In the present work we propose a dynamical mathematical model of the lung cells inflammation process in response to SARS-CoV-2 infection. In this scenario the main protease Nsp5 enhances the inflammatory process, increasing the levels of NF kB, IL-6, Cox2, and PGE2 with respect to a reference state without the virus. In presence of the virus the translation rates of NF kB and IkB arise to a high constant value, and when the translation rate of IL-6 also increases above the threshold value of 7 pg mL$^{-1}$ s$^{-1}$ the model predicts a persistent over stimulated immune state with high levels of the cytokine IL-6. Our model shows how such over stimulated immune state becomes autonomous of the signals from other immune cells such as macrophages and lymphocytes, and does not shut down by itself. We also show that in the context of the dynamical model presented here, Dexamethasone or Nimesulide have little effect on such inflammation state of the infected lung cell, and the only form to suppress it is with the inhibition of the activity of the viral protein Nsp5. To that end, our model suggest that drugs like Saquinavir may be useful. In this form, our model suggests that Nsp5 is effectively a central node underlying the severe acute lung inflammation during SARS-CoV-2 infection. The persistent production of IL-6 by lung cells can be one of the causes of the cytokine storm observed in critical patients with COVID19. Nsp5 seems to be the switch to start inflammation, the consequent overproduction of the ACE2 receptor, and an important underlying cause of the most severe cases of COVID19.

**Keywords:** SARS-CoV-2 infection, interleukin 6, NFKB, NSP5, COX2, SARS-CoV-2 interactome, nonlinear dynamics of inflammation

**INTRODUCTION**

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus is an intracellular infectious agent whose replication cycle depends on the host’s cell structures and functions. In particular, it uses the translational apparatus of different types of infected cells to express its proteins (Nakagawa et al., 2016). SARS-CoV-2 causes the Coronavirus Disease 2019 (COVID19) that has infected over 241,000,000 persons and killed over 4,900,000 worldwide since the end of 2019. No specific and effective therapeutic drugs to defeat SARS-CoV-2 infection have been developed yet (Díaz, 2020a), although several effective vaccines have been developed and are being applied. Therefore, it is necessary to continue the search for effective therapeutic agents that complement the use of vaccines, to minimize the risk of death from COVID19.
SARS-CoV-2 virion is formed by four proteins: spike (S), envelope (E), membrane (M) and nucleocapside (N) that enclose the virus genome (McBride and Fielding, 2012), which consists of a positive-sense nonsegmented single stranded mRNA [(+)ssRNA] of ∼30 kb. The open reading frames 1a (orf1a) and 1b (orf1b) are located near the 5′UTR of the (+)ssRNA and they code for the polyproteins pp1a and pp1ab. Maturation of these polyproteins results in 11 nonstructural proteins (Nsp) from the orf1a segment (Nsp1 to Nsp11) and 5 nonstructural proteins from the orf1b segment (Nsp12 to Nsp16). Nsp proteins form the replication-transcription complex (RTC) a set of nested subgenomic minus-strands of RNA [(-)sgRNA] are synthesized in a process of discontinuous transcription. These (-) sgRNAs serve as the templates for the production of subgenomic mRNAs from which the structural proteins E, M, N and S, together with the accessory proteins orf3a, orf6, orf7a, orf7b, orf8, orf9b, orf9c and orf10 are synthesized. The presence of viral (+)ssRNA initiates theTLR3 and TLR4 mediated immune response, which increases the concentration of free NFκB in cytoplasm. Cytoplasmic NFκB enters the nucleus where promotes the transcription of a series of target genes. Viral protein Nsp5 inhibits the inhibition exerted by HDAC2 on nuclear NFκB transcription factor, enhancing the synthesis of inflammatory cytokines like IL-6 (Figure drawn with Biorender template https://app.biorender.com/).

