

# COVID-19 immunotherapy

## A mathematical model

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### Abstract

The pandemic caused by SARS-CoV-2 is responsible for a terrible health devastation with profoundly harmful consequences for the economic, social and political activities of communities on a global scale. Extraordinary efforts have been made by the world scientific community, who, in solidarity, shared knowledge so that effective vaccines could be produced quickly. However, it is still important to study therapies that can reduce the risk, until group immunity is reached, which, globally, will take a time that is still difficult to predict. On the other hand, the immunity time guaranteed by already approved vaccines is still uncertain. The current study proposes a therapy whose foundation lies in the important role that innate immunity may have, by preventing the disease from progressing to the acute phase that may eventually lead to the patient's death. Our focus is on NK cells and their relevant role.

Natural killer cells (NK) are considered the primary defence lymphocytes against virus-infected cells. They play a critical role in modulating the immune system. Preliminary studies in COVID-19 patients with severe disease, suggest a reduction in the number and function of NK cells, resulting in decreased clearance of infected and activated cells and unchecked elevation of inflammation markers that damage tissue. SARS-CoV-2 infection distorts the immune response towards a highly inflammatory phenotype. Restoring the effector functions of NK cells has the potential to correct the delicate immune balance needed to effectively overcome SARS-CoV-2 infection.

## 1 Preliminaries. Biological information.

The prevention and control of virus infections involves a complex interplay between diverse cell types of the innate and adaptive immune systems. Natural killer cells (NKs) are a type of innate lymphoid cell that play an important role

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in immune defense against infection in both mice and humans. The contribution of NK cells to cytolytic killing of virus-infected cells is well-established and well-described in immunology textbooks (see [12],[15],[13],[32]).

Likewise, the importance of early and potent production of pro-inflammatory cytokines like interferon gamma (IFN- $\gamma$ ) by NK cells is widely accepted. More recently, there is increasing evidence that NK cells play a key regulatory role in shaping adaptive immune responses to control infection. Nevertheless, NK cells also have been shown to kill both APC (antigen-presenting cells) and virus-specific T-cells, producing anti-inflammatory cytokines, like interleukin-10 (IL-10), suppressing immunity.

Finally, a series of recent intriguing studies have questioned the innate nature of NK cells by advancing the concept of long-lived memory NK cells that can contribute to viral control during latent infections or following re-infection. In fact, NK cells serve to contain virus infections, while the adaptive immune response is generating antigen-specific cytotoxic T-cells (CTLs), and antibodies (Abs) that can clear the infection.

According to Zheng & al. [1], the total number of NK and CD8+T cells (CTL) was decreased markedly in patients with SARS-CoV-2 infection. The function of NK and CTLs was exhausted with the increased expression of NKG2A in COVID-19 patients. NKG2A is a self-inhibitory receptor on NK cells, that suppress their killing activity. On the other hand, patients convalescing after therapy, the number of NK and CD8+T was restored with reduced expression of NKG2A. These results suggest that the functional exhaustion of cytotoxic lymphocytes is associated with SARS-CoV-2 infection. Hence, SARS-CoV-2 infection may break down antiviral immunity at an early stage. We postponed the discussion, and a possible explanation of this phenomenon to section 4.

This means that it is essential that, in the face of a COVID-19 virus attack, the innate immune response must be sufficiently effective, and that, in particular, the number of NK active cells is high up to the point of triggering the elimination of infected cells and also stimulate the action of macrophages and the differentiation of T-cells into CTL cells specific for the virus-associated antigen.

There is, however, another side of the coin. While cytokines and chemokines are important in the immune control of virus infections, their contribution can be harmful as they can potentiate an acute fatal phase of infection called cytokine storm syndrome (CSS) (see [4], [5], [6]) which has been identified as one of the possible causes of death in COVID-19 patients. CSS is a form of systemic inflammatory response syndrome that occurs when large numbers of white blood cells, including B-cells, T-cells, NK's, macrophages, dendritic cells, and monocytes are activated and release inflammatory cytokines, which activate more white blood cells in a positive feedback loop of pathogenic inflammation.

A vast body of evidence provides support to a central role of promotion of massive inflammatory infiltrates with significantly higher levels of cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IL-1, IL-6, IL-12, IL-18), and chemokines (MIP-2, MIP-1a/b, MCP-1), causing hypercytokinemia <sup>1</sup>. Therefore, particularly early on, potent

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<sup>1</sup>potentially fatal immune reaction, consisting of a positive feedback loop between cytokines and immune cells, with highly elevated levels of various cytokines.

inflammatory antiviral activity may be dangerous to the host, rather than protective, due to the deleterious impact on lung pathology.

The aim of this work is essentially to study the role of innate immunity in fighting infection and, in particular, the role of NK cells. In a later study, already underway, we will complement the model considering adaptive immunity in the acute phase of the infection, with the possible appearance of cytokine storm (CSS).

## 2 Strategies to Enhance NK Cell Function for the Treatment of Infections

### Blockade of immune checkpoints. NKG2A blocking.

Among the most promising approaches to immunotherapy is the blockade of immune checkpoints (see [14]). Immune checkpoints refer to inhibitory pathways hardwired into the immune system that are crucial for maintaining self-tolerance and for modulating the duration and amplitude of physiological immune responses in peripheral tissues in order to minimize collateral tissue damage. This has been studied mainly in cancer immunotherapy since it is now clear that tumors choose certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T-cells that are specific for tumor antigens [14]. Unprecedented improvements in tumor control have been achieved using therapeutic antibodies (Abs) that block immune inhibitory checkpoint receptors [14]. In particular, monoclonal antibodies (mAbs) directed against the PD-1/PDL-1 axis (programmed-cell death protein 1/programmed-cell death ligand 1) in monotherapy or combination therapy, have been approved for the treatment of several indications, including metastatic melanoma, non-small-cell lung cancer, kidney cancer, bladder cancer, Hodgkin lymphoma, and solid tumors.

Haanen et al. [3] examine targeting of a novel immune checkpoint, NKG2A, that can be expressed on both NK cells and on CD8+T, as we will see in a moment. NK cells are essential in the early immune response against viral infections, in particular through clearance of virus-infected cells. Let us detail this point.

One key aspect of NK cell function is their ability to kill cells that have down-regulated MHC-I, a process known as missing-self recognition. As NK cells express self-inhibitory receptors that bind to MHC-I molecules, cells with down-regulated MHC-I activate NK cells and become targets of NK cell-mediated cytotoxicity (see Fig. 1).

NK cells defend against viruses or other pathogens, because they are able to distinguish infected cells from uninfected healthy cells ([32], [13],[12]). In fact, NK cells distinguish infected and stressed cells from healthy normal cells, and NK cell function is regulated by a balance between signals that are generated from inhibitory receptors (ex. NKG2A) and activating receptors (ex. NKG2D). These receptors recognize molecules on the surface of other cells and generate inhibitory or activating signals that inhibit or promote NK responses (see Fig.

