Stochastic dynamics of receptor-virus interaction: the case of natural killer cells

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It has recently been suggested that natural killer cells can display adaptive features in response to viral infections, a very different view from their widely accepted, but now obsolete, innate nature. Human cytomegalovius seems to be a key ingredient for the observation of such adaptive responses, inducing a bistable response of a particular subset expressing the NKG2C activating receptor and memory after infection. Here we present a mathematical model of human cytomegalovius induced proliferation and long-time persistence of a natural killer cell subset (NKG2C$^{\mathbf{bright}}$), based on flow cytometry measurements from blood samples of infected and non-infected healthy donors. Our model takes the form of a parabolic partial differential equation describing the time-evolution of the distribution of receptors in a population of natural killer cells. The model assumes that the cell proliferation rate increases with receptor expression during viral infection, and that the virus response takes the form of a transient viral load dynamics with a long-persistent latency stage. These assumptions allow us to interpret the persistence of the NKG2C$^{\mathbf{bright}}$ subset as a non-equilibrium steady state that arises from a specific interplay between production and degradation of receptors, cell differentiation and death.

1 INTRODUCTION

Natural killer (NK) cells are a type of lymphocytes commonly associated with the innate immune system [1]. Despite this accepted role, it has recently been argued that NK cells may also exhibit features that are usually attributed to the adaptive immune system, such as memory of specific antigens after viral infection [2] [3]. This claim concurs with evidence that certain viruses alter specific NK-cell subsets, inducing proliferation and modifying cytotoxic functions [4]. Infection by human cytomegalovirus (HCMV) represents a particular situation in which a subset of NK-cells with high levels of the NKG2C receptor (NKG2C^{bright}) is stimulated to proliferate. Direct correlations have been found between cell surface density of NKG2C and NKG2C^{bright} cell number, suggesting that NKG2C is involved in the differentiation and expansion of this subset [5] [11]. Moreover, this subset seems to persist at high levels over years, raising the question of whether it corresponds to a kind of memory-like collective behavior, possibly associated to the latency of HCMV [12]. There are additional observed features this subset seems to display, such as an increased production of IFN-γ or reduced apoptosis rate. The ligands involved in the triggering of signal pathways are still unknown, and the only data available is phenomenological: information about the host's immune response can only be assessed through its effect on blood samples.

From the point of view of the virus, things become more complicated: real time clinical data is hard to obtain, and therefore it is difficult to keep track of its dynamics and effect in short time intervals. A particular case [13] in which data is available comes from a 3-month-old girl in which immune functions had been monitored for about two months. In the early onset of infection, it was observed that lymphocyte subsets causing the leukocytosis were extremely unbalanced: 90% were CD56^{dim} NK cells, 10% were B cells, while T cells were virtually absent. Moreover, increased values of cytokines (IFNγ) were correlated between viral load. As the viral load decreased, cytokine levels and NK cell numbers also decreased, until the virus could not be detected anymore by the 6th week. By then, cytokine levels were normal and NK cell number also dropped. However, certain subsets of NK cells, in which NKG2C^{bright} is included, remained at high levels after the infection. Such a receptor specific proliferation seems to be a fingerprint of the HCMV [4] [5], and not observed for other viruses. Moreover, the proliferating subset seems to remain at high values for months, at least. How can we explain such behaviour, considering that the virus is latent and supposedly inactive?

Mathematical models of the interaction of viruses with the immune system abound in the literature [6] [7]. Sadly, most of them focus on the dynamics of populations of immune cells (mostly T and B cells) when pathogens are present, and usually ignore the dynamics of cell surface receptors. The aim of this paper is to tackle this very problem in the context of NK cells: how receptors are distributed among the population and how this distribution is altered when external factors such as a virus come into play. We restrict our study to the NKG2C receptor and the effect of HCMV on their expression. To study such dynamics, the process of expression of the NKG2C gene must be adressed. Due to its random nature, gene expression is usually formulated as a stochastic process in which a gene in a cell is transcripted into mRNA molecules and then translated into proteins [8]. One is then usually interested in the *probability distributions* of such proteins, that is, the quantity that gives the probability that a randomly chosen cell has a given amount of proteins. Such function can be obtaind as a solution of a *master equation* [9] [10] corresponding to the stochastic process. However, master equations are usually very difficult to deal with, even in the stationary state. An alternative approach is the use of *stochastic differential equations* (SDE) in which proteins are treated as continuous variables

subject to a noise source. In such approach, the stochastic part must be introduced by hand, as opposed to the master equation in which noise is implicit in the formulation. We will see that the appropiate choice of the noise sources and their strengths, together with biologically relevant approximations, will allow us to construct a relatively simple model in which analytical solutions for the distribution of receptors can be obtained.

