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A hybrid stochastic–deterministic computational model accurately describes spatial dynamics and virus diffusion in HIV-1 growth competition assay

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HIGHLIGHTS

- ▸ We simulate growth competition assays used to determine the ex vivo fitness of HIV-1.
- ▸ Our model accounts for viral diffusion via a deterministic diffusion model.
- ▸ Cellular dynamics are simulated via a stochastic Markov chain model.
- ▸ We estimate experimentally a cell's probability of infection based on viral density.
- ▸ The model captures observed variation in the fitness difference between two viruses.

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ABSTRACT

We present a new hybrid stochastic–deterministic, spatially distributed computational model to simulate growth competition assays on a relatively immobile monolayer of peripheral blood mononuclear cells (PBMCs), commonly used for determining ex vivo fitness of human immunodeficiency virus type-1 (HIV-1). The novel features of our approach include incorporation of viral diffusion through a deterministic diffusion model while simulating cellular dynamics via a stochastic Markov chain model. The model accounts for multiple infections of target cells, CD4-downregulation, and the delay between the infection of a cell and the production of new virus particles. The minimum threshold level of infection induced by a virus inoculum is determined via a series of dilution experiments, and is used to determine the probability of infection of a susceptible cell as a function of local virus density. We illustrate how this model can be used for estimating the distribution of cells infected by either a single virus type or two competing viruses. Our model captures experimentally observed variation in the fitness difference between two virus strains, and suggests a way to minimize variation and dual infection in experiments.

1. Introduction

Fitness is defined as the replicative capacity of an organism in its environment, where survival of fittest drives the evolution of a genetically diverse population (Domingo and Holland, 1997). In a host–pathogen relationship, higher replicative fitness of a pathogen is often associated with virulence, however, restriction factors within the host, such as the immune system, can counter and limit pathogen replication and survival (Casadevall and Pirofski, 2001). With human immunodeficiency virus type 1 (HIV-1), the immune system cannot contain or eradicate the infection due in part to the specific infection of CD4+ T cells. Several recent studies now suggest that ex vivo HIV-1 fitness, defined as the ability of the virus to replicate in CD4+ T cells in culture, is predictive of the rate of disease progression in patients infected with HIV-1 (Troyer et al., 2005; Blaak et al., 1998; Quiñones-Mateu et al., 2000; Navis et al., 2007; Kouyos et al., 2011). First, HIV-1 isolates from patients classified as long-term survivors have significantly lower ex vivo fitness than HIV-1 isolates from patients who progressed to disease (Blaak et al., 1998; Quiñones-Mateu et al., 2000; Navis et al., 2007). Second, ex vivo replicative fitness increases during the time-course of HIV-1 infection (Kouyos et al., 2011) and correlates with both increasing viral

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loads and genetic diversity, as well as decreasing CD4+ T cell counts within a patient (Troyer et al., 2005). Finally, the rare condition of elite HIV-1 suppression, where virus levels are undetectable during infection, appears related to infection with a very weak HIV-1 isolate in terms of ex vivo replicative fitness (Lobritz et al., 2011; Miura et al., 2009a, 2009b, 2010).

Growth competition assays are considered the most accurate method to quantify ex vivo replicative fitness of HIV-1 isolates (Collins et al., 2004; Prado et al., 2004). While several different assays have been developed, the basic concepts are similar (Quiñones-Mateu et al., 2000; Ball et al., 2003; Dykes et al., 2006; Dykes and Demeter, 2007). The appropriate cell culture is first infected with two or more isolates/strains of the same virus species. The production of viruses is then monitored over time to maximize replication. The proportions of the virus strains used to inoculate the culture are then compared to the relative production of each virus strain during the experiment to compute their relative fitness (Quiñones-Mateu et al., 2000). As fitness has become a major focus in experimental HIV-1 research, the development of mathematical tools to characterize and estimate fitness parameters in the context of growth competition experiments has become essential. One of the motivations of this work is to set up a computational framework to study how differences in viral traits, such as the infection rate per virion, the rate of virus production per integrated provirus, and the death rate of infected cells, translate into differences in relative fitness, and to better understand the intrinsic uncertainty in estimating fitness.