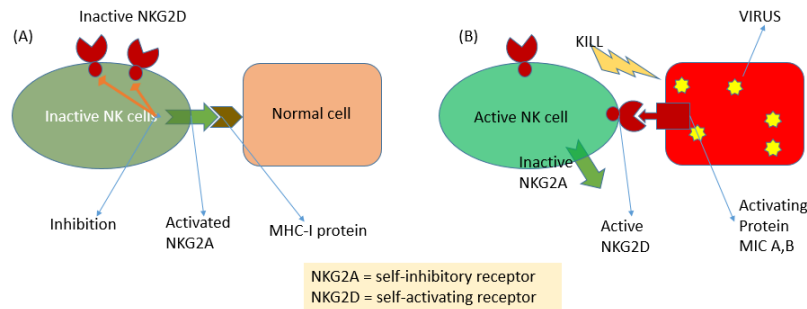


Figure 1: How an *NK* cell recognizes its target. An *NK* cell preferentially attacks infected host cells because these cells have on their surface both activating proteins (MIC-A,B) and, in some cases, abnormally low levels of MHC-I proteins. (A) - the high levels of MHC-I proteins, found on normal host cells, activate self-inhibitory receptors on the *NK* cell (ex. NKG2A) that suppress their killing activity. (B): in contrast, the activating proteins on infected cells bind to self-activating receptors on the *NK* cell (ex. NKG2D) and stimulate their killing activity.

1). The activating receptors stimulate protein kinases that phosphorylate downstream signaling substrates, while inhibitory receptors stimulate phosphatases that counteract the kinases. In general, the inhibitory receptors recognize ligands on healthy normal cells (Fig. 1(A)), while activating receptors recognize ligands on infected and injured cells (Fig. 1(B)). But, be warned, there is a possible violation of this rule. In fact, SARS-CoV-2 can contradict this rule as a self-defense mechanism (see section 4)!

Still another aspect – *NK* cells are able to eliminate virally infected cells in an antigen-independent manner. In fact, as we have just seen, *NK* cells are activated and respond to a variety of different stimuli, including stress-induced proteins, mismatched MHC-I, and missing/down-regulated MHC-I [7]. Viral infections have developed evasion tactics to avoid T-cell recognition. While T-cells and B-cells possess a single antigen receptor that dominates their development and activation, and signals initiated through these antigen receptors are augmented by costimulatory molecules, *NK* cells, in contrast, do not possess one dominant receptor, but instead rely on a vast combinatorial array of receptors to initiate effector functions.

omitted: Finally, one last point – B-cell and T-cell lymphocytes were once considered the only cells in the immune system that could generate memory responses. However, recent data indicate that some *NK* cells can also generate a memory response to antigen. The evidence for this remarkable property came from experiments showing that *NK* cells expressing a receptor that binds a viral protein could transfer memory of this antigen exposure to naive animals that had not been previously exposed or infected. These observations show that at least some *NK* cells possess the developmental machinery to become memory cells, in other words to increase their longevity (although perhaps not as long as B-cell and T-cell lymphocytes) and improve their response times.

## Cytokine activation of NK-cells

Another important function of NK cells is the production of inflammatory cytokines, such as interferon-gamma ( $\text{IFN-}\gamma$ ) and tumor necrosis factor alpha ( $\text{TNF-}\alpha$ ). These cytokines recruit T-cells to the site of infection and impact the function and maturation of myeloid cells. The time course of viral infection can be seen in Fig. 2, adapted from [13].

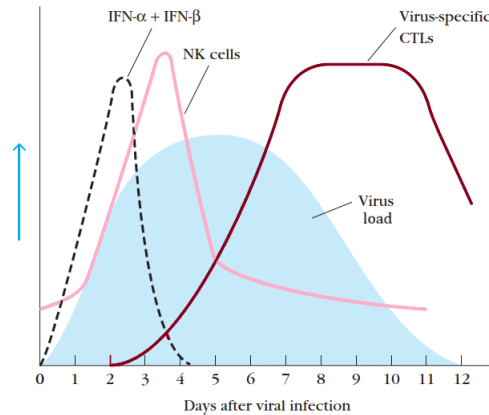


Figure 2: Time course of viral infection.  $\text{IFN-}\alpha$ ,  $\text{IFN-}\beta$ , and the cytokines  $\text{TNF-}\alpha$  and  $\text{IL-12}$  are released from virus-infected cells soon after infection (dashed curve). These cytokines stimulate the NK cells, quickly leading to a rise in the NK-cell population (rose curve) from the basal level, which together control virus replication but do not eliminate the virus. NK cells help contain the infection during the period required for generation of CTLs (brown curve). Virus elimination is accomplished when virus-specific  $\text{CD8+T}$  and neutralizing antibodies are produced. Without NK cells, the levels of some viruses are much higher in the early days of the infection, and the infection can be lethal unless treated vigorously with antiviral compounds. Figure adapted from [13]. The blue shaded region represents Viral load (also known as Viral titer) – a numerical expression of the quantity of virus in a given volume of plasma.

Interferons (IFNs) are a family of cytokines that have been identified through their central role in response to viral infections. In particular,  $\text{IFN-}\gamma$  is a cytokine that plays a central role in immune system signaling, modulating innate and adaptive immune responses to viral and bacterial challenges.  $\text{IFN-}\gamma$  is secreted predominantly by T-cells and NK cells.  $\text{IFN-}\gamma$  signals as an antiparallel homodimer through the high-affinity ( $\text{IFN-R1}$ ) and low-affinity ( $\text{IFN-R2}$ ) receptors. These receptors are present on a very wide range of immune system and other cells, including  $\text{CD4+}$  and  $\text{CD8+}$  T cells, B-cells, NK cells, dendritic cells (DCs), macrophages, platelets, and others.

Production and release of  $\text{IFN-}\gamma$  by NK cells is typically stimulated by the combination of  $\text{IL-12}$  and  $\text{IL-18}$ . Also in  $\text{CD8+}$  T cells and  $\text{CD4+}$  T helper cells the combination of  $\text{IL-12}$  and  $\text{IL-18}$  promotes production and release of  $\text{IFN-}\gamma$  (see Fig. 4). It is important to note that neither  $\text{IL-12}$  nor  $\text{IL-18}$  alone are sufficient to induce elevated production of  $\text{IFN-}\gamma$  from NK cells.

The ability of NK cells to kill target cells can be enhanced by interferons or certain cytokines. NK cells capacity of killing infected cells is increased 20– to

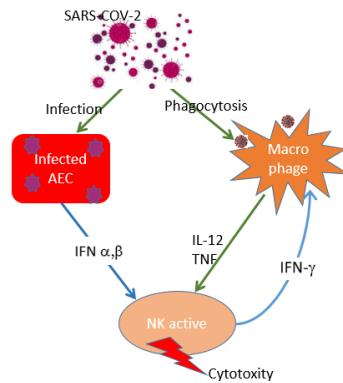


Figure 3: Cytokine activation of NK-cells. Adapted from [32].

