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Model of influenza A virus infection: dynamics of viral antagonism and innate immune response

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Abstract

Viral antagonism of host responses is an essential component of virus pathogenicity. The study of the interplay between immune response and viral antagonism is challenging due to the involvement of many processes acting at multiple time scales. Here we develop an ordinary differential equation model to investigate the early, experimentally-measured, responses of human monocyte-derived dendritic cells to infection by two H1N1 influenza A viruses of different clinical outcome: pandemic A/California/4/2009 and seasonal A/New Caledonia/20/1999. Our results reveal how the strength of virus antagonism, and the time scale over which it acts to thwart the innate immune response, differ significantly between the two viruses, as is made clear by their impact on the temporal behavior of a number of measured genes. The model thus sheds light on the mechanisms that underlie the variability of innate immune responses to different H1N1 viruses.

1. Introduction

Despite advances in treatment and vaccination, influenza A virus remains a major international health concern [1]. Influenza virus strains vary in their pathogenicity, and epidemiological features. The causes of these differences are not fully understood and may include a combination of viral dynamics, previous immunity, and the virus specific effects

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of antagonism on the innate immune response (for reviews see [2, 3, 4]). The interplay between these two opposing forces—the immune response and the virus strategy to evade it—is complex and involves multiple processes acting at different time scales. This complexity makes the study of the early dynamics of response to viral infection and the comparison between different viral strains challenging.

Many cellular components are involved in the innate immune response to infection, including epithelial barriers, phagocytes such as neutrophils and macrophages (the first line of defense against microbes if they breach these barriers), natural killer cells, and dendritic cells. Dendritic cells (DCs) are particularly interesting because they possess the unique capability of triggering and directing adaptive cell-mediated immune responses in a way that is dependent on their innate immune responses to microbes. In that way, differences in the early response may have a major impact on the immune response following infection.

The first steps of DCs early immune response upon virus detection are interferon $\text{IFN}\beta$ production, its secretion and binding to interferon receptors, and subsequent activation of signal transduction pathways that trigger the coordinated induction of a diverse set of genes, the so-called ISGs (Interferon stimulated genes) that establish an antiviral state in uninfected cells and in infected cells. While many details of this complex process have not been fully characterized, the main ingredients have been identified. We developed a theoretical model for the early-time dynamics of the DC immune response to different viral strains based on key steps in the virus-DC interaction, and compared our results with our recent experimental measurements. Viral entry into the cell is detected by a cytoplasmic protein RIG-I. This detection leads initially to the activation of a number of transcription factors, which are responsible for the induction of genes, such as $\text{TNF}\alpha$, and most importantly $\text{IFN}\beta$. Protein $\text{IFN}\beta$ is secreted, and binds in an autocrine and paracrine manner to its cognate receptors on cell surfaces. Its binding activates the Jak/Stat pathway. This pathway, via phosphorylation and dimerization of STAT1 and STAT2, leads to the induction of many genes, some of which give rise to antiviral proteins such as OAS and MX1, others to RIG-I which is instrumental in detecting the virus, and to IRF7, which in infected cells enhances $\text{IFN}\beta$ induction and is the transcription factor of $\text{IFN}\alpha$.

Influenza A virus has developed multiple strategies to thwart DCs cellular response to infection. One of its main weapons in evading DC response is the viral non-structural NS1 protein coded by one of the eight negative-sense segments of the viral RNA [5, 6]. NS1 is a multifunctional protein that interferes with the immune response at many levels [7], starting with the detection of viral presence by the cell. It reduces the level of $\text{IFN}\beta$ induction [8], prevents dendritic cell maturation and consequently the activation of adaptive immunity [9]. In the early stages of infection, the NS1 protein counteracts the immune response at two main points, by limiting on the one hand virus detection, and by interfering on the other with antiviral gene induction by the host cell. The virus escapes detection because the NS1 protein prevents in different ways [3] the activation of the cytoplasmic viral sensor RIG-I. It blunts antiviral gene induction through binding to the host CPSF protein [10, 11] which is essential for pre-mRNA processing, and also to proteins of the nuclear export system, blocking nuclear export [12]. Lack of activation of RIG-I leads to curtailment of the signaling pathways that activate $\text{NF}\kappa\text{B}$, IRFs, and AP-1, which participate in the formation

of the enhanceosome responsible for IFN β induction. The suppression of IFN β induction is a serious blow to the cell's antiviral response, since it entails reduced secretion of IFN β , and therefore diminished paracrine and autocrine signaling. For many strains of influenza A virus such as pandemic A/California/4/2009, this appears to be the principal aspect of their antagonistic function [3, 13]. For others such as seasonal A/New Caledonia/20/1999, there is, in addition, inhibition of pre-mRNA processing and nuclear mRNA export and therefore curtailment of cytoplasmic mRNA levels. There is a time window of opportunity for the host cell to mount a strong response before the virus can produce enough NS1 proteins to block the anti-viral functions of the host cell. Therefore, both understanding this competing dynamics in the first few hours after infection and extracting the differences between flu strains are of great interest and therefore the focus of our modeling study.

In order to shed light on the interplay, for different viruses, between the processes involved in NS1 antagonism and the immune response, we studied the infection of human monocyte-derived DCs by two influenza A H1N1 viruses, either the pandemic swine-origin A/California/4/2009 (Cal/09), or the seasonal A/New Caledonia/20/1999 (NC/99). We developed an ordinary differential equation (ODE) model applicable to both types of viral infection. The model embodies the contest between the cell's defense system following viral entry and detection, and the virus' attempts to thwart it. We tested the model results against experimental time course data for a reduced set of genes. Our model is—to the best of our knowledge—the first one to incorporate viral antagonism at the scale of intracellular interactions. This ODE model enabled us to explain the differences in the immune response to the two viruses, which are illustrated in Figure 1 by the time courses of IFN β gene expression for Cal/09 and NC/99. It allowed us to extract the specific features, such as strength and time scale, of NS1 antagonism for each viral strain, and its impact on gene expression in IFN β induction and in IFN β signaling pathways.

2. Methods

2.1. Model description and assumptions

The present model is built from one [14, 15] we developed for understanding data on infection of human monocyte-derived DCs with influenza virus PR8 after pretreatment with IFN β , or with Newcastle Disease Virus (NDV) expressing Nipah virus proteins [16, 17]. The similarity of the equations in the two models is the consequence of a common early immune response of DCs. The equations differ by the ways viral antagonism acts, because the latter does so very differently for Nipah chimeras and influenza A viruses.

Our model comprises main elements of the innate immune network of genes and proteins in DCs. The schematic of the innate immune response network described by the ordinary differential equation (ODE) model is shown in Figure 2, where, additionally, the points of viral NS1 protein antagonist activity are marked. There are three compartments, external medium, cytoplasm and nucleus, and a positive feedback loop associated with IFN β/α , and TNF α . The cellular protein RIG-I detects viral intrusion and initiates a signaling pathway that leads to the induction of TNF α and IFN β . Upon secretion the latter activates the Jak/Stat pathway through phosphorylation of Tyk2/JAK1*, followed by phosphorylation of STAT, formation of dimers, translocation to the nucleus, and induction of IRF7 which

contributes to IFN α/β induction. There is induction as well of SOCS which impedes STAT phosphorylation, and therefore the upstream activation of the interferon-induced signaling pathway.

