MODELLING VIRAL INFECTION ON POPULATION AND INTRACELLULAR LEVEL

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ABSTRACT
This work presents a novel, two-scale model of viral infection evolution in a cell population taking into account immunological response based on Interferon-β. Infection dynamics is analyzed on a population level, using standard compartmental approach and on an intracellular level, describing biochemical processes taking place inside cells during infection. Additionally, an optimization problem has been stated, aiming at finding best therapy protocols using Interferon-β.

KEY WORDS
Modelling, viral infection, signalling pathways

1. Introduction
Understanding dynamics of viral infection is key to successful fight against diseases and effective therapy development. Though mathematical modelling cannot provide precise guidelines for therapy design, through qualitative analysis of existing models it can suggest what kind of knowledge is missing (if the model cannot reproduce experimental results) or what is the most promising course of therapy to be tested experimentally.

While mathematical models of viral infections have been developed for many years, usually they were developed to describe only the processes at a population level (e.g., [1-4]) or dealt with intracellular processes leading to virus replication on a single cell level (e.g., [5]). Moreover, usually they rarely took into account both control (i.e. therapy) and responses of immunological system.

Mathematical models describing both intracellular processes and their influence on cell populations enable much deeper analysis of viral infection dynamics, and, ultimately, protocols of therapy. Cascades of biochemical processes taking place inside cells are initiated here by virus infection of a cell and by binding of a particular cytokine, called Interferon-β (IFN-β), to its specific receptor on a cell surface. These processes involve virus replication and production of components needed for that, creation or degradation of protein complexes, activation of enzymes and usually lead to activation or repression of transcription of specific genes. This results in production of new proteins (or their disappearance, if the genes are repressed) which may affect earlier stages of the cascade, thus creating positive or negative feedback loops or activate other signaling pathways. Thus, the dynamics of cell population may be affected, since the newly activated pathways may lead to apoptosis or stop cell division. Additionally, some types of proteins synthesized in these pathways are released to the extracellular environment, where they act as ligands for neighbouring cells. This actually is the case of the IFN-β pathway involved in immune system response.

This paper presents a model of viral infection that takes into account the immunodefense system acting through Interferon-β (IFN-β). First, the standard population models are discussed and the mechanism of viral replication is briefly described. In the subsequent sections the biological background for modeling IFN-β pathway is introduced, followed by ODE models of processes taking place at the population and cellular level. Finally, the applicability of the model and its limitations are discussed.

In order to keep the same notation throughout the text, original notation from cited papers has been changed.

2. Modelling viral infection on population level
The simplest model of viral infection of a cell population describes dynamics of uninfected cells, infected cells and virus, whose average amounts are denoted \( N_0 \), \( N_1 \) and \( P \), respectively [1]:

\[
\begin{align*}
\frac{dN_0}{dt} &= \lambda - dN_0 - bPN_0 \\
\frac{dN_1}{dt} &= bPN_0 - d_1N_1 \\
\frac{dP}{dt} &= kN_1 - d_2P
\end{align*}
\] (1)

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where \( \lambda, d, b, d_i, k \) and \( d_e \) are model parameters.

If a therapy is applied, it is either directed at eliciting immune response from the organism infected or at targeted killing of infected cells. Let us assume the latter case, easier to incorporate in a mathematical model (one of the ways of including the former is presented later in the paper). One must take into account that such therapy can also affect healthy cells, though to lesser extent that the infected ones. Then, denoting control variable by \( U \), the simplest model takes the following form:

\[
\frac{dN_0}{dt} = \lambda - (\alpha U + d)N_0 - bP_e N_0 \quad (2)
\]

\[
\frac{dN_1}{dt} = bP_e N_0 - (\alpha U + d_1)N_1 \quad (3)
\]

\[
\frac{dP_e}{dt} = kN_1 - d_e P_e \quad (4)
\]

Here, the terms \( \alpha U \) represent drug effect rather then its concentration. In order to move modeling closer to clinical application, i.e. link these effects with drug concentration, in such model, intracellular processes activated by the drug, must be taken into account. These involve, among others, the immune system response. One of the few models addressing this problem has been recently published in [6], with immune mechanisms acting through a cytokine called Interferon-\( \beta \) incorporated into he population model. However, it is reasonable to consider also intracellular processes leading to virus replication.