2 ASSESSING EXPERIMENTAL DISTRIBUTIONS

There seems to be at least three distinctive features in the HCMV-NK cell interaction:

- Proliferation and increasement of the total number of NK cells.
- Reshaping of the NKG2C subset.
- Memory of the NKG2C^{bright} subset after infection which lasts for years.

Such effects are reflected in measurements obtained by the group of one of us (M. L. B) in which some subjects are infected by HCMV and others not. The data is in the form flow cytometry measurements coming from 78 blood samples of different donor profiles, classified as HCMV$^+$ (infected) and HCMV$^-$ (not infected), as well as by their zygosity with respect to the NKG2C gene: 21 HCMV$^+$ homozygous, 11 HCMV$^+$ hemizygous, 3 HCMV$^+$ nullzygous, 29 HCMV$^-$ homozygous, 9 HCMV$^-$ hemizygous and 5 HCMV$^-$ nullzygous. The observed signal of the flow cytometry measurements corresponds to cell fluorescence, which is proportional to the NKG2C receptor concentration. Nullzygous profiles provide a way to discern between true NKG2C signal and *background* noise coming from the cytometer (Figure 1), as such donors do not express the NKG2C gene.

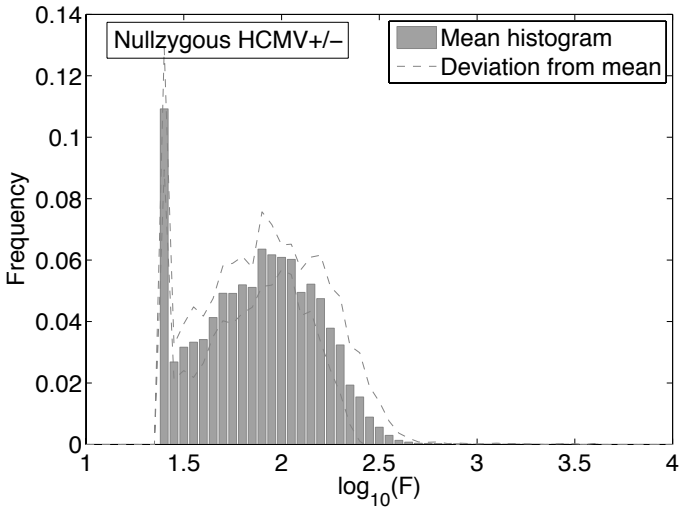

Figure 1: Mean normalized histogram from all (both infected and uninfected) nullzygous donors. Since such profiles do not express NKG2C, the obtained signal must be background noise from the cytometer.

For non-nullzygous donors (Figure 2), there is signal above the threshold value $\theta = 10^{2.6}$ both in HCMV$^{+/-}$ donors. Unfortunately, part of this cells remain hidden behind the background signal, and therefore their fluorescense cannot to be measured. The NKG2C^{dim} subset (low signal) is present in both HCMV$^{+/-}$ individuals, which is understood as a consequence of the balance between the production of receptors and their degradation when no external factors are present. For HCMV$^+$ donors there is a peak at high receptor concentration, the NKG2C^{bright} subset, indicating that HCMV must have, directly or indirectly, a role in such expansion. Notice that in this subset standard deviation is large because some of the individuals do not display an increasement in the NKG2C^{bright} subset. When plotted individually, histograms show high variability, possibly due to heterogeneity in the viral load and on the particular immune response of each subject.

Infected individuals seem to display bimodal distributions, thus suggesting a positive feedback mechanism in the single-cell receptor dynamics. One hypothesis is that it appears as a result of cytokines produced by the NK cells themselves due to the interaction with HCMV infected cells. Such cytokines in turn induce a response in NK cells to produce more receptors, in such a way a bimodal profile is produced. Other models have been proposed [**?**], assessing the nature of the feedback mechanism, whether is pre-transcriptional, post-transcriptional or in both regimes. However, clear evidence of such a mechanism still remains elusive, thus allowing some freedom to study other possible options for the bimodal profiles.