100–fold when NK cells are exposed to  $IFN-\alpha$  and  $IFN-\beta$ , or to  $IL-12$ , a cytokine produced by dendritic cells and macrophages during infection by many types of pathogens.  $IL-12$ , acting in synergy with the cytokine  $IL-18$ , produced by activated macrophages, can also stimulate NK cells to secrete large amounts of interferon  $IFN-\gamma$ , and this is crucial in controlling some infections before the  $IFN-\gamma$  produced by activated CTL ( $CD8+T$ -cells) becomes available. E. Prompetchara et al. [20], describes a study of 41 hospitalized patients with high-levels of proinflammatory cytokines, including  $IL-2$ ,  $IL-7$ ,  $IL-10$ , and  $TNF-\alpha$  observed in the COVID-19 severe cases. These findings are in line with SARS in that the presence of lymphopenia (abnormally low level of lymphocytes in the blood) and cytokine storm syndrome (CSS) may have a major role in the pathogenesis of COVID-19. This CSS can initiate viral sepsis and inflammatory-induced lung injury which lead to other complications including pneumonitis, acute respiratory distress syndrome (ARDS), respiratory failure, shock, organ failure and potentially death. We will explore this in an ongoing study, to be submitted soon.

Several unrelated viruses, including influenza virus, respiratory syncytial virus, and human immunodeficiency virus, can directly interfere with NK cell functionality, through infection of these cells. Viral infection can lead to immune suppression, either by downregulation of the cytotoxic function or by triggering apoptosis, leading to depletion of NK cells.

On the other hand, a vast body of evidence provides support to a central role of exaggerated production of  $IFN-\gamma$  in causing hypercytokinemia and signs and symptoms of HLH<sup>2</sup>.

<sup>2</sup>WIKI [https://en.wikipedia.org/wiki/Hemophagocytic\\_lymphohistiocytosis](https://en.wikipedia.org/wiki/Hemophagocytic_lymphohistiocytosis)  
Hemophagocytic lymphohistiocytosis (HLH), is an uncommon hematologic disorder seen more often in children than in adults. It is a life-threatening disease of severe hyperinflammation caused by uncontrolled proliferation of activated lymphocytes and macrophages, characterized by proliferation of morphologically benign lymphocytes and macrophages that secrete high amounts of inflammatory cytokines. It is classified as one of the cytokine storm syndromes. There are inherited and non-inherited (acquired) causes of hemophagocytic lymphohistiocytosis (HLH).

### 3 Mathematical Modeling

Our model will focus in the role of innate immunity (cells and cytokines included), in fighting infection and, in particular, the role of NK cells. In a later study, already underway, we will complement the model considering adaptive immunity in the acute phase of the infection, with the possible appearance of cytokine storm (CSS). Figure 4 shows a schematic diagram of the proposed model dynamics and the associated equations are given in the text. The ODE's modeling methodology owes much to previous work by Avner Friedman, Ruby Kim, Ami Radunskaya, Denise Kirschner, among others. Specifically, we use (i). the distinction between internal virus load  $V_{int}$ , within infected cells, and external or free virus load  $V_{free}$ , (ii). Hill's kinetic laws to model activation/inhibition factors, (iii). parameter values due to the lack of experimental data. The significance, units and simulation values of the parameters used in the equations below, can all be seen in section 8.

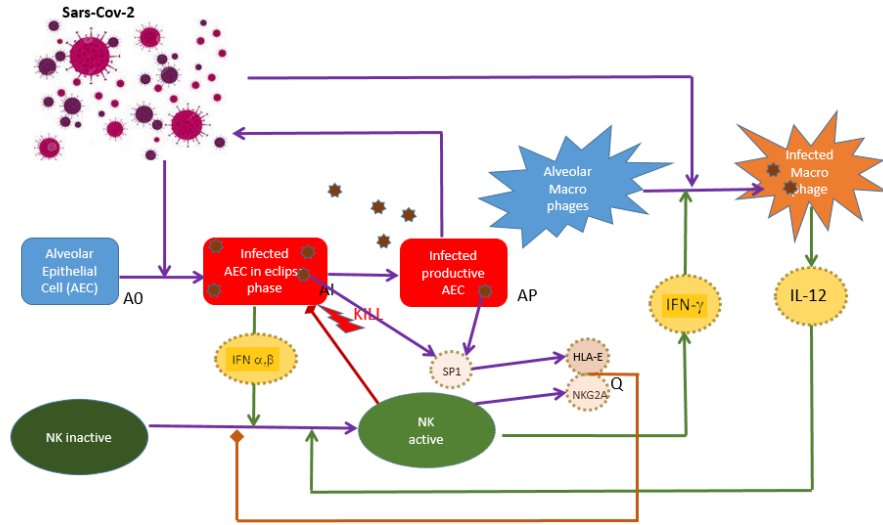


Figure 4: Schematic diagram of viral dynamics and innate response.

Previous studies have shown that SARS-CoV-2 predominantly infects airway and alveolar epithelial cells (AECs) and macrophages. SARS-CoV-2 uses the same entry receptor, angiotensin-converting enzyme (ACE2), as SARS-CoV, for infection, suggesting the likelihood of the same set of cells being targeted and infected. We start with a classic viral dynamic model, with eclipse phase [19], [36], where: (i). Target Cells (AECs) that are susceptible to infection,  $A_0$ , become infected, ( $A_I$ ), upon interaction with infectious free viral particles,  $V_{free}$ , at rate  $\beta_1 V_{free}$ , according to mass-action law, where  $\beta_1$  is the infection rate constant; (ii). Target cells,  $A_0$ , have a constant supply rate,  $s$ , and natural death rate,  $\mu$ ; (iii). An eclipse phase, in which  $A_I$  cells remain infected, the virus is replicated in the usual way, but not yet released into the plasma. These infected cells evolve into a productive state,  $A_P$ , in which they explode, releasing virions at the rate  $p$  virions/cell. (iv). Virus is cleared at rate  $c$  per virion, which corresponds to a half-life  $t_{1/2} = \ln(2)/c$ , and (v). An infected cell has an

average lifespan of  $1/\delta$ , where  $\delta$  is its death rate, and thus produces an average of  $p_m = p/\delta$  virions during its lifetime. Infected cells ( $A_I$ ) can be killed by interaction with active Natural Killers ( $N_{at}$ ). See [5], [6] and so we have the following first three equations (See Fig. 4):

$$\frac{dA_0}{dt} = s - \beta_1 A_0 \cdot V_{free} - \mu A_0 \quad (1)$$

$$\frac{dA_I}{dt} = \underbrace{\beta_1 A_0 \cdot V_{free}}_{\text{Infection}} - \underbrace{\beta_2 N_{at} \cdot A_I}_{\text{Killing}} - \delta A_I \quad (2)$$

$$\frac{dA_P}{dt} = \delta A_I - \underbrace{\beta_2 N_{at} \cdot A_P}_{\text{Killing}} - \eta A_P \quad (3)$$

We have assumed a constant supply of  $A_0$ -cells, like in [47]. We distinguish between two populations of virus: free virus,  $V_{free}$ , in plasma, and internal virus,  $V_{int}$ , in alveolar and macrophages infected cells. We neglect the internal virions in infected macrophages that are killed by phagocytosis. The equation for the free virus, has a term coming from the bursting of  $A_P$ -cells, with burst number  $p$  virions/ $A_P$ .  $V_{int}$  have terms corresponding to virus proliferation in  $A_I$  and  $A_P$  cells, and subtracting terms corresponding to killing of  $A_I$  and  $A_P$  cells by activated NK-cells,  $N_{at}$ .