3. Models of viral replication

In [5] authors presented a simple, lumped ODE model of a generic virus replication in a single cell. The mechanisms included in the model were: conversion of virus genome \([\text{gen}]\) into new virus templates \([\text{tem}]\), production of viral structural proteins \([\text{struct}]\) from the templates, release of virus genome from the cell, after it has been packaged within structural proteins, and degradation of each of the three components. The equations governing dynamics of these processes are the following:

\[
\frac{d[\text{tem}]}{dt} = k_1[\text{gen}] - k_2[\text{tem}] \quad (5)
\]

\[
\frac{d[\text{gen}]}{dt} = k_3[\text{tem}] - k_4[\text{gen}][\text{struct}] \quad (6)
\]

\[
\frac{d[\text{struct}]}{dt} = k_5[\text{tem}] - k_6[\text{struct}] - k_4[\text{gen}][\text{struct}] \quad (7)
\]

Though that model does not represent accurately information flow (the same virus genome can be ether used for replication process or released from the cell, for example), it is very convenient for analysis of dynamic properties of the system describing viral infection. Due to its simplicity, it can be easily incorporated into a population model. In [7] a model describing both intracellular processes was presented. It contained a segregated subsystem for the population level, and a modified subsystem (5) for description of intracellular processes. This paper uses a modified viral replication model from [7] but segregation is left out from the extracellular level, in order to simplify the model. Moreover, it is assumed that the viruses that enter the cell can only serve as templates for production of new virions, and that new virions that are produced cannot initiate replication process in the cell they have been produced. Therefore, the virus population is divided into three compartments: extracellular virus \( P_e \), intracellular virus that infected cells \([P_i]\) and intracellular virus that has been produced, but not yet released \([P_{new}]\). The change of intracellular virus is given by

\[
\frac{d[P]}{dt} = k_{in} P_e N_0 - k_0[P_i] \quad (6)
\]

Following assumption from [7] production of new virus templates and structural proteins are catalyzed by two enzymes, \([\text{enzyme1}]\) and \([\text{enzyme2}]\), respectively, whose dynamics is given by

\[
\frac{d[\text{enzyme1}]}{dt} = k_1[P_i] - k_{1\text{deg}[\text{enzyme1}]} \quad (7)
\]

\[
\frac{d[\text{enzyme2}]}{dt} = k_2[P_i] - k_{2\text{deg}[\text{enzyme2}]} \quad (8)
\]

The production of new viruses to be released into extracellular space is then given by

\[
\frac{d[P_{new}]}{dt} = k_{\text{prod}[\text{tem}]} - k_{\text{release}[P_{new}]}[\text{struct}] \quad (9)
\]

Finally, the extracellular virus dynamics is given by

\[
\frac{d[P_e]}{dt} = -k_{inf} P_e N_0 - k_c P_e + k_{\text{release}[P_{new}]}[\text{struct}] \quad (10)
\]

Now, equations (6)-(10) can be combined with (2)-(3) to give whole system description for both population and intracellular levels. However, the issue of pharmacodynamics remains unresolved then. Moreover, if intracellular processes concerning virus replication have to be taken into account [7] then equally important is including other processes initiated in a cell during viral infection.
4. Immune response mediated by IFN-β

Interferons (IFNs) are very important components of the immunodefense system. Their role and elements of interferon-induced signaling pathways are subjects of ongoing research (see e.g. reviews [8], [9]). Interferon family of proteins is divided into two classes of molecules: type I, comprising IFN-α, IFN-ω, IFN-κ, and IFN-β, and type II, to which IFN-γ belongs. This work deals with virus-induced actions signaling of IFN-β, which is produced by most cell types.