The following assumptions have been introduced in the model. We do not distinguish STAT1 and STAT2, SOCS1 and SOCS3, and do not introduce explicitly the transcriptional trimer ISGF3 that comprises IRF9 besides the STAT dimer [3, 4] in order to avoid the introduction of numerous unknown parameters while preserving the basic mechanistic structure of the response. We take into account a possible Jak/Stat pathway-independent induction of SOCS [18], but neglect the indirect influence of TNF α on the Jak/Stat pathway through induction of IFN β with the help of IRF1 [19], as well as interferon-independent pathways for induction of interferon-stimulated genes (ISGs) [20].

IRF7 is singled out for two reasons. It is the transcription factor of the IFN α gene, and it contributes strongly to the formation of the IFN β transcription complex [21]. More importantly, the measured time course of IRF7 gene expression after viral infection is typical of similar genes induced by the same trimeric complex (ISGF3), genes such as RIG-I, and the antiviral genes MX1 and OAS [18, 22]. This is seen in our experimental data on IRF7, MX1, DDX58 (the gene that codes for the cytoplasmic viral sensor RIG-I), OAS1/2, and EIF2AK2 (PKR) (Figure 3), all of them genes induced in the Jak/Stat pathway by interferon signaling. The data show that, for the same infecting virus, the time course behavior of any one of these genes is similar. Furthermore, the relative magnitude of gene expression levels for the two viral infections (NC/99 and Cal/09) is approximately maintained across the genes. Thus, for the sake of model simplification, IRF7 can be taken as the representative gene of this class of interferon-stimulated genes important in the immune response.

2.2. Model equations

The equations of the model represent the network and its interactions shown in Figure 2. They describe the temporal behavior of IFN β/α mRNA and TNF α mRNA and their respective proteins in the environment, of STAT mRNA and its protein denoted STAT, of the phosphorylated STAT dimer STATP2n in the nucleus, of SOCS mRNA, and of IRF7 mRNA and activated IRF7 protein IRF7Pn in the nucleus. There are thus 12 network components and equations. Apart from the crucial factors in the equations that describe the antagonism carried by the viral NS1 protein, the equations are similar to those in reference [15], which includes a detailed derivation based on an extensive review of the literature.

In the present model, NS1 antagonism of the immune response is characterized by expressions IC1 and IC2, explained below (section 2.3.), that embody the principal, dual aspect of viral antagonism of both interferon induction and mRNA preprocessing. The equations are:

$$dIFN\beta_m/dt = (r_0 * IC1 + k_{15} IRF7Pn) * IC2 - IFN\beta_m * \log(2)/\tau_1 \quad (1)$$

$$dIFN\beta_{env}/dt = \gamma C v_{max2} IFN\beta_m / (K_2 + IFN\beta_m) \quad (2)$$

$$dSTATP_{2n}/dt = k_5 TJ * STAT / (2(K_5 + STAT)) - STATP_{2n} * \log(2) / \tau_3 \quad (3)$$

$$dSOCS_m/dt = (r_3 * IC_1 + k_8 STATP_{2n}) * IC_2 - SOCS_m * \log(2) / \tau_4 \quad (4)$$

$$dIRF\gamma_m/dt = (k_{11} STATP_{2n} + k_{14} IRF\gamma P_n) * IC_2 - IRF\gamma_m * \log(2) / \tau_6 \quad (5)$$

$$dIRF\gamma P_n/dt = k_{12} IRF\gamma_m * IC_1 \quad (6)$$

$$dIFN\alpha_m/dt = k_{16} IRF\gamma P_n * IC_2 - IFN\alpha_m * \log(2) / \tau_8 \quad (7)$$

$$dIFN\alpha_{env}/dt = \gamma C v_{max17} IFN\alpha_m / (K_{17} + IFN\alpha_m) \quad (8)$$

$$dTfN\alpha_m/dt = \left(r_1 * IC_1 + \frac{r_{20} * TfN_{env}}{K_{20} + TfN_{env}} \right) * IC_2 - TfN\alpha_m * \log(2) / \tau_9 \quad (9)$$

$$dTfN_{env}/dt = \gamma C v_{max19} TfN\alpha_m / (K_{19} + TfN\alpha_m) \quad (10)$$

$$dSTAT_m/dt = (r_4 * IC_1 + k_{26} STATP_{2n}) * IC_2 - STAT_m * \log(2) / \tau_{12} \quad (11)$$

$$dSTAT/dt = k_{26} STAT_m - STAT * \log(2) / \tau_{13} \quad (12)$$

The 12 species names denote the corresponding concentrations in μM , C is the experimental concentration of cells in the medium which is 5×10^5 cells/ml. The factor $\gamma = 10^9/N_A$ (where N_A is Avogadro's number and equal to 6.023×10^{23} molecules/mol) in equations (2), (8) and (10) is a conversion factor from numbers per ml for the right-hand sides of these equations to concentrations in μM for the left-hand sides. TJ is a quantity that describes binding of $IFN\alpha/\beta$ in the environment to their receptors and the effect of the negative feedback loop associated with SOCS. The expression of TJ is

$$TJ = TJ_{tot} \frac{IFN\beta_{env} + IFN\alpha_{env}}{K_3 + IFN\beta_{env} + IFN\alpha_{env}} \frac{1}{1 + \frac{K_9 SOCS_m}{\delta}} \quad (13)$$

The constants have the same values for the two viruses Cal/09 and NC/99, with $TJ_{tot} = 10^{-4}$ μM , $K_3 = 4.3 \times 10^{-3}$ μM , $K_9 = 7.8 \times 10^2$, and $\delta = 0.0003$ μM . Initial values are zero for all molecules, except for STAT proteins, for which the concentration is taken to be 0.1 μM .

Each model equation for gene expression is composed of a production term on which NS1 antagonism acts, and a degradation term characterized by a half-life. As an example, consider equation (1) for $IFN\beta$ mRNA induction, the gene whose expression is most crucial

to the innate immune response. The induction of interferon- β mRNA contains an early response term represented in a very simple way by $r0$ that occurs due to the constitutive presence in the cell of all of the components of the IFN β enhanceosome (the higher $r0$, the faster the induction of IFN β m). The degradation term is characterized by a half-life τ_I , in such way that the longer the half-life, the slower the degradation of IFN β m. The amount of IFN β m as a function of time is the result of the combined effect of production and degradation. The viral antagonism of the production process is denoted by the factor IC1 defined below (expression (14)) (the lower the IC1 value, the greater the antagonism and its reduction of $r0$). In addition, there is a positive feedback due to IRF7Pn that is included in the second term. Since the production of mRNA by both processes is curtailed by the CPSF binding mechanism of NS1, both terms are multiplied by the viral antagonism described by IC2 also defined below (expression (15)). The two antagonisms, embodied in the terms IC1 and IC2, depend on the time-dependent concentration of the NS1 protein (expression (16)), which we infer for each viral strain from NS1 mRNA measurements (Figure 4).