Ordinary differential equation based models have been developed to estimate the replication dynamics of two competing virus variants (Goudsmit et al., 1997; Bonhoeffer et al., 2002; Marée et al., 2000). Wu et al. (2006) extended the basic framework proposed by previous models to estimate viral kinetic and fitness parameters for the flow cytometry-based growth competition assay developed by Dykes and Demeter (2007). The model was further improved by Miao et al. (2008) by allowing for dual infections of target cells as well as a time-varying target cell population. Differential equation models assume that free virus and susceptible and infected cells are uniformly distributed, and as such, may not be appropriate for systems with more complicated spatial distribution (Funk et al., 2005; Beauchemin et al., 2005; Beauchemin, 2006). The model that we propose is designed to simulate the cellular and viral dynamics of a growth competition assay where the cell culture is composed of a relatively immobile monolayer of susceptible peripheral blood mononuclear cells (PBMCs). Due to inherent spatial structure, the assumption that the system is well-mixed may not be appropriate. Fig. 1 shows a microscope image of a cell culture infected with the NL4-3GFP HIV-1 strain. Some spatial heterogeneity due to the clustering of infected cells (green) is evident.

To account for the inhomogeneous distribution of infected and uninfected cells and the spatial dynamics of free virus particles, we turn to cellular automaton models, which have been previously employed to study both influenza and HIV-1 in vivo (Beauchemin et al., 2005; Strain et al., 2002; Zorzenon dos Santos and Coutinho, 2001). In particular, compared to previously published models of growth competition experiments, the novel features of the present one include considering the diffusion of populations of free virus particles explicitly and accounting for spatial constraints in cell division. Furthermore, we account for retroviral interference in multiple infections of target cells, a phenomenon first investigated mathematically in the stochastic–deterministic models of Dixit and Perelson (2004, 2005). Retroviral interference is a known limitation for super infection of an infected target cell by the resident HIV-1 via down-regulation of the CD4 virus receptor for host cell entry (Piguet et al., 1999). We also incorporate the inherent delay in the virus production process between the infection of a cell and the subsequent production of new virions, explored by Banks and Bortz (2003) via delay differential equation models.

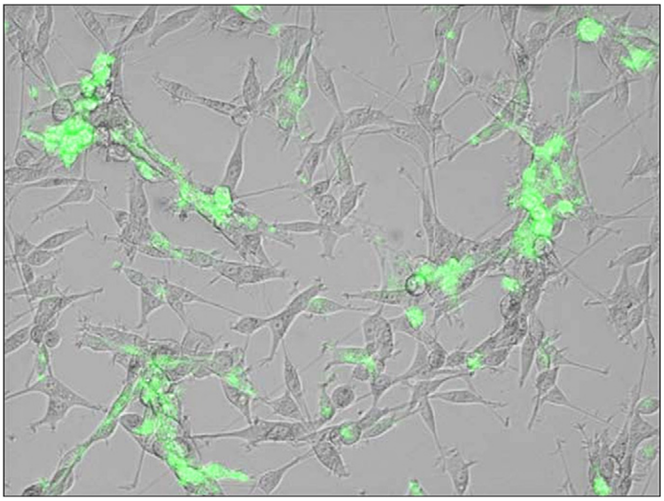

Fig. 1. *Microscope image of cell culture infected with NL4-3GFP.* U87 CD4 CXCR4 cells were plated a density of 150,000 cells per well in 6 well plates in growth media [DMEM, 15% FBS, 100 mg/ml Puromycil, 0.2 mg/ml G418, 100 U/ml Penicillin, 100 μg/ml streptomycin]. 24 h after plating, the cells were infected with NL4-3GFP (Weber et al., 2006) at an MOI of 0.005. The inoculum was washed away 24 h after infection. The image was captured 72 h after infection using a Motic AE31 microscope equipped with a QICAM, using QCapture Pro software at 20 × magnification. Infected (green) cells appear to cluster together. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

We demonstrate the use of this model for estimating the distributions of cells infected by either one or two virus types, and explore the impact of initial virus inoculum and rate of diffusion on simulation results. Although the simulations are performed with dual competitions in mind, an extension to multiple virus competition experiments is straightforward.

2. Experiments

MT4 cells were plated at a density of 10,000 cells in 96 well plates in growth media [RPMI 1640, 10% fetal bovine serum (FBS), 100 U/ml Penicillin, 100 μg/ml streptomycin]. Approximately 18 h after plating NL4-3 virus was added to the cells at a range of MOIs from 4.5×10^{-4} to 0. After 18 h of infection the inoculum was washed and fresh growth media was added to the wells. Quantification of virus growth kinetics was measured by reverse transcriptase activity every 18 h for 10 days as previously described (Ball et al., 2003).

3. Models

The cellular automaton, developed on Matlab R2011b, is supported on a two-dimensional square lattice, which simulates the cell culture. Each pixel can be either empty or occupied by a cell, which in turn can be in one of four possible states: uninfected, infected but not yet infectious, infectious, or dead. An infectious cell actively produces virus particles, which are typically released continually rather than in a large burst upon cell lysis. The system is updated using time increments of 1 h to simplify the comparison of computed and experimental results. The state of each pixel is updated at the end of each time step by means of a Markov transition matrix, described below.