It has been found that IFN-β, after binding to its respective receptor on a cell surface, activates a series of signaling pathways that result in upregulation of a large number of genes [10]. They include genes coding proteins that are subsequently used to elicit antiviral response in one of the following, distinct ways [11]: (i) stop translational processes in infected cells, disabling viral replication; (ii) induce apoptosis in cells than cannot fight the infection, thus reducing their lifespans; (iii) induce cell surface display of intracellular peptides in the context of Major Histocompatibility Complex I enabling recognition of infected cells by Natural Killer cells; (iv) elicit resistance to the virus in uninfected cells; (v) release protein IFN-β to the extracellular environment to relay information about infection and amplify cells’ responses.

Various viruses suppress the defense mechanisms of cells, however this paper concentrates on the case in which the immunodefense either works properly, or the only mechanism that is interrupted is production of IFN-β.

Since in virus infection of higher organisms it is the average response of the population that is important, and not individual cell responses, it is assumed that the cell population is homogeneous. Extracellular environment is ideally mixed fluid and therefore no diffusion is considered.

5. Simplified model of IFN-β activated pathway and viral infection

In this section, the mathematical model of signaling pathways activated by IFN-β will be briefly introduced.

Due to complexity of signaling networks and their intertwining, we constrained our analysis to only the most important processes. Seven basic processes were included in the model formulation: formation and dissociation of protein complexes, phosphorylation and dephosphorylation of molecules, gene transcription, translation of mRNA and degradation of molecules.

The following notation has been used:

- Variables names correspond to proteins, complexes and transcripts they represent; for greater clarity they are put in brackets;
- Variables denote cytoplasmic molar concentrations if no subscripts are present, while nuclear concentration is represented by subscript n;
- Concentration of mRNA transcripts is denoted by t subscript and always refers to cytoplasmic concentration; the transport of mRNA to the cytoplasm is assumed to be very fast in relation to other processes and therefore neglected in the model;
- Phosphorylated form of proteins is indicated by p subscript;
- Subscripts active and inactive are used to distinguish respective states of the molecules;
- Activation is a binary variable, equal to 1 for the time of IFN stimulation, 0 otherwise;
- \( k_v \) – ratio of cytoplasmic and nuclear volumes;
- All other symbols not mentioned above are model parameters.

It is assumed that the structure of signaling pathways is identical in all three cell types.

The most important molecules mediating cell responses after IFN (both type I and II) stimulation are STAT (Signal Transducer and Activator of Transcription) proteins. In particular, two members of this family of proteins, STAT1 and STAT2, mediate the responses taken into account in the analyzed pathway. Binding of IFN-β to a cell receptor results in phosphorylation of STAT proteins. Subsequently, phosphorylated STATs form hetero- and homodimers. In cytoplasm, STAT1|STAT2 heterodimers form a complex with an IRF9 protein, called ISGF3. Both STAT1 dimers and ISGF3 complex are very rapidly transported into the nucleus, where they serve as active transcription factors. In the presented model the STAT2 protein exists in dimer with IRF9, therefore directly after phosphorylation the ISGF3 complex can be formed. STATs are dephosphorylated by phosphatases both in the nucleus and in cytoplasm. Dephosphorylation results in dissociation of complexes leading to nuclear export of STATs and making them available to subsequent phosphorylation/ dephosphorylation cycles [12]. Denoting by Activation function of extracellular IFN-β we have:

\[
\frac{d(STAT1)}{dt} = k_{\text{prod}} \cdot (STAT1) - k_{\text{deg}} \cdot (STAT1) - i_{\text{i1}} \cdot (STAT1) + e_{1i} \cdot (STAT1)_{p} \\
- Activation \cdot \frac{k_{\text{phos}} \cdot (STAT1)}{1 + k_{\text{phos}} \cdot (STAT1)} + k_{\text{dep}} \cdot (STAT1)_{p} \\
+ 2k_{\text{transl}} \cdot (STAT1)_{p} \cdot (STAT1)_{p} + k_{\text{ISGF3}} \cdot (ISGF3)
\]