2.3. Viral antagonist factors

The viral antagonist factors that appear in the equations above are denoted IC1 and IC2. IC1 represents NS1 protein antagonism in the pathway leading to IFN β induction which is activated after viral detection by RIG-I. IC2 represents NS1 protein curtailment of cytoplasmic mRNA levels due to viral inhibition of pre-mRNA processing and blocking of mRNA nuclear export. In the absence of antagonism, the factors IC1 and IC2 which multiply the production terms in the model equations are equal to 1. In the presence of antagonism, the smaller their value, the stronger the corresponding antagonism is in suppressing production.

IC1 and IC2 depend on time, because their expression depends on the concentration of the viral NS1 protein (denoted NS) which depends on time (equation (16)). We use the following Hill-type equations for IC1 and IC2:

$$IC1 = \frac{1 + sp * (NS/\delta_1)^{n1}}{1 + (NS/\delta_1)^{n1}} \quad (14)$$

$$IC2 = \frac{1 + sv * (NS/\delta_2)^{n2}}{1 + (NS/\delta_2)^{n2}} \quad (15)$$

Here δ_1 and δ_2 act as thresholds for the action of the antagonism. At saturation values of NS, IC1 and IC2 respectively reach the values of sp and sv . Pre-mRNA processing inhibition is considered very weak for Cal/09 NS1 [13] and therefore we take $IC2=1$ for Cal/09 infection.

The inhibitory factors IC1 and IC2 depend on the concentration of the NS1 protein (NS), which is not measured in our experiments. However its temporal behavior can be estimated from the measured NS1 mRNA (Figure 4).

For the growth in time of NS1 protein (NS) we use the following Hill function form

$$NS = r5 * t^{n3} / (bm^{n3} + t^{n3}) \quad (16)$$

The value of $r5 = 1 \mu\text{M}$. The value (in hours) of bm , which describes how fast NS1 protein rises, is estimated from the time behavior of the two viral NS1 mRNAs in Figure 4. Here one sees that the rise of Cal/09 NS1 mRNA is slower and occurs later than the rise of NC/99 NS1 mRNA. The fixed values of $n3$ and bm in expression (16) reflect this difference (see Table 1), and are taken to be respectively equal to 5 and 6 hr for Cal/09, and 8 and 4.5 hr for NC/99.

2.4. Simulations and Parameter values

The immune response gene levels were obtained experimentally from luminosity measurements (see Appendix B), whereas the model ones are expressed in terms of concentration. Since there is no accurate calibration of luminosities, the comparison of model results and experimental data requires the use of normalized expression levels. We therefore divide, for both viruses, each gene level at each time point by the corresponding Cal/09 gene expression level at the last measured time point after infection, which is 8 hours.

For all parameters in the equations, except those associated with NS1 antagonism, the values found previously for NDV-Nipah virus infection of human DCs [15] serve as calibration. We fit the model results to experimental measurements of the gene expression levels of IFN β , IFN α , TNF α , STAT and IRF7. Of the total number of parameters in the model for either virus, most are estimated (see Table 1). The number of parameters varied to achieve fits to the data is reasonably small, 10 parameters for 35 data points for Cal/09 corresponding to the measurements of five genes at eight time points, and 13 parameters for 40 data points for NC/99 obtained from the corresponding measurements of the same five genes. The number of independent data points is smaller for Cal/09 because of the normalization to their eight hour time point for each gene expression level. A comprehensive summary of the parameters fitted and estimated can be found in Table 1.

The fitting for each viral infection proceeds in stages that rely on comparison of model simulation and of experimental results, with the aim of reducing the differences between them across time for the five genes considered. The fitting procedure is designed to obtain qualitative fits to the data for parameter values in a biologically-reasonable region of parameter space, with emphasis on the time scales involved in the temporal development of gene expression, in particular those that characterize the two NS1 antagonism factors IC1 and IC2 (see section 2.3.). For parameters common to this and our previous work, the starting values are taken from our previous investigations of human DC infections by NDV-Nipah protein chimeras [15]. Many parameters are kept fixed (see Table 1), such as half-lives; we identified by inspection of the model equations and the experimental data those parameters that can be expected to play a dominant role in fitting the model results to the data, and varied them manually. There are 10 parameters that are varied for Cal/09 infection and 13 for NC/99 infection (see Table 1) with 3 less for Cal/09 because IC2=1 (see section 2.3.). The 13 parameters are the rate of IRF7 translation, the rates of gene induction (for

IFN β , IRF7, IFN α , TNF α , and STAT) which in the model determine the early linear rise of gene production, as well as the strengths, thresholds and exponents for the two factors IC1 and IC2 that define NS1 antagonism and counteract the early linear rise of gene expression levels. The parameter values for IC2, which represents the expression of NS1 antagonism to interferon signaling operative for viral strain NC/99 and not for Cal/09, are mainly determined by the model fits to IRF7 and STAT mRNA expression levels. For these two genes only IC2 antagonism enters directly, because their expression is not part of the IFN β induction pathway but is stimulated through interferon signaling. There is also in expression (13) an additional fitted parameter, namely the Michaelis-Menten constant K_3 , which controls the impact of secreted interferon on downstream gene expression. One obtains in the end a reasonably good fit of the model to experimental results in a domain of parameter values restricted by the above mentioned calibration, though presumably not the best possible one in a much larger parameter space.

We followed our informed manual fit by the use of an evolutionary algorithm to check whether the parameter values obtained as described above belonged to the family of optimal solutions when the antagonism parameters for IC1 and IC2 (sv , sp , δ_I , δ_2 , $n1$, and $n2$) are varied in our model over a wide range of parameter values. For this purpose we used a standard genetic algorithm [23] with a fitness function equal to the least-square distance between model and experimental results. Briefly, we generated a starting set of vectors with the parameter values, tested their fit to the experimental data using the least-square cost function, and retained the solutions with the lowest value. These solutions were in turn mutated by changing the values of the parameters, and checked for an improvement in the fit of the model to the data. At each iteration the best solutions were saved and mutated again. The algorithm stopped when no more improvement in fitting the experimental data was achieved. The range of parameter values explored for Cal/09 are $n1 = [0.1, 3.16]$, $\delta_I = [0.01, 10]$, and $sp = [0.03, 30]$. The range of parameter values explored for NC/99 are $n1 = [0.5, 15.81]$, $\delta_I = [0.01, 10]$, $sp = [0.01, 10]$, $n2 = [0.5, 15.81]$, $\delta_2 = [0.04, 40]$, and $sv = [0.03, 30]$. The results from the evolutionary algorithm support our choice of time scales and relative magnitudes of the parameters for each virus, in particular those parameter values that occur in expressions (14) and (15) of IC1 and IC2 and which characterize the two ways NS1 antagonism acts.

Viral antagonism determines to a large extent the temporal behavior of gene expression levels, which therefore can show a high sensitivity to certain parameters associated with viral antagonism. In Appendix A we study the sensitivity of IFN β and IRF7 with respect to parameters of the antagonist factors IC1 and IC2. This sensitivity is itself a time-dependent quantity because it depends on the characteristic behavior in time of IC1 and IC2 (Appendix A).