$$\frac{dV_{free}}{dt} = \underbrace{\eta \cdot p \cdot A_P \cdot \vartheta_{int}^m}_{\text{Bursting}} - \beta_1 A_0 \cdot V_{free} - \beta_4 M_0 \cdot V_{free} - c V_{free} \quad (4)$$

$$\begin{aligned} \frac{dV_{int}}{dt} = & \underbrace{r V_{int}(1 - \vartheta_{int}^m)}_{\substack{\text{Virus proliferation} \\ \text{in } A_P \text{ and } A_I\text{-cells}}} + \underbrace{\beta_1 A_0 \cdot V_{free}}_{V_{free} \rightarrow V_{int}} + \underbrace{\beta_4 M_0 \cdot V_{free}}_{V_{free} \rightarrow V_{int}} \\ & - \eta \cdot p \cdot A_P \cdot \vartheta_{int}^m - \underbrace{p_1 \beta_2 N_{at} \cdot (A_I + A_P) \cdot \vartheta_{int}^m}_{\substack{\text{Killing of } V_{int} \in A_I \cup A_P \\ \text{before bursting}}} \end{aligned} \quad (5)$$

where we have defined  $\vartheta_{int}^m$  by

$$\vartheta_{int}^m = \left( \frac{V_{int}^m}{V_{int}^m + (p A_P)^m} \right)$$

Here  $p_1 < p$ , and we assume that, in the average,  $p_1 = (p/2)$  virions/ $A_I, A_P$ .

Inactive NKs (Natural Killer cells),  $N_{in}$ , are activated through the positive mediation of Interferons  $IFN-\alpha$  and  $IFN-\beta$ ,  $I_{\alpha,\beta}$ , which, in turn, are segregated by infected AECs,  $A_I$  and  $A_P$ . Moreover the activation of NKs is also stimulated by cytokine  $IL-12$ ,  $I_{12}$ , which, in turn, are segregated by infected alveolar macrophages,  $M_I$  (see Fig. 4). On the other side, as we have said before, SARS-CoV-2 defends itself, inactivating active NK cells, presumably, increasing the



cytotoxic self-inhibitor NKG2A, as we will see soon. Therefore, we obtain four more equations:

$$\begin{aligned} \frac{dN_{\text{at}}}{dt} = & \underbrace{k_1 N_{\text{in}} \cdot \left( \frac{I_{\alpha\beta}}{K_{\alpha\beta} + I_{\alpha\beta}} \right)}_{\substack{I_{\alpha\beta}\text{-activated} \\ \text{production of } N_{\text{at}}\text{-cells}}} + \underbrace{k_2 N_{\text{in}} \cdot \left( \frac{I_{12}}{K_{12} + I_{12}} \right)}_{\substack{I_{12}\text{-activated} \\ \text{production of } N_{\text{at}}\text{-cells}}} + \\ & -\beta_2 N_{\text{at}} \cdot (A_I + A_P) - \mu_1 N_{\text{at}} \end{aligned} \quad (6)$$

$$\begin{aligned} \frac{dN_{\text{in}}}{dt} = & k_3 - k_1 N_{\text{in}} \cdot \left( \frac{I_{\alpha\beta}}{K_{\alpha\beta} + I_{\alpha\beta}} \right) + \\ & -k_2 N_{\text{in}} \cdot \left( \frac{I_{12}}{K_{12} + I_{12}} \right) - \mu_2 N_{\text{in}} \end{aligned} \quad (7)$$

$$\begin{aligned} \frac{dM_I}{dt} = & \underbrace{k_4 M_0 \cdot \left( \frac{I_\gamma}{K_\gamma + I_\gamma} \right)}_{\substack{I_\gamma\text{-activated} \\ \text{production of } M_I\text{-cells}}} + \underbrace{\beta_4 M_0 \cdot V_{\text{free}}}_{\text{Infection}} - \mu_3 M_I \end{aligned} \quad (8)$$

$$\frac{dM_0}{dt} = k_0 - k_4 M_0 \cdot \left( \frac{I_\gamma}{K_\gamma + I_\gamma} \right) - \beta_4 M_0 \cdot V_{\text{free}} - \mu_4 M_0 \quad (9)$$

The equation for IL-12 is

$$\frac{dI_{12}}{dt} = k_{12} M_I - \mu_{12} I_{12} \quad (10)$$

IFN- $\gamma$ ,  $I_\gamma$ , is produced by active NK cells,  $N_{\text{at}}$ :

$$\frac{dI_\gamma}{dt} = k_\gamma N_{\text{at}} - \mu_\gamma I_\gamma \quad (11)$$

Similarly, IFN- $\alpha$  and IFN- $\beta$ ,  $I_{\alpha\beta}$ , are produced by  $A_I$  and  $A_P$ , and this production is activated by  $V_{\text{int}}$ :

$$\frac{dI_{\alpha\beta}}{dt} = k_{\alpha\beta} (A_I + A_P) \cdot \vartheta_{\text{int}}^m - \mu_{\alpha\beta} I_{\alpha\beta} \quad (12)$$

## 4 Inhibition of NK cytotoxicity by the complex NKG2A/HLA-E

As a self-inhibitory receptor, NKG2A has been demonstrated to induce NK cell exhaustion in chronic viral infections. Notably, NKG2A expression, on NK and CD8+T-cells, results in functional exhaustion of NK and CD8+T-cells. In patients infected with SARS-CoV-2, NKG2A expression was increased significantly on NK and CD8+T cells compared with that in HCs (healthy controls). Thus, blocking NKG2A, releases both T-cell and NK cell effector functions. In fact, André et al. [2] shows that blocking the inhibitory NKG2A receptor enhances both natural killer (NK) and CTL (CD8+T) effector functions in mice and humans.

In an interesting study, Daria Bortolotti and collaborators [55] evaluated the possible role of SARS-CoV-2 spike proteins in modifying NK cell functions. Their conclusion is that SARS-CoV-2 Spike 1 Protein (SP1), when presented by infected lung epithelial cells,  $A_I$  and  $A_P$ , via HLA-E molecules, modifies NK Cell cytotoxicity. They successfully test the hypothesis that the resulting inactivity of NK cells to kill  $A_I$  and  $A_P$  cells, expressing SP1, was determined by HLA-E/NKG2A binding. They evaluated the effect of SARS-CoV-2 spike proteins in the control of NK cell activation, considering SP1, which is involved in the attachment of the virion to the cell membrane by interacting with ACE2 receptor, and SP2 that mediates the fusion of the virion.

Since the presence of intracellular SP1 protein in lung epithelial cells induced a decrease in the cytotoxic activity of NK cells, we analyzed the possible factors involved in the modification of NK cell status. Since viral proteins are commonly recognized and degraded by the proteasome inside the infected cells, we hypothesized that SP1 peptides might be presented to NK cells. Intracellular peptide presentation is performed by human leukocyte antigen (HLA) class I molecules, which are expressed by all nucleated cells. Epithelial lung cells can express also non-classical HLA-E molecule. HLA-E binds peptides primarily derived from specific signal sequences and interacts with NKG2A/CD94 NK cell inhibitory receptors. There was a significant increase in HLA-E protein and mRNA expression in the presence of SP1 protein.