(10)
The newly synthesized mRNA molecules serve as a template for producing IRF1 and IRF7 proteins. These, together with constitutively expressed IRF3, undergo posttranslational modifications making them active and subsequently are imported into the nucleus. Once there, they play an important role in initiating transcription of late genes, IFN-β, STAT1, LMP2 and TAP1 among others. However, to activate transcription of IFN-β, dsRNA, which is a product of viral actions inside a cell, is required. Proteins IRF3 and IRF7 are phosphorylated by it and the rate of phosphorylation is assumed to be proportional to the viral load of a cell.

\[
\frac{d(I_{RF1})}{dt} = k_{\text{trans}} \cdot (IRF1_t) - k_{d_{1}, d_{1}} \cdot (IRF1) - i_{11} \cdot (IRF1) + e_{11} \cdot (IRF1)_n
\]

\[
\frac{d(I_{RF3})}{dt} = k_{\text{prod} \_ IRF3} - k_{d_{IRF3}} \cdot (IRF3) - k_{a_{IRF3}} \cdot (IRF3)[\text{tem}] + e_{17} \cdot (IRF3)_n
\]

\[
\frac{d(I_{RF7})}{dt} = k_{\text{trans} \_ IRF7} \cdot (IRF7_t) - k_{d_{IRF7}} \cdot (IRF7) - k_{a_{IRF7}} \cdot (IRF7)[\text{tem}] + e_{17} \cdot (IRF7)_n
\]

Once in nucleus, STAT1 dimers and ISGF3 activate transcriptions of so called early genes. To this group IRF1 and IRF7 genes belong.

\[
\frac{d(I_{RF1})}{dt} = v_{11t} \cdot (STAT1_t \cdot STAT1_t) - k_{l_{1}, d_{1}} \cdot (IRF1) - i_{11} \cdot (IRF1)
\]

\[
\frac{d(I_{RF7})}{dt} = v_{77t} \cdot (ISGF3)_n - k_{l_{1}, d_{1}} \cdot (IRF7)
\]
The complex of unphosphorylated STAT1 and IRF1 activates transcription of LMP2 and TAP1 genes. Their products, in turn, contribute to the rate of killing infected cells by NK cells. Moreover, IRF1 (or, more accurately IRF1|CBP complex whose dynamics is omitted here) is the transduction factor for STAT1 gene, thus creating a positive feedback loop. Phosphorylated IRF3 and IRF7 are weak and strong activators of IFN-β transcription, respectively [13]. Therefore:

$$\frac{d(STAT1)}{dt} = v_{\text{const}} + v_{\text{tr}} \cdot (IRF1)_{n} - k_{\text{tr}} \cdot (STAT1)_{n}$$

(32)

$$\frac{d(TAP1)}{dt} = v_{\text{tr}} \cdot (IRF1 | STAT1)_{n} - k_{\text{tr}} \cdot (TAP1)_{n}$$

(33)

$$\frac{d(IFN\beta)}{dt} = -k_{\text{deg}} \cdot (IFN\beta)_{n} + v_{\text{phosph}} \cdot (IFN\beta)_{n} + v_{\text{phosph}} \cdot (IFN\beta)_{p}$$

(34)

The equations for IRF1|CBP and IRF1|STAT1 complexes are not shown here, due to lack of space. According to the experimental results, dynamics of TAP1 is similar to the dynamics of LMP2, so only one of those genes and their products is explicitly modeled.

Finally, the products of those late genes, linking the population-level and cell-level models are given by

$$\frac{d(TAP1)}{dt} = -k_{\text{deg}} \cdot (TAP1) + k_{\text{trans}} \cdot (TAP1)$$

(35)

$$\frac{d(IFN\beta)}{dt} = -k_{\text{deg}} \cdot (IFN\beta) - e_{\text{phosph}} \cdot (IFN\beta) + k_{\text{trans}} \cdot (IFN\beta)$$

(36)

IFN-β produced inside cells is subsequently released to the extracellular environment, contributing to the immunological response to the viral infection. Proteins like LMP2 and TAP1, in turn, play their role inside cells, inducing cell resistance to infection. Therefore, they provide link to the model describing cell population dynamics and the system given by equations (2)-(3) should be modified to reflect this.