All simulations were performed in MATLAB (<http://www.mathworks.com>) with the *ode15s* solver for the differential equations, and the genetic algorithm functions of the Optimization Toolbox for the evolutionary algorithms.

2.5. Data analysis: infected and uninfected cells

Infections of human dendritic cells by influenza A are not productive [24], and thus the amount of infected cells depends exclusively on the initial infection and remains stable during the experiment. The model considered (see section 2.2) describes infected cells. However, experimentally the system contains both infected and uninfected cells, and measurements have contributions from both varieties for those genes expressed as a result of interferon signaling. This poses a problem for comparing model results to experimental data which is resolved below.

In our experimental measurements multiplicity of infection (MOI) is equal to 1. At MOI=1, close to 40% of the cells are uninfected. Uninfected cells get stimulated through paracrine signaling by the cytokines secreted by the infected cells as part of their immune response. Any experimental measurement of interferon stimulated genes (ISGs) has contributions from both infected and uninfected cells. The contribution from uninfected cells can dominate ISG gene expression whenever their number is high. NS1 antagonism of ISG mRNA nuclear processing and export, when present, acts in infected cells only, and thus enhances even more the relative contribution to ISG expression from uninfected cells. Comparison of model with experiment for the two viruses, Cal/09 and NC/99, therefore requires a method for estimating the contribution from uninfected cells to the measured expression levels of ISGs, of which there are two in the model, namely IRF7 and STAT1. We obtain this estimate in a semi-quantitative way. As a first approximation, and neglecting the effect of other pathways, we suppose that, for Cal/09, infected and uninfected cells, which bathe in the same cytokine environment, have similar expression levels for ISGs, because of the lack of CPSF binding by the Cal/09-encoded NS1 [18]. If this is a reasonable assumption, the expression levels of ISGs in DCs infected by Cal/09 should only weakly depend on the number of infected versus uninfected cells, i.e. on the value of MOI. A comparison of interferon stimulated gene IP10 expression levels for MOI=0.5, 1 and 2 is consistent with this assumption (data not shown). We then posit that uninfected cells behave the same way for NC/99 as for Cal/09. One neglects thus the differences that may exist between the two viruses as to the magnitude and time dependence of the respective cytokine environments and their impact on cell receptors. One is then led to the prescription that for NC/99 infections, for any ISG, one should subtract from the experimental data the 40% contribution coming from Cal/09 measurements, which represents an estimate of the contribution to each measured gene from the uninfected cells. The same procedure is applied to Cal/09 itself. The remainder of gene expression level for the two ISGs in the model, IRF7 and STAT1, is the amount due to infected cells, which is the amount compared to the numerical solutions of our model. Despite its approximate character, we believe our method for extracting the contribution of uninfected cells to be adequate for our purpose, which is the comparison for the two viruses considered of the mechanisms of NS1 antagonism and their development in time after infection.

3. Results

We constructed an ODE model with a limited set of equations (see Methods section) and fitted it separately to experimental data obtained for two influenza A H1N1 viral strains,

Cal/09 and NC/99. The experimental methods are described in Appendix B. Gene expression levels were measured for IFN β , IFN α , TNF α , STAT1 (STAT) and IRF7, and the corresponding amounts were computed with the model. The resulting parameter values of the model (see Table 1) allowed us to compare the action of viral antagonism for the two strains.

After virus entry into the cell, antagonism to the induction of IFN β , the initiator of the cellular antiviral response, is first to manifest itself, when the viral NS1 protein attempts to prevent virus detection by the cell's sensor (i.e. RIG-I), and disrupt the activation of transcription factors that lead to IFN β induction. This antagonism is described in the model by factor IC1 (expression (14), and Figure 5). Once transcribed from the viral gene, the NS1 protein acts to inhibit mRNA nuclear production. It does so for NC/99, but not for Cal/09. This antagonism is represented in the model by factor IC2 (expression (15) and Figure 5). The succession in time of the two antagonisms for NC/99 is implemented in the model by choosing different thresholds of NS1 protein concentration at which the corresponding antagonism becomes effective. The two threshold factors control the point in time when NS1 protein concentration (see expression (16)) reaches a level where the viral antagonism embodied in factors IC1 and IC2, shown in Figure 5, starts inhibiting gene induction. In the model the threshold is smaller for IFN β induction antagonism (factor IC1) than for mRNA processing antagonism ($\delta_1 = 0.1 \mu\text{M}$ in (14) for IC1, $\delta_2 = 0.4 \mu\text{M}$ in (15) for IC2; see Figure 5 and Table).

The temporal behavior of IC1 and IC2, with the corresponding parameter values, is given in Figure 5 for infections by NC/99 and Cal/09. For IFN β and TNF α , gene expression levels depend on the product IC1*IC2 (equations (1) and (9)), shown as well in Figure 5 for NC/99 (for Cal/09, IC1*IC2=IC1 as noted in section 2.3.). The shape of IC1*IC2 in Figure 5 illustrates the fact that for NC/99 infection viral antagonism reaches its maximum strength in a short time scale, shown by a sharp drop at about 3.5 hours and determined by IC1. In contrast, for Cal/09 infection viral antagonism acts on a much longer time scale than for NC/99, as indicated by a decrease of IC1 between early and late times that is much slower in comparison. Beyond 4 hours the level of viral inhibition, measured by the smallness of IC1*IC2, is also much higher for NC/99 than for Cal/09 infection.

Clearly the time scales that characterize the inhibitory behavior of IC1, IC2 and IC1*IC2, are closely associated with the location in time of the peak of IFN β expression for NC/99 infection and of the plateau for Cal/09 infection. Thus, the time of about 3.5 hours at which NS1 antagonism of IFN β starts being fully expressed for NC/99 infection (see Figure 5), coincides with the time of peak IFN β expression. It must however be noted that the measured temporal shapes of genes (see in particular IFN β and TNF α) are not determined only by the time scales associated with NS1 antagonism, though this antagonism is essential to break the initial rise of gene induction. Other factors in the model that influence gene expression levels over the time period considered are the gene half-life, and at very early times, the rate of gene production. For IFN β and TNF α genes the values of the model parameters that enter IC1 and IC2 are the same for infection by NC/99 or by Cal/09. NS1 antagonism, embodied in the product IC1*IC2, acts thus with the same strength and over the identical time scale for both infections. Therefore the difference in temporal behavior

observed for the two genes is mainly due to the difference in their production rates, with the production rate of TNF α (r_I in (9)) close to an order of magnitude smaller than that of IFN β (r_0 in (1)) (see Table 1).

The model simulations of experimental data (Figures 6) lead to the following insights for viral infection by pandemic Cal/09 and seasonal NC/99, and for the comparison of their respective antagonisms of the innate immune response.