3 METHODS

Chemical reactions ocurring inside cells make gene expression a stochastic process [14]. Both the random transcription of mRNA and the subsequent translation to proteins generates noise which propagates through the whole network [15]. In addition, fluctuations in the amounts or states of other cellular components lead indirectly to variation in the expression of a particular gene and thus represent extrinsic noise [16]. In NK cells, the expression of receptors such as NKG2C are no exception. Stochastic effects of gene expression are reflected in the long tails of the receptor histograms and their large deviation from the mean. Such features cannot be explained without incorporating random effects to their expression.

We consider a simplified system of NK cells producing and degrading receptor proteins (the words *preteins* and *receptors* will be used interchangeably through the remaining text) through simple chemical reactions and then we allow the cells to proliferate in response to HCMV. First we examine the situation in which the virus is not present and then we move to the case in which the individual in infected and NK cells respond subsequently.

3.1 Receptor dynamics in absence of HCMV

In the simplest model of gene expression, a gene (in our case the NKG2C) is copied (transcripted) into messenger RNA (mRNA) molecules, which serve as templates from which proteins are produced (translated) by the ribosome. Here we follow this simplistic attitude and consider only the dynamics of NKG2C. Receptors are assumed to be produced constitutively with rate α and degraded linearly with rate β:

$$A \xrightarrow{\alpha} X \xrightarrow{\beta} \emptyset \tag{1}$$

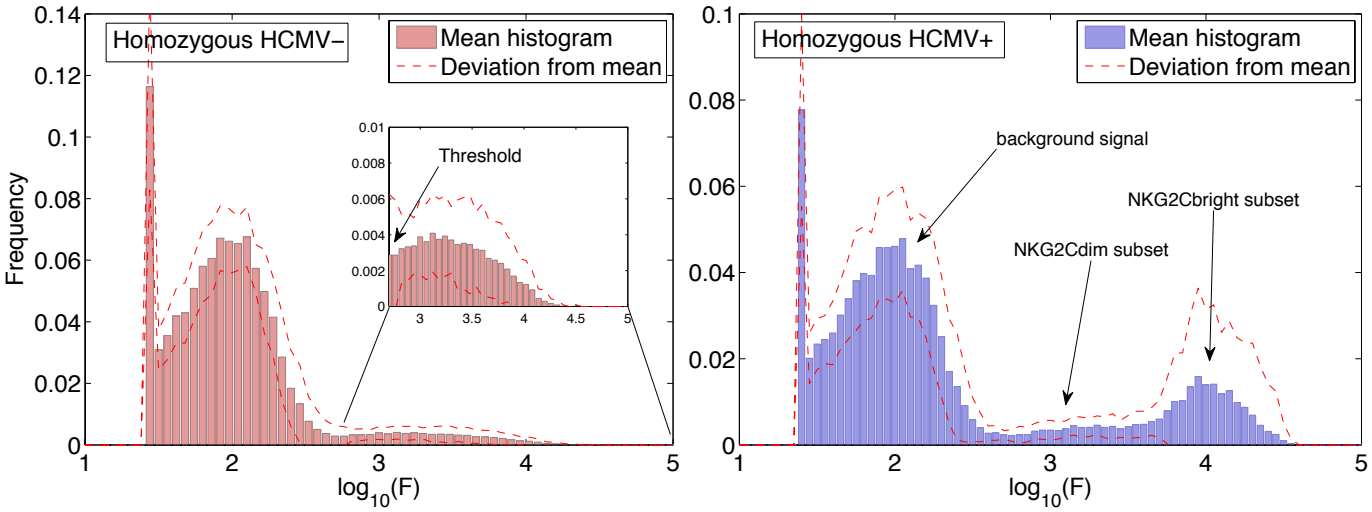

Figure 2: (Left) Mean normalized histogram for seronegative homozygous donors. The NKG2C^{dim} subset represents the basal state in which receptors are produced and degraded without external influences. Notice that part of this subset may be hidden behind the background signal, and thus unable to be measured. (Right) Mean normalized histogram for seropositive homozygous donors. The NKG2C^{bright} subset emerges when HCMV is present, thus evidencing its effect on the expression of the NKG2C receptor.