SARS-CoV-2 virion is formed by four proteins: spike (S), envelope (E), membrane (M) and nucleocapside (N) that enclose the virus genome (McBride and Fielding, 2012), which consists of a positive-sense nonsegmented single stranded mRNA [(+)ssRNA] of ∼30 kb. The open reading frames 1a (orf1a) and 1b (orf1b) are located near the untranslated 5′ region (5′UTR) of the positive single stranded RNA [(+)ssRNA] and they encode for the polyproteins pp1a and pp1ab.

When SARS-CoV-2 virion infects the organism, S protein binds with high affinity to the surface receptor Angiotensin-Converting Enzyme 2 (ACE2), highly abundant in the lung alveolar type II cells and other cell types (Hamming et al., 2004; Gordon et al., 2020), and forms a molecular complex that begins the process of fusion of the virion envelope with the host cell membrane. Finally, viral (+)ssRNA is released into the host cytoplasm (Letko et al., 2020) (Figure 1).

The process of translation occurs in the cytoplasm and produces a set of viral polyproteins whose maturation results in 11 nonstructural proteins (Nsp) from the orf1a segment (Nsp1 to Nsp11) and 5 nonstructural proteins from the orf1b segment (Nsp12 to Nsp16). Nsp proteins form the replication-transcription complex (RTC) in a double-membrane vesicle where a set of nested subgenomic minus-strands of RNA [(-)sgRNA] are synthesized in a process of discontinuous transcription. These (-) sgRNAs serve as the templates for the production of subgenomic mRNAs from which the structural proteins E, M, N and S, together with the accessory proteins orf3a, orf6, orf7a, orf7b, orf8, orf9b, orf9c and orf10 are synthesized (Sevajol et al., 2014; Díaz, 2020a; Dongwan et al., 2020). SARS-CoV and SARS-CoV-2 genomes have ~79% of homology (Forster et al., 2020), and most of the set of structural and nonstructural proteins are practically the same. However, the virus species differ in the accessory proteins orf8, orf8a, orf8b, orf9c and orf10 (Bartlam et al., 2005; Gordon et al., 2020).

Viral proteins are inserted into the host molecular machinery to modify and redirect a great number of host cell functions towards the production of additional viral particles (Masters, 2006; Wu et al., 2020). Experimental analysis of the interaction of viral and host proteins, or interactome, by Gordon and collaborators (Gordon et al., 2020) has been a fundamental contribution to understand the form in which SARS-CoV-2 virus takes control of the host molecular network to produce new virions and propagate the infection (Hekman et al., 2020). Gordon and collaborators (2020) cloned, tagged and expressed 26 viral proteins in human cells using affinity-purification mass...
spectrometry to identify the human proteins physically associated with each other. They found around 332 SARS-CoV-2-human protein-protein interactions that constitute the virus interactome. From these results, the construction of the network representation of the interactome is possible. In the particular case of the Gordon interactome, the analysis of its undirected network model indicates a modular free-scale hierarchical type of structure in which proteins orf8, N and Nsp7 are the main hubs (Díaz, 2020a).

Despite all the molecular knowledge rapidly accumulated on SARS-CoV-2 itself and the infection or inflammation processes in which it is involved in human cells, we still lack dynamical models to understand which are the key nodes underlying severe inflammation processes in some cases. Mathematical and computational dynamic network models are useful tools to integrate data, find experimental holes and also propose or understand the underlying dynamics mechanisms involved in the complex viral/human networks involved during SARS-CoV-2 infection (Breitling, 2010; Díaz, 2020a; Enciso et al., 2020; Weinstein et al., 2020). The number of nodes, the number of connections of each node to its neighbours, and the distribution of these connections in the network determine its complexity, structure and dynamical properties (Kumar et al., 2020).