Denote by  $N$  the concentration of NKG2A protein, in  $N_{\text{at}}$ -cells,  $H$  the concentration of  $H = \text{HLA-E}$  protein, in  $A_I \cup A_P$ -cells, and finally  $Q$  (the concentration of) the complex

$$Q = \text{NKG2A/HLA-E}$$

More concretely HLA-E is induced by SP1 protein of internal virions on infected alveolar cells  $A_I$  and  $A_P$  (see below).  $N = \text{NKG2A}$  is expressed on the surface of activated NK cells,  $N_{\text{at}}$ -cells. Hence,  $N$  is given by

$$N = \rho_N N_{\text{at}} \quad (13)$$

where  $\rho_N$  is the ratio between the mass of one  $N$ -protein to the mass of one  $N_{\text{at}}$  cell. Now,  $H = \text{HLA-E}$  is expressed on the surface of infected  $A_I$  and  $A_P$  cells. Following the conclusion of [55], SARS-CoV-2 Spike 1 Protein (SP1), when presented by infected lung epithelial cells,  $A_I$  and  $A_P$ , via HLA-E molecules, modifies NK Cell cytotoxicity. Hence, the concentration  $H$  of HLA-E is up-regulated by  $V_{\text{int}}$ . We assume that its dynamics follows the ODE:

$$\frac{dH}{dt} = \rho_H r V_{\text{int}} \cdot (1 - \vartheta_{\text{int}}^m) - \mu_H H - k_Q \rho_N N_{\text{at}} \cdot H - k_Q (N \cdot H) \cdot \text{Inhib}_B \quad (14)$$

The complex  $Q$  results from the reaction  $N + H \xrightarrow{k_Q} Q$ , which leads to mass-action equation:

$$\frac{dQ}{dt} = k_Q N \cdot H - k_Q \rho_N N_{\text{at}} \cdot H \quad (15)$$

This  $Q = \text{NKG2A/HLA-E}$  complex inhibits the activation  $N_{\text{in}} \rightarrow N_{\text{at}}$ , by a Hill factor

$$\text{Inhib}_Q = \frac{K_Q^m}{K_Q^m + Q^m} \quad (16)$$

since NKG2A is a self-inhibitor of of NK cytotoxicity. We model the inhibition effect of  $\text{Inhib}_Q$ , by Hill's law, as usual (see [34], [47]). As  $\text{Inhib}_Q = \frac{1}{1 + (Q/K_Q)^m}$ , the constant  $K_Q$  is the half-saturating, in the sense that when  $Q \rightarrow K_Q$ ,  $\text{Inhib}_Q \rightarrow 1/2$ . When  $Q \rightarrow \infty$ ,  $\text{Inhib}_Q \rightarrow 0$  (max inhibition) and when  $Q \rightarrow 0$ ,  $\text{Inhib}_Q \rightarrow 1$  (no inhibition). Hence equation (6) for  $dN_{\text{at}}/dt$ , must be modified by:

$$\begin{aligned} \frac{dN_{\text{at}}}{dt} = & k_1 N_{\text{in}} \cdot \left( \frac{I_{\alpha\beta}}{K_{\alpha\beta} + I_{\alpha\beta}} \right) \cdot \text{Inhib}_Q + \\ & + k_2 N_{\text{in}} \cdot \left( \frac{I_{12}}{K_{12} + I_{12}} \right) \cdot \text{Inhib}_Q + \\ & - \beta_2 N_{\text{at}} \cdot (A_I + A_P) - \mu_1 N_{\text{at}} \end{aligned} \quad (17)$$

## 5 Treatment with NKG2A–blocker

The new aspects of interaction between SARS-CoV-2 SP1 protein and the host cells, described in the previous section, might have important implications in the pathogenesis of COVID-19, providing opportunities for developing new therapies against SARS-CoV-2. In particular, counteracting the cellular stress, targeting the SP1 protein or using NKG2A–blockers, might represent new strategies to enhance the innate immune response at the early stage of the disease, inducing mucosal immunity that might lead to a long-term protection against infection.

**Monalizumab** is a monoclonal antibody developed to bind to NKG2A receptors expressed on T and NK cells, which could reestablish a broad anti-viral response. It is a checkpoint inhibitor. The natural ligand of NKG2A is HLA-E (HLA class I histocompatibility antigen, alpha chain E), which is frequently over-expressed in tumor diseases, transplants and virus-infected. Binding of HLA-E to NKG2A leads to the inhibition of activation and cytotoxic activity of T and NK cells.

The antibody drug **Monalizumab** is aimed at blocking the binding of NKG2A to HLA-E, and thus activate T and NK cells and their cytotoxic cell responses, enhancing the immune function of the body and re-establishing their immune response. In fact, blocking the binding of NKG2A to HLA-E, inhibits the  $Q$ -inhibitor of activation  $N_{\text{in}} \rightarrow N_{\text{at}}$ , and so enhances NK cytotoxicity.

### Equation for NKG2A–blocker **Monalizumab**

We assume that **Monalizumab** is injected intradermally in three doses, in days 2, 16 and 30, by an amount of  $\theta$  pg/mL (the effective dose level of the drug), providing a source  $I_\theta$ :

I have changed  $M(\theta)$  by  $M_\theta$ ,  $I(\theta)$  by  $I_\theta$ , etc., as suggested.

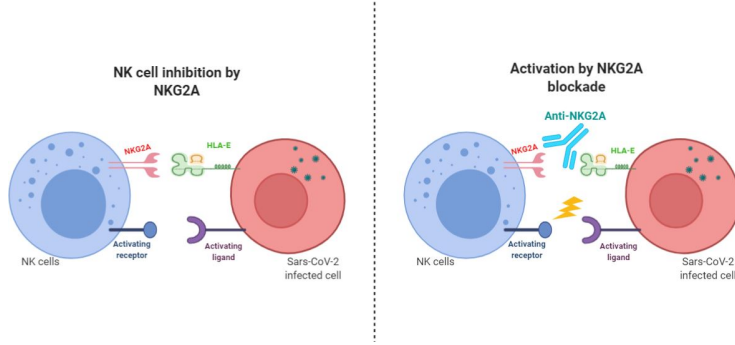


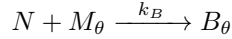
Figure 5: Monalizumab is aimed at blocking the binding of NKG2A to HLA-E.

$$modified\ I_\theta(t) = \begin{cases} \theta & \text{if } 2 \leq t \leq 3 \\ \theta & \text{if } 16 \leq t \leq 17 \\ \theta & \text{if } 30 \leq t \leq 31 \\ 0 & \text{otherwise} \end{cases} \quad (18)$$

and a corresponding concentration  $M_\theta$ , given by:

$$\frac{dM_\theta}{dt} = I_\theta(t) - \delta_M M_\theta - k_B \rho N_{at} \cdot M_\theta \quad (19)$$

Let  $B_\theta$  be the complex  $B_\theta = M_\theta/NKG2A$ , that results from the process of NKG2A-blocking:



which leads to mass-action equation:

$$\frac{dB_\theta}{dt} = k_B N \cdot M_\theta = k_B \rho N_{at} \cdot M_\theta \quad (20)$$

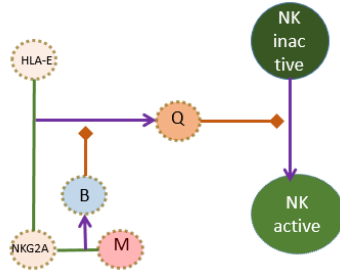


Figure 6: Treatment with NKG2A-blocker  $B$ .