Let us denote by $N_{0}$, $N_{1}$, $N_{2}$ the average number of cells that are infection susceptible, infected and resistant to infection, correspondingly. The compartmental model describing cell population dynamics is then given by the following:

$$\frac{dN_{0}}{dt} = \lambda - d(TAP1)N_{0} - a_{0}N_{2}N_{0} - b_{0}U_{\text{ext}}$$

$$\frac{dN_{1}}{dt} = -\lambda_{1}N_{1} - k_{\text{tr}}(TAP1)N_{1} + a_{0}N_{2}N_{0}$$

(37)

$$\frac{dN_{2}}{dt} = -\lambda_{2}N_{2} + b_{0}U_{\text{int}}$$

where

$$U = U_{\text{int}} + U_{\text{ext}}$$

(38)

is IFN-β concentration in extracellular environment. Taking into account that IFN-β is not only a protein produced by the cells, but also the active drug component used in antiviral and anticancer therapies [14], its total amount comprises both the externally added $U_{\text{ext}}$ and $U_{\text{int}}$ stemming from intracellular production of IFN-β:

$$\frac{dU_{\text{int}}}{dt} = k_{s}e_{\text{phosph}}(IFN\beta) - k_{\text{deg}} \cdot e_{\text{phosph}}U_{\text{int}}$$

(39)

In fact, $\lambda_{i}$ may also depend on the outcome of the intracellular processes and therefore be functions of both $P_{i}$ and $U$, but in this model they are assumed to be constant. For the sake of simplicity, this influence is reflected only in values of those parameters, varying among compartments. The term $a_{0}N_{2}N_{0}$ describes the rate of infection of cells of type 0.

The basic aim of analysis of model given by equations (4)-(39) is to (i) determine stability conditions showing under what circumstances the cells can successfully defend themselves and (ii) solve an optimization problem that leads to development of an optimal treatment protocol with externally added IFN. For the latter, the optimization goal is to reduce the infected population and amount of viruses in a given time horizon $T$, taking into account cumulative negative effects of therapy. Therefore, the performance index to be minimized is defined as:

$$\min_{U_{\text{ext}}} J = N_{2}(T) + P_{e}(T) + \int_{0}^{T} (U_{\text{ext}} + U_{\text{int}})dt$$

(40)

with $0 \leq U_{\text{ext}} \leq U_{\text{max}}$

The solution to this problem depends on the form of the function Activation in (10), (11), (14), (15) which remains to be determined experimentally. If it linearly depends on IFN-β concentration, then the optimal solution will be bang-bang control. However, if this will prove to be a sigmoidal function of IFN-β concentration, then we will obtain smooth optimal control, not applicable in clinical practice.

6. Conclusion

The paper presents a complex, two-scale model of viral infection in the population of homogenous cells coupled with the model of intracellular signalling pathways involved in fight against infection. Due to its nonlinear character and large number of parameters it can be used only for semiqualitative numerical analysis of infection dynamics. However, with the advance of experimental techniques and increasing availability of experimental data more and more parameters will be possible to identify, and, ultimately, allow for full analysis of such system as well as for mathematically based design of therapy protocols.
Actually, this model should be treated only as a general framework not as a set of equations to be numerically solved. Instead, one should first analyze intracellular subsystem given by (10)-(36) and then substitute it with a model of much lower order, exhibiting similar dynamics. Then other processes can be taken into account and even structured, segregated population model can be introduced. The advantage of building the complex model first is to be able to use the same methodology in case when viruses try to evade the immune response of a cell population. Additionally, experimental data, if such exist, can be used for partial identification of model parameters so that at least part of them have biological interpretation.

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References