In this network, the viral main protease Nsp5 is the main protease (Mpro) involved in the assembly of the polyprotein cluster that translates viral RNA, and in the post-translational modification of viral proteins through its ADP ribose phosphatase activity (Nakagawa et al., 2016). SARS-CoV-2 Nsp5 has 96% sequence homology with its SARS-CoV counterpart, and its level of phylogenetic conservation by sequence and structure is high having high homogeneity in its volume and electrostatic profile throughout the Coronaviridae family. Interest in elucidating the functional structure of this protease has resulted in new reports of 3D structures showing that Nsp5 is a complex formed by two equal protomers (A and B) each consisting of 306 residues structured in three domains: I Chymotrypsin-like domain (residues 8–101), Picornavirus 3C protease-like domain II (residues 102–184), and Globular Cluster domain III (residues 201–303). Domains I and II have an antiparallel β-barrel structure and associate with their counterparts to form a dimer, which loop residues 185–200 connect to domain III made up of five α-helices. The substrate binding site is found in the antiparallel β-barrels located between domains I and II, forming a catalytic pocket composed of four subsites (S1, S2, S3 and S4) that exhibit charged residues that include the nucleophilic sulphur atom of Cys145, which forms a dyad with the imidazole ring of His 41. Therefore, the blocking one of these residues with a small molecule could inhibit the enzymatic activity of Nsp5 (Mukherjee et al., 2011; Pillaiyar et al., 2016; Konwar and Sarma, 2021).

There are several crystallographic structures of Mpro with peptidomimetic inhibitors that show interactions with the enzyme at S3-S4 subsites in SARS-CoV-2, SARS-CoV and MERS. This type of inhibitor incorporates an imidazole ring of cyclic ketoamides with five-components that binds covalently to Cys145 or its equivalent in the different isoforms of the enzyme. Although these types of molecules are not optimal for drug development, due to proteolytic degradation, they have made possible to describe the dynamics of the enzymatic mechanism of this active site for the search and development of molecules with better pharmacodynamic parameters. An example of viable candidates for clinical implementation is boceprevir and telaprevir, whose mechanism of action consists of blocking the catalytic dyad through the covalent binding to the sulphur atom of Cys145 (Pan et al., 2012).

In the SARS-CoV-2 molecular network, Nsp5 has a special role in lung inflammation process through its link with Histone Deacetylase 2 (HDAC2) (Díaz, 2020a; Gordon et al., 2020; Hekman et al., 2020) (Figures 1, 2), suggesting that Nsp5 represses HDAC2 inhibitory action on NF-κB (Figure 1), and thus enhances the transcription of the pro-inflammatory genes targeted by the Nuclear Factor κB (NF κB) (Figures 1, 2) (Wagner et al., 2015). Such role distinguishes SARS-CoV and SARS-CoV-2. The SARS-CoV virus uses N protein to promote the sustained transcription of the Cyclooxygenase 2 (Cox2) enzyme by binding directly to the NF-κB transcriptional regulatory elements, and to the CCAAT/enhancer binding proteins of the gene Cox2 (Yan et al., 2006). Hence, SARS-CoV and SARS-CoV-2 produce severe acute respiratory syndrome through different molecular mechanisms.

The change from a hub (N protein in SARS-CoV) to a poor connected protein (Nsp5 in SARS-CoV-2) as the underlying...
Inactivation of HDAC2 by Nsp5 produces a sustained increase in the production of IL-6 and NFκB, and NFκB and IκB. A negative feedback loop between PGE2 and IκB is observed in critical COVID19 patients (Leisman et al., 2020). However, none of these have shown to be really effective for preventing or reverting acute inflammatory cases after SARS-CoV-2 infection.

In this work, we propose a dynamical mathematical model that suggests that the three feedback loops mentioned above constitute a minimal circuit for the inflammatory process in the epithelial lung cells during SARS-CoV-2 infection (Figure 2), and we use an ordinary differential equations (ODEs) continuous model to explore the effect of Nsp5 in the qualitative dynamics of this circuit in which this viral protein acts as an enhancing perturbation in the phase space of this dynamical system (Diaz, 2020b) (Figure 2). For the model of the circuit, we chose as main molecular components the proteins NFκB, IκB, IL-6, Cox2, and PGE2. In this model, Nsp5 prevents the movement of HDAC2 into the nucleus allowing an increase in NFκB transcriptional activity. The input that turns on the circuit in lung cells is a signal from monocytes, lymphocytes, and other immune cells that activates IL-6 production. We propose that this model represents the basic set of interactions that settles the inflammatory process in the epithelial lung cells during SARS-CoV-2 infection.
SARS-CoV-2, and that it is a first approximation to understand the main dynamical features of this response.