As we said before, Monalizumab, or any other NKG2A-blocker, is aimed at blocking the binding of NKG2A to HLA-E, and this is achieved by blocking NKG2A. We model this effect by a Hill factor  $Inhib_B = \frac{K_B^m}{K_B^m + B^m}$ , which inhibits  $Q$ , and thus activate NK cells and their cytotoxic response. Under treatment the equation (15) for  $Q$ , must be modified by the following one:

$$\frac{dQ}{dt} = k_Q (N \cdot H) \cdot Inhib_B \quad (21)$$

Conclusion: Under treatment we have a new system of differential equations, formally equal to the “control” system (without treatment), but with new variables,  $A_0(\theta)$ ,  $A_I(\theta)$ ,  $A_P(\theta)$ ,  $N_{at}(\theta)$ , and so one. The equations are

- Final equations without treatment: Equations (1) to (12), with (6) substituted by (17), tacking account of the inhibition, by SARS-CoV-2 virus, of NK-activation.
- Final equations with  $M$ -treatment: formally, the same as before, with new variables as we have said above, where, for simplicity we omit the  $\theta$ -dependence, with (15), for  $Q$ , substituted by (21), tacking account of the inhibition, by the blocker  $B$ , of  $Q$ -activation, together with equations (19) for  $M$ , and (20) for  $B$ . We must subtract the terms  $k_B(\rho_N N_{at} \cdot M_\theta)$  and  $k_Q(\rho_N N_{at} \cdot H) \cdot \text{Inhib}_B$ , in the  $N_{at}$ -ODE (17), to guarantee mass conservation. Therefore, the final equation for  $N_{at}$ , under treatment, must be

$$\begin{aligned} \frac{dN_{at}}{dt} = & k_1 N_{in} \cdot \left( \frac{I_{\alpha\beta}}{K_{\alpha\beta} + I_{\alpha\beta}} \right) \cdot \text{Inhib}_Q + k_2 N_{in} \cdot \left( \frac{I_{12}}{K_{12} + I_{12}} \right) \cdot \text{Inhib}_Q + \\ & -\beta_2 N_{at} \cdot (A_I + A_P) - \mu_1 N_{at} - k_B(\rho_N N_{at} \cdot M_\theta) + \\ & -k_Q(\rho_N N_{at} \cdot H) \cdot \text{Inhib}_B \end{aligned} \quad (22)$$

## 6 Simulations and Results

We have used the software COPASI <http://copasi.org/>, for numerical integration of our equations. The parameters were found through a detailed revision of the bibliography. Some of them were adjusted to reproduce more faithful the already known dynamics of SARS-CoV-2 virus load (VL, without treatment with NKG2A-blockers, as far as we know).

This type of modeling, by ignoring the spatial component, as well as factors intrinsically stochastic to the molecular regimens, implies a very drastic simplification of the complexity of biological phenomena at the cellular and molecular scale. However, they can be useful as first approximations and, in any case, they provide an alternative in silico simulation process, with the advantage of being able to lead to simulations of quantifiable observable dynamics, as well as possible control through appropriate therapies.

The paradigm of immunotherapy treatment, in this case of the NKG2A/HLA-E checkpoint blocking, seems very promising for effective therapies for SARS-CoV-2, preventing the disease from evolving to severe acute states. As we have already said, this work must be deepened, taking into account the decisive role of the adaptive immune system and consideration of the serious risk factor - the cytokine storm.

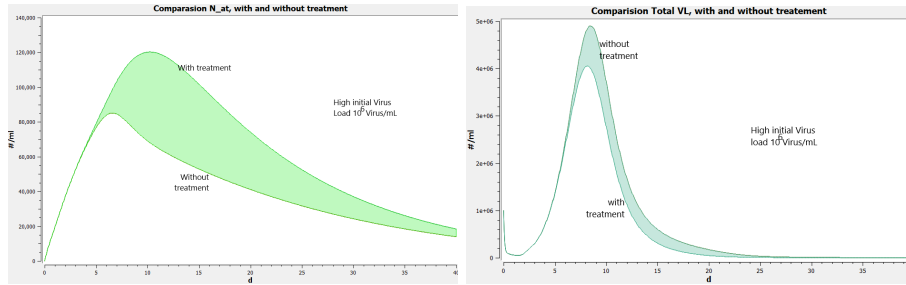


Figure 7:  $N_{at}$  and total Viral load evolution, for high initial  $V \approx 10^6$  virions/mL.

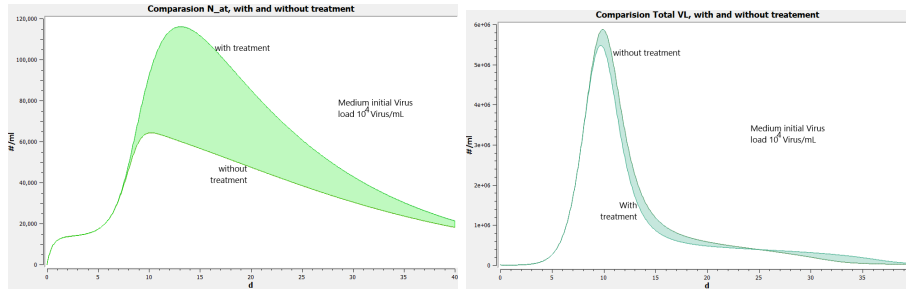


Figure 8:  $N_{at}$  and total Viral load evolution, for medium initial  $V \approx 10^4$  virions/mL.

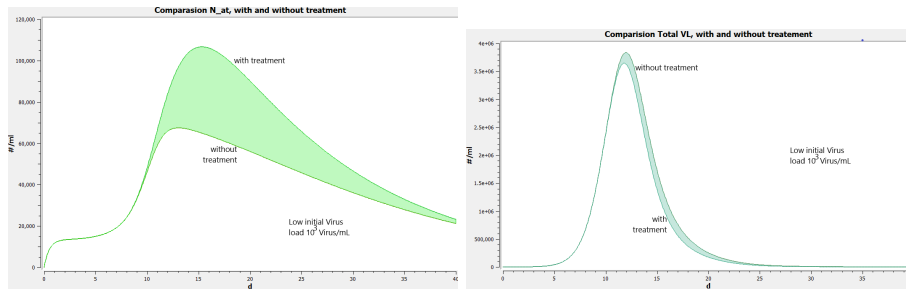


Figure 9:  $N_{at}$  and total Viral load evolution, for low initial  $V \approx 10^3$  virions/mL.