where A is an infinite reservoir of mRNA capable to be translated into receptors X. The nature of each parameter must be adressed: the production rate of receptors α encodes both transcription of an active gene and mRNA translation into receptors. On the other hand, the rate of receptor degradation, β, can be computed if one knows the receptor half-life time T and the cell cycle time τ, in which the cell volume doubles. Assuming symmetric division, we can assume that receptor concentration halves at each cell division, and thus $\beta = \ln 2/T + \ln 2/\tau$. Treating the concentration of NKG2C $x = [X]$ as a continuous variable, then reaction kinetics and the law of mass action allow us to write the following differential equation:

$$\dot{x} = \alpha - \beta x \tag{2}$$

which has the stable fixed point $x^* = \alpha/\beta$. Fluctuations must be included in order to account for the heterogeneity of receptor distributions, a consequence of the stochastic nature of gene expression. They can be introduced either in the production rate as additive noise and/or through the degradation rate, as multiplicative noise. If additive gaussian noise is introduced in the form $\alpha \to \alpha(1+\sigma\xi)$, with autocorrelation $\langle \xi(t)\xi(t')\rangle = \delta(t-t')$ and σ the noise strength, then it can be shown that the steady state distribution of receptors in an infinite number of realizations is normally distributed with mean $\mathbb{E}[x] = \alpha/\beta$ and variance $\mathrm{Var}[x] = (\alpha\sigma)^2/2\beta = \frac{\alpha\sigma^2}{2}\mathbb{E}[x]$. However, experimental distributions are far from being normal distributions, thus eliminating the possibility that additive noise dominates the system. On the other hand, if noise is introduced in the degradation rate $\beta \to \beta(1+\sigma\xi)$, it acts as multiplicative noise as receptor degradation is proportional to its concentration. Such term could be attributed to random cell growth/division and random receptor half-lifes.

$$\dot{x} = \alpha - \beta x(1+\sigma\xi) \tag{3}$$

It is well-stablished [20] that proteins are usually produced in bursts, in which an mRNA molecule is translated into a few protein molecules before its degradation [21]. In our model, simulations show that such bursting behaviour can be achieved by finely tuning the noise strength σ, as shown in **??**. As σ increases, fluctuations away from the degradation rate β allow the cell to produce large amounts of proteins in a short time interval, only by rapidly decrease their number due to degradation.

The assumptions made on the nature of of $\xi(t)$ imply [9] [22] that the receptor concentration is described by a Markov process $X(t)$ whose probability density function $p(x,t|x_0)$, where x_0 is the receptor concentration at $t = 0$, satisfies the following Fokker-Planck equation (in the sense of Itô):

$$\frac{\partial p}{\partial t} = -\frac{\partial[f(x)p]}{\partial x} + \frac{1}{2}\frac{\partial^2[\nu(x)p]}{\partial x^2} \tag{4}$$

where the drift $f(x)$ and diffusion $\nu(x)$ coefficients associated with the process are

$$f(x) = \alpha - \beta x, \quad \nu(x) = (\beta\sigma x)^2 \tag{5}$$

In the absence of the virus, we hypothesize that the stationary distribution of such equation gives the experimental distributions of seronegative donors, which can be used to estimate the values of α, β and σ. Equation (4) is of singular type, and its boundaries $x = 0$ and $x = \infty$ are natural boundaries for the diffusion process. This circumstance, together with the fact that $\nu(x) > 0$ in $(0,\infty)$ and $\nu'(x)$ and $f(x)$ are defined and continuous therein, guarantees that the sole initial condition $p(x,t=0|x_0) = \delta(x-x_0)$ (expressing the statement that at the initial time the whole probability mass is concentrated around the initial receptor concentration x_0) uniquely determines the transition probability density function of the process. The stationary solution corresponding to setting the time derivative of p to zero:

$$p_s(x) = \frac{N}{\nu(x)}\exp\left(\int_0^x \frac{f(s)}{\nu(s)}ds\right) \tag{6}$$

The stationary solution of the Fokker-Planck equation has the

form of an inverse gamma distribution:

$$p_s(x) = \frac{\theta^k}{\Gamma(k)} x^{-k-1} e^{-\frac{\theta}{x}} \tag{7}$$

where $k = 2/\beta\sigma^2 + 1$ and $\theta = 2\alpha/(\beta\sigma)^2$. The mean and variance of the distribution are:

$$\mathbb{E}[x] = \frac{\alpha}{\beta}, \quad \mathrm{Var}[x] = \frac{\alpha^2}{\beta^2}\left(\frac{\beta\sigma^2/2}{1-\beta\sigma^2/2}\right) \tag{8}$$