3.1. Cal/09 infection shows mild IFN β induction antagonism

The comparison of the data (symbols) for IFN β , IFN α , TNF α , STAT1, and IRF7 mRNAs with a model simulation (continuous line) is shown in Figure 6. The simulation provides a reasonable fit to the data, except perhaps for IFN α for which however the data are noisy with low expression levels relative to control (data not shown). The ISGs, STAT1 and IRF7, are fully expressed since NS1 lacks the CPSF binding and mRNA nuclear export antagonism. The experimental data for these two genes have been adjusted, as described in section 2.5., in order to eliminate the contribution to their expression level from uninfected cells. The leveling-off after four hours of IFN β , and TNF α , whose shapes are similar, reflects the impact of NS1 antagonism of the interferon induction pathway. This antagonism is relatively weak and delayed in time with a maximum reached beyond 4 hours after infection (see Figure 5), a result consistent with the slow and late rise of Cal/09 NS1 mRNA (see Figure 4) when compared to that of NC/99. The strength of the antagonism, as manifested by the function IC1 (expression (14)), is four times greater for NC/99 than for Cal/09 (Figure 6).

3.2. NC/99 infection shows strong antagonism of both IFN β induction and nuclear mRNA

What is striking in the results for NC/99 for both IFN β and TNF α (see Figure 6) is the very strong early induction starting at 2 hours, combined with a rapid decrease after 3 hours. This behavior of IFN β induction, reflects the early contest—within one to two hours after infection—between on one hand detection of viral entry by the cell, and on the other hand production of the viral antagonistic protein NS1.

The other important result in Figure 6 is the low level of STAT1 and IRF7 for NC/99 when compared with Cal/09, particularly at late time points. The experimental data for these two genes have been adjusted, as described in section 2.5., in order to eliminate the contribution to their expression level from uninfected cells. The peak seen at 4.5 hours in the model simulation is associated with the “turning-on” of the inhibition carried by IC2 (see Figure 5). The simulation results lie above the experimental points at later times, an effect which may be influenced by the approximate subtraction of the contribution from uninfected cells as we have described above (Methods section). Clearly, mRNA export inhibition is strong in infected cells, leading to reduced levels of cytoplasmic mRNA. One infers that the ISG experimental expression levels shown in Figure 3 are dominated by the contribution from uninfected cells.

The equality in our simulations of the values of IFN β for the two viruses close to 8 hours is a result of higher early production of IFN β and faster viral antagonism for NC/99 leading to

the same level of IFN β as for Cal/09, where early production is lower and antagonism weaker. If further experiments were to confirm that beyond eight hours after infection IFN β expression levels follow nearly equal plateaus, our model, which does not show this feature, would need to be extended to take into account additional processes that contribute to gene regulation.

3.3. Interferon induction antagonism is stronger for NC/99 than for Cal/09

Antagonism of IFN β induction for NC/99 is much stronger than for Cal/09, because the maximum strength of that antagonism is larger and is reached sooner for NC/99 (see Figure 5, and compare the values of in Table 1 of parameters sp that controls the strength, and nI that controls the steepness of the increase in equation (14)). A level of 50% of antagonistic effect by the NS1 protein is achieved shortly after 3 hours after infection for NC/99 infection, and about 1.5h later for Cal/09 (see Figure 5).

4. Discussion

We have proposed an ODE model that extracts what we believe are the principal features of the early time contest between cellular innate immune response in human dendritic cells and viral NS1 protein antagonism after infection by two influenza A H1N1 viruses, pandemic Cal/09 and seasonal NC/99. Our results show that Cal/09, which lacks mRNA nuclear antagonism, exhibits weak IFN β induction antagonism, whereas NC/99 has both strong IFN β induction antagonism and mRNA nuclear antagonism. We have proposed a phenomenological way to characterize viral antagonism (equations (14) and (15)) that allows us to put these results on a quantitative footing, through the parameter values that determine rates of gene induction and the strength of NS1 antagonism and time scales over which it acts.

The model also gives general insight into the two main features of early viral NS1 antagonism, namely antagonism of both interferon induction and interferon signaling processes. For the first process, it takes expression levels of IFN β as representative, for the second one, those of IRF7 (see Figure 6). The temporal behavior of these genes differs greatly between the two viruses. An inspection—informed by the model results—of those two shapes provides hints for a general characterization of viral antagonism and early immune response in dendritic cells. The interferon levels show a strong correlation between early production, which is the cell's immune response, and NS1 antagonism and its characteristic time and strength. The earlier NS1 antagonizes IFN β production, the earlier the maximum or flattening of IFN β expression. Thus, from the results in Figure 6, NS1 antagonism occurs over a faster time scale and more strongly for NC/99 than Cal/09. In the model, the two main NS1 antagonisms act multiplicatively in the interferon induction pathway, but not so for ISGs in the Jak/Stat pathway where only the mRNA export inhibition matters. Here the distinction in the experimental measurements between the contributions of infected and uninfected cells is crucial, since only in the former mRNA export inhibition operates. One can predict that if for infected cells expression levels of ISGs are low—if they can be extracted from the data, or the data is obtained at such high MOI that the number of uninfected cells is small—the relevant virus has weak CPSF binding and nuclear export blockage of mRNA.

Our model incorporates a number of simplifications. We have taken the IRF7 gene expression as a representative of a large class of genes induced by the same transcription factor after Jak/Stat pathway activation. We model the cellular processes of influenza A infection, with emphasis on what is believed to be the main weapon of influenza A viral antagonism, namely the viral NS1 protein. NS1 mediates its antagonism predominantly through the disruption on one hand of the IFN β induction pathway, and on the other hand of cytoplasmic mRNA accumulation. These antagonistic activities stand as summaries of a number of different mechanistic processes that underlie them [3, 4]. We do not consider other forms of NS1 antagonism, such as its inhibition of elongation initiation factor [25], or its role in apoptosis [26]. By introducing these simplifications, one avoids the introduction in the model of many unknown parameters unconstrained by experimental results. The model organization relies on the assumption that the two NS1 protein antagonisms considered act only at the gene production level, while gene degradation rates (or equivalently half-lives) remain unaffected with their values fixed at biologically reasonable levels. Although virus infection can lead to increased mRNA degradation [2], the corresponding processes, involving antiviral proteins whose function can also be antagonized by NS1, are not part of the simple model presented here.

As future work, one could envisage the decomposition of the immune response to viral intrusion into different segments, the development of separate models for each segment as more detailed experimental observations become available, and the subsequent agglomeration of the separate segments into a complete description. Such a decomposition would most certainly include—as we have done here—one segment concerning IFN β induction after viral infection, and a second one about induction in the Jak/Stat pathway of interferon stimulated genes, that would consider separately the contributions of infected and uninfected cells.

In summary, our modeling effort has highlighted the role of the time course of IFN β induction as a sensitive indicator of the contest between its early production following virus entry detection and NS1 antagonism, clarifying the time scales involved, and also the role of IRF7 (or a similar ISG) in revealing the strength of nuclear mRNA inhibition. The mathematical approach is useful, although limited to a small number of genes, because we believe, as we have argued, that it encapsulates typical behavior of many more genes than occur in the equations. This approach could be potentially used to establish a classification of influenza H1N1 viruses in terms of the dynamics associated with NS1 antagonism.

