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Modelling and analysis of dynamics of viral infection of cells and of interferon resistance

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Abstract

Interferons are active biomolecules, which help fight viral infections by spreading from infected to uninfected cells and activate effector molecules, which confer resistance from the virus on cells. We propose a new model of dynamics of viral infection, including endocytosis, cell death, production of interferon and development of resistance. The novel element is a specific biologically justified mechanism of interferon action, which results in dynamics different from other infection models. The model reflects conditions prevailing in liquid cultures (ideal mixing), and the absence of cells or virus influx from outside. The basic model is a nonlinear system of five ordinary differential equations. For this variant, it is possible to characterise global behaviour, using a conservation law. Analytic results are supplemented by computational studies. The second variant of the model includes age-of-infection structure of infected cells, which is described by a transport-type partial differential equation for infected cells. The conclusions are: (i) If virus mortality is included, the virus becomes eventually extinct and subpopulations of uninfected and resistant cells are established. (ii) If virus mortality is not included, the dynamics may lead to extinction of uninfected cells. (iii) Switching off the interferon defense results in a decrease of the sum total of uninfected and resistant cells. (iv) Infection-age structure of infected cells may result in stabilisation or destabilisation of the system, depending on detailed assumptions. Our work seems to constitute the first comprehensive mathematical analysis of the cell-virus-interferon system based on biologically plausible hypotheses.

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1. Introduction

We present a mathematical model for the dynamics of viral infection *in vitro*, including infection, cell death, production of interferon and development of resistance. The dynamics of the model can be understood as a combat between the invading virus particles and the ability of the immune system to react to the invasion by producing substances conferring resistance to virus. We concentrate on the case, in which the supply of unexposed cells ceases at the moment of infection. This corresponds to conditions prevalent in cell culture experiments.

The model is motivated by experiments involving vesicular stomatitis virus, [4,18] and respiratory syncytial virus (RSV) [15] including unpublished experimental results performed in Dr. Allan Brasier's laboratory of the University of Texas Medical Branch in Galvestone. The model proposed by us consists of five ordinary differential equations and despite its complexity can be analysed mathematically, yielding conclusions regarding existence, asymptotics and local stability of equilibria. In an extended version, the model involves infection-age structure of cells and is formulated by means of a partial differential equation.

For a eukaryotic virus to successfully infect and propagate in cultured cells several events must occur: The virion must identify and bind its cellular receptor, become internalised, uncoat, synthesise viral proteins, replicate its genome, assemble progeny virions, and exit the host cell. The virions bud off from the cell, gaining an envelope from the cell membrane as they exit. The new viral particle infects another cell to repeat the cycle. Usually, during the repeated process of self-replication, the virus destroys host's cells.

While these events are taking place, intrinsic host defenses activate in order to defeat the virus. The first-line defense against viruses is based on innate immunity. This includes, among others, activation of the interferon system, induction of apoptosis, and attempted elicitation of immune responses via chemokine and cytokine production. Interferons are a family of active biochemical species, which help to fight viral infections by spreading from infected to uninfected cells and triggering production of effector molecules. There are three major classes of interferons: alpha (α), beta (β), and gamma (γ). All classes of interferons are very important in the course of RNA virus infections. They are triggered when abnormally large amounts of double-stranded RNA (dsRNA) are found in a cell. The interferons interact with receptors located on the membrane of uninfected cells, which leads to the activation of the reactions cascade in the uninfected cells and production of specific proteins. These latter when activated confer on cells resistance from the virus [15].

In the current paper, we analyse the dynamics of a model of a laboratory viral infection of a cell culture. The model involves wild-type, infected and resistant cells, viral particles and interferon molecules. The basic variant corresponds to a perfectly mixed liquid culture, and therefore, it is expressed in the terms of a system of five nonlinear ordinary differential equations. For this system, we obtain global analytic results, by taking advantage of a partial conservation law and nonnegativity of solutions. In particular, we investigate asymptotic stability and influence of virus mortality and interferon activity on dynamics. We support our analytical results by numerical simulations.

A next step towards a better understanding of the role of intracellular processes in the infection spread is to structure the model by infection age, i.e., the time elapsed after a cell was infected: After endocytosis, infection develops as an autonomous process within the infected cell. The processes on the intracellular level are very complicated, consisting both of the replication cycles of virus and the signalling pathway leading to the synthesis of interferon. They take place in different time intervals after the infection of the cell. Understanding these processes is especially important for developing drug therapies targeting specific virus components or stimulating synthesis of IFN. In this paper we do not explicitly consider intracellular processes. However, we differentiate among the intracellular stages of infection for infected cells using an additional variable describing the age of infection. The corresponding model involves a transport type partial differential equation for infected cells. Partial global stability results can be obtained by virtue of another partial conservation law. However, most results are obtained using the linearised analysis of a delay-differential equation, derived from the original structured problem. Application of the relatively little-known Mikhailov criterion allows us to investigate, among other, the relationship between structure and stability. We show that the time "ordering" of virus production and infected cells' mortality can have (de-)stabilising effects at the cell population level.

2. Hypotheses of the model

We consider a model for the dynamics of viral infection, which involves wild-type (i.e., unexposed to virus), infected and resistant cells, as well as particles of virus (virions) outside cells, and molecules of interferon, the substance

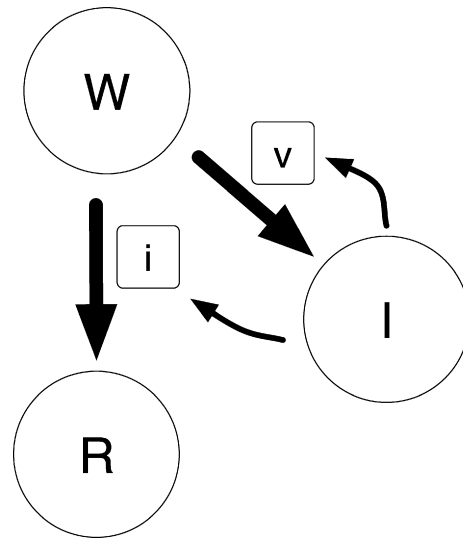


Fig. 1. Flowchart of the basic model: wild-type cells (W) may become infected cells (I) if exposed to the action of virions (v), or they may become resistant cells (R) under the influence of interferon (i). Virions and interferon are produced by the infected cells. Infection and transition to resistance are represented by thick arrows, whereas multiplicative interactions are represented by thin arrows.

released by infected cells, which boosts the resistance of wild-type cells. The general scheme of the transitions between different cell states is presented in Fig. 1. In the current paper, we do not consider the spatial spread of infection. However, in one version of the model, we incorporate the infected cells' age of infection.

The dynamics of virus-cell-interferon interactions is described as follows:

1. Wild-type cells (W)

(a) Wild-type cells are not replenished once the culture is exposed to virus.

(b) No natural mortality of wild-type cells is considered.

Hypotheses 1(a) and 1(b) reflect the situation, in which no proliferation is observed during the experiment. In this case we do not consider death of the wild-type cells either. This is acceptable, since we are interested in the process on the short-time scale.

(c) Wild-type cells are infected by the virions (denoted by v) at a rate, which is proportional to the frequency of encounters Wv .

(d) Wild-type cells turn to resistant cells (R) at a rate, which is proportional to the product Wi , where i is the level of interferon molecules.

2. Infected cells (I)

(a) Infected cells are eliminated by internalised virions at a rate proportional to their own number (i.e., their lifetimes are exponentially distributed).

(b) According to the law of mass action, infected cells are produced at a rate, which is proportional to the product Wv (see 1(c)).

3. Resistant cells (R)

(a) Resistant cells are produced at a rate, which is proportional to the product Wi , where i is the level of interferon molecules (see 1(d)).

(b) Similarly as wild-type cells, resistant cells do not die in the model with no supply of new cells (see 1(a)). As mentioned above, this corresponds to a different experimental situation and the chosen time-scale of experiments.

4. Interferon molecules (i)

(a) Interferon molecules are produced at a rate proportional to the number of infected cells.

(b) Interferon molecules are used up by interacting with wild-type cells in order to boost their immunity, at a rate proportional to the product Wi (see 3(a)).

(c) Interferon molecules are degraded at a rate proportional to their number.

5. Extra-cellular virus particles (free virions) (v)

- (a) Free virions, apart from their initial supply, are released by infected particles at a rate, which is proportional to the number of these latter. We do not explicitly model processes of viral replication within cells.
- (b) Free virions are eliminated in the process of infecting the wild-type cells at a rate proportional to the product Wv (see 2(b)). One virion is sufficient to infect a cell. However, usually more virions enter the cell, therefore; more virions are consumed by one cell and we consider independent rates for virus removal by consumption and for infection of cells.
- (c) Free virions are degraded at a rate proportional to their own number. We separately consider the case, when there is no degradation of virions. Whereas *in vivo* free virions can be degraded by reactive oxygen species produced by macrophages, no such possibility is known *in vitro* (Dr. Allan Brasier, personal communication). Therefore, the case with no virus outflux other than that resulting from endocytosis (entry into cells) seems to be relevant for the *in vitro* model.

Remark 2.1. In this model we suppose that after endocytosis, the infection within a cell develops as an autonomous process, and therefore the re-infections of an already infected cell play no role. In the case of RSV infection, cells once infected can be reinfected again and the influence of possible re-infections on the dynamics of the infection is not known (personal communication by Dr. Allan Brasier). However, in this paper we assume that the virus reproduces within the cell at such a rate that further infections by other virions can be neglected and we do not consider infection-degree structure. Moreover, one virion suffices to infect a cell. By allowing α_1 unequal to α_3 , we allow a cell to be infected by more than one virion “at a time.”

The hypotheses above can be readily translated into a system of five ordinary differential equations for variables W , I , R , i and v , each being a function of time t . In the model with structure, infected cells are parameterised by their infection-age, i.e., the time elapsed since the cell became infected, which we denote by a . Cells of different infection-age produce interferon and release virions at different rates. The evolution of the infection-age distribution of cells, $I(t, a)$, is described by a transport-type partial differential equation.

3. ODE model

The process described above is modelled by the following system of equations:

$$W' = -\alpha_1 v W - \alpha_2 i W, \quad (3.1)$$

$$I' = -\mu_I I + \alpha_1 v W, \quad (3.2)$$

$$R' = \alpha_2 i W, \quad (3.3)$$

$$i' = -\mu_i i + \alpha_i I - \alpha_3 i W, \quad (3.4)$$

$$v' = -\mu_v v + \alpha_v I - \alpha_4 v W, \quad (3.5)$$

with initial conditions

$$(W, I, R, i, v)(0) = (W_0, I_0, R_0, i_0, v_0), \quad (3.6)$$

production rates:

α_v = number of virions produced per infected cell per unit of time,

α_i = quantity of interferon produced per infected cell per unit of time,

conversion rates:

α_1 = fraction of wild-type cells converted to infected cells per virion per unit of time,

α_2 = fraction of wild-type cells converted to resistant cells per quantity of interferon per unit of time,

removal rates due to other causes (mortality, decay, washout):

μ_I = fraction of infected cells removed per unit of time,

μ_i = fraction of interferon removed per unit of time,

μ_v = fraction of virus removed per unit of time,

and consumption rates:

α_3 = fraction of interferon consumed per wild-type cell per unit of time,

α_4 = fraction of virus consumed per wild-type cell per unit of time.

For the modelling of experiments in which the initial population consists only of healthy cells and infection is started by adding virus to the cell culture one should keep in mind the choice of initial conditions $I_0 = R_0 = i_0 = 0$. Taking $i_0 > 0$ simulates an experiment with an initial amount of IFN added from outside (IFN pre-treatment). To satisfy assumption 5(b) one should have $\alpha_4 \geq \alpha_1$ and similarly $\alpha_3 \geq \alpha_2$, though for our analytical computations this does not play a role. To reduce the number of parameters, we rescale system (3.1)–(3.5) using, $\tilde{v} := \alpha_1 v$, $\tilde{i} := \alpha_2 i$, $\tilde{\alpha}_v := \alpha_1 \alpha_v$, $\tilde{\alpha}_i := \alpha_2 \alpha_i$. Omitting “ $\tilde{\cdot}$ ” for simplicity we obtain the following rescaled system:

$$W' = -vW - iW, \tag{3.7}$$

$$I' = -\mu_I I + vW, \tag{3.8}$$

$$R' = iW, \tag{3.9}$$

$$i' = -\mu_i i + \alpha_i I - \alpha_3 iW, \tag{3.10}$$

$$v' = -\mu_v v + \alpha_v I - \alpha_4 vW. \tag{3.11}$$

The interpretations of the new quantities are:

- v fraction of wild-type cells infected per unit of time,
- i fraction of wild-type cells becoming resistant per unit of time,
- α_v fraction of wild-type cells infected per unit of time by all virions produced in one unit of time by one infected cell,
- α_i fraction of wild-type cells made resistant per unit of time by all interferon that is produced per unit of time by one infected cell.

3.1. Existence, uniqueness and asymptotic behaviour

Local existence and uniqueness of nonnegative solutions follow from Lipschitz continuity and nonnegativity of the right-hand side in a standard way, see e.g. Corollary 5.2 in the appendix of [17], or a more general proof of nonnegativity in the structured model. Further, we show monotonicity properties. We also prove that, if all parameters are strictly positive, the infection will ultimately die out. The special case $\mu_v = 0$ is separately analysed in Section 3.1.1.

Theorem 3.1. *Suppose that all initial conditions are nonnegative and define the total number of cells as*

$$N(t) := W(t) + R(t) + I(t).$$

Then

- (a) N, W, R, I, i and v are nonnegative,
- (b) N and W are nonincreasing and R is nondecreasing,
- (c) N, W, R, I, i and v , as well as the derivatives of all five components are uniformly bounded and, if $\mu_I > 0$, so is $\int_0^t I(s) ds$,
- (d) all solutions exist for all times, and
- (e) W tends to a finite limit and R tends to a finite nonzero limit. At least one of the two, v or W , tends to zero. If $\mu_I > 0$, then I tends to zero. If additionally $\mu_v > 0$, respectively $\mu_i > 0$, then also v , respectively i tends to zero. If all three $\mu_I, \mu_v, \mu_i > 0$, then $W(\infty) > 0$. In particular, if all parameters are strictly positive, all five components tend to finite limits, W and R tend to nonzero limits and I, i and v tend to zero.

