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Quantifying viral dynamics of highly and less pathogenic simian/human immunodeficiency viruses from in vitro experimental data

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Abstract—When virologists have interests in the kinetics of virus replication, they perform certain experiments using cell cultures. To reveal the features of virus replication, certain parameters (e.g., the levels of viral components and activities) at certain time points are usually measured. The obtained data, which we call it “snap-shot” data, might reflect one of the aspects of virus replication. However, virus infection is the complex phenomenon that consists of the consecutive interactions with viruses, their target cells, and infected cells. Therefore, it would be difficult to elucidate the overall features of dynamic and complex phenomenon based on “snap-shot” data. In order to completely decompose and quantify the dynamics of virus infection, combining mathematical modeling, mathematical analysis, and numerical simulation with the experimental data is a powerful way. Here we introduce a method to “quantitatively” investigate the dynamics of virus infection in in vitro cell culture and discuss the potential of the combinatorial analyses with experimental and computational virology for understanding highly and less viral pathogenesis.

1. Introduction

Historically, the study of the highly and less pathogenic simian/human immunodeficiency virus (SHIV) has provided important information for the understanding of human immunodeficiency virus type-1 (HIV-1) pathogenesis. For example, it was clarified in an SHIV animal study that co-receptor usage determined by the HIV-1 env gene affects the virus’ cell tropism (preference for specific target cell populations), and thus its pathogenesis, in vivo [1, 2, 3]. Furthermore, infections with highly pathogenic SHIV strains in animal models have exhibited stable clinical manifestations in most infected animals, similar to an aspect of infection course in human HIV infections [4, 5]. One of the highly pathogenic SHIV strains, SHIV-KS661, which has the env gene of HIV-1 89.6 and predominantly uses CXCR4 as the secondary receptor for its infection [2], causes an infection that systemically depletes the CD4+ T cells of rhesus macaques within 4 weeks after infection [6, 7]. In observations by our group in recent years, the intravenous infection of rhesus macaques with SHIV-KS661 has consistently resulted in high viremia and CD4+ T cell depletion, followed by malignant morbidity as a result of severe chronic diarrhea and wasting after 6 to 18 months [8]. On the other hand, less pathogenic strain, SHIV-#64 (which also predominantly uses CXCR4 as the secondary receptor for its infection), does not cause the severe symptom in vivo. That is, SHIV-#64 infected macaques do not show the systemically depletes the CD4+ T cells after infection because the viral replication is suppressed by host immune response [6]. Despite this well-developed in vivo model, the detailed kinetics of SHIV-KS661 remains unclear. Quantifying and understanding viral kinetics will provide us with novel insights about the pathogenesis of SHIV (and HIV-1), for example, by enabling the quantitative comparison of the replicative capacity of those different strains.

Here, we combined a relatively simple mathematical model of SHIV infection in HSC-F cells with an in vitro experimental system which allows for the measurement of both total and infectious viral load and the concentration of target and infected cells [9]. We infected HSC-F – a CD4+ T cell line established from cynomolgus monkey – in vitro with SHIV-KS661/SHIV-#64 at two different multiplicities of infection (MOI) and measured the concentration of Nef-negative (susceptible/target) and Nef-positive (infected/virus producing) HSC-F cells [cells/ml], and the total [RNA copies/ml] and infectious [TCID50/ml] viral load daily over 10 days. With this abundant and diverse data, we were able to fully parameterize the dynamic model and determine robust estimates for viral kinetics parameters, thus quantifying the infection cycle. Our in vitro quantification system for SHIV-KS661/SHIV-#64 should be a valuable complement to the well-developed in vivo model and can be used to significantly improve the understanding of SHIV and HIV-1 pathogenesis.