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References

1. Talon J, Salvatore M, O'Neill RE, Nakaya Y, Zheng H, Muster T, Garcia-Sastre A, Palese P. Influenza A and B viruses expressing altered NS1 proteins: A vaccine approach. *Proc Nat Acad Sciences*. 2000; 97:4309–4314.
2. Garcia-Sastre A. Induction and evasion of type I interferon responses by influenza viruses. *Virus Res*. 2011;10.1016/j.virusres.2011.10.017

3. Hale BG, Albrecht RA, Garcia-Sastre A. Innate immune evasion strategies of influenza viruses. *Future Microbiol.* 2010; 5:23.10.2217/fmb.09.108 [PubMed: 20020828]
4. Randall RE, Goodbourn ST. Interferon and viruses: An interplay between induction, signalling, antiviral responses and viral countermeasures. *J Gen Virol.* 2008; 89:1–47. [PubMed: 18089727]
5. Kochs G, Garcia-Sastre A, Martinez-Sobrido L. Multiple anti-interferon actions of the influenza A virus NS1 protein. *J Virol.* 2007; 81:7011–7021. [PubMed: 17442719]
6. Haye K, Bourmakina S, Moran T, Garcia-Sastre A, Fernandez-Sesma A. The NS1 protein of a human influenza virus inhibits type I interferon production and the induction of antiviral responses in primary human dendritic and respiratory epithelial cells. *J Virol.* 2009; 83(13):6849–6862. [PubMed: 19403682]
7. Hale BG, Randall RE, Ortin J, Jackson D. The multifunctional NS1 protein of influenza A viruses. *J Gen Virol.* 2008; 89:2359–2376. [PubMed: 18796704]
8. Hayman A, Comely S, Lackenby A, Murphy S, McCauley J, Goodbourn S, Barclay W. Variation in the ability of human influenza A viruses to induce and inhibit the IFN β pathway. *Virology.* 2006; 347:52–64. [PubMed: 16378631]
9. Fernandez-Sesma A, Marukian BJ, Ebersole S, Kaminski D, Park MS, Yuen T, Sealfon SC, Garcia-Sastre A, Moran TM. Influenza virus evades innate and adaptive immunity via the NS1 protein. *J Virol.* 2006; 80:6295–6304. [PubMed: 16775317]
10. Nemeroff ME, Barabino M, Li Y, Keller W, Krug RM. Influenza virus NS1 protein interacts with the cellular 30 kDa subunit of CPSF and inhibits 3' end formation of cellular pre-mRNA. *Mol Cell.* 1998; 1:991–1000. [PubMed: 9651582]
11. Noah DL, Twu KY, Krug RM. Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAs. *Virol.* 2003; 307:386–395.
12. Satterly N, Tsai PL, VanDeursen J, Nussenzweig DR, Wang Y, Faria PA, Levay A, Levy DE, Fontoura BMA. Influenza virus targets the mRNA export machinery and the nuclear pore complex. *Proc Nat Acad Sciences.* 2007; 104:1853–1858.
13. Hale BG, Steel J, Medina RA, Manicassamy B, Ye J, Hickman D, Hai R, Schmolke M, Lowen AC, Perez DR, Garcia-Sastre A. Inefficient control of host gene expression by the 2009 pandemic H1N1 influenza A virus NS1 protein. *J Virol.* 2010; 84:6909–6922. [PubMed: 20444891]
14. Qiao L, Phipps-Yonas H, Hartmann B, Moran TM, Sealfon SC, Hayot F. Immune response modeling of interferon beta pretreated influenza virus-infected human dendritic cells. *Biophys J.* 2010; 98:505–514. [PubMed: 20159146]
15. Seto J, Qiao L, Guenzel C, Xiao S, Shaw ML, Hayot F, Sealfon SC. Novel Nipah virus immune-antagonism strategy revealed by experimental and computational study. *J of Virology.* 2010; 84(21):10965–10973. [PubMed: 20739535]
16. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol.* 2002; 76:11476–11483. [PubMed: 12388709]
17. Shaw ML, Garcia-Sastre A, Palese P, Basler CF. Nipah virus V and W proteins have a common STAT1 binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. *J Virol.* 2004; 78:5633–5641. [PubMed: 15140960]
18. Pauli EK, Schmolke M, Wolff T, Viemann D, Roth J, Bode JG, Ludwig S. Influenza A virus inhibits type I IFN signaling via Nf κ B-dependent induction of SOCS-3 expression. *PloS Path.* 2008; 4(1):e1000196.
19. Yarilina A, Park-Min KH, Antoniv T, Hu X, Ivashkiv LB. TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon-response genes. *Nat Immunol.* 2008; 9(4):378–387. [PubMed: 18345002]
20. Hasan M, Koch J, Rakheja D, Pattnaik AK, Brugarolas J, Dozmorov I, Levine B, Wakeland EK, Lee-Kirsch MA, Yan N. Trex1 regulates lysosomal biogenesis and interferon-independent activation of antiviral genes. *Nat Immunol.* 2013. 2012 Jan; 14(1):61–71.10.1038/ni.2475
21. Marie I, Durbin JE, Levy DE. Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor-7. *EMBO J.* 1998; 17:6660–6669. [PubMed: 9822609]

22. Phipps-Yonas H, Seto J, Sealfon SC, Moran TM, Fernandez-Sesma A. Interferon β pretreatment of conventional and plasmacytoid human dendritic cells enhances their activation by influenza virus. *PloS Pathogens*. 2008; 4(10):e1000193. [PubMed: 18974865]
23. Multi-objective Optimization using Evolutionary Algorithms. John Wiley & Sons; 2001.
24. Bender A, Albert M, Reddy A, Feldman M, Sauter B, Kaplan G, Hellman W, Bhardway N. The distinctive features of influenza virus infection of dendritic cells. *Immunobiol*. 1998; 198(5):552–567.
25. Katze MG, Krug RM. Translational control in influenza virus-infected cells. *Enzyme*. 1990; 44:265–277. [PubMed: 2133654]
26. Herold S, Ludwig S, Pleschka S, Wolff T. Apoptosis signaling in influenza virus propagation, innate host response, and lung injury. *J Leuk Biol*. 2012; 92:75–82.
27. Borderia AV, Hartmann BM, Fernandez-Sesma A, Moran TM, Sealfon SC. Antiviral-activated dendritic cells: a paracrine-induced response state. *J Immunology*. 2008; 181(10):6872–81. [PubMed: 18981106]
28. Hartmann BM, Li W, Jia J, Patil S, Marjanovic N, Martinez-Romero C, Albrecht RA, Hayot F, Garcia-Sastre A, Wetmur JG, Moran TM, Sealfon SC. Mouse Dendritic Cell (DC) Influenza Virus Infectivity Is Much Lower than That for Human DCs and Is Hemagglutinin Subtype Dependent. *J G Virol*. 2013; 10.1128/JVI.0.02980-12

Appendix A

Using IFN β and IRF7 gene expression levels as representatives of respectively the interferon induction and the interferon signaling pathway, we study their sensitivity to NS1 antagonism parameters sp , δ_1 in IC1 (interferon induction antagonism given by expression (14)) and sv , δ_2 in IC2 (cytoplasmic mRNA inhibition given by expression (15)). The parameters sp and sv describe the strength of NS1 antagonism, while the parameters δ_1 and δ_2 describe the thresholds of NS1 concentrations, which, since the latter depend on time, determine the time scale over which the antagonism acts. From the variations in time of IC1 and IC2 in Figure 5, it is clear that from early to late times the strength of antagonism changes significantly, and the time scale over which this change takes place is characteristic of virus and type of antagonism. One should therefore expect that parameter sensitivity can be large, and that this sensitivity is more or less depending on the time development of gene expression. A consequence is that, overall, the parameters that define NS1 antagonism are well constrained by the experimental time course measurements.