Proof. Adding Eqs. (3.7)–(3.9) yields

$$W'(t) + R'(t) + I'(t) + \mu_I I(t) = 0. \quad (3.12)$$

It follows that N is nonincreasing and, after integration, that N , W , R and I and, if $\mu_I > 0$, also $\int_0^t I(s) ds$ are uniformly bounded. The monotonicity of W and R follows from (3.7) and (3.9). Next, note that from (3.10), one obtains after integration that

$$i(t) \leq i_0 + \alpha_i \int_0^t I(s) ds,$$

which guarantees the uniform boundedness of i . Analogously, we obtain the uniform boundedness of v . The uniform boundedness of the derivatives then follows via (3.7)–(3.11). The boundedness of solutions guarantees global existence in the standard way. The limit statement for R follows from the boundedness and monotonicity of R . From (3.7) we obtain

$$W(t) = W_0 e^{-\int_0^t (i(s)+v(s)) ds}, \quad (3.13)$$

which implies that W tends to a finite limit. If both $\int_0^t i(s) ds$ and $\int_0^t v(s) ds$ are uniformly bounded in t this limit is positive; if at least one of them is unbounded this limit is zero. From (3.8), we obtain

$$\int_0^t v(s)W(s) ds = I(t) - I_0 + \mu_I \int_0^t I(s) ds. \quad (3.14)$$

The uniform boundedness of $\int_0^t I(s) ds$ together with the nonnegativity of I implies that $\int_0^\infty I(s) ds$ converges if $\mu_I > 0$. Since also $I'(t)$ is uniformly bounded, one can conclude that $I(t) \downarrow 0$ for $t \uparrow \infty$. The limit behaviour and nonnegativity of $I(t)$ and $\int_0^t I(s) ds$ ensure that also the integral on the left-hand side of (3.14) converges. As $(vW)'$ is bounded, one can then conclude that vW tends to zero and hence either v or W or both tend to zero. Integrating (3.11) leads to

$$v(t) + \alpha_4 \int_0^t W(s)v(s) ds + \mu_v \int_0^t v(s) ds = v_0 + \alpha_v \int_0^t I(s) ds. \quad (3.15)$$

If $\mu_I > 0$ and $\mu_v > 0$, from the uniform boundedness of $\int_0^t I(s) ds$, we conclude that $\int_0^t v(s) ds$ is uniformly bounded and v tends to zero by the uniform boundedness of v' . One analogously proves the statement for i . Hence it follows from (3.13) that $\mu_I, \mu_v, \mu_i > 0$ implies that $W(\infty) > 0$. \square

3.1.1. Subcase: No virus decay

Theorem 3.1(e) leaves three possible cases of asymptotic behaviour for v and W : (i) the population of wild-type cells becomes extinct and the virus stays in the environment, (ii) the population of wild-type cells persists and the population of virions becomes extinct, or (iii) both, population of wild-type cells and virus, become extinct. Moreover, we gave conditions under which situation (ii) is found. We will now give conditions under which situation (i) is found. We consider a special case, where there is no uptake of virions from extracellular space other than by endocytosis and infection of the cell, a distinctive possibility (Dr. Allan Brasier, *personal communication*). In this case, i.e., for $\mu_v = 0$, we show that wild-type cells may become extinct.

Lemma 3.2. *If $\mu_I > 0$ and $\mu_v = 0$, the size of the virus population converges to a limit. If additionally $\alpha_v - \alpha_4 \mu_I \geq 0$, then $v(\infty) \geq v_0$ and wild-type cells become extinct.*

Proof. As $\mu_v = 0$, combining (3.15) with (3.14), we obtain

$$v(t) - v_0 = (\alpha_v - \alpha_4 \mu_I) \int_0^t I(s) ds + \alpha_4 I_0 - \alpha_4 I(t). \quad (3.16)$$

As $\mu_I > 0$ implies that $I(t)$ tends to zero at infinity, we conclude that if $\alpha_v - \alpha_4\mu_I \geq 0$, then $v(t) \geq v_0$ for sufficiently large t and, since the right-hand side converges, so does v . Then, by Theorem 3.1(e), we obtain that $W(\infty) = 0$. If $\alpha_v - \alpha_4\mu_I < 0$, we can merely conclude that v tends to a limit. \square

Remark 3.3. Numerical studies (see Figs. 7 and 10) indicate that if $\alpha_v - \alpha_4\mu_I < 0$, then also for $\mu_v = 0$ it may occur that $W(\infty) > 0$, which yields $v(\infty) = 0$. Also (3.16) shows that, depending on initial conditions, it cannot be excluded that $v(\infty) = 0$.

3.2. Effects of interferon synthesis

In this section we give analytical results on how the incorporation of resistance against the virus affects the spread of viral infection. We show that if the efficiency of the virus is bounded in a way to be specified in Theorem 3.4 the limit number of healthy cells increases when incorporating interferon synthesis. For $\alpha_i = 0$, both models proposed in this paper describe an infection in a population of cells that are not able to produce interferon. Since in the system there is no feedback from resistant cells (see Fig. 1) it is intuitively clear that changes in the value of the parameter α_i do not influence the qualitative behaviour of solutions W , I and v . This also becomes apparent in the stability analysis below. However, as it can be seen in numerical simulations, these changes alter the limit value of the number of resistant cells R and therefore, also the total number of non-infected cells, and the number of cells that become infected (see Figs. 8 and 10). For $\alpha_i > 0$, as follows from Theorem 3.1(b) and (e), $R(t) = \int_0^t W(s)i(s) ds$ is a monotonously increasing function, which is bounded from above and tends to a positive constant. The total number of healthy cells, $W + R$, is monotonously decreasing and also tends to a positive limit, which is greater than the limit for the wild-type cells in the model with $\alpha_i = 0$. Analytically this is evident for the case $\mu_v = 0$ and $\alpha_v - \mu_I\alpha_4 \geq 0$, in which independently of IFN dynamics all wild-type cells become extinct and the virus remains in the system (Lemmas 3.2 and 4.7 below). Then, for $\alpha_i = 0$, also all uninfected cells become extinct, while for $\alpha_i > 0$, the total number of healthy cells $W(t) + R(t) \rightarrow R(\infty) > 0$.

For $\mu_v > 0$ we establish the following analytical result.

Theorem 3.4. Suppose that $\mu_I, \mu_v > 0$, $R_0 = i_0 = 0$ and that either

- (i) $\alpha_v < \alpha_4\mu_I$, or
- (ii) $\alpha_v > \alpha_4\mu_I$, and $(\alpha_v - \alpha_4\mu_I)(W(\infty) + R(\infty)) < \mu_v\mu_I$.

Then, the limit of the total number of uninfected cells $W(\infty) + R(\infty)$, is larger if $\alpha_i > 0$ than if $\alpha_i = 0$.

Proof. Adding (3.7) and (3.9) leads to

$$(W + R)' = -vW \geq -v(W + R).$$

Therefore, in the limit for $t \rightarrow \infty$

$$W(\infty) + R(\infty) \geq W_0 e^{-\int_0^\infty v(s) ds}. \tag{3.17}$$

After integration of (3.12) we obtain

$$W(t) + R(t) + I(t) = W_0 + I_0 - \mu_I \int_0^t I(s) ds,$$

which in the limit $t \rightarrow \infty$ and recalling that $I(\infty) = 0$ yields

$$\int_0^\infty I(s) ds = \frac{W_0 + I_0 - W(\infty) - R(\infty)}{\mu_I}. \tag{3.18}$$

We combine (3.15) with (3.14) and, recalling that also $v(\infty) = 0$, we obtain

$$\int_0^{\infty} v(s) ds = \frac{\alpha_v - \alpha_4 \mu_I}{\mu_v} \int_0^{\infty} I(s) ds + \frac{v_0}{\mu_v} + \frac{\alpha_4 I_0}{\mu_v}. \quad (3.19)$$

Substituting (3.18) into (3.19) and the resulting equation into (3.17) leads to

$$\begin{aligned} W(\infty) + R(\infty) \geq & W_0 \exp\left(- (W_0 + I_0) \frac{\alpha_v - \alpha_4 \mu_I}{\mu_v \mu_I} - \frac{v_0}{\mu_v} - \frac{\alpha_4 I_0}{\mu_v}\right) \\ & \cdot \exp\left\{\frac{\alpha_v - \alpha_4 \mu_I}{\mu_v \mu_I} [W(\infty) + R(\infty)]\right\}. \end{aligned} \quad (3.20)$$

The latter can be written as

$$[W(\infty) + R(\infty)] \exp\{-C_1 [W(\infty) + R(\infty)]\} \geq C_0, \quad (3.21)$$

where C_1 and C_0 are constants defined by

$$\begin{aligned} C_1 &= \frac{\alpha_v - \alpha_4 \mu_I}{\mu_v \mu_I}, \\ C_0 &= W_0 \exp\left[- (W_0 + I_0) \frac{\alpha_v - \alpha_4 \mu_I}{\mu_v \mu_I} - \frac{v_0}{\mu_v} - \frac{\alpha_4 I_0}{\mu_v}\right]. \end{aligned}$$

Next, consider the model with $\alpha_i = 0$. Then, since $i_0 = 0$ and $R_0 = 0$, one has $i(t) = 0$ and $R(t) = 0$ for all $t \geq 0$. Then similar considerations as before lead to the relation

$$W_-(\infty) \exp[-C_1 W_-(\infty)] = C_0, \quad (3.22)$$

where W_- denotes the density of wild-type cells in the model with $\alpha_i = 0$. Therefore, combining (3.21) with (3.22) we obtain

$$[W(\infty) + R(\infty)] \exp\{-C_1 [W(\infty) + R(\infty)]\} \geq W_-(\infty) \exp[-C_1 W_-(\infty)]. \quad (3.23)$$

For $C_1 < 0$, the function $f(u) = ue^{-C_1 u}$ is monotone increasing, and therefore (3.23) yields that

$$W(\infty) + R(\infty) \geq W_-(\infty).$$

For $C_1 > 0$, the function $f(u) = ue^{-C_1 u}$ is monotone increasing for $C_1 u < 1$, i.e., if

$$(\alpha_v - \alpha_4 \mu_I) [W(\infty) + R(\infty)] < \mu_v \mu_I. \quad \square$$

In the remainder of the subsection we interpret conditions (i) and (ii) of Theorem 3.4. First note that (i) is equivalent to

$$\frac{\alpha_v}{\mu_I} W < \alpha_4 W.$$

The left-hand side equals the number of cells out of a wild type cell population W that is expected to be infected per unit of time by all virus expected to be produced by one infected cell during its lifetime. The right-hand side is the fraction of virus consumed per unit of time by the wild type cell population W . In this sense we may call

$$\left(\frac{\alpha_v}{\mu_I} - \alpha_4\right) W$$

the efficiency rate of a virus disregarding (virus) decay in cell population W , such that condition (i) is equivalent to saying that, (even) disregarding its decay, a virus is not efficient in any wild type cell population. Condition (ii) then means that, disregarding decay, virus is efficient for all wild type cell populations but the efficiency rate of virus, disregarding its decay in a population whose number equals the limit number of all healthy cells for infinite times is less than the actual decay rate for the virus. Hence the conclusion of the theorem can be summarised as: The synthesis of interferon results in a larger amount of healthy cells in the end, given that the efficiency of the virus is within certain bounds.

3.3. Equilibria and linearised stability

Model (3.7)–(3.11) has a trivial equilibrium $(0, 0, 0, 0, 0)$ and a two-dimensional space of disease-free equilibria $(\bar{W}, 0, \bar{R}, 0, 0)$.

Without virus, interferon and infected cells, wild-type cells and resistant cells (given there are some) have neither in- nor outflows and hence are decoupled; therefore any initial population remains in equilibrium. From (3.7) it follows that there is no endemic equilibrium: Without virus and interferon, the wild-type cells are in equilibrium, but as soon as there is either of those, the wild-type cells have only outflow and hence cannot be in equilibrium. We will see that for all equilibria all eigenvalues are real, two eigenvalues are zero and two are negative. The remaining eigenvalue is negative for the trivial equilibrium, whereas for the disease-free equilibria the sign of the remaining eigenvalue depends on the equilibrium and on the choice of parameters. The Jacobi matrix of the linearised system around $(\bar{W}, 0, \bar{R}, 0, 0)$ is given as

$$\begin{pmatrix} 0 & 0 & 0 & -\bar{W} & -\bar{W} \\ 0 & -\mu_I & 0 & 0 & \bar{W} \\ 0 & 0 & 0 & \bar{W} & 0 \\ 0 & \alpha_i & 0 & -\mu_i - \alpha_3 \bar{W} & 0 \\ 0 & \alpha_v & 0 & 0 & -\mu_v - \alpha_4 \bar{W} \end{pmatrix}.$$

The characteristic equation can then be computed as

$$\lambda^2(-\mu_i - \alpha_3 \bar{W} - \lambda)[(-\mu_I - \lambda)(-\mu_v - \alpha_4 \bar{W} - \lambda) - \alpha_v \bar{W}] = 0$$

and we obtain

Lemma 3.5. *All roots of the characteristic equation are real, two roots are zero, two roots are negative and the remaining root is negative, if and only if*

$$\frac{\alpha_v \bar{W}}{\mu_I} < \mu_v + \alpha_4 \bar{W}. \tag{3.24}$$

Proof. The last factor of the characteristic polynomial set to zero gives

$$\lambda^2 + (\mu_I + \mu_v + \alpha_4 \bar{W})\lambda + (-\mu_I)(-\mu_v - \alpha_4 \bar{W}) - \alpha_v \bar{W} = 0,$$

from which it follows that

$$\lambda_{4,5} = -\frac{\mu_I + \mu_v + \alpha_4 \bar{W}}{2} \pm \sqrt{\frac{(\mu_I + \mu_v + \alpha_4 \bar{W})^2}{4} + \alpha_v \bar{W} - \mu_I(\mu_v + \alpha_4 \bar{W})}.$$

The expression under the square root is always positive, which shows that λ_4 and λ_5 , and therefore all eigenvalues, are real. Moreover, we see that λ_5 is negative. Finally, λ_4 is negative if and only if

$$\alpha_v \bar{W} < \mu_I(\mu_v + \alpha_4 \bar{W}). \quad \square$$

We will interpret (3.24) in Section 5. However, it is obvious from the equation for W that even a small perturbation of the steady state, i.e., for example $v = \epsilon > 0$ leads to a decrease of W , hence a new \bar{W} must be smaller than the perturbed one. Therefore, after a small perturbation W and R do not come back to the previous steady states, whereas v , I and i do (see Fig. 10 upper panel for an illustration of the “stability” of a steady state in comparison with the instability presented in the lower panel).