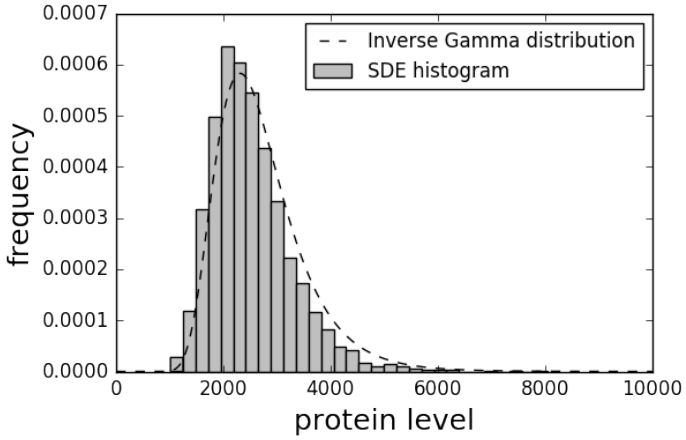

Figure 3: Histogram obtained from 5000 realizations of the process (3) and comparison with the analytical solution of the Fokker-Planck equation (4), an inverse gamma distribution (7). The parameters are $a = 0.1m/s$, $b = 0.001s^{-1}$, $\beta = 0.0004s^{-1}$ and $\sigma = 20$.

In fact, we could extend this notion and consider the *density of cells* $n(x,t)$ with receptor x at time t and write:

$$\frac{\partial n}{\partial t} = -\frac{\partial\left[f(x)n\right]}{\partial x} + \frac{1}{2}\frac{\partial^2\left[v(x)n\right]}{\partial x^2} - \mu(x)n + \lambda(x,t) \tag{9}$$

where μ is the death rate of cells and $\lambda(x,t)$ is a source of progenitors into the pool of NKG2C cells. Then the integral $N(t) = \int_0^\infty n(x,t)dx$ is interpreted as the total number of cells. For $\lambda(x,t) = 0$, the following change of variables

$$\hat{n}(x,t) = n(x,t)e^{\mu(x)t} \tag{10}$$

allow us to write the equation for $\hat{n}(x,t)$:

$$\frac{\partial n}{\partial t} = -\frac{\partial\left[f(x)\hat{n}\right]}{\partial x} + \frac{1}{2}\frac{\partial^2\left[v(x)\hat{n}\right]}{\partial x^2} \tag{11}$$

3.2 The effect of the HCMV

Since we will compare our results to experimental (serological) data, we are interested in the *distribution of receptors* in the cells present in a given amount of blood. Let $n(x,t)$ be the distribution of receptors x in a given amount of blood at time t. The total number of cells is then $N(t) = \int_0^\infty n(x,t)dx$. The equation describing the dynamics of such distribution is

$$\frac{\partial n}{\partial t} = -\frac{\partial(fn)}{\partial x} + \frac{(\beta\sigma)^2}{2}\frac{\partial}{\partial x}\left[\frac{\partial(x^2 n)}{\partial x}\right] - \mu_0 n + \lambda(x,t) \tag{12}$$

MODEL PARAMETERS

α	Production rate of receptors
β	Degradation rate of receptors
σ	Noise strength
v_1	Latency state of the virus
v_2	Rate at which viral load increases
v_3	Rate at which viral load decreases
λ_0	Basal proliferation rate of NK cells
μ_0	Apoptosis rate of NK cells
ε	Maximum cell proliferation increment
k	Receptors at half-maximum growth rate
κ	Maximum number of receptors in a cell
p	Hill coefficient
δ	Regulation rate of the population

Where $f(x) = \gamma - \beta x$ is the single-cell receptor dynamics, μ_0 is the natural death rate of cells and $\lambda(x,t)$ is an external source of cells given by

$$\lambda(x,t) = \frac{\lambda_0}{1+(x/\kappa)^p}\left(1 + \frac{V(t)}{k_v + V(t)}\frac{\varepsilon x^p}{k^p + x^p}\right) \tag{13}$$

Here $V(t)$ represents the effect of the virus as a *viral load*: if $T > 0$ is the time of infection, then

$$V(t) = \begin{cases} 0 & \text{when } t < T \\ v(t-T) & \text{when } t > T \end{cases}, \text{ with } v(t) = v_1 + v_2 t e^{-v_3 t} \tag{14}$$