2. Material and Methods

2.1. In vitro experiment

Each experiment was performed using 2 wells of a 24-well plate with a total suspension volume of 2 ml (1
ml per well) and an initial cell concentration of $6.46 \times 10^6$ cells/ml in each well. Because the initial cell concentration is close to the carrying capacity of 24-well plates and the doubling time of HSC-F cells is not short, the population of target cells, in the absence of SHIV-KS661/SHIV-#64 infection, changes very little on the timescale of our experiment (data not shown). We can therefore neglect the effects of potential regeneration of HSC-F cells when constructing the mathematical model.

Cultures of HSC-F cells were inoculated at different MOIs ($2.0 \times 10^3, 2.0 \times 10^5$; MOI = TCID$_{50}$/cell) of SHIV-KS661/SHIV-#64 and incubated for 4 h at 37°C. After inoculation, cells were washed to remove the infectious medium and placed in fresh medium. Subsequently, the culture supernatant was harvested daily for 10 d, along with a small fraction of the cells (5.5%) for counting of viable and infected cells. The remaining cells placed in a fresh, virus-free, medium. Separate experiments (not shown) determined that free virus was not completely removed, but that virus concentration in the supernatant dropped to 14.6% of its value prior to this sampling and washing procedure. Harvested culture supernatants were frozen and stored at -80°C until they were assayed via RT-PCR and TCID$_{50}$ titration.

2.2. Mathematical model

To describe the in vitro kinetics of the SHIV-KS661/SHIV#64 viral infection in our experimental system, we expanded a basic mathematical model widely used for analyzing viral kinetics [9, 10, 11]. The following equations are our extended model:

$$\frac{dx}{dt} = -\beta x v_J - dx \quad (1)$$

$$\frac{dy}{dt} = \beta x v_J - ay \quad (2)$$

$$\frac{dv_J}{dt} = pky - r_J v_J - r_{RNA} v_J \quad (3)$$

$$\frac{dv_{NI}}{dt} = (1-p)ky + r_J v_J - r_{RNA} v_{NI} \quad (4)$$

where $x$ and $y$ are the number of target (susceptible) and infected (virus-producing) cells per ml of medium, $v_J$ and $v_{NI}$ are the number of RNA copies of infectious and non-infectious virus per ml of medium, respectively. Parameters $d$, $a$, $r_{RNA}$, and $\beta$ represent the death rate of target cells, the death rate of infected cells, the degradation rate of viral RNA, and the rate constant for infection of target cells by virus, respectively. We assume that each infected cell releases $k$ virus particles per day (i.e., $k$ is the viral production rate of an infected cell), of which a fraction $p$ are infectious and $1-p$ are non-infectious. Infectious virions lose infectivity at rate $r_J$, becoming non-infectious. Implicit in Eqs.(1)-(4) is the assumption that once a cell is infected by infectious virus it immediately begins producing progeny virus. We also tested a variant of the model which incorporates an “eclipse” phase of infection to represent the cell’s period of latency prior to virus production. We found, however, that including this phase did not significantly improve the fit of the model to the data and led to very similar extracted parameter values (data not shown). Therefore, in all further analyses, this phase was omitted in favor of the simpler model formulation.

2.3. Data fitting

To fit the observed viral load data – consisting of RNA copies/ml and TCID$_{50}$/ml – and to account for the partial removal of cells and virus due to sampling, we transformed Eqs.(1)-(4) into the following scaled model:

$$\frac{dx}{dt} = -\beta_{50} x v_{50} - dx - \delta x \quad (5)$$

$$\frac{dy}{dt} = \beta_{50} x v_{50} - ay - \delta y \quad (6)$$

$$\frac{dv_{RNA}}{dt} = ky - r_{RNA} v_{RNA} - r_J v_{RNA} \quad (7)$$

$$\frac{dv_{50}}{dt} = k_{50} v_J - r_{50} v_{50} - r_J v_{50} - r_{NI} v_{50} \quad (8)$$