If we assume that there is no virus degradation, i.e., $\mu_v = 0$, for $\alpha_v - \mu_i \alpha_4 > 0$ in Lemma 3.2 it was shown that W tends to zero and hence no positive steady states \bar{W} are stable.

Applying linearised stability analysis to our model we refer to the following result from the monograph by Codrington and Levinson [1, Theorem 13.4.1] (as amended in Problem 11 to Chapter 13): *Consider ODE system in n dimensions $x' = Ax + f(t, x)$, where f is continuous in (t, x) for small $|x|$ and $t \geq 0$; moreover for any $\epsilon > 0$*

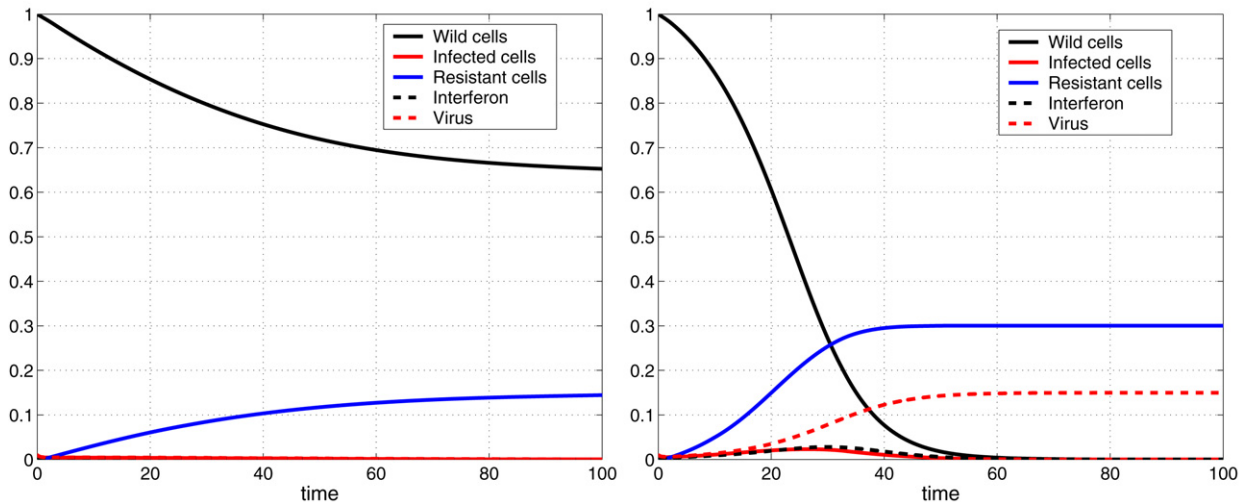


Fig. 2. Simulations with parameters $\alpha_3 = 1, \alpha_4 = 1, \mu_I = 1, \mu_i = 0.9, \alpha_i = 1.5, \alpha_v = 1.2, \alpha_2 = 1, \alpha_1 = 1$ initial value $W_0 = 1$ and $v_0 = 0.01$, and $\mu_v = 0.2$ on the left-hand side and $\mu_v = 0$ on the right-hand side.

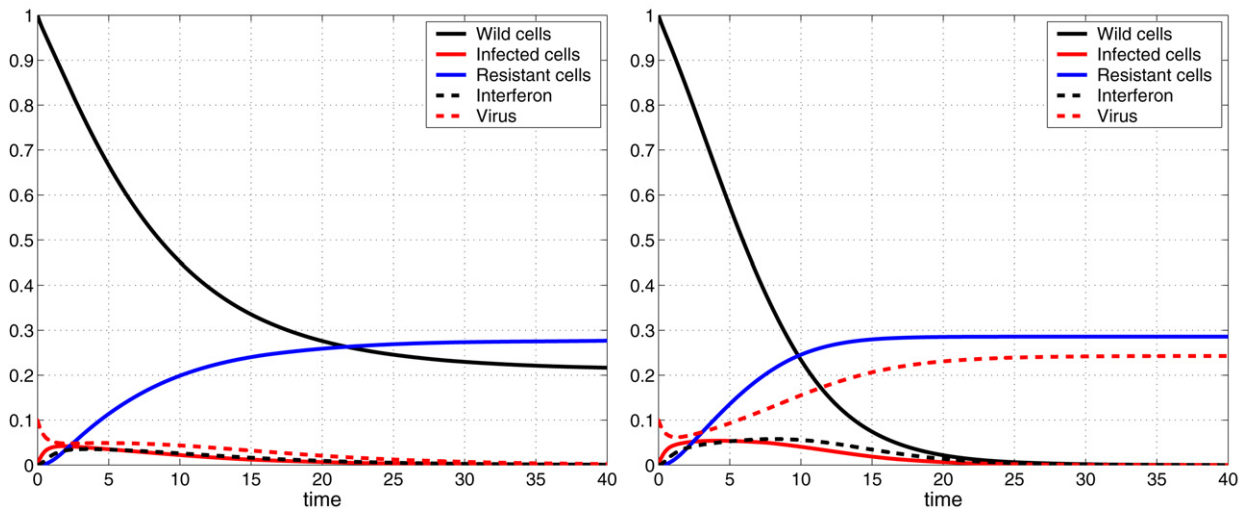


Fig. 3. Simulations with parameters as in Fig. 2 and initial condition $v_0 = 0.1$.

there exists a δ and T such that for $t \geq T$, it holds $|f(t, \tilde{x}) - f(t, x)| \leq |\tilde{x} - x|$, for $|x|, |\tilde{x}| \leq \delta$. It is assumed that $f(t, 0) = 0$, but not necessarily that there are no other equilibria in the vicinity of $x = 0$ (indeed, they may be present, for example if $f(t, x) = 0$ for x in the null-space of matrix A). Then, let us assume that k characteristic roots of A have negative real parts and $n - k$ have zero real parts. Then, for any large t_0 there exists in \mathbb{R}^n space a real k -dimensional manifold S containing the origin such that any solution φ of the system above with $\varphi(t_0) \in S$ satisfies $\varphi(t) \rightarrow 0$, as $t \rightarrow \infty$. Moreover, there exists an η such that any solution φ near the origin but not on S at $t = t_0$ cannot satisfy $|\varphi(t)| \leq \eta, t \geq t_0$ and $\limsup_{t \rightarrow \infty} [t^{-1} \ln|\varphi(t)|] < 0$. If f is analytic in x for each $t \geq 0$ and $|x|$ small, then S is an analytic manifold.

The above provides a rigorous framework for carrying out the linearised analysis in the presence of a continuum of equilibria, as it was done above, in which case there usually occur zero (or at least zero real value) eigenvalues of the “linear part” (matrix A) of the right-hand side of the system. The limit solutions demonstrated to exist in Section 3.1 are also the non-isolated equilibrium solutions considered in the present section.

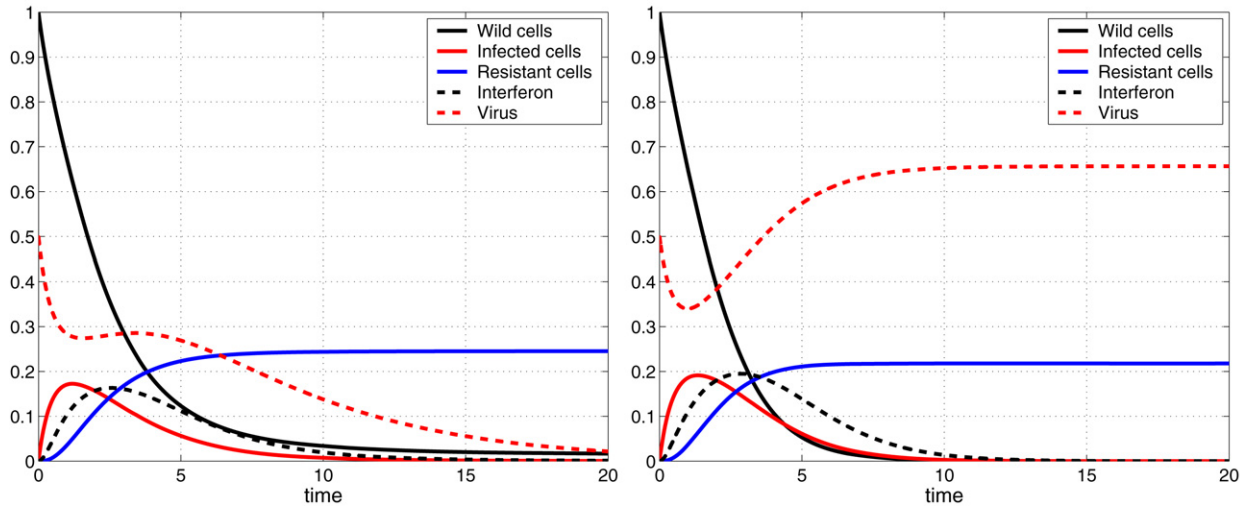


Fig. 4. Simulations with parameters as in Fig. 2 and initial condition $v_0 = 0.5$.

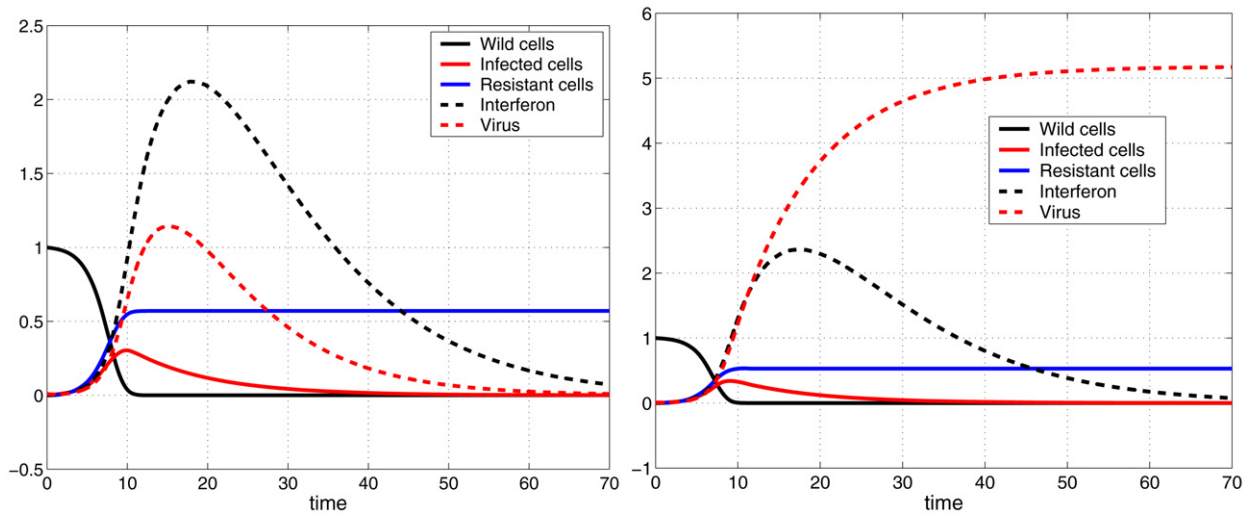


Fig. 5. Simulations with small decay of infected cells and IFN. Parameters: $\alpha_3 = 1, \alpha_4 = 1, \mu_I = 0.1, \mu_i = 0.1, \alpha_i = 1.5, \alpha_v = 1.2, \alpha_2 = 1, \alpha_1 = 1$, initial value $W_0 = 1$ and $v_0 = 0.01$, and $\mu_v = 0.2$ on the left-hand side and $\mu_v = 0$ on the right-hand side.

3.4. Numerical simulations

We performed a series of numerical simulations for the ODE version of the model, which help to visualise qualitative and quantitative properties of the trajectories. These properties will largely carry over to the model with structure. Since the death rate μ_v of the virus is an important discriminative parameter, most graphs are arranged in pairs, the left-hand panel corresponding to $\mu_v > 0$ and the right-hand panel to $\mu_v = 0$. Figs. 2–4 depict the influence of initial conditions of the wild-type cells (W_0) and of the virus (v_0) on the asymptotics. We observe that, although $W(\infty) > 0$ when $\mu_v > 0$, it may become very small when v_0 is large. Notice however a high number of resistant cells in all cases. For the case $\mu_v = 0$, we observe that $W(\infty) = 0$ for the current parameter values, which is one of the possibilities in this case (see Lemma 3.2). Fig. 5 depicts a variant of the above with lower death rates of infected cells and lower decay rate of the interferon. Notice prominent transients of interferon level.