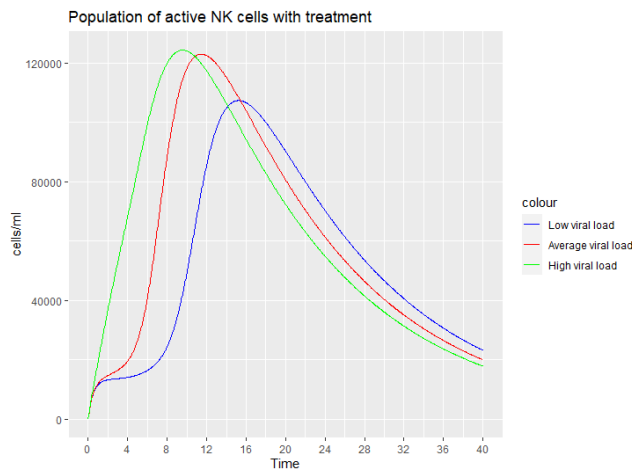


Figure 10: Comparison of active NK cell concentration, with different initial viral loads and treatment. Initial high, medium and low viral loads are  $10^6$ ,  $10^4$  and 1000 virions/mL, respectively.

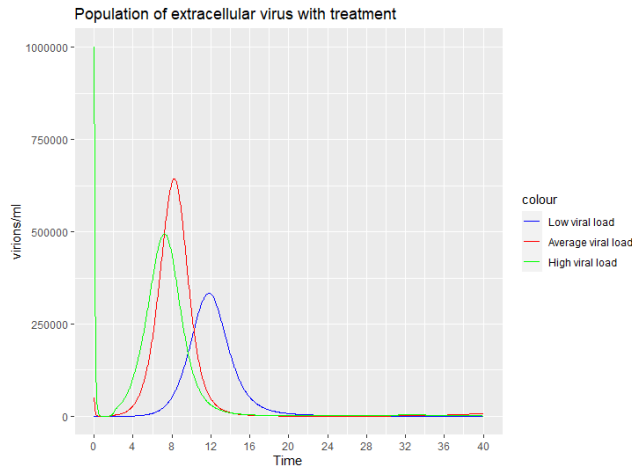


Figure 11: Comparison of the extracellular virus concentration,  $V_{free}$ , with different initial viral loads and treatment. Initial high, medium and low viral loads are  $10^6$ ,  $10^4$  and 1000 virions/mL, respectively.

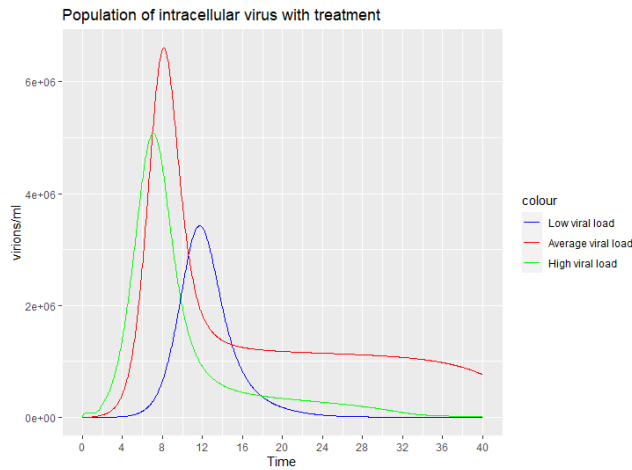


Figure 12: Comparison of the intracellular virus concentration,  $V_{int}$ , with different initial viral loads and treatment. Initial high, medium and low viral loads are  $10^6$ ,  $10^4$  and 1000 virion/mL, respectively.

## 7 Discussion

Regarding the population of active NK cells, one of the observations resulting from our simulations, was that, with a high initial viral load, the initial growth of active NKs was very fast, with a higher peak, than with other viral loads. This is reasonable, since if initially the concentration of virions is very high, they infect healthy cells more quickly, so there are more infected cells and, consequently, the larger the population of active NK cells will be. Another observation was about slowing growth when the viral load was low. This may be due to the fact that the load is very low and the time of infection by the virus is shorter in this case than with higher viral loads.

As for the intra or extracellular virus, it was observed that initially the free virus with high viral load decreases. This may be due to the fact that the virus begins to infect healthy cells and there is a decrease in the extracellular virus and an increase in the intracellular virus.

Note that it was also observed that the virus peak (both extracellular and intracellular) with intermediate initial load exceeds the virus peak when the initial load is high. Although there is no scientific explanation, there is a study that shows that low viral loads cause higher peaks than high viral loads [68]!, which goes according to the results obtained in the simulations of Fig. 11 and 12.

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## 8 Supplementary material

Given the impossibility of accessing serological data, it makes some sense to follow the main parameters used in previous studies on SARS viruses. However, SARS-CoV-2 has different characteristics that lead to different temporal dynamics and degrees of severity than those mentioned. On the other hand, it is too early to have reliable serological data on SARS-CoV-2. In this context, the parameters were assumed within a logic of plausibility maintaining the parameters of previous studies, namely production and decay rates  $\beta_i, \mu_i, etc.$ , and some were adjusted to the infection onset and to the most readily accessible observables, namely VL peak, eclipse time, infection time, etc.

We postpone to a future work the simulation with combined treatment with anti-virals like, for example, Remdisivir.

Dynamic variables	Name	Units
$A_0$	Susceptible epithelial cell	$A_0/\text{mL}$
$A_I$	Infected epithelial cells in elipse (non-productive) phase	$A_I/\text{mL}$
$A_P$	Infected epithelial cells in productive phase	$A_P/\text{mL}$
$V_{\text{free}}$	Free virus particles in plasma	$V_{\text{free}}/\text{mL}$
$V_{\text{int}}$	Internal virus particles in $A_I \cup A_P$ cells	$V_{\text{int}}/\text{mL}$
$N_{\text{in}}$	Inactive NK cells	$N_{\text{in}}/\text{mL}$
$N_{\text{at}}$	Active NK cells	$N_{\text{at}}/\text{mL}$
$M_0$	Macrophages	$M_0/\text{mL}$
$M_I$	Infected Macrophages	$M_I/\text{mL}$
$I_{12}$	IL-12	$\text{pg}/\text{mL}$
$I_\gamma$	IFN- $\gamma$	$\text{pg}/\text{mL}$
$I_{\alpha\beta}$	IFN- $\alpha \cup$ IFN- $\beta$	$\text{pg}/\text{mL}$
$N$	NKG2A protein	$\text{pg}/\text{mL}$
$H$	HLA-E protein	$\text{pg}/\text{mL}$
$Q$	Complex NKG2A/HLA-E	$\text{pg}/\text{mL}$
$M_\theta$	anti-NKG2A with dose $\theta$	$\text{pg}/\text{mL}$
$B_\theta$	Blocking Complex NKG2A/ $M_\theta$	$\text{pg}/\text{mL}$