Our hypothesis is that Nsp5 leads to the sustained overproduction of IL-6, Cox2, PGE2, and NF kB, in the epithelial lung cells, which is a necessary but not a sufficient condition for a cytokine storm. In the particular case of epithelial lung cells, we found that the computational and mathematical analysis of the model supports our hypothesis, and that Nsp5 effectively enhances NF kB, IL-6, Cox2 and PGE2 production during the time that remains bounded to HDAC2. Furthermore, the model predicts the existence of an over stimulated immune state (OSIS) in which the amount of IL-6 produced by the infected lung cells is high, and such state becomes autonomous and robust, independent of any stimulus from external sources like monocytes, and lymphocytes. Hence this OSIS cannot be completely shut down by classical anti-inflammatory drugs like Nimesulide and Dexamethasone, and can be possibly beaten only with specific inhibitors of Nsp5 (Xun et al., 2020).

MODEL

Immune response to infections involves complex spatio-temporal processes involving multiple molecular components, non-linear interactions and cell-cell interactions. In consequence, the immune response to infections is a multidimensional, multiscale and multilevel dynamical process in which innate and acquired immunity mechanisms are involved to protect the organism against pathogens (Efimiev et al., 2016). In this scenario, the number of variables that become both sufficient and necessary for a detailed enough description of the spatio-temporal dynamics of the immune system is tremendously high. Some computational integrative efforts have been made in this direction (Danos et al., 2007), but imply very large systems that cannot be modeled with continuous quantitative models that are, in turn, necessary if we want to understand which are key nodes driving the system towards the OSIS. The lack of quantitative experimental data and knowledge of the values of most of the model parameters are additional challenges to achieve such dynamic models of the immune system in response to specific infectious diseases. Thus, most models of the immune system have been qualitative (Efimiev et al., 2016; Martinez-Sanchez et al., 2018).

A possible approach implies the use of ordinary differential equations (ODEs) qualitative modeling that is oriented to determine all the possible trajectories of a dynamical system along its n-dimensional phase space (with $n > 0$), when the system is subject to a set of initial conditions and parameters values. High dimensional nonlinear dynamical systems can exhibit a variety of coexisting dynamical behaviors that determine the structure of their phase space (Strogatz, 2015). However, the structure of the phase space of biological nonlinear dynamical systems is highly dependent on the set of parameters values. Variations in the value of one or more parameters can drive a drastic change in phase space generating a different qualitative dynamics of the system. This process is known as bifurcation, and it is important to find the possible bifurcations in the system and to identify which parameter or parameters are the responsible of these changes (Strogatz, 2015) (Supplementary Material S1).

In order to avoid the problem due to the high dimensionality and complexity of the immune response to SARS-CoV-2 infection, and to use the minimum number of parameters in the model that we are proposing in this work, we assume that the interaction between nodes IL-6, NF kB, IkB, Cox2 and PGE2 settles on the main dynamical features of the inflammation process in lung epithelial cells due to the presence of the Nsp5 viral protein. In the proposed circuit shown in Figure 2, IL-6, NF kB, Cox2 and PGE2 define three feedback loops (Figures 3A,B): a positive feedback loop between NF kB and IL-6 (Figure 3A); a positive feedback loop between NF kB and IkB (Figure 3A); and a negative feedback loop between PGE2 and IkB (Figure 3B).