Fig. 6 depicts a qualitatively different picture emerging when the coefficient α_4 equal to the relative consumption of virions in the process of infection, is increased. We observe sharp decay of the virus to near-0 levels and emergence of

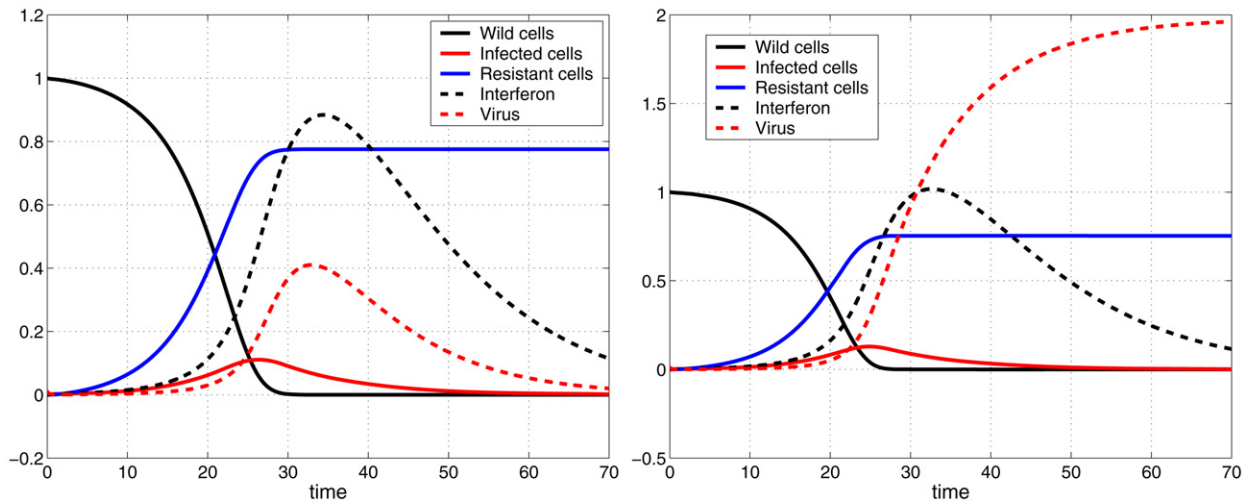


Fig. 6. Simulations with parameters as in Fig. 5 and higher consumption of the virions during infection $\alpha_4 = 4$, initial value $W_0 = 1$ and $v_0 = 0.1$, and $\mu_v = 0.2$ on the left-hand side and $\mu_v = 0$ on the right-hand side.

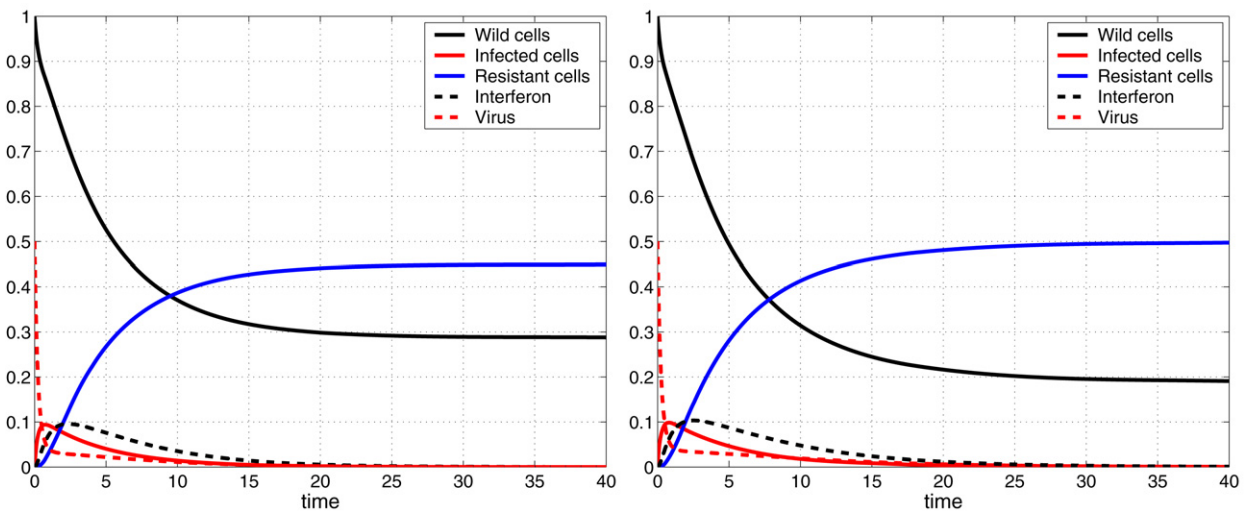


Fig. 7. Simulations with parameters as in Fig. 5 and higher decay of the infected cells, $\mu_I = 0.5$. Initial value $W_0 = 1$ and $v_0 = 0.5$, and $\mu_v = 0.2$ on the left-hand side and $\mu_v = 0$ on the right-hand side.

a large population of resistant cells, with limit of wild-type cells $W(\infty)$ very low or equal to zero depending whether $\mu_v > 0$ or $\mu_v = 0$. If higher decay rates μ_I of infected cells is assumed, we consistently observe $W(\infty) > 0$.

Figs. 8 and 9 depict the situation when interferon is not produced. In such case no resistant cells arise. The wild-type cell population decays in both cases. The total number of uninfected cells, $W(\infty) + R(\infty)$, is greater when interferon is produced, than when it is not produced (as in Theorem 3.4).

Finally, Fig. 10 compares the dynamics of the system with linearly “stable” versus unstable initial condition.

4. Infection-age structured model

Under the assumptions at the end of Section 2, the model described by (3.7)–(3.11) can be generalised in the following way:

$$W'(t) = -v(t)W(t) - i(t)W(t), \tag{4.1}$$

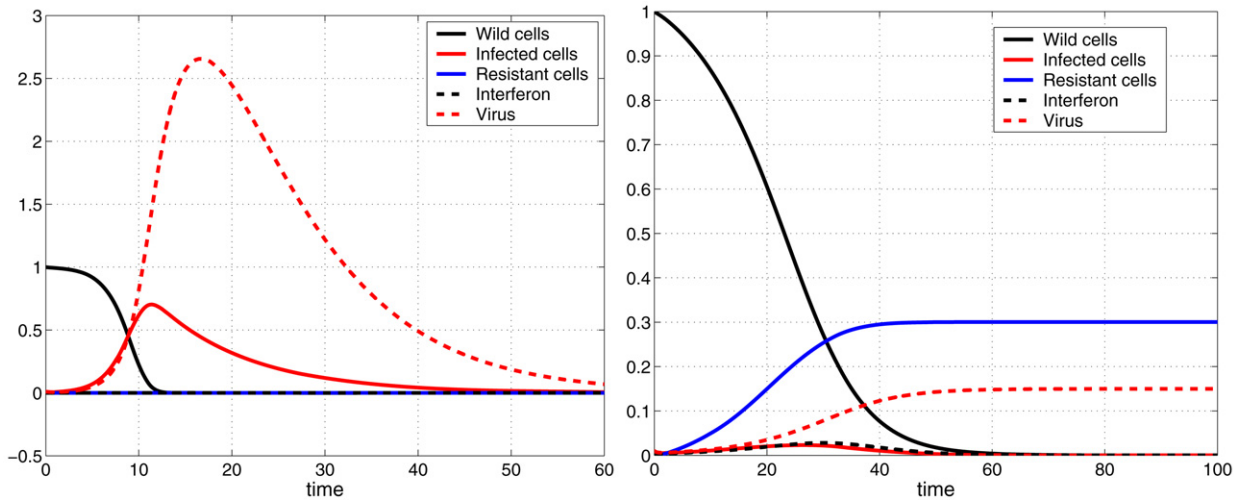


Fig. 8. Simulations with parameters as in Fig. 5 and no IFN synthesis, initial value $W_0 = 1$ and $v_0 = 0.5$, and $\mu_v = 0.2$ on the left-hand side and $\mu_v = 0$ on the right-hand side.

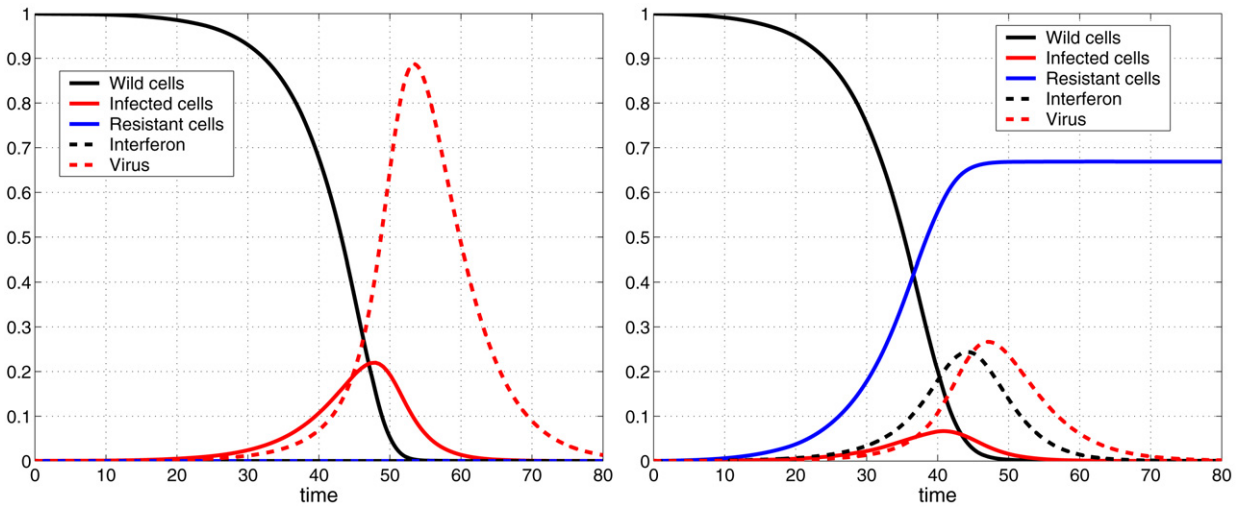


Fig. 9. Simulations illustrating the role of IFN synthesis in the unstable case for $v_0 = 0.001$ and $\mu_v = 0.2$. Simulations presented on the left-hand side correspond to the model without IFN synthesis, i.e., $\alpha_i = 0$ while simulations on the right-hand side correspond to a large IFN synthesis $\alpha_i = 2$.

$$\frac{\partial I(t, a)}{\partial t} + \frac{\partial I(t, a)}{\partial a} = -\mu_I(a)I(t, a), \quad a > 0, \tag{4.2}$$

$$R'(t) = i(t)W(t), \tag{4.3}$$

$$i'(t) = -\mu_i i(t) + \int_0^\infty \alpha_i(a)I(t, a) da - \alpha_3 i(t)W(t), \tag{4.4}$$

$$v'(t) = -\mu_v v(t) + \int_0^\infty \alpha_v(a)I(t, a) da - \alpha_4 v(t)W(t), \tag{4.5}$$

for $t > 0$ with boundary condition

$$I(t, 0) = v(t)W(t), \quad t > 0, \tag{4.6}$$

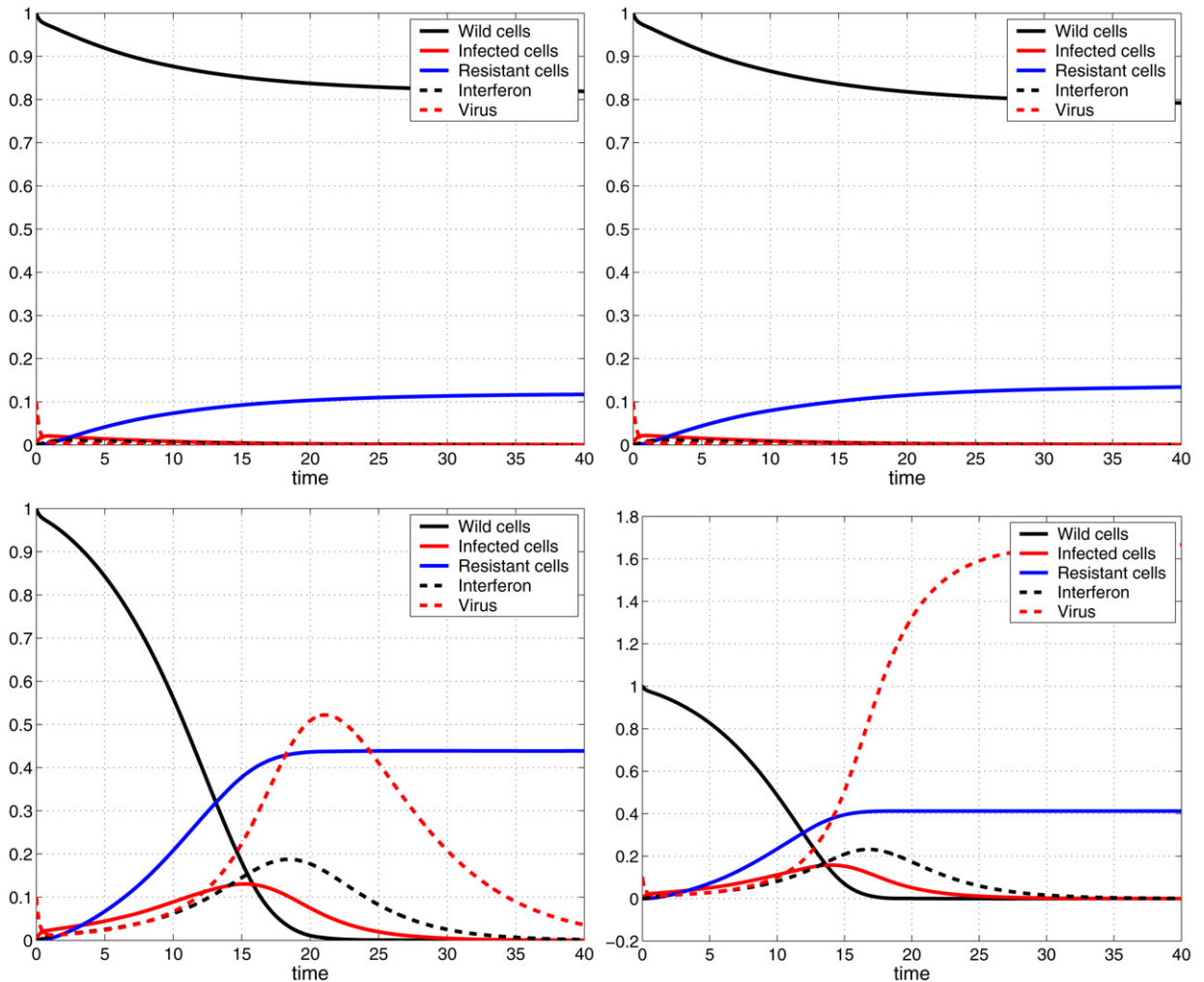


Fig. 10. Simulations illustrating local “stability” (in the upper panel) and instability (in the lower panel). Simulations were performed for the parameters: $\alpha_3 = 1, \alpha_4 = 4, \mu_I = 0.3, \mu_i = 0.4, \alpha_i = 0.8$ and initial concentration $W_0 = 1, v_0 = 0.1$ and for the different μ_v rates: $\mu_v = 0.2$ on the left-hand side, and $\mu_v = 0$ on the right-hand side. Simulations presented in the upper panel were performed for $\alpha_v = 0.8$, for which the stability condition is fulfilled while the simulations presented in the lower panel correspond to the unstable case with $\alpha_v = 2$.

and initial conditions

$$W(0) = W_0, \quad R(0) = R_0, \quad i(0) = i_0, \quad v(0) = v_0, \tag{4.7}$$

$$I(0, a) = I_0(a), \quad a \geq 0. \tag{4.8}$$

One can show that the structured model (4.1)–(4.8) is equivalent to the unstructured model (3.7)–(3.11) if defining $I(t) := \int_0^\infty I(t, a) da$ in the unstructured model and if the infection age dependent functions $\mu_I(a), \alpha_v(a)$ and $\alpha_i(a)$ are constant on $[0, \infty)$. Indeed, integrating Eq. (4.2) in respect to a and applying the boundary condition (4.6) we obtain the equation describing the dynamics of the total number of infected cells,

$$\frac{d}{dt} \int_0^\infty I(a, t) da = - \int_0^\infty \mu_I(a) I(a, t) da + v(t)W(t), \tag{4.9}$$

which is equivalent to Eq. (3.8) for a constant parameter μ_I .

