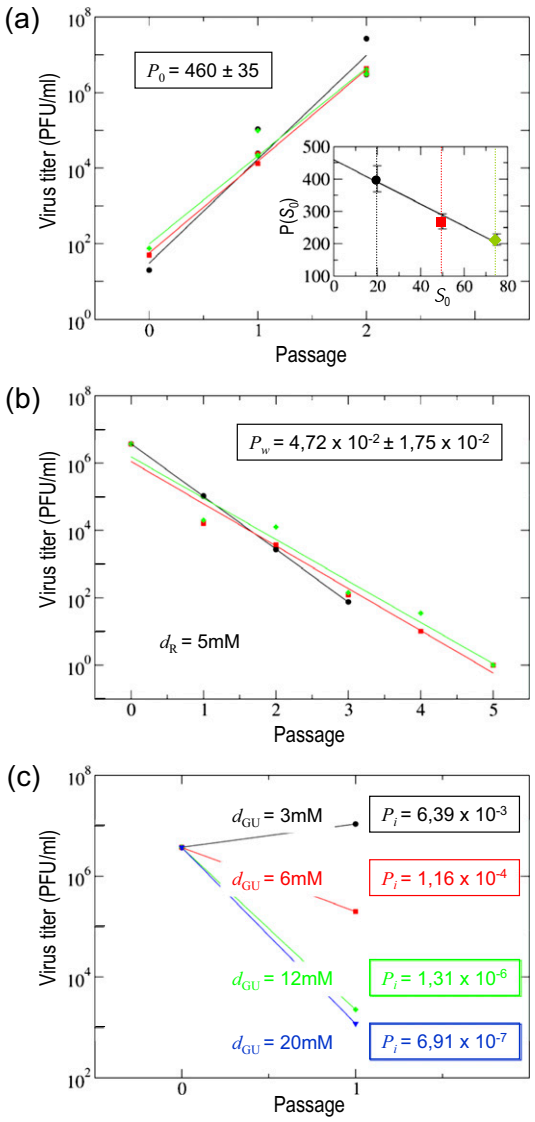

Fig. S1. A case example of determination of model parameters from experimental data. (*A*) Natural growth of the population. Three different sets of experiments using triplicates for each condition have been used. The titer of the virus (main plot) has been determined starting with different initial conditions [that is, initial population sizes S_0 = 20 (black circles), S_0 = 50 (red squares), and S_0 = 75 (green diamonds) at two consecutive passages]. Least squares regression with exponential functions yields the productivity $P(S_0)$, which depends on S_0. Representation of the obtained productivities as a function of S_0 (*Inset*) allows for the extrapolation to $S_0 \to 1$, which finally yields the basal productivity $P_0 = 460 \pm 35$. (*B*) Decrease of the population in presence of the mutagen without the inhibitor. We show the exponential decay in the viral titer for three independent realizations of the experiment with a dose of mutagen $d_R = 5$ mM. The slopes of the curves have been averaged to obtain an estimation of the productivity P_w in the presence of the mutagen, yielding $P_w = m^G(1 - w)^G = 4.72 \times 10^{-2} \pm 1.75 \times 10^{-2}$. (*C*) Population growth in the presence of the inhibitor and absence of the mutagen. Productivity in the presence of the inhibitor has been determined using a single passage to avoid confounding effects caused by the appearance of resistant forms. The slope of each curve corresponds to $P_i = im^G(1 - w_0)^G = i^G P_0$. All productivities are measured in units of pfu per milliliter and per passage.