where $v_{RNA} = v_J + v_{NI}$ is the total concentration of viral RNA copies, $v_{50} = a v_J$ is the infectious viral load expressed in TCID$_{50}$/ml, and $\alpha$ is the conversion factor from infectious viral RNA copies to TCID$_{50}$. Since the measure of 1 TCID$_{50}$ corresponds to an average of 0.68 infection events (by Poisson statistics), we have $0<\alpha \approx 1.47$ TCID$_{50}$ per RNA copies of infectious virus. Parameters $\beta_{50} = \beta/\alpha$ and $k_{50} = apk$ are the converted infection rate constant and production rate of infectious virus, respectively. At each sampling time, the concentration of Nef-negative and Nef-positive HSC-F cells must be reduced in our model by 5.5% and the viral loads (RNA copies and TCID$_{50}$) by 85.4% to account for the experimental harvesting of cells and virus. These losses were modeled in Eqs.(5)-(8) by approximating the sampling of cells and virus as a continuous exponential decay, yielding a rate of $\delta = 0.057$ per day for cell harvest and $r_J = 1.93$ per day for virus harvest. We found that a model which implements the sampling explicitly, as a punctual reduction at each sampling time, similar to the model in [12], did not significantly improve the quality of the fit (data not shown).

Of the seven free model parameters remaining, three of them ($d$, $r_J$, $r_{RNA}$) were determined by direct measurements in separate experiments (see below). The remaining four parameters ($\beta_{50}$, $a$, $k_{50}$, $k_{50}$) along with 8 initial ($t=0$) values for the variables (two at each of the four MOI values) were determined by fitting the model to the data as follows: We simultaneously fit Eqs.(5)-(8) to the concentration of Nef-negative and Nef-positive HSC-F cells and the infectious and total viral loads at two different MOIs using nonlinear least-squares regression
obtained 68/65 data points for quantifying SHIV-KS661/SHIV-#64 viral kinetics in vitro, respectively. Having fixed the values of the rates of virion decay ($r_r$ and $r_{RNA}$) and the target cell death rate ($d$) using separate experiments, we estimated the values of the four remaining unknown parameters ($\beta_{50}$, $a$, $k$, $k_{50}$) by fitting the model in Eqs.(5)-(8) to the full in vitro dataset simultaneously.

From the directly fitted parameters, we calculated a number of important derived quantities as follows: The half-life of SHIV-KS661/SHIV-#64 infected cells (i.e., $log2/a$) is 11.1/11.9 h, respectively. The viral burst sizes of SHIV-KS661/SHIV-#64, which is the total number of virus produced by an infected cell during its lifetime (i.e., $k/a$ for total viral burst size and $k_{50}/a$ for infectious viral bust size), are $3.00 \times 10^7/2.46 \times 10^6$ RNA copies and 0.256/0.0262 TCID_{50}, respectively. The basic reproductive numbers of SHIV-KS661/SHIV-#64, which has the form $R_0 = \beta_{50}/(a+\delta)(r_{r}+r_{RNA}+r_{d})$ and is interpreted as the number of newly infected cells generated by a single infectious cell at the start of the infection, are 5.06/5.36, respectively.

Interestingly, we found the SHIV-KS661/SHIV-#64 show similar cytopathic (i.e., the half-life of infected cells) and total viral burst size in spite of those pathogenesis in vivo are completely different. The above results let us guess that viral cytopathogenesis and total viral replicability do not contribute in vivo viral pathogenesis. However, on the other hand, our analysis revealed that the infectious viral burst size of each strain is different about 10 times but their basic reproductive numbers are still similar between SHIV-KS661 and SHIV-#64. This strongly suggests that the difference of SHIV-KS661 and SHIV-#64 is ability to produce infectious virus and the ability leads to the different viral pathogenesis in vivo, which is determined during early viral infection. By quantifying very early phase of in vivo SHIV-KS661/SHIV-#64 infection, we might be able to conclude how different viral pathogenesis in rhesus macaques is led. But these analyses combined in vivo and in vitro experiments are our next research (actually doing now).

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References