Structuring the density of infected cells with respect to infection age a allows incorporating variability of the production rates of virus and IFN as well as variability of the decay rate of infected cells. This variability results

from intracellular dynamics and is observed in experiments. In particular, we may assume that new virions and IFN molecules are produced by the infected cell only in some bounded intervals of infection age $[h_{v1}, h_{v2}]$ for virus and $[h_{i1}, h_{i2}]$ for IFN, which results in bounded supports of the functions $\alpha_v(a)$ and $\alpha_i(a)$. In what follows we assume that $\text{supp } \alpha_v \subset [0, h]$ and $\text{supp } \alpha_i \subset [0, h]$ for some $h > 0$, so that in the integrals in (4.4) and (4.5) we may take upper bounds equal to h .

To analyse the nonlinear PDE model, we reformulate the problem as a delay-differential equation. In this formulation we can use the theory established for such equations in [2]. We will first establish existence and uniqueness in Section 4.1. Next, we consider asymptotic behaviour in Section 4.2 and finally we analyse equilibria and stability in Section 4.3.

4.1. Existence and uniqueness, and reformulation as a Delay-Differential Equation

We decouple the equations for W , R , i and v from the equation for infected cells and reformulate the problem as a delay-differential equation (DDE). Integrating (4.2), (4.6) and (4.8) along characteristics we obtain an expression for $I(t, a)$ in terms of v and W (for details see Appendix A). For a general introduction to this method for this type of equations we refer to III.4 in [13].

$$I(t, a) = \begin{cases} v(t - a)W(t - a)e^{-\int_0^a \mu_I(\alpha) d\alpha} & \text{for } t > a \geq 0, \\ I_0(a - t)e^{-\int_{a-t}^a \mu_I(\alpha) d\alpha} & \text{for } a \geq t \geq 0. \end{cases} \tag{4.10}$$

The expression suggests to replace the initial condition for I by initial conditions for v and W on $[-h, 0]$, or, in other words, extend the boundary condition (4.6) to $[-h, \infty)$. This includes the possibility that infected cells emerge from wild-type cells becoming infected at times in $[-h, \infty)$. The reformulation of (4.1)–(4.8) then results in

$$I(t, a) = v(t - a)W(t - a)e^{-\int_0^a \mu_I(\alpha) d\alpha}, \quad t > 0, a \geq 0, \tag{4.11}$$

$$W'(t) = -v(t)W(t) - i(t)W(t), \tag{4.12}$$

$$R'(t) = i(t)W(t), \tag{4.13}$$

$$i'(t) = -\mu_i i(t) + \int_0^h \alpha_i(a)v(t - a)W(t - a)e^{-\int_0^a \mu_I(\alpha) d\alpha} da - \alpha_3 i(t)W(t), \tag{4.14}$$

$$v'(t) = -\mu_v v(t) + \int_0^h \alpha_v(a)v(t - a)W(t - a)e^{-\int_0^a \mu_I(\alpha) d\alpha} da - \alpha_4 v(t)W(t) \tag{4.15}$$

for $t > 0$ and

$$(W, R, i, v)(t) = (W^0, R^0, i^0, v^0)(t), \quad t \in [-h, 0], \tag{4.16}$$

where the right-hand side is a vector of assumed functions on $[-h, 0]$. Note that (4.12)–(4.16) is a closed system decoupled from (4.11) and a solution of (4.12)–(4.16) leads to a solution of (4.11) and hence to a solution of the entire system.

Remark 4.1. The transport equation formulation of the structured problem (4.1)–(4.8) is not fully equivalent to the DDE formulation (4.11)–(4.16). Indeed, let us inspect expressions (4.10) for $a > t \geq 0$. We would like to replace $I_0(\tau) \exp[-\int_\tau^a \mu_I(\alpha) d\alpha]$ with $v(-\tau)W(-\tau) \exp[-\int_0^a \mu_I(\alpha) d\alpha]$, where $\tau = a - t \in (0, a]$. Equating these, we obtain the condition

$$v(-\tau)W(-\tau) = I_0(\tau)e^{\int_0^\tau \mu_I(\alpha) d\alpha}. \tag{4.17}$$

Accordingly, for each $\tau > 0$, given initial conditions $(v(-\tau), W(-\tau))$ we obtain $I_0(\tau)$. The opposite causes some problems: Given $I_0(\tau)$, we can obtain $v(-\tau)W(-\tau)$, which is satisfied by infinitely many functions $v(-\tau)$ and $W(-\tau)$, $\tau > 0$. However, it is not guaranteed that if $v(-\tau)$ and $W(-\tau)$ are continuous, $v(-0) = v(0)$ nor $W(-0) = W(0)$, where $v(0)$ and $W(0)$ are initial conditions for v and W in the original system. Therefore, it is possible that the structured system leads to a DDE system, with discontinuous initial conditions $v(-\tau)$ and $W(-\tau)$. This does not constitute a difficulty, provided that the maximum delay h is finite, since the DDE solutions are continuous for $t \geq h$.

We use the standard notation for functional differential equations, see [6]

$$x_t(\theta) := x(t + \theta),$$

where $t > 0$, $\theta \in [-h, 0]$ and $x_t \in C[-h, 0]$. Then (4.12)–(4.15) can be written as

$$W'(t) = -v_t(0)W_t(0) - i_t(0)W_t(0),$$

$$R'(t) = i_t(0)W_t(0),$$

$$i'(t) = -\mu_i i_t(0) + \int_0^h A_i(a)v_t(-a)W_t(-a) da - \alpha_3 i_t(0)W_t(0),$$

$$v'(t) = -\mu_v v_t(0) + \int_0^h A_v(a)v_t(-a)W_t(-a) da - \alpha_4 v_t(0)W_t(0),$$

where we introduced $A_j(a) := \alpha_j(a)e^{-\int_0^a \mu_l(\alpha)d\alpha}$, $j \in \{i, v\}$ and $a \in [0, h]$. With $x := (W, R, i, v)$, this has the form

$$x'(t) = F(x_t), \quad t \geq 0 \tag{4.18}$$

where for $\varphi := (W, R, i, v) \in C([-h, 0], \mathbb{R}^4)$, one has

$$F(\varphi) = \begin{pmatrix} -v(0)W(0) - i(0)W(0) \\ i(0)W(0) \\ -\mu_i i(0) + \int_0^h A_i(a)v(-a)W(-a) da - \alpha_3 i(0)W(0) \\ -\mu_v v(0) + \int_0^h A_v(a)v(-a)W(-a) da - \alpha_4 v(0)W(0) \end{pmatrix}. \tag{4.19}$$

Let us define $\varphi^0 := (W^0, R^0, i^0, v^0)$, then (4.16) becomes

$$x_0 = \varphi^0, \tag{4.20}$$

where the subscript 0 refers to the new notation, and our original system can now be reformulated as (4.11) and (4.18)–(4.20). To guarantee existence and uniqueness of solutions we make the mathematical setting a bit more precise. For DDE of the form (4.18), (4.20) existence and uniqueness of a local solution is guaranteed if

$$F : C([-h, 0], \mathbb{R}^4) \rightarrow \mathbb{R}^4$$

is locally Lipschitz, see Section VII.6 in [2].

In Section 5 we will consider examples of discontinuous functions α_i and α_v . The integrals in (4.19) should therefore be understood as Riemann–Stieltjes integrals as we will explain now. The dual space of $C[-h, 0]$, which will also play a role in the linearisation below, can be represented by $NBV([-h, 0], \mathbb{R}^4)$, the Banach space of normalised functions of bounded variation via the Riemann–Stieltjes integral: Here we call a function A of bounded variation *normalised*, if $A(0) = 0$ and if $A(s-0) = A(s)$ on $(-h, 0)$, i.e., if A is continuous from the left on $(-h, 0)$. Then we assume that α_i , α_v and μ_l are L^1 and that the functions

$$a \mapsto \int_0^a \alpha_j(\alpha)e^{-\int_0^\alpha \mu_l(\sigma)d\sigma}, \quad j \in \{i, v\},$$

are absolutely continuous and thus normalised functions of bounded variations. Hence these functions can be paired with the continuous function $a \mapsto v(-a)W(-a)$ via the Riemann–Stieltjes integral and the resulting integrals equal the ones in (4.19). For simplicity however, for the time being, we keep the notation in (4.19) also for discontinuous α_i and α_v .

Next, one can show that F is locally Lipschitz on $C([-h, 0], \mathbb{R}^4)$. From boundedness of solutions follows global existence, see [2, Proposition VII 2.2], or, for a result related to the boundedness of F , Proposition VII 6.5. Then, in summary we obtain similar results as in the ODE model.

Theorem 4.2. *Suppose that all initial conditions are nonnegative and define the total number of cells as*

$$N(t) := W(t) + R(t) + N_I(t)$$

with the total number of infected cells as

$$N_I(t) := \int_0^\infty I(t, a) da,$$

then

- (a) N, W, R, N_I, i and v are nonnegative,
- (b) N and W are nonincreasing and R is nondecreasing,
- (c) N, W, R, N_I and $\int_0^t \int_0^\infty \mu_I(a) I(s, a) da$ are uniformly bounded,
- (d) if μ_I is bounded away from zero, then $\int_0^t N_I(s) ds$ is uniformly bounded. If additionally α_v , respectively α_i is bounded, so is v , respectively i . Elements of L^1 are equivalence classes; therefore when we call them bounded, we mean that their representatives are bounded outside a set of measure zero,
- (e) all solutions exist for all $t \geq 0$.

Proof. First, integration of (4.12) yields

$$W(t) = W(0)e^{-\int_0^t (v(s)+i(s)) ds}, \tag{4.21}$$

from which follows that W is nonnegative and nonincreasing. Suppose now that v becomes negative and choose the first t where this happens, i.e., such that $v(t) = 0$ and $v'(t) < 0$. Then, on the other hand from (4.15) one obtains that

$$v'(t) = \int_0^h \alpha_v(a)v(t-a)W(t-a)e^{-\int_0^a \mu_I(\alpha) d\alpha} da \geq 0,$$

which is a contradiction. Using then nonnegativity of v and W , nonnegativity of i follows from (4.14) in a similar way as for v . Moreover, since i and W are nonnegative, by (4.13) R is nonnegative and nondecreasing. Finally, nonnegativity of N_I is clear by Eq. (4.9). Then, also N is nonnegative. To show the remaining properties note first that

$$N'_I(t) = - \int_0^\infty \mu_I(a) I(t, a) da + v(t)W(t). \tag{4.22}$$

Next, by adding (4.12), (4.13) and (4.22) we obtain that

$$W'(t) + R'(t) + N'_I(t) + \int_0^\infty \mu_I(a) I(t, a) da = 0,$$

from which follows that N is nonincreasing. After integration, we obtain

$$W(t) + N_I(t) + R(t) + \int_0^t \int_0^\infty \mu_I(a) I(s, a) da ds = W_0 + N_I(0) + R_0, \tag{4.23}$$

which implies uniform boundedness of W, R, N_I, N and $\int_0^t \int_0^\infty \mu_I(a) I(s, a) da ds$.

This latter implies that $\int_0^t N_I(s) ds$ is bounded, because μ_I is bounded away from zero. The boundedness of i then follows after integration of (4.4) and using the boundedness of α_i and $\int_0^t N_I(s) ds$. The boundedness of v follows similarly. \square

For a sharper version of (d), see Theorem 4.5 below.

4.2. Asymptotic behaviour

In Theorem 3.1(e) we analysed asymptotic behaviour for the unstructured model under the assumptions of strict positivity of parameters. We show here that the result can be generalised to the structured case if one replaces positivity by pointwise positivity of the corresponding functions. In this way, we also generalise the result to biologically relevant cases, where virus production and infected cells' mortality have disjoint support, cases that will be considered in more detail in Section 5. We start with a technical result that will be used to prove Theorem 4.5, our main result in this subsection.

Proposition 4.3. *Suppose that there exists a nonnegative function $g \in L^\infty$, defined on $[0, M]$ for some $M > 0$, a constant $C_1 > 0$ and a function $f \in L^\infty$, such that*

$$f(a) \leq C_1(\mu_I * g)(a), \quad (4.24)$$

for all $a \geq 0$, where $(\mu_I * g)(a) := \int_0^M g(\tau)\mu_I(\tau + a) d\tau$, then there exists a constant C_2 such that

$$\int_0^t \int_0^\infty f(a)I(s, a) da ds \leq C_2 \int_0^{t+M} \int_0^\infty \mu_I(a)I(s, a) da ds. \quad (4.25)$$

Remark 4.4. Integrals in expression (4.25) exist, since f is essentially bounded and $I(t, \cdot)$ is integrable.

