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Modeling of innate immune responses of cells for vaccine production

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ABSTRACT

The virus replication *in vitro*, in particular in bioreactors, for vaccine production (for instance influenza A virus) has been previously studied using a distributed population balance model (Sidorenko et al., 2008). The solution of such models can be obtained with kinetic Monte Carlo simulations. Here we extend the previous model structure in two ways. First we replace the constant infection reaction constant by a function taking into account the *heterogeneity of cells* in a typical bioreactor environment. Second, we take into account the observed response of a cell to a virus attack, an *immune response* of the system. The immune response in a bioreactor can be related to the production of a signal protein like an interferon. Parallel to the cell dynamics also the interferon concentration will be modeled. We will study this as an additional parameter playing an important role for the infection probability. The dynamical evolution of the cell population, the total virus number via replication, and its dependency on the initial conditions will be studied here. It can be shown that the extended model can be used to improve experimental data interpretation in several ways. The previous artificial introduction of a time lag is no longer necessary in the proposed model. Also different scenarios of virus replication with low and high yield can now be interpreted consistently within one model approach.

1. Introduction

The immune response of living tissues *in vivo* is governed by complex mechanisms at several levels, from a systemic to an individual cell level. One of these mechanisms is the innate immune response of cells against infections. Whereas an immune response of the whole organism is adaptive by targeting invasions originating by previously encountered pathogens, the innate immune response of individual cells is non-adaptive and represents an early defense against viral infections (Medzhitov and Janeway, 1997). The innate immune response is not only active *in vivo* but also *in vitro*. A relevant example is a cell culture for the production of vaccines (Seitz et al., 2010). In such cases, the understanding of the immune response of the cells is relevant for the implementation of an optimized vaccine production: the modulation of the number of viral particles may depend on the control over the innate immune response of the cells. Appropriate modeling and simulation of different scenarios of the replication dynamics will help to understand the replication process and therefore improve overall efficiency.

Influenza A virus replication has been studied using Madin-Darby canine kidney (MDCK) cells in bioreactors for some time (Möhler et al., 2005). The MDCK cell line shows a high susceptibility for infection by influenza A viruses and is, therefore, widely used for the isolation of influenza viruses. It also has been shown recently that MDCK cells show a significant innate immune response against viral infections (Seitz et al., 2010). This immune response is regulated by the induction and expression of proteins called interferons (IFN). It has been shown that phosphorylated viral DNA is detected by a cytoplasmic protein (GTPase) which in turn activates a signal cascade inducing the activation of the interferon factor, the nuclear factor κB and an activator protein. These three factors activate a promoter that allows the secretion of IFN. Subsequently, IFN binds to the interferon-α receptor, which stimulates antiviral genes in the cell (Haller et al., 2006; Isaacs and Lindenmann, 1957; Takaoka and Yanai, 2006; Takeuschi and Akira, 2009). It could also be shown that viruses can suppress the action of IFN, a mechanism that should be helpful for virus proliferation. Here, viruses make use of a protein (NS1) that inhibits the induction of IFN, which prevents the sensing of viral RNA in the host. In this particular case, the IFN induction and its suppression by NS1 seems to be dependent on the virus strain and the cell type (Haller et al., 2006).

The overall interferon-mediated defense mechanism of cells against virus is well known (Francoeur et al., 1980; Haseltine

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et al., 2008; Lam et al., 2005; Ludwig and Planz, 2008; Medzhitov and Janeway, 1997; Takaoka and Yanai, 2006). Already some studies on the dynamical aspects of IFN reaction on cells have been carried out. A model for the dynamics of the infection spread in humans, considering an eclipse phase, where virus yield is delayed and where populations of infected cells are in different productive and unproductive phases, has been developed (Baccam et al., 2006). Also, spatially distributed models with cell monolayers have been studied by Howat et al. (2006). An ideally mixed cell population with a variable cell infectivity due to an immune response was studied previously by us (Diaz Ochoa et al., 2009). Nevertheless, a number of open questions about the kinetics and dynamics of the antiviral response and its implication on the virus replication in a heterogeneous cell population in bioreactors remain. The effect of IFN on the cells internal response to the virus infection and the virus replication is of high interest. In order to study it in more detail a distributed population is defined, where the replication mechanism is represented by an internal level of viral expression in the cell. This mechanism can help to shed light on the observed outer dynamics. Also, such a representation can be compared to newer experimental measurement techniques, for example by relating the internal virus protein concentration to a fluorescence intensity by flow cytometry (Schulze-Horsel et al., 2008). From a biotechnological process point of view it makes sense to explore the detailed mechanisms that the interferons trigger in the cell. Our modeling approach will be a step-by-step method by adding new model aspects. We will start with the model of Sidorenko et al. (2008) and add new details including the innate immune response in order to investigate its influence on the overall virus replication dynamics.