Parameters	Description	Units
$s$	Production rate of susceptible $A_0$ -cells	$(A_0/\text{mL})/\text{day}$
$\beta_1$	Virus infection rate of $A_0$ -cells	$(V_{\text{free}}/\text{mL})^{-1}/\text{day}$
$\mu$	Natural death rate of susceptible $A_0$ -cells	$\text{day}^{-1}$
$\beta_2$	Killing rate of infected $A_I$ -cells by active NK cells	$(N_{\text{at}}/\text{mL})^{-1}/\text{day}$
$\delta$	Death rate of infected $A_I$ -cells	$\text{day}^{-1}$
$\eta$	Death rate of $A_P$ -cells	$\text{day}^{-1}$
$p$	burst number	virions/ $A_P$
$\beta_4$	Virus infection rate of $M_0$ -cells	$(V_{\text{free}}/\text{mL})^{-1}/\text{day}$
$c$	Virus death rate	$\text{day}^{-1}$
$r$	Growth rate of internal virus	$\text{day}^{-1}$
$k_1$	Activation rate of NK cells from $N_{\text{in}}$ -cells up-regulated by IFN- $\alpha$ and IFN- $\beta$	$\text{day}^{-1}$
$k_2$	Activation rate of NK cells from $N_{\text{in}}$ -cells up-regulated by IL-12	$\text{day}^{-1}$
$k_3$	Production rate of $N_{\text{in}}$ -cells	$(N_{\text{in}}/\text{mL})/\text{day}$
$\mu_1$	Death rate of $N_{\text{at}}$ -cells	$\text{day}^{-1}$
$\mu_2$	Death rate of $N_{\text{in}}$ -cells	$\text{day}^{-1}$
$K_{\alpha\beta}$	$I_{\alpha,\beta}$ half-saturation in the positive up-regulation of NK-cell activation	$I_{\alpha,\beta}/\text{mL}$
$K_{12}$	IL-12 half-saturation in the positive up-regulation of NK-cell activation	$I_{12}/\text{mL}$
$K_\gamma$	IFN- $\gamma$ half-saturation in the positive up-regulation of $M_0$ -activation	$I_\gamma/\text{mL}$
$k_4$	Activation rate of $M_I$ -cells, from inactive $M_0$ -cells, modified by IFN- $\gamma$	$\text{day}^{-1}$
$\mu_3$	Death rate of $M_I$ -cells	$\text{day}^{-1}$
$k_0$	Production rate of $M_0$ -cells	$(M_0/\text{mL})/\text{day}$
$\mu_4$	Death rate of $M_0$ -cells	$\text{day}^{-1}$
$k_{12}$	Production rate of IL-12 by $M_I$ -cells	$(\text{pg}/M_I)/\text{day}$
$\mu_{12}$	Decay rate of IL-12	$\text{day}^{-1}$
$k_\gamma$	Production rate of IFN- $\gamma$ by active NK-cells	$(\text{pg}/N_{\text{at}})/\text{day}$
$\mu_\gamma$	Decay rate of IFN- $\gamma$	$\text{day}^{-1}$
$k_{\alpha\beta}$	Production rate of IFN- $\alpha$ and IFN- $\beta$ by $A_I$ and $A_P$ -cells	$(\text{pg}/A_{I,P})/\text{day}$
$\mu_{\alpha\beta}$	Decay rate of IFN- $\alpha$ and IFN- $\beta$	$\text{day}^{-1}$

Table 1: Table with the description of the parameters used in the model of differential equations.

Parameters	Value	Units	References
$s$	$4 \times 10^3$	$(A_0/\text{mL})/\text{day}$	[47]
$\beta_1$	$1.17228 \times 10^{-6}$	$(V_{\text{free}}/\text{mL})^{-1}/\text{day}$	[8]
$\beta_2$	$3.72316 \times 10^{-6}$	$(N_{\text{at}}/\text{mL})^{-1}/\text{day}$	adjusted
$\beta_4$	$2.77351 \times 10^{-7}$	$(V_{\text{free}}/\text{mL})^{-1}/\text{day}$	[47]
$\mu$	0.14	$\text{day}^{-1}$	[8]
$c$	10	$\text{day}^{-1}$	[61]
$p$	400	virions/ $A_P$	[65]
$r$	2.5	$\text{day}^{-1}$	[47]
$\delta$	4	$\text{day}^{-1}$	[61]
$\eta$	2	$\text{day}^{-1}$	[61]
$k_1, k_2$	0.52	$\text{day}^{-1}$	[66]
$k_0$	0	$\text{day}^{-1}$	assumed
$k_3$	0	$(N_{\text{in}}/\text{mL})^{-1}/\text{day}$	assumed
$k_4$	0.4	$\text{day}^{-1}$	[47]
$\mu_1$	0.07	$\text{day}^{-1}$	[66]
$\mu_2$	0.0412	$\text{day}^{-1}$	[67]
$\mu_3$	0.05	$\text{day}^{-1}$	[34]
$\mu_4$	0.008	$\text{day}^{-1}$	[34]
$k_3$	0	$\text{day}^{-1}$	assumed
$k_4$	0.4	$\text{day}^{-1}$	[47]
$k_{12}$	0.0008	$(\text{pg}/M_I)/\text{day}$	[47]
$k_{\alpha\beta}$	0.0029	$(\text{pg}/A_{I,P})/\text{day}$	[47]
$k_\gamma$	0.02	$(\text{pg}/N_{\text{at}})/\text{day}$	[47]
$K_{\alpha\beta}$	75	pg/mL	[34]
$K_{12}$	75	pg/mL	[34]
$K_\gamma$	75	pg/mL	[34]
$\mu_{12}$	1.188	$\text{day}^{-1}$	[47]
$\mu_{\alpha\beta}$	2.77	$\text{day}^{-1}$	[47]
$\mu_\gamma$	3	$\text{day}^{-1}$	[47]
$k_Q$	0.77	$(\text{pg}/\text{mL})^{-1}/\text{day}$	calculated
$K_Q$	3.63838	pg/mL	calculated
$\mu_H$	1.28	$\text{day}^{-1}$	[8]
$k_B$	0.6	$(\text{pg}/\text{mL})^{-1}/\text{day}$	calculated
$K_B$	4	pg/mL	assumed
$\rho_H$	$8.415 \times 10^{-5}$	$(\text{pg}/\text{virion})/\text{day}$	calculated
$\rho_N$	$1.5 \times 10^{-7}$	pg/ $N_{\text{at}}$	calculated
$\delta_M$	0.0346	$\text{day}^{-1}$	calculated
$\theta$	400	pg/mL	assumed
$m, n$	2		

Table 2: Table with the description of the parameters used in the numerical simulation of differential equations.

IC's	Value	Range	Units	References
$A_0(0)$	$4 \times 10^5$		$A_0/\text{mL}$	[50]
$A_I(0)$	1		$A_I/\text{mL}$	
$A_P(0)$	1		$A_P/\text{mL}$	
$V_{\text{free}}(0)$	$10^6$	$[600, 10^6]$	$V_{\text{free}}/\text{mL}$	[50]
$V_{\text{int}}(0)$	10		$V_{\text{free}}/\text{mL}$	[50]
$N_{\text{in}}(0)$	$3 \times 10^5$		$N_{\text{in}}/\text{mL}$	[38]
$N_{\text{at}}(0)$	0		$N_{\text{at}}/\text{mL}$	–
$M_0(0)$	$10^6$		$M_0/\text{mL}$	[34]
$M_I(0)$	1		$M_I/\text{mL}$	[34]
$I_{12}(0)$	6	$[0 - 9.5]$	$\text{pg}/\text{mL}$	[43]
$I_{\alpha\beta}(0)$	20	$[0 - 20]$	$\text{pg}/\text{mL}$	[41]
$I_\gamma(0)$	30	$[0 - 60]$	$\text{pg}/\text{mL}$	[41]

Table 3: Initial conditions used in the simulations.

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