Proof of Proposition 4.3. Using (4.24), we estimate

$$\int_0^t \int_0^\infty f(a)I(s, a) da ds \leq C_1 \int_0^t \int_0^\infty (\mu_I * g)(a)I(s, a) da ds = C_1 \int_0^t \int_0^\infty \int_0^M g(\tau)\mu_I(a + \tau)I(s, a) d\tau da ds. \quad (4.26)$$

Applying the property of the solution $I(t, s)$,

$$I(s + \tau, a + \tau) = I(s, a)e^{-\int_a^{a+\tau} \mu_I(\eta) d\eta},$$

which is a consequence of (4.11), we can estimate the right-hand side of (4.26) as

$$\begin{aligned} C_1 \int_0^t \int_0^\infty \int_0^M g(\tau)\mu_I(a + \tau)I(s + \tau, a + \tau)e^{\int_a^{a+\tau} \mu_I(\eta) d\eta} d\tau da ds \\ \leq C_2 \int_0^t \int_0^\infty \int_0^M g(\tau)\mu_I(a + \tau)I(s + \tau, a + \tau) d\tau da ds, \end{aligned}$$

where $C_2 = C_1 e^{\int_0^h \mu_I(\eta) d\eta}$. Changing the variables $a' = a + \tau$ and $s' = s + \tau$, the last term can then be estimated as

$$\begin{aligned} C_2 \int_0^M g(\tau) \int_\tau^{t+\tau} \int_\tau^\infty \mu_I(a')I(s', a') da' ds' d\tau &\leq C_2 \int_0^M g(\tau) \int_0^{t+M} \int_0^\infty \mu_I(a')I(s', a') da' ds' d\tau \\ &= C_2 \|g\| \int_0^{t+M} \int_0^\infty \mu_I(a')I(s', a') da' ds', \end{aligned}$$

by which we conclude that (4.25) holds for an appropriately re-defined constant C_2 . \square

In the following theorem we call a function *eventually bounded away from zero*, if there exist $K, \delta > 0$, such that $f(t) \geq \delta$ for all $t \geq K$. If K can be chosen as zero, then f is *bounded away from zero*.

Theorem 4.5. *R(t) tends to a positive value as $t \rightarrow \infty$. If condition (4.24) holds for $f = \alpha_v$, respectively $f = \alpha_i$, then v , respectively i tend to zero. If it holds for both choices then W tends to a positive limit. If μ_I is eventually bounded away from zero, then $\int_0^t N_I(s) ds$ is bounded and N_I tends to zero.*

Proof. The assertion that R tends to a positive limit follows from Theorem 4.2. Next, integrating (4.5), yields

$$v(t) + \alpha_4 \int_0^t W(s)v(s) ds + \mu_v \int_0^t v(s) ds = v_0 + \int_0^t \int_0^\infty \alpha_v(a)I(s, a) da ds, \tag{4.27}$$

from which follows that

$$\mu_v \int_0^t v(s) ds \leq \int_0^t \int_0^\infty \alpha_v(a)I(s, a) da ds + v_0. \tag{4.28}$$

Hence, to conclude that $\int_0^t v(s) ds$ is uniformly bounded, it is sufficient to assure the boundedness of $\int_0^t \int_0^\infty \alpha_v(a)I(s, a) da ds$. Boundedness holds by Proposition 4.3 and boundedness of $\int_0^t \int_0^\infty \mu_I(a)I(s, a) da ds$, which was shown in Theorem 4.2(c). Analogously one shows that $\int_0^t i(s) ds \rightarrow \int_0^\infty i(s) ds < \infty$ as $t \rightarrow \infty$. Then, by (4.21), we obtain that $W(\infty) > 0$. Finally, applying Proposition 4.3 to $f = 1$ (compare Remark 4.6), we conclude that there exists a constant C_2 such that

$$\int_0^t \int_0^\infty I(s, a) da ds \leq C_2 \int_0^{t+M} \int_0^\infty \mu_I(a)I(s, a) da ds.$$

Therefore, the previous argument yields that $\int_0^\infty I(t, a) da$ tends to zero for t tending to ∞ . \square

Remark 4.6. (a) For $f = \alpha_v$, the estimate (4.25) is automatically fulfilled if there exists a pointwise estimate $\alpha_v(a) \leq C\mu_I(a)$ for some constant C , which can be found if μ_I is bounded away from zero on the support of α_v . The condition formulated in Proposition 4.3, however, is significantly weaker. It is e.g. also fulfilled for the choice of parameters in Lemma 5.1 below.

(b) For $f = 1$, condition (4.24) is equivalent to the existence of $M, c > 0$, such that for all $a \geq 0$

$$\int_0^M \mu_I(\tau + a) d\tau \geq c.$$

This condition is automatically fulfilled if μ_I is eventually bounded away from zero.

4.2.1. Subcase: No virus decay in the structured model

Similarly as in the ODE case, for a special case of the model, where $\mu_v = 0$, i.e., when there is neither natural degradation of virions nor uptake other than by endocytosis, we obtain the following result.

Lemma 4.7. *Suppose that $\mu_v = 0$, μ_I is eventually bounded away from zero and that the function $f(a) = \alpha_v(a) - \alpha_4\mu_I(a)$ is nonnegative and fulfils (4.24), then v converges to a positive limit $v(\infty) \geq v(0)$ and W converges to zero.*

Proof. First note that similarly as in the unstructured model, one obtains

$$v(t) - v_0 = \int_0^t \int_0^\infty [\alpha_v(s) - \alpha_4\mu_I(s)]I(\tau, s) ds d\tau + \int_0^t N_I(s) ds. \tag{4.29}$$

The function on the right-hand side is then positive and increasing and by Proposition 4.3 the right-hand side is dominated by

$$C \int_0^{t+M} \int_0^\infty \mu_I(s) I(\tau, s) ds d\tau + \int_0^t N_I(s) ds$$

for some constant C . This expression is bounded as shown in the proof of Theorem 4.5. Hence, the right-hand side converges to a positive limit and the statement follows. Finally, then W converges to zero by (4.21). \square

4.3. Equilibria and linearised stability

An equilibrium for a DDE of the form (4.18) is a constant function \bar{x} with $F(\bar{x}) = 0$. The resulting conditions are

$$\begin{aligned} -\bar{v}\bar{W} - \bar{i}\bar{W} &= 0, \\ \bar{i}\bar{W} &= 0, \\ -\mu_i\bar{i} + \bar{v}\bar{W}\bar{A}_i - \alpha_3\bar{i}\bar{W} &= 0, \\ -\mu_v\bar{v} + \bar{v}\bar{W}\bar{A}_v - \alpha_4\bar{v}\bar{W} &= 0, \end{aligned}$$

defining $\bar{A}_j := \int_0^h A_j(a) da$, $j \in i, v$, which we supplement by the condition

$$\bar{I}(a) = \bar{v}\bar{W}e^{-\int_0^a \mu_I(\alpha) d\alpha}.$$

We obtain the same structure of equilibria for the DDE as for the ODE, i.e., a plane of disease-free steady states of the form

$$(\bar{W}, 0, \bar{R}, 0, 0).$$

For the case of delay-differential equations, the center manifold corresponding to 0-real part eigenvalues of the linearised problem, has been discussed for example in Chapter 10, Section 4 of Hale's book [6] (see also [2]). Without further discussion, the behaviour of the DDE problem in the center manifold can be reduced to that of an ODE. This allows using stability analysis along the lines of Theorem 14.4.1 in [1], as discussed in Section 3.3.

The characteristic equation can be defined as

$$\det(zI - DF(\bar{\varphi})e_z) = 0, \quad e_z(\theta) = e^{z\theta}, \quad \theta \in [-h, 0],$$

where the Riesz-representation theorem explains the notation: As the map $F : C([-h, 0], \mathbb{R}^4) \rightarrow \mathbb{R}^4$ is differentiable, we can represent its derivative by an element of the dual space. We represent the dual space of $C([-h, 0], \mathbb{R}^4)$ by $NBV([-h, 0], \mathbb{R}^4)$ as defined in Section 4.1 via the Riemann–Stieltjes integral. Then by Riesz' theorem, there exists a matrix-valued function $A \in NBV([-h, 0], \mathbb{R}^{4 \times 4})$ such that

$$\begin{aligned} (DF)(\bar{\varphi})\varphi &= \int_{[-h, 0]} \phi(s) d_s A(s) \quad (4\text{-vector}), \\ (DF)(\bar{\varphi})e^z &= \int_{[-h, 0]} e^{zs} d_s A(s) \quad (4 \times 4\text{-matrix}). \end{aligned}$$

We quote Theorem 6.8 from [2].

Theorem 4.8. *The steady state $\bar{\varphi}$ is*

- (i) *unstable, if $\text{Re } \lambda > 0$ for some root λ of the characteristic equation,*
- (ii) *locally exponentially stable, if $\text{Re } \lambda < 0$ for all roots λ of the characteristic equation.*

In our case the NBV-matrix becomes for $a \in [-h, 0]$

$$A(a) = \left(\begin{array}{cc|cc} (-\bar{v} - \bar{i})\delta_0(a) & 0 & -\bar{W}\delta_0(a) & -\bar{W}\delta_0(a) \\ \bar{i}\delta_0(a) & 0 & \bar{W}\delta_0(a) & 0 \\ -\bar{v} \int_0^{-a} A_i(\alpha) d\alpha - \alpha_3 \bar{i}\delta_0(a) & 0 & (-\mu_i - \alpha_3 \bar{W})\delta_0(a) & -\bar{W} \int_0^{-a} A_i(\alpha) d\alpha \\ -\bar{v} \int_0^{-a} A_v(\alpha) d\alpha - \alpha_4 \bar{v}\delta_0(a) & 0 & 0 & (-\mu_v - \alpha_4 \bar{W})\delta_0(a) - \bar{W} \int_0^{-a} A_v(\alpha) d\alpha \end{array} \right).$$

We are now interested in the stability of disease-free equilibria $(\bar{W}, \bar{R}, 0, 0)$. The characteristic equation then has the form

$$\det \left(\begin{array}{cc|cc} z & 0 & \bar{W} & \bar{W} \\ 0 & z & -\bar{W} & 0 \\ 0 & 0 & z + \mu_i + \alpha_3 \bar{W} & -\bar{W} \int_0^h A_i(\alpha) e^{-z\alpha} d\alpha \\ 0 & 0 & 0 & z + \mu_v + \alpha_4 \bar{W} - \bar{W} \int_0^h A_v(\alpha) e^{-z\alpha} d\alpha \end{array} \right) = 0.$$

For the trivial equilibrium, the above yields the nonpositive eigenvalues

$$0, 0, -\mu_i, -\mu_v$$

as in the ODE case. For the disease free equilibrium, we have zero and negative eigenvalues $0, 0, -\mu_i - \alpha_3 \bar{W}$. To determine the remaining eigenvalues, we investigate the roots of

$$z + \mu_v + \alpha_4 \bar{W} - \bar{W} \int_0^h A_v(\alpha) e^{-z\alpha} d\alpha = 0. \tag{4.30}$$

Note first that if z is a zero of (4.30), then so is \bar{z} . We state a result taken from [5], which is a consequence of the Mikhailov criterion of asymptotic stability. The proof can be found in Appendix A. We include it, because the original proof in [5] is split into a proof for discrete delays and additional features, whereas we here explicitly use a priori bounds for the spectrum and Lebesgue’s dominated convergence theorem. For another criterion of exponential asymptotic stability of characteristic equations for delay-differential equations, see [16]. Let $\Delta = \Delta_{\omega \in [0, \infty)} \arg \gamma(\omega)$ denote the change of the argument of a curve γ in \mathbb{C} , taking into account windings and orientation.

Lemma 4.9. *Let $Q(z) = z + \alpha + \beta \int_0^h A(a) e^{-za} da$ and suppose that*

- (i) Q has no zeros on the imaginary axis,
- (ii) there exist real numbers k_1, k_2 , such that $|\operatorname{Re} z| \leq k_1, |\operatorname{Im} z| \leq k_2$ for all zeros with $\operatorname{Re} z > 0$,

then the number of zeros in the right half-plane is equal to $\frac{1}{2} - \frac{\Delta}{\pi}$.

In particular, if (i) and (ii) are met, we can conclude that all roots are located in the left half-plane, if and only if $\Delta = \frac{\pi}{2}$. To apply this result, we first give conditions under which Q as defined by the left-hand side of (4.30) has no zeros on the imaginary axis.

Lemma 4.10. *Suppose that either of the following conditions hold:*

- (a) $\bar{W} \int_0^h A_v(a) da < \mu_v + \alpha_4 \bar{W}$,
- (b) $\bar{W} \int_0^h A_v(a) da < \pi$ and $\bar{W} \int_{-h}^0 A_v(a) da \neq \mu_v + \alpha_4 \bar{W}$,

then Q has no zeros on the imaginary axis.

Proof. $Q(i\omega) = 0$ if and only if $\operatorname{Re} Q(i\omega) = 0$ and $\operatorname{Im} Q(i\omega) = 0$, i.e., if and only if

$$0 = \bar{\mu} - \int_0^h \bar{A}_v(a) \cos(\omega a) da, \quad 0 = \omega + \int_0^h \bar{A}_v(a) \sin(\omega a) da,$$

where $\bar{\mu} := \mu_v + \alpha_4 \bar{W}$, $\bar{A}_v(a) := \bar{W} A_v(a)$. If condition (a) is met, then $\operatorname{Re} Q(i\omega) > 0$ for all ω and hence $Q(i\omega) \neq 0$ for all ω . Now suppose that the conditions in (b) are met. The second condition ensures that $\operatorname{Re} Q(0) \neq 0$ and hence that $Q(0) \neq 0$. Next, for $\omega \in (0, \pi]$, it is clear that $\operatorname{Im} Q(i\omega) > 0$, such that also in this case $Q(i\omega) \neq 0$. For $\omega > \pi$, the first condition in (b) guarantees that $\operatorname{Im} Q(i\omega) > 0$. Hence, altogether we have shown that $Q(i\omega) \neq 0$ for all $\omega \geq 0$ and since, as we mentioned, zeros of Q come in complex conjugate pairs, we conclude that Q has no zeros on the imaginary axis. \square

Next, we prove that Q satisfies condition (ii) in Lemma 4.9.