The development of our model will preserve the general representation of the system. The risk of infection (ROI) will be individually distributed to each cell, as the cells will differ from each other in a bioreactor environment. Through the IFN concentration the ROI will also depend on the overall infection status of the whole cell population. Our approach will not take into account a spatial distribution of cells or IFN as the bioreactors are typically ideally mixed systems on the time scales of the virus replication dynamics, i.e. 24 up to 80 h. Within a discretized kinetic Monte Carlo approach one can study cell populations with a fixed number of cells as a well selected and descriptive subsystem of the whole bioreactor. In our model we will therefore take into account external (IFN concentration as well as virus concentration) and internal factors (internal virus expression) to study the interplay of these factors on cell infection, replication and virus yield.

The present model is motivated by experimental observations where the virus replication dynamics in MDCK cells depends on a number of process conditions like multiplicity of infection, virus strain or concentration and type of interferons (Müller et al., 2008; Schulze-Horsel et al., 2009; Seitz et al., 2010). Our model may shed some light on possibly important aspects in virus replication processes and could help experimentalist to guide them in their research on questions about experimental strategies as well as process optimizations.

The manuscript is divided into the following parts. In Section 2 we review the basic model and introduce our model extensions. We will introduce the concept of the risk of infection, which will be our fundamental parameter in the modeling of the innate immune response. In Section 3 the simulation of the model is shortly described to be followed by a number of simulation results. In Section 4 we discuss our results by comparing them to previous modeling approaches as well as to experimental observations. Final Section 5 is devoted to conclusions and outlook.

2. Model

2.1. Basic model

We will describe a cell population in an ideally mixed bioreactor as a discretized and predefined subsystem with a number of cells C. The experiments for virus replication and virus yield are performed once the cell division reaches a low rate. Despite this division process takes place, it is slow enough, such that the cell population can be approached to a constant number. The underlying biotechnological process uses adherent cells on microcarriers in a batch reactor system. Typically cell growth in these systems has already reached a final state where the complete surface of the microcarriers is covered with cells. We will model our system like a selected volume of such a bioreactor with a fixed number of microcarriers, where the attached cells are in constant contact with the surrounding solution containing virus particles or IFN. The spatial distribution of the cell and virus population is non-relevant in our model. All cells may *interact* with all other cells via distribution and exchange of virus or IFN in the surrounding solution. Virus and IFN are also assumed to be uniformly distributed in our model system.

The status of each single cell i will be represented as one of the three assumed biological states: uninfected $S_{i,U}$, infected $S_{i,I}$ or apoptotic $S_{i,A}$ (an additional state $S_{i,F}$ described cells that has an active immune response will be introduced in the next subsection). Counting the total number of cells in each class at every time step t will give us the distribution of uninfected, infected and apoptotic cells in our system, respectively: $Z_U(t)$, $Z_I(t)$ and $Z_A(t)$. In the initial state all cells are uninfected. The total number of cells will be constant over time: $C = Z_U(t)+Z_I(t)+Z_A(t)$, whereas the numbers in each class will change according to the system dynamics of infection, apoptosis etc. introduced below.

In Fig. 1 the different states as well as the transitions and their rates are given. In the model, replication and release of viruses in the infected cell is occurring, which is related to changes in the protein levels inside the cell. The internal status of an infected cell is described with the class number J_i, which is related to the number of viral proteins inside a cell (and in turn to the fluorescence signal in the experiments). Every infected cell has at least $J_i = 1$, otherwise it would not be an infected cell. The internal status J_i may increase over time. Previous investigations showed that this number never increased above 100 within the simulated times (Sidorenko et al., 2008). It can therefore be

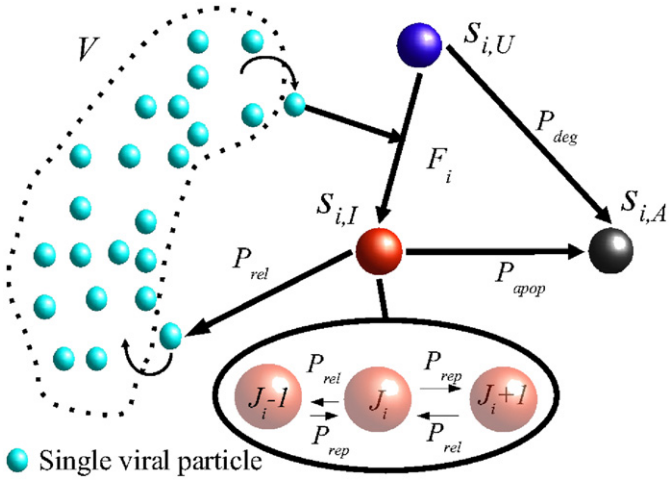

Fig. 1. Basic transitions between cellular states. Here the transition from an uninfected to an infected state depends on the adsorption of viral particles from the virus population V (set of viruses inside the dotted figure).