We compute, using a finite difference approximation, the normalized sensitivity to small changes in the j^{th} parameter of the temporal behavior of the i^{th} gene in our ODE model, using the formula

$$S_{ij}(t) = \frac{p_j}{y_i(t, p_j)} \frac{\delta y_i(t, p_j)}{\delta p_j} \quad (\text{A.1})$$

where p_j is one of the parameters considered, and $y_i(t, p_j)$ stands for the expression of gene i of our ODE model at time point t , which depends on the value of parameter p_j . It is necessary to consider time-dependent sensitivities since we are modeling early time, time-varying behavior. The results (Figures A.1 and A.2) show that in most cases sensitivities increase from early to late times. For IFN β m and NC/99 infection (Figure A.1, left panel), the most striking aspect is the precipitous increase of δ_1 sensitivity at 3 hours with a

maximum close to 4 hours, which reflects the predominant role played in IFN β m production by the threshold value in IC1. In contrast sensitivities to the two parameters in IC2 occur later and are lower. For IFN β m and Cal/09 (Figure A.2, left panel) sensitivity to sp is strong at late times, reflecting the impact at later times of IFN β induction antagonism. As to IRF7m, sensitivity to IC2 parameters for NC/99 (Figure A.1, right panel) is strong. Its dominant feature is high sensitivity to δ_2 which peaks at 5 hours for NC/99 infection, which is the time when mRNA antagonism starts inhibiting expression of interferon stimulated genes. For IRF7m and Cal/09 (Figure A.2, right panel), for which there is no dependence on the parameters of IC2, sensitivity dependence on both sp and δ_I is weak.

Appendix B

Experimental Methods

B.1. Differentiation of Dendritic Cells

All human research protocols for this work have been reviewed and approved by the IRB of the Icahn School of Medicine at Mount Sinai. Monocyte-derived dendritic cells (DCs) were obtained from two anonymous healthy human blood donors as described before [27]. Briefly, human peripheral blood mononuclear cells were isolated from buffy coats by Ficoll density gradient centrifugation and positive immunomagnetic purification for CD14+ cells followed by a 5 day incubation with 500 U/ml hGM-CSF (Preprotech, Rocky Hill NJ) and 1000 U/ml hIL-4 (Preprotech, Rocky Hill NJ). The parameters of the model presented correspond to one donor. Similar results and parameters were obtained for the second donor (data not shown).

B.2. Viral Infection

DCs from donors were infected in triplicate with the influenza strains A/California/04/09, A/New Caledonia/20/1999, or allantoic fluid for 20 minutes in RPMI medium at 37°C. In order to achieve comparable levels of infectivity, titers for each viral strain were determined in DCs at 8h post-infection by an anti-NP (Influenza A nuclear protein) mouse monoclonal antibody (ab20921;Abcam) conjugated to the FITC fluorophore. After infection, cells were centrifuged to remove the viral inoculation media, and resuspended in culture medium. Samples were fixed with RNeasy Protect Cell Reagent (Qiagen, Valencia, CA) at 0 (control), 2, 2.4, 3.2, 4, 5, 6, 7, and 8 hours post-infection.

B.3. Gene Expression Measurements

For viral genes, RNA expression was quantified using quantitative RT-PCR. CDNA was synthesized from total RNA using AffinityScript MultiTemp RT (Agilent Santa Clara CA) with an oligo(dT)18 primer. Real-time PCR was performed using PlatinumTaq DNA polymerase (Invitrogen, Carlsbad CA) and SYBR-green (Invitrogen, Carlsbad CA) on an ABI7900HT thermal cycler (Applied Biosystems, Foster City CA). A robust global normalization algorithm, using expression levels of the housekeeping genes ribosomal protein S11 (*rps11*), β -actin (*actb*), and α -tubulin (*tuba*), was used for all experiments, as described elsewhere [27]. In brief, all crossing threshold (Ct) values were first adjusted by median difference of all samples from *actb*. Each individual sample was then further

corrected by the median Ct value of the three corrected housekeeping control for that sample. Finally, nominal copy numbers were calculated by assuming 2500 molecules of *actb* mRNA per cell, and an amplification efficiency of 93% using the difference in Ct value (Ct method). PCR primer sequences for viral NS1 used are GGTGATGCCCCATTCCTTGA for the sense and GGCATGAGCATGAACCAGTC for the antisense.

Levels of expression of other genes were measured as follows. Total RNA from samples was isolated using Rneasy Plus Micro Kit Qiagen, Valencia, CA) or a Rneasy 96 BioRobot 8000 Kit with on-column DNase treatment Qiagen, Valencia, CA), according to the manufacturers protocol. RNA was quantified using a ND-1000 spectrophotometer (NanoDrop, Wilmington DE). The RNA integrity of all samples was confirmed using an Agilent Bioanalyzer 2100 (Agilent Santa Clara CA), according to the manufacturers protocol. Gene expression analysis was performed utilizing the human HT-12 v4

Expression BeadChip arrays (Illumina San Diego CA). A control for infectivity levels for each virus was achieved via staining of the viral nuclear protein NP as previously described [28]. Assays were processed at the Yale Center for Genome Analysis; the subset of genes of interest were assayed using the Illumina iScan (Illumina San Diego CA), and data for gene expression obtained.

B.4. Signal Processing

Data was first quantile-normalized to their luminosity value for each probe. Since multiple probes are available for each gene, we measured the quality of the signal associated to each gene by calculating the signal to noise ratio (SNR) in dBs as follows,

$$S = \frac{1}{N} \sum_{n=1}^N g[n]^2 \quad (17)$$

$$A = \frac{1}{N} \sum_{n=1}^N a[n]^2 \quad (18)$$

$$SNR = 10 \log \left(\frac{S}{A} \right) \quad (19)$$

where $g[n]$ is the sequence including the luminosity values for all time points for a specific gene and for all 3 replicates, $a[n]$ is the sequence including the luminosity values for all time points for the allantoic fluid for all 3 replicates, N is the total number of samples for that gene sequence, S is the average signal power for a particular gene, and A is the noise for that gene as determined by the power of the allantoic fluid signal.

- We study two influenza A H1N1 viral strains with different clinical outcomes
- We model the dynamics of the interplay between viral antagonism and the innate immune response.
- We extract the dynamic features of viral antagonism and innate immune response from time course gene expression measurements for a set of selected genes.
- We find that the strength and time scale of action of viral antagonism is significantly different between the two viruses.

Figure 1.

Experimental gene expression levels for IFN β in cells infected by NC/99 (●), and Cal/09 (■) as a function of time. For visual clarity, linear segments are drawn connecting the data points. Allantoic fluid (gray line) provides the negative control. A control at 0h (○) is also depicted.

Figure 2.

Schematic of the model considered, where the points of application of NS1 antagonism are highlighted. There are three compartments, medium, cytoplasm and nucleus, and positive feedback loops associated with IFN β , IFN α , and TNF α .