However, the distribution $n(x,t)$ is not normalized, and the total number of cells N follows the differential equation:

$$\dot{N} = \frac{d}{dt}\int n(x,t)dx = -\mu_0 N + \int \lambda(x,t)dx \tag{15}$$

where the last equality is obtained using the no-flux boundary conditions. Thus, a normalized distribution can be obtained by solving the new partial differential equation

$$\frac{\partial n}{\partial t} = -\frac{\partial(fn)}{\partial x} + D\frac{\partial}{\partial x}\left[x\frac{\partial(xn)}{\partial x}\right] - \mu_0 n + \lambda(x,t) - \dot{N} \tag{16}$$

Then $\dot{N} = 0$ and therefore $N(t) = N_0$, which without loss of generality can be set to $N_0 = 1$.

We consider first the case $\lambda(x,t) = \lambda(x)$ is time independent, which could be associated to the biological situation in which the virus suddenly appears for $t > T$ and it is a constant. Then the previous system has the simple form:

$$\dot{N} = A - \mu_0 N \tag{17}$$

where $A \equiv \int \lambda(x)dx \neq 0$. The solution is

$$N(t) = N_0 e^{-\mu_0 t} + \frac{A}{\mu_0}(1 - e^{-\mu_0 t}) \tag{18}$$

Then the total number of cells will eventually settle to the stationary state $N^* = A/\mu_0$.

4 RESULTS

5 DISCUSSION

The structured population model we propose seems to be compatible with the flow cytometry data of HCMV infected patients. Moreover, the model can reproduce the high phenotypic variability of steady state distributions just by the tuning a few parameters. We claim that initial (without virus) distributions and parameters such as ε, i.e. the maximum proliferation rate, are the main responsible for this heterogeneity. As we have seen, this parameter may vary up to two orders of magnitude, corresponding to expansions larger than a 100-fold. Although this may seem very large, experiments in mice [3] show that NK cells bearing the virus-specific Ly49H receptor (the corresponding to NKG2C receptor in mice) may proliferate up to 100-fold in the spleen and 1,000-fold in the liver after infection. Other sources of phenotypic variability may be λ_0, which corresponds to a basal (without virus) source of NK cells into the blood stream, or k_1, the threshold value of receptors in which NK cells appear in the blood stream. Other parameters such as the production α and degradation rate β of receptors can be kept constant, as there is no noticeable variability in the distribution upon their tuning as long as they remain in a biologically conceivable range.

One of the assumptions implemented in the mathematical model is that the virus does not affect the cell itself, but instead promotes only a differentiation of stem cells into NKG2C^{bright} cells. Thus, the dynamics of single-cell receptors are not affected by the infection. Without evidence of this happening, we chose the simplest possible situation: basal production and lineal degradation of receptors.

Of course, the effect of the virus is decisive in the steady state distributions of cells. On one hand, the functional form (18) is implemented because we want the immune system to be very sensitive of the presence of the virus, thus allowing a faster response. This kind of function can be interpreted biologically as the probability of encounter between infected cells and NK cells (see [23] for a discussion, focused on dendritic cells). On the other hand, there is the assumption of viral latency. This behaviour is well established in the case of HCMV, but it is still unknown whether this latency is necessary for the maintenance of the NKG2C^{bright} subset. In our model, due to the functional implementation of the virus, latency is necessary for the perpetuation of the subset, but a small quantity of virus is enough to trigger the immune response. This is supported by evidence [**?**] of the disappearance of the NKG2C^{bright} subset in HCMV negative hematopoietic stem cell transplant recipients, indicating that the persistence of such subset is dependent on the effect of the chronic viral infection, which probably promotes a continuous replenishment of newly differentiated cells to the blood stream.

A different matter concerns the duration of the infection. By infection, we mean the time interval in which the viral load is still higher that the latent state v_1. Since infection is subclinical, there is still no evidence of how much time does it need the immune system to control infection, although there has been estimated that it may be of the order of weeks. Since in our model viral dynamics and NK cell dynamics are uncoupled, we cannot infer on the matter. However, in the model we assume a duration of 4-5 weeks until infection is controlled, which is a typical, conceivable duration for such viruses. Changing the duration of infection does not affect much to the final results because due to the sensitivity of the virus implementation, there is a threshold in which increasing the viral load makes almost no effect to the proliferation.

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