IEICE Proceeding Series

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

Shingo Iwami

Vol. 1 pp. 37-40
Publication Date: 2014/03/17
Online ISSN: 2188-5079

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

Shingo Iwami† ‡

†Department of Biology, Kyushu University
6-10-1 Hakozaki, Higashi-ku, Fukuoka, 812-8581 Japan
‡ Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency
4-1-8 Honcho, Kawaguchi-shi, Saitama, 332-0012 Japan
Email: siwami@kyushu-u.org

**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 is consisted 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 combinational 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 [$TCID_{50}$/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 $6.46\times10^6$ 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 ($2.0\times10^{-4}$, $2.0\times10^{-5}$; MOI = TCID50/cell) of SHIV-KS661/SHIV-#64 and incubated for 4 h at 37˚C. After inoculation, cells were washed to remove the infection medium and placed in fresh media. 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:

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

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

$$\frac{dv_I}{dt} = pky - r_I v_I - r_{RNA} v_I \quad (3)$$

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

where $x$ and $y$ are the number of target (susceptible) and infected (virus-producing) cells per ml of medium, $v_I$ 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 $r_I$, 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:

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

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

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

$$\frac{dv_{50}}{dt} = k_{50} y - r_I v_{50} - r_{RNA} v_{50} - r_c v_{50} \quad (8)$$

where $v_{RNA}=v_I+v_{NI}$ is the total concentration of viral RNA copies, $v_{50}=\alpha v_I$ 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 $0<\alpha\leqq1.47$ $TCID_{50}$ per RNA copies of infectious virus. Parameters $\beta_{50}=\beta/\alpha$ and $k_{50}=\alpha pk$ 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 $r_c=1.93$ 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 ($d$, $r_I$, $r_{RNA}$) were determined by direct measurements in separate experiments (see below). The remaining four parameters ($\beta_{50}$, $a$, $k$, $k_{50}$) 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

(FindMinimum package of *Mathematica8.0*) which minimizes the following objective function:

$$SSR = \sum_{j=1}^{2}\left[\sum_{i=1}^{10}\left\{\log x_j(t_i) - \log x_j^e(t_i)\right\}^2 + \sum_{i=1}^{10}\left\{\log y_j(t_i) - \log y_j^e(t_i)\right\}^2 + \sum_{i=1}^{10}\left\{\log v_{RNAj}(t_i) - \log v_{RNAj}^e(t_i)\right\}^2 + \sum_{i=1}^{10}\left\{\log v_{50j}(t_i) - \log v_{50j}^e(t_i)\right\}^2\right]$$

where $x_j(t_i)$, $y_j(t_i)$, $v_{RNAj}(t_i)$, and $v_{50j}(t_i)$ are the model-predicted values for Nef-negative cells, Nef-positive cells, total RNA viral load and infectious ($TCID_{50}$) viral load, given by the solution of Eqs.(5)-(8) at measurement time $t_i$ ($t_i$=0,1,2,⋯,9 d). Index $j$ is a label for the MOI of the two experiments (i.e., for MOI: $2.0\times10^{-4}$ and $2.0\times10^{-5}$). The variables with superscript "*e*" are the corresponding experimental measurements of those quantities. Experimental measurements below the detection limit were excluded when computing the *SSR*. Alternative fits with various weights on the infectious viral load to account for larger errors in the $TCID_{50}$ value, were also performed, but these did not significantly alter the extracted parameter values (data not shown).

**3. Results and Discussion**

The rates at which SHIV-KS661/SHIV-#64 virions lose infectivity, $r_I$, and the rate at which their viral RNA degrades, $r_{RNA}$, were each estimated directly in separate experiments. Linear regressions were performed to fit $logv_{RNA}(t)=logv_{RNA}(0)-r_{RNA}t$ and $logv_{50}(t)=logv_{50}(0)-r_It$ to those data, yielding values of $r_{RNA}=0.09/0.16$ per day and $r_I=0.87/0.99$ per day, respectively. These correspond to an RNA viability half-life of *7.7/4.3* d and an infectious virion half-life of *19.1/16.8* h, respectively. The death rate of target cells, $d$, was also estimated directly, in a mock infection experiment where Nef-negative (target) HSC-F cells were exposed to the culture conditions of the experiment without virus (data not shown). A linear regression was performed to fit $logx(t)=logx(0)-(d+\delta)t$ to the time course data, yielding $d \fallingdotseq 0$ per day.