Figure 3.

Experimental gene expression levels for IRF7, MX1, DDX58, OAS1, OAS2, and EIF2AK2 in cells infected by NC/99 (●), and Cal/09 (■) as a function of time. For visual clarity, linear segments are drawn connecting the data points. Allantoic fluid (gray line) provides the negative control. A control at 0h (○) is also depicted.

Figure 4.

RT-PCR measurements of NS1 mRNA expression levels as a function of time after infection by NC/99 (●), and Cal/09 (■). Allantoic fluid (gray line) serves as a negative control.

Figure 5.

NS1 protein antagonistic effect as a function of time for Cal/09 (top) and NC/99 (bottom). The solid gray line represents IC1 (IFN β induction antagonism) and the dashed gray line represents IC2 (nuclear mRNA antagonism). Their expressions are $IC1 = [1 + sp (NS/\delta_1)^{n1}] / [1 + (NS/\delta_1)^{n1}]$ with parameter values $sp=0.3$, $\delta_1=0.1 \mu\text{M}$, $n1=1$ for Cal/09 and $sp=0.1$, $\delta_1=0.1 \mu\text{M}$, $n1=5$ for NC/99, and $IC2 = [1 + sv (NS/\delta_2)^{n2}] / [1 + (NS/\delta_2)^{n2}]$ with parameter values $sv=0.1$, $\delta_2=0.4 \mu\text{M}$, $n2=5$ for NC/99 and $IC2=1$ for Cal/09 since there is no nuclear mRNA antagonism. The product $IC1*IC2$ for NC/99 is also shown (solid black line). The time dependence of the curves is due to the time-varying amount of NS1 protein (expression (16)) in the expressions for IC1 and IC2 given above. The smaller IC1 and IC2, the stronger the antagonism, since both terms multiply production terms in the model equations.

Figure 6.

Comparison of experimental data (symbols) and model simulations (lines) for infection with NC/99 (●, 3 replicates, solid lines) or Cal/09 (■, 3 replicates, dashed lines) for IFN β , IFN α , TNF α , STAT1 (STAT), and IRF7 mRNAs as a function of time after infection. The time at which IFN β induction antagonism (IC1) and nuclear mRNA antagonism (IC2) reach their respective midpoint in strength also indicated. The data for IRF7 and STAT1 represent the contributions from infected cells only, obtained from the experimental measurements as explained in section 2.5. The data, in each figure, are normalized by the corresponding 8 hr time point expression level for Cal/09.

Figure A.1.

NC/99 infection: time behavior of normalized parameter sensitivity of NS1 antagonism for IFN β_m and IRF7 $_m$. Parameters are sp , sv , δ_1 , and δ_2 , which characterize NS1 antagonism (see expressions (14) and (15) of IC1 and IC2, and Figure 5).

Figure A.2.

Cal/09 infection: time behavior of normalized parameter sensitivity of NS1 antagonism for IFNβ_m and IRF7_m. Parameters are sp and δ_1 , which characterize NS1 antagonism (see expression (14) of IC1). For Cal/09, there is no dependence on parameters sv , and δ_2 since IC2=1.

Model Parameters

List of parameter values for each process described in the model for the different viral strains: Cal/09, and NC/99. Estimated parameters are in black, parameters fitted to the experimental data are in red. Our estimates of parameter values are taken from [15], where they are obtained from the literature or biological considerations, and to which we refer for details. The exceptions are our estimates for parameters v_{max2} , v_{max19} , and r_{20} , which are relevant to secreted proteins: their values are obtained in [15] from the model fits. The value of sv for NC/99 is 0.1 for all genes except for IFN α , for which it takes a value of 0.3.

Table 1

Process	Parameter	Value		Units	Remarks
		Cal/09	NC/99		
IFNb mRNA induction	r_0	1×10^{-3}	3×10^{-3}	mM.h ⁻¹	Fitted
	k_{15}	3600×10^{-8}		h ⁻¹	Estimated
IFNb mRNA degradation	t_1	2.5		h	Estimated
IFNb/a translation and secretion	$v_{max2/17}$	20.1×3600		molecules.h ⁻¹ .cell ⁻¹	Estimated
	$K_{2/17}$	2×10^{-3}		mM	Estimated
	TJ_{tot}	1×10^{-4}		mM	Estimated
	K_3	4.3×10^{-3}	4.3×10^{-3}	mM	Fitted
	d	3×10^{-4}		mM	Estimated
[TJ]	K_9	7.8×10^2		-	Estimated
	k_5	3600		h ⁻¹	Estimated
Formation of STATP2n	K_5	1×10^{-2}		mM	Estimated
STATP2n dephosphorylation	t_3	0.56		h	Estimated
	r_3	1×10^{-7}		mM.h ⁻¹	Estimated
SOCS mRNA Induction	k_8	3600×10^{-6}		h ⁻¹	Estimated
SOCS mRNA degradation	t_4	0.46		h	Estimated
IRF7 mRNA induction	k_{11}	3600×10^{-7}	3600×10^{-7}	h ⁻¹	Fitted
	k_{14}	$3600 \times 8.9 \times 10^{-11}$		h ⁻¹	Estimated
IRF7/mRNA degradation	t_6	1		h	Estimated
IRF7 translation	k_{12}	3600×0.1	3600×1	h ⁻¹	Fitted
IFN α mRNA induction	k_{16}	3600×10^{-4}	$3600 \times 2 \times 10^{-4}$	h ⁻¹	Fitted
IFN α mRNA degradation	t_8	2		h	Estimated

Process	Parameter	Value		Units	Remarks
		Cal/09	NC/99		
TNF α mRNA induction	r_1	1×10^{-4}	2.5×10^{-4}	$\text{mM} \cdot \text{h}^{-1}$	Fitted
	r_{20}	1×10^{-3}		$\text{mM} \cdot \text{h}^{-1}$	Estimated
TNF α mRNA degradation	K_{20}	6×10^{-4}		mM	Estimated
	t_9	2		h	Estimated
TNF α translation and secretion	$v_{\text{max}19}$	46×3600		$\text{molecules} \cdot \text{h}^{-1} \cdot \text{cell}^{-1}$	Estimated
	K_{19}	4×10^{-3}		mM	Estimated
STAT mRNA induction	r_4	1×10^{-6}		$\text{mM} \cdot \text{h}^{-1}$	Estimated
	k_{26}	$3600 \times 5 \times 10^{-6}$	$3600 \times 5 \times 10^{-6}$	h^{-1}	Fitted
STAT mRNA degradation	t_{12}	1		h	Estimated
STAT translation	k_{28}	3600×10^{-1}		h^{-1}	Estimated
STAT degradation	t_{13}	15		h	Estimated
NSI Protein	r_5	1		mM	Estimated
	n_3	5	8	-	Estimated
	b_m	6	4.5	h	Estimated
	sp	0.3	0.1	-	Fitted
IFN β induction antagonism (IC1)	d_1	0.1	0.1	mM	Fitted
	n_1	1	5	-	Fitted
	sv	-	0.1/0.3	-	Fitted
	d_2	-	0.4	mM	Fitted
nuclear mRNA antagonism (IC2)	n_2	-	5	-	Fitted