These feedback loops are distributed between three compartments:

1) IL-6 is secreted into the extracellular medium where it acts in paracrine and exocrine form. IL-6 binds to its receptor IL6R at the cell surface. Once the complex IL6-IL6R is formed, it activates IKK in the cytoplasm (Wang et al., 2003). PGE2 is also secreted into this compartment where it binds to EP2 and EP4 receptors at the cell surface, activating Protein Kinase B (Akt) in the cytoplasm (Cho et al., 2014).

2) IKK, free NF kB, and the complex IkB-NF kB are located in the cytoplasm, where IkB is degraded by ubiquitination. Protein Cox2 is located in the endoplasmic reticulum (ER).

3) Free NF kB enters the nucleus where induce the transcription of IL6, Cox2, IKB and NF kB genes. Transcriptional activity of nuclear NF kB is inhibited by HDAC2, and enhanced by Nsp5.

In this form, the model cell consists of three compartments: extracellular medium, cytoplasm and nucleus. We assume that the ratio of the cytoplasmatic volume to external volume ($V_{cyt}/V_{out}$) is one, and that the ratio of the cytoplasmatic volume to the nuclear volume ($V_{cyt}/V_{nuc}$) is 2. In each compartment the concentration of the molecules are measured in pg mL$^{-1}$.

In the circuit of Figure 2, the rate of production of free cytoplasmatic NF kB depends on the rate of its release from the IkB-NF kB complex, which is proportional to the product of the concentrations of IkB and IKK (Figure 3A), its own rate of production due to nuclear NF kB transcriptional activity inhibited by HDAC2 (Figures 3A, 3A), the rate at which is recaptured by IkB (Figure 3A), and the rate of transport into the nucleus:

$$\frac{d(nf)}{dt} = V_{nf}^{max} \cdot p_{nf}(t) + k_{iikb} \cdot ikb nf - k_{4nf} \cdot ikb - D \cdot nf$$  (1)

where $nf$ is the concentration of free cytoplasmatic NF kB, $V_{nf}^{max}$ is the maximum rate of translation of the NF kB gene, $p_{nf}(t)$ is the probability that the NF k B gene is in its active state (on), $ikb$ is the concentration of IKK, $ikb nf$ is the concentration of IkB-NF k B complex, $k_{iikb}$ and $k_{4nf}$ are rate constants and $D$ is the transport constant (Table 1).

We assume that the concentration of IKK activated by IL-6 is proportional to the external concentration of the cytokine:
In the reference state the translation velocities are: $V_{\text{cyt}} = k_1 \cdot V_{\text{Nuc}} - k_{11} \cdot p_{\text{g2}} \cdot V_{\text{ibf}} - k_{\text{ibf}} \cdot n_{\text{f}}$.

\[
\frac{d(nf)}{dt} = V_{\text{max},nf} p_{\text{g2}}^{\text{max}}(t) + k_1 \cdot il6 \cdot \text{ibf} + k_{11} \cdot p_{\text{g2}} \cdot \text{ibf} - k_4 \cdot n_{\text{f}} \cdot il6 - D \cdot nf
\]

where $k_4$ and $k_{11}$ are the new rate constants (Table 1).

The rate at which the complex IxB-NFκB is broken in presence of IKK depends on the rate at which IKK and Akt remove IxB from the complex under the action of IL-6 and PGE2, and the rate at which the complex is formed again:

\[
\frac{d(\text{ibf})}{dt} = k_4 \cdot nf - k_{10} \cdot p_{\text{g2}} \cdot \text{ibf}
\]

\[
\frac{dnf}{dt} = D \left( V_{\text{max}} \right) n_{\text{f}} - k_{\text{deg}} n_{\text{f}}
\]

where $n_{\text{f}}$ is the nuclear concentration of NFκB and $k_{\text{deg}}$ is a rate constant.