Lemma 4.11. *There exist real numbers k_1, k_2 , such that*

$$|\operatorname{Re} z| \leq k_1, \quad |\operatorname{Im} z| \leq k_2$$

for all solutions z of (4.30) with $\operatorname{Re} z > 0$.

Proof. In the notation of the proof of Lemma 4.10 we have $Q(z) = 0$ if and only if

$$z + \bar{\mu} - \int_0^h \bar{A}_v(\alpha) e^{-z\alpha} d\alpha = 0.$$

For $z = \mu + i\nu$ this is equivalent to

$$\mu + \bar{\mu} - \int_0^h \bar{A}_v(\alpha) e^{-\mu\alpha} \cos(\nu\alpha) d\alpha = 0, \quad \nu + \int_0^h \bar{A}_v(\alpha) e^{-\mu\alpha} \sin(\nu\alpha) d\alpha = 0.$$

Then for $\mu > 0$ we obtain

$$|\mu| \leq |\bar{\mu}| + \left| \int_0^h \bar{A}_v(\alpha) e^{-\mu\alpha} \cos(\nu\alpha) d\alpha \right| \leq |\bar{\mu}| + \int_0^h |\bar{A}_v(\alpha)| d\alpha,$$

$$|\nu| \leq \int_0^h |\bar{A}_v(\alpha)| d\alpha.$$

Then, since zeros of (4.30) come in complex conjugate pairs, the proof is complete. \square

Lemma 4.12. *If*

$$\bar{W} \int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da < \mu_v + \alpha_4 \bar{W}, \tag{4.31}$$

then all possible roots of Q lie in the left half plane, whereas if

$$\mu_v + \alpha_4 \bar{W} < \bar{W} \int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da < \pi, \tag{4.32}$$

then exactly one root lies in the right half plane.

Proof. First note that in either case $Q(0) = \bar{\mu} - \int_0^h \bar{A}_v(a) da$, recall the definitions in the proof of Lemma 4.10, and Q has no zeros on the imaginary axis by Lemma 4.10. Next, if (4.31) holds, we have $\arg Q(0) = 0$ and $\operatorname{Re} Q(i\omega)$ is always positive, which shows that there are no windings, such that $\arg Q(i\infty) = \frac{\pi}{2}$ and hence $\Delta = \frac{\pi}{2}$. If (4.32) holds, one has $\arg Q(0) = \pi$ and again there are no windings because in the proof of Lemma 4.10 we showed that $\operatorname{Im} Q(i\omega)$ is positive for all $\omega > 0$. Hence $\Delta = -\frac{\pi}{2}$ so that the number of roots in the right half-plane is exactly one. \square

Remark 4.13. The exponential stability result Corollary 7.4.1 in [6] requires that the real parts of the roots of Q remain uniformly separated from zero. This is satisfied if these roots do not have finite accumulation points. However, the Mikhailov criterion (see Lemma 4.9 and [5]) only assumes that the real parts remain negative, if $\Delta = \pi/2$. To understand that the latter is sufficient to ensure the former, it is necessary to consider spectral properties of the semigroup $T(t)$ in $C([-h, 0])$ generated by the solution x_t of the Cauchy problem $x'(t) = \int_{[-h, 0]} d_s A(s)x_t(s)$, $x_0(s) = \phi(s)$, such that $T(t)\phi = x_t$. It is known [6, Lemma 7.1.1] that $T(t)$, $t \geq 0$, is strongly continuous and it is compact for $t \geq h$. Therefore, its spectrum consists of isolated eigenvalues with the possible exception of $\{0\}$ [9, Theorem 5.7]. The roots of Q are eigenvalues of the infinitesimal generator A of $T(t)$, $A = \lim_{t \rightarrow 0^+} (1/t)[T(t) - I]$ [6, Lemma 7.2.1]. However, see [9, Theorem 16.7.2], the point spectrum of $T(t)$ is composed of elements $e^{\lambda t}$, where λ is an eigenvalue of A plus possible $\{0\}$. Therefore, the spectrum of A cannot have finite accumulation points.

5. Interpretation of linearised stability results and comparison of structured and unstructured models

We interpret and compare the results obtained from linearised stability for structured and unstructured models. In both, there exists a two-dimensional space of equilibria given as

$$(\bar{W}, 0, \bar{R}, 0, 0)$$

for all \bar{W} and \bar{R} . Also, in both cases, there exist two zero eigenvalues and one negative eigenvalue. In the unstructured model one additional negative eigenvalue $-\mu_I$ exists, whereas in the structured model it does not, since the infected population is decoupled. As we have seen in Lemmas 3.5 and 4.12, in both models the sign of other possible roots of the characteristic equation depends on parameters. Therefore, if (3.24) or (4.31) is fulfilled we speak of a *stabilising effect* in the corresponding model. If for a set of parameters (4.31) is satisfied, but (3.24) is not, we say that *structure has a stabilising effect*. In an analogous manner we define *destabilising effects*. Next let us note that both left-hand sides represent the number of wild-type cells infected per unit of time by all virions expected to be produced by one infected cell during its lifetime. Both right-hand sides represent the virus outflow in terms of decay and internalisation by the wild-type cells. Hence, in both models the stabilising effect results from a trade off between the efficiency of the virus and its outflow. For example, if \bar{W} is small, the virus is not effective, since there are not many encounters with wild-type cells, neither is there much consumption of virus, such that the virus decay dominates and we obtain a stabilising effect. Other causes for a stabilising effect are α_v becoming small or μ_I , μ_v or α_4 becoming large, i.e., small virus production or high infected cells' mortality, virus mortality or virus consumption. Analogously we obtain destabilising effects in each model.

When comparing the models, the interpretation becomes easier, if we divide the inequalities by α_1 and \bar{W} and restore the meaning α_v had before scaling. Then introducing structure has a stabilising effect, if

$$\int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da < \frac{1}{\alpha_1} \left(\frac{\mu_v}{\bar{W}} + \alpha_4 \right) < \frac{\alpha_v}{\mu_I}. \quad (5.1)$$

For appropriate values of α_1 , α_4 , μ_v and \bar{W} , this is the case if

$$\int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da < \frac{\alpha_v}{\mu_I}. \quad (5.2)$$

Analogously, we obtain that introducing structure has a destabilising effect if

$$\frac{\alpha_v}{\mu_I} < \frac{1}{\alpha_1} \left(\frac{\mu_v}{\bar{W}} + \alpha_4 \right) < \int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da < \frac{\pi}{\alpha_1 \bar{W}}. \quad (5.3)$$

Note that if

$$\frac{\alpha_v}{\mu_I} < \int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da \quad (5.4)$$

holds, we can first choose \bar{W} small enough for the last inequality in (5.3) to hold and then adjust μ_v so that all inequalities hold. In this sense we can say that structure has a destabilising effect if (5.4) holds. The new interpretation is that structure has a stabilising (respectively, destabilising) effect if the expected lifetime virus production of an infected cell is lower (respectively, higher) in the structured model than in the unstructured model. In the structured model the effect depends on the time intervals after infection in which virus production occurs and in which the mortality of infected cells is high. (Recall that the infected cells' mortality rate μ_I merely corresponds to decay caused by infection, e.g., apoptosis, and that for all cell-types we disregard natural mortality.) The following caricature examples exploit this idea and provide conditions under which structure has a stabilising or destabilising effect.

In our first example we show that the introduction of structure can have a destabilising effect if virus production is early and the infected cells' mortality is late even if (i) the cumulative virus production, given a cell does not die, is lower in the structured than in the unstructured model, and (ii) the expected lifetime of the infected cell is shorter in the structured model than in the unstructured model.

Let $\chi_A(a)$ denote the indicator function of a set A .

Lemma 5.1. *Suppose that*

$$\alpha_v(a) = \alpha_v^s \chi_{[0, a_1]}(a), \quad \mu_I(a) = \mu_I^s \chi_{[a_1, h]}(a),$$

for some constants μ_I^s , α_v^s (the superscript s standing for "structure"), $\alpha_v^s \in (\alpha_v, h)$, and (for sufficiently large h)

$$a_1 \in \left(\frac{1}{\mu_I^s} \left(\frac{\alpha_v^s}{\alpha_v} - 1 \right)^{-1}, h \right), \quad (5.5)$$

then, one can find a parameter μ_I , such that

$$a_1 + \frac{1}{\mu_I^s} < \frac{1}{\mu_I} < \frac{\alpha_v^s}{\alpha_v} a_1, \quad (5.6)$$

and (5.4) holds, even though for h large enough

$$(i) \quad \int_0^h \alpha_v(a) da < \alpha_v h, \quad (ii) \quad \int_0^h e^{-\int_0^a \mu_I(s) ds} da < \frac{1}{\mu_I}.$$

Proof. First, condition (i) is guaranteed since for large enough h we have

$$\alpha_v^s a_1 < \alpha_v h. \quad (5.7)$$

Then, one computes that

$$\int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da = \alpha_v^s a_1,$$

such that (5.4) is equivalent to

$$\alpha_v^s a_1 > \frac{\alpha_v}{\mu_I}. \quad (5.8)$$

Moreover,

$$\int_0^h e^{-\int_0^a \mu_I(s) ds} da = a_1 + \frac{1}{\mu_I^s} (1 - e^{-\mu_I^s (h-a_1)}) \rightarrow a_1 + \frac{1}{\mu_I^s}$$

for $h \uparrow \infty$. Thus, (ii) is fulfilled for h large enough if

$$a_1 + \frac{1}{\mu_I^s} < \frac{1}{\mu_I}. \quad (5.9)$$

Hence (5.8) and (5.9) are guaranteed because, as (5.5) holds, we can find a μ_I that fulfils (5.6), from which (5.8) and (5.9) follow. \square

In our next example, we show that the introduction of structure can also have a stabilising effect, if the mortality of the infected cell is early and the virus production late, even if (i) the cumulative virus production given the cell does not die is higher in the structured model, and (ii) the expected lifetime of the infected cell is higher in the structured model.

Lemma 5.2. *Suppose that*

$$\alpha_v(a) = \alpha_v^s \chi_{[a_1, h]}(a), \quad \mu_I(a) = \mu_I^s \chi_{[0, a_1]}(a), \tag{5.10}$$

where $\alpha_v^s > \alpha_v$, $a_1 < h$ and h are such that

$$\alpha_v^s(h - a_1) > \alpha_v h, \tag{5.11}$$

μ_I^s is large enough such that

$$e^{-a_1 \mu_I^s} \left[(h - a_1) \left(\frac{\alpha_v^s}{\alpha_v} - 1 \right) + \frac{1}{\mu_I^s} \right] < \frac{1}{\mu_I^s}, \tag{5.12}$$

then one can find a parameter μ_I , such that

$$\frac{\alpha_v^s}{\alpha_v} e^{-a_1 \mu_I^s} (h - a_1) < \frac{1}{\mu_I} < \frac{1}{\mu_I^s} (1 - e^{-\mu_I^s a_1}) + e^{-\mu_I^s a_1} (h - a_1) \tag{5.13}$$

and (5.2) holds even though for large enough h

$$(i) \int_0^h \alpha_v(a) da > \alpha_v h, \quad (ii) \int_0^h e^{-\int_0^a \mu_I(s) ds} da > \frac{1}{\mu_I}.$$

Proof. One has

$$\int_0^h \alpha_v(a) e^{-\int_0^a \mu_I(s) ds} da = \alpha_v^s e^{-a_1 \mu_I^s} (h - a_1),$$

such that (5.2) becomes

$$\alpha_v^s e^{-a_1 \mu_I^s} (h - a_1) < \frac{\alpha_v}{\mu_I}. \tag{5.14}$$

Next, (i) becomes (5.11) and is therefore satisfied. Moreover,

$$\int_0^h e^{-\int_0^a \mu_I(s) ds} da = \frac{1}{\mu_I^s} (1 - e^{-\mu_I^s a_1}) + e^{-\mu_I^s a_1} (h - a_1).$$

Then, condition (ii) can be formulated as

$$\frac{1}{\mu_I^s} (1 - e^{-\mu_I^s a_1}) + e^{-\mu_I^s a_1} (h - a_1) > \frac{1}{\mu_I}. \tag{5.15}$$

Next, note that if (5.12) holds, so does the outer inequality in (5.13) and hence we can find a μ_I such that (5.13) holds. As (5.13) holds, so do (5.14) and (5.15), such that (5.2) and condition (ii) are satisfied and the proof is complete. \square

The following example demonstrates that delaying and shortening the time of new virus synthesis lead to a stabilising effect of structure.

Lemma 5.3. *Suppose that the decay and production parameters take the form*

$$\mu_I(a) = \mu_I, \quad \alpha_v(a) = \alpha_v, \quad (5.16)$$

$$\text{supp } \alpha_v = [h_1, h_2] \subset [0, \infty). \quad (5.17)$$

Then, for h_1 sufficiently large or h_2 sufficiently small, there are steady states $(\bar{W}, 0, \bar{R}, 0, 0)$, which are unstable for the unstructured model, but stable for the structured model.