Time-course *in vitro* experimental data were collected over 10 days, consisting of the concentrations of Nef-negative and Nef-positive HSC-F cells [cells/ml], the total SHIV-KS661/SHIV-#64 viral load [RNA copies/ml], and the infectious viral load [$TCID_{50}$/ml]. At each daily measurement, almost all of the culture supernatant (85.4%) was removed for viral counting; a small percentage of cells (5.5%) were removed for counting and FACS analysis, and the remaining cells placed in a fresh medium. The experiment was repeated for two different values of the initial viral inoculum (MOI). In total, we 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 ($r_I$ 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 burst size), are $3.00\times10^4/2.46\times10^4$ RNA copies and *0.256/0.0262* $TCID_{50}$, respectively. The basic reproductive numbers of SHIV-KS661/SHIV-#64, which has the form $R_0 = \beta_{50}k_{50}x_0/((a+\delta)(r_I+r_{RNA}+r_C))$ 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 bust 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).

**Acknowledgments**

This work was supported, in part, by JST PRESTO program.

**References**

[1] Harouse JM, Gettie A, Tan RC, Blanchard J, and Cheng-Mayer C: Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. Science 1999, 284:816-819.

[2] Matsuda K, Inaba K, Fukazawa Y, Matsuyama M, Ibuki K, Horiike M, Saito N, Hayami M, Igarashi T, and Miura T: *In vivo* analysis of a new R5 tropic SHIV generated from the highly pathogenic SHIV-KS661, a derivative of SHIV-89.6. Virology 2010, 399:134-143.

[3] Nishimura Y, Igarashi T, Donau OK, Buckler-White A, Buckler C, Lafont BA, Goeken RM, Goldstein S, Hirsch VM, and Martin MA: Highly pathogenic SHIVs and SIVs target different $CD4^+$ T cell subsets in rhesus monkeys explaining their divergent clinical courses. Proc. Natl. Acad. Sci. USA 2004, 101:12324-12329.
[4] Reimann KA, Li JT, Veazey R, Halloran M, Park IW, Karlsson GB, Sodroski J, and Letvin NL: A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. J. Virol. 1996, 70:6922-6928.
[5] Shinohara K, Sakai K, Ando S, Ami Y, Yoshino N, Takahashi E, Someya K, Suzaki Y, Nakasone T, Sasaki Y, Kaizu M, Lu Y, and Honda M: A highly pathogenic simian/human immunodeficiency virus with genetic changes in cynomolgus monkey. J. Gen. Virol. 1999, 80:1231-1240.
[6] Kozyrev IL, Ibuki K, Shimada T, Kuwata T, Takemura T, Hayami M, and Miura T: Characterization of less pathogenic infectious molecular clones derived from acute-pathogenic SHIV-89.6p stock virus. Virology 2001, 282:6-13.
[7] Miyake A, Ibuki K, Enose Y, Suzuki H, Horiuchi R, Motohara M, Saito N, Nakasone T, Honda M, Watanabe T, Miura T, and Hayami M: Rapid dissemination of a pathogenic simian/human immunodeficiency virus to systemic organs and active replication in lymphoid tissues following intrarectal infection. J. Gen. Virol. 2006, 13:11-20.
[8] Inaba K, Fukazawa Y, Matsuda K, Himeno A, Matsuyama M, Ibuki K, Miura Y, Koyanagi Y, Nakajima A, Blumberg RS, Takahashi H, Hayami M, Igarashi T, and Miura T: Small intestine $CD4^+$ cell reduction and enteropathy in simian/human immunodeficiency virus KS661-infected rhesus macaques in the presence of low viral load. J. Gen. Virol. 2010, 91:773-781.
[9] Iwami S, Holder BP, Beauchemin CAA, Morita S, Tada T, Sato K, Igarashi T, and Miura T: Quantification system for the viral dynamics of a highly pathogenic simian/human immunodeficiency virus based on *in vitro* experiment and a mathematical model. Retrovirology. 2012, 9: 18.
[10] Nowak MA and May RM: Virus dynamics, Oxford University Press; 2000.
[11] Perelson AS: Modeling viral and immune system dynamics. Nat. Rev. Immunol. 2001, 2: 28-36.
[12] Beauchemin CA, McSharry JJ, Drusano GL, Nguyen JT, Went GT, Ribeiro RM, and Perelson AS: Modeling amantadine treatment of influenza A virus *in vitro*. J. Theor. Biol. 2008, 254:439-451.