Proof. Assuming (5.16), then condition (4.31) is equivalent to

$$\frac{\alpha_v}{\mu_I} \bar{W} (e^{-h_1 \mu_I} - e^{-h_2 \mu_I}) < \mu_v + \alpha_4 \bar{W}. \quad (5.18)$$

Assuming that $h_2 < \infty$ leads to $e^{-h_1 \mu_I} - e^{-h_2 \mu_I} < 1$. Therefore, it is clear that (5.18) is weaker than (3.24), i.e., for a steady state \bar{W} , for which (3.24) is satisfied also (5.18) is satisfied for every choice of h_1 and h_2 . On the other hand, if \bar{W} does not satisfy (3.24), then we can choose parameters h_1 and h_2 in such a way that condition (5.18) is satisfied. This means that there are steady states, which are unstable for the unstructured model, but stable if h_1 is sufficiently large or h_2 sufficiently small. \square

The example described in Lemma 5.3 can be interpreted by saying that the unstructured model corresponds to the “maximal” time interval $[0, \infty)$ of virus synthesis by a single infected cell. Possible stabilising effects of structure are especially evident and interesting in the model with no virus mortality, i.e., if $\mu_v = 0$. As we have seen in Section 3.1.1, in the unstructured model, for $\alpha_v > \alpha_4 \mu_I$ the solution $W(t)$ tends asymptotically to zero and hence there cannot exist any positive stable steady states \bar{W} . However, there exist parameters h_1 and h_2 such that (5.18) is satisfied for some positive \bar{W} . Hence, in this case $v(t)$ tends to zero. The asymptotic behaviour of the solutions of the structured system is thus qualitatively different from the behaviour of the solutions of the corresponding unstructured model and while the solution $W(t)$ of the ODE systems tends asymptotically to zero (and $v(t)$ to some positive constant), the solution $W(t)$ of the structured model tends to some positive constant (and $v(t)$ tends to zero). This means that the viral infection can be defeated by prolonging the replication process, i.e., delaying the beginning of the release of virus from a cell, and shortening the time period when a single cell releases new virions. It also shows that in some cases the description of the viral infection using the unstructured model is not sufficient.

6. Discussion

There are two examples of experimental systems, which are employed to follow dynamics of viral infections. One is liquid culture, where viral particles and cells interact under conditions of almost perfect mixing, and where spatial heterogeneity and spread effects do not have to be taken into account. Another type are planar cultures, where it is possible to introduce virions approximately at a point source [4,18]. Under such conditions, propagation dynamics and infection dynamics followed by interferon production and development of resistance can be observed as distributed over the plane of the culture.

The model considered in the present paper accounts for the essential features of viral infection *in vitro*, such as cell infection, death, production of interferon, and development of resistance. We assume that wild-type cells are not replenished. One version of the model involves infection-age structure of infected cells. Mathematically, this variant requires an additional transport-type partial differential equation to model the infection-age structure in infected cells. However, as shown in the paper, the transport process can be reduced to distributed delay terms in two of the model equations. Therefore, the model with structure can be analysed using local linearised stability results for the functional (delay type) differential equation system [6].

More specifically, the methods we used to analyse our models involve both global and local methods. There can be derived a conservation law for the model without structure, application of which guarantees that the solutions converge to limit values as $t \rightarrow \infty$ (Theorem 3.1). The same conservation law allows to conclude that unexpectedly, in the case with virus mortality, there is always a residual population of wild-type cells. When the virus mortality rate is equal to zero, this is not necessarily the case (Lemma 3.2). The conservation law can be extended to the structured case, under some additional hypotheses concerning supports of age-dependent mortality and infectivities (Theorem 4.5). This

law is mathematically interesting, since it is not a complete law as frequently used in the epidemics theory, however together with nonnegativity, it provides upper bounds, which sufficiently constrain the solutions.

Let us notice that the system, both in the unstructured and structured versions, is somewhat unusual in that it does not have unique equilibrium points. The limits to which the system is converging strongly depend on initial conditions. For example, the nonzero limit for the wild-type cells may be very low, so that numerically it may be confused with a zero limit. This property has an impact on the linearised stability. Attracting properties are limited to the subspace spanned by eigenvectors corresponding to nonzero eigenvalues, while the solution slides along the complementary subspace (Section 3.3). In the case of the structured model, considerations of linearised stability can be done using an extension of the Mikhailov criterion (Lemma 4.9).

Conditions of stability, which we obtained in this paper, seem to have interesting biological interpretations. First of all, the influence of structure can be both stabilising and destabilising. As follows from the discussion in Section 5, structure can have a stabilising (respectively, destabilising) effect even if the expected lifetime virus production of an infected cell is higher (respectively, lower) in the structured model than in the unstructured model (Lemmas 5.1 and 5.2). Also, delaying and shortening the time of new virus synthesis lead to a stabilising effect of structure (Lemma 5.3). These results illustrate the importance of the dynamics of the process of virus proliferation and death of the infected cells. In the ODE system, duration of these processes can be understood as being described by exponentially distributed random variables. Our results indicate that this is not always sufficient and illustrate the need to understand these processes.

In [3] a classical SIR-type model based on the Kermack–McKendrick model is proposed and analysed. As these models are designed for individuals and not for cells the mechanisms of infection and resistance are incorporated rather implicitly; susceptible become infected at a rate proportional to their encounters with infected. Infected become resistant at a constant rate. This constitutes a major difference with respect to our model, where only susceptible may become resistant. Finally, in [3], the influx of susceptible occurs at a constant rate whereas it is disregarded in our model. What follows from this assumption, is the existence of an always stable endemic equilibrium. One can show that the equilibrium structure and stability is strongly dependent on the modelling of the influx of susceptibles; the endemic equilibrium becomes unstable when the influx of susceptibles is modelled as proportional to their present number. Furthermore, it does not exist when the influx is assumed to equal to 0, i.e., in our case.

One of the important elements of the model is the presence of a mechanism of interferon-induced virus resistance. Interferon can be produced only by infected cells and confers resistance (in our model a complete resistance) on wild-type cells. This is a very specific mechanism, see a discussion further on. It is interesting that setting the interferon production rate to zero does not qualitatively change the behaviour of the system (Section 3.2). However, it reduces the total number of wild-type and resistant cells as we can deduce from comparison of Figs. 6 and 8. The simulated trajectories of the ODE model generally indicate a strong dependence on initial conditions and parameter values. Although the model we consider is biologically not very detailed, it displays the dynamics that one expects from the biological system.

Recently, a model of viral infection has been developed by Haseltine et al. [7]. The model proposes to take into account both the intracellular and extracellular levels of information. One way of performing this task in a deterministic setting is to derive cell population balances from the equation of continuity. The authors apply such a balance to obtain a two-level model of a viral infection. They then use numerical simulation to demonstrate cell culture and in vivo responses given a variety of experimental conditions. They compare these responses to those obtained from applying other commonly used models. Haseltine's model involves infection age structure, but also spatial spread, at the definition level, not considered when analyzing the model. The model is successfully fitted to simulated data. Having too many equations, however, it seems too complicated to allow analytical approaches.

In his unpublished thesis, Haseltine [8] devised an even more complex model of viral infection, involving interferon (IFN) production. This model involved IFN production by wild-type cells previously exposed to IFN secreted by infected cells, resulting in their resistance to viral infection. Interestingly, IFN autoregulation in epithelial cells is not confirmed by laboratory experiments. For example, in type II airway epithelial cells (A549), IFN β stimulation at saturating concentrations of up to 100 IU/ml produces no detectable IFN expression, whereas in response to RSV infection, the same cells induce IFN β expression by over a 100-fold, which is biologically active [10,14,19]. The lack of IFN autoregulation has been confirmed in HeLa epithelial cells, where IFN stimulation induces less than a 2-fold induction of IFN β gene expression. Therefore, in this model we have omitted IFN autoregulation from this model to more accurately understand the dynamics of infection in epithelial cells. Incorporating this particular mechanism

into the model leads to solutions with qualitative behaviour different than in our model. Therefore, excluding this mechanism is important for an understanding of the dynamics of infection.

Perelson and his co-authors published a number of modelling and data analysis papers, concerning various viral infections (mainly HIV and hepatitis), in which interferons play a major role, albeit mostly as therapeutic agents. However, the importance of the interferon transduction pathways is noticed at least on two occasions: In [12] it is mentioned in the context of comparing response to treatment by White and African American patients with hepatitis C that “the mechanism(s) explaining this difference in treatment effectiveness between races is unclear but could include differences in IFN pharmacokinetics and IFN signal transduction pathways (. . .).” Also, in a recent paper concerning HIV infection [11], it is mentioned that “in mice cytokines, interferons released during the course of an immune response can induce memory T cells to undergo a single division irrespective of their antigen specificity.” These remarks underscore the importance of a correct model of interferon action.

In the paper by Lam et al. [18], focal infection experiments were reported, designed to gain insights into how the innate responses can limit spread of recombinant vesicular stomatitis viruses (VSV) on baby hamster kidney (BHK) and delayed brain tumor (DBT) cell monolayers. It was observed that rates of infection spread were affected by addition of anti-interferon antibody: the antibody enhanced the spread of virus although it did not affect its growth. This demonstrated that viral spread was inhibited by cellular antiviral activities, i.e., interferon production. These observations, although in a different experimental context (monolayer as opposed to liquid culture) are consistent with the dynamics of our model.

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Appendix A

A.1. Integration along the characteristics

In this appendix we provide the details of the reformulation of the structured population model as a delay-differential equations.

First note that cells are either infected at time $t_0 > 0$ or have infection age $a_0 \geq 0$ at time zero. Consider first the cells that at time zero have infection age $a_0 \geq 0$. The amount of these cells evolves according to (4.2) and (4.8), which is equivalent to the ODE

$$\begin{aligned} \frac{d}{dt} I(t, a_0 + t) &= -\mu(a_0 + t) I(t, a_0 + t), \quad t > 0, \\ I(0, a_0) &= I_0(a_0). \end{aligned}$$

This ODE is solved as

$$I(t, a_0 + t) = I_0(a_0) e^{-\int_0^t \mu(a_0 + s) ds}, \quad t \geq 0.$$

Consider now the cells that at time t have infection age $a \geq t$, then we can redefine $a_0 := a - t \geq 0$ and obtain

$$I(t, a) = I_0(a - t) e^{-\int_{a-t}^a \mu(\alpha) d\alpha}, \quad a \geq t \geq 0.$$

Suppose now that a cell gets infected at time $t_0 > 0$. For this cell (4.2) and (4.6) are equivalent to

$$\begin{aligned} \frac{d}{dt} I(t, t - t_0) &= -\mu(t - t_0) I(t, t - t_0), \quad t \geq t_0, \\ I(t_0, 0) &= v(t_0) W(t_0), \end{aligned} \tag{A.1}$$

which we integrate to obtain

$$I(t, t - t_0) = v(t_0)W(t_0)e^{-\int_{t_0}^t \mu(s-t) ds}, \quad t \geq t_0. \tag{A.2}$$

If we redefine $a = t - t_0 \geq 0$, we obtain

$$I(t, a) = v(t - a)W(t - a)e^{-\int_0^a \mu(s) ds}, \quad t > a \geq 0. \tag{A.3}$$

Hence, in summary we have shown (4.10).

Proof of Lemma 4.9. Let $C := C_0 \cup C_+$ denote the closed oriented curve defined by a closed semicircle into the right half-plane in the following way: C_0 denotes the straight line from ir to $-ir$ and C_+ the semicircle from $-ir$ to ir with radius r into the right half plane. The principle of the argument implies that for the total change of the argument along this curve, one has

$$\frac{1}{2\pi} \Delta_C \arg Q = N_z - N_p,$$

where N_z and N_p denote the number of zeros and poles inside C , respectively. Since $N_p = 0$, this is., in obvious notation, equivalent to

$$\frac{1}{2\pi} (\Delta_{C_+} \arg Q + \Delta_{C_0} \arg Q) = N_z.$$

As roots of Q come in complex conjugate pairs, if $\Delta_{C_0}^\infty$ and $\Delta_{C_+}^\infty$ denote the changes of the argument as r tends to infinity, one has

$$\Delta_{C_0}^\infty = -2\Delta.$$

Next note that the number N_z^∞ of zeros inside the circle as r tends to infinity is well defined by condition (ii). Therefore it holds that

$$N_z^\infty = \frac{1}{2\pi} \Delta_{C_+}^\infty \arg Q - \frac{\Delta}{\pi} \tag{A.4}$$

and it remains to compute $\Delta_{C_+}^\infty \arg Q$. We do this by using that for our curve C_+ , we have

$$\frac{1}{2\pi} \Delta_{C_+} \arg Q = \frac{1}{2\pi i} \int_{C_+} \frac{Q'(z)}{Q(z)} dz. \tag{A.5}$$

Let us parametrise C_+ by $z = re^{i\phi}$, $\phi \in (-\frac{\pi}{2}, \frac{\pi}{2})$, then

$$\begin{aligned} \int_{C_+} \frac{Q'(z)}{Q(z)} dz &= \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{izQ'(z)}{Q(z)} \Big|_{z=re^{i\phi}} d\phi \\ &= i \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{z(1 - \beta \int_0^h A(a)ae^{-za} da)}{z + \alpha + \beta \int_0^h A(a)e^{-za} da} \Big|_{z=re^{i\phi}} d\phi \\ &= i \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{e^{i\phi}(1 - \beta \int_0^h A(a)ae^{-ar \cos \phi} e^{-ari \sin \phi} da)}{e^{i\phi} + \frac{\alpha}{r} + \frac{\beta}{r} \int_0^h A(a)e^{-ar \cos \phi} e^{-ari \sin \phi} da} d\phi. \end{aligned}$$

Now observe that

$$|e^{-ar \cos \phi} e^{-ari \sin \phi}| \leq 1, \quad \forall r > 0, \phi \in \left[-\frac{\pi}{2}, \frac{\pi}{2}\right], a \in [0, h], \tag{A.6}$$

and thus

$$\frac{1}{r} \int_0^h A(a)e^{-ar \cos \phi} e^{-ari \sin \phi} da \rightarrow 0 \tag{A.7}$$

as $r \uparrow \infty$ uniformly for $\phi \in [-\frac{\pi}{2}, \frac{\pi}{2}]$. Next note that

$$aA(a)e^{-ar \cos \phi} e^{-ari \sin \phi} \rightarrow 0 \quad (\text{A.8})$$

as $r \uparrow \infty$ almost everywhere for $(a, \phi) \in [0, h] \times [-\frac{\pi}{2}, \frac{\pi}{2}]$ and is also bounded. Therefore by Lebesgue's dominated convergence theorem, we obtain

$$\int_{C_+} \frac{Q'(z)}{Q(z)} dz \rightarrow i\pi \quad \text{as } r \uparrow \infty. \quad (\text{A.9})$$

Combining (A.4), (A.5) and (A.9) we obtain $N_z^\infty = \frac{1}{2} - \frac{\Delta}{\pi}$, which completes the proof. \square

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