The probabilities of activation of genes $IL6$, $Cox2$, $IxB$ and $NFκB$ (Figures 3A,B) are given by:

**Table 1** Equations and parameters of the basic model of inflammation.

| Basic inflammation circuit | Parameters |
|----------------------------|------------|
| $d(nf)/dt = V_{\text{max},nf} p_{\text{g2}}^{\text{max}}(t) + k_1 \cdot il6 \cdot \text{ibf} + k_{11} \cdot p_{\text{g2}} \cdot \text{ibf} - k_4 \cdot n_{\text{f}} - D \cdot nf$ | $k_i = 0.5 \text{ pg mg}^{-1} \text{s}^{-1}$; $k_j = 5 \text{ pg mg}^{-1} \text{s}^{-1}$; $D = 1 \text{ s}^{-1}$ |
| $d(\text{ibf})/dt = k_4 \cdot nf - k_{10} \cdot p_{\text{g2}} \cdot \text{ibf}$ | $k_{\text{deg}} = 2 \text{ s}^{-1}$; $(\frac{\text{V}_{\text{act}}}{\text{V}_{\text{max}}}) = 2$ |
| $dnf/dt = D \left( V_{\text{max}} \right) n_{\text{f}} - k_{\text{deg}} n_{\text{f}}$ | $k_5 = 5 \text{ pg mg}^{-1} \text{s}^{-1}$; $k_6 = 0.5 \text{ pg mg}^{-1} \text{s}^{-1}$ |
| $r = 30 \text{ pg mg}^{-1} \text{s}^{-1}$; $D_2 = 2 \text{ s}^{-1}$; $\eta = 1 \text{ pg mg}^{-1}$ | $k_7 = 0.1 \text{ Number of Molecules}^{-1} \text{s}^{-1}$; $k_{\lambda} = 0.035 \text{ s}^{-1}$ |
| $k_{10} = 0.5 \text{ pg mg}^{-1} \text{s}^{-1}$; $k_{11} = 0.5 \text{ s}^{-1}$; $a(f) = 10 \text{ pg mg}^{-1} \text{s}^{-1}$ | $k_{12} = 0.4 \text{ s}^{-1}$ |
| $k_1 = 0.5 \text{ pg mg}^{-1} \text{s}^{-1}$; $k_2 = 5 \text{ pg mg}^{-1} \text{s}^{-1}$; $D = 1 \text{ s}^{-1}$ | $k_{13} = 0.8 \text{ s}^{-1}$; $k_{14} = 0.8 \text{ pg mg}^{-1} \text{s}^{-1}$; $k_{15} = 3 \text{ s}^{-1}$ |

ikk = $\gamma_1 \cdot il6$, where $\gamma_1$ is a constant of proportionality. In a similar form, PGE2 increases the rate of cleavage of the complex IxB-NFκB by phosphorylation of the IKKa subunit by Akt (Bai et al., 2009) (Figure 3B). Thus, we assume that the concentration of Akt is proportional to the external concentration of PGE2: $akt = \gamma_2 \cdot p_{\text{g2}}$. Substituting both expressions in Eq. 1 we finally obtain:

\[
\frac{d(nf)}{dt} = V_{\text{max},nf} p_{\text{g2}}^{\text{max}}(t) + k_1 \cdot il6 \cdot \text{ibf} + k_{11} \cdot p_{\text{g2}} \cdot \text{ibf} - k_4 \cdot n_{\text{f}} \cdot il6 - D \cdot nf
\]
According to the rate equation:

\[ \frac{dp_{IL6}^{m}(t)}{dt} = k_5 \left( \frac{nf^*}{hd^* + 1} \right) (1 - p_{IL6}^{m}(t)) - k_{-2} p_{IL6}^{m}(t) \]  
(5)

\[ \frac{dp_{IFN\beta}^{m}(t)}{dt} = k_3 \left( \frac{nf^*}{hd^* + 1} \right) (1 - p_{IFN\beta}^{m}(t)) - k_{-3} p_{IFN\beta}^{m}(t) \]  
(6)

\[ \frac{dp_{Cox2}^{m}(t)}{dt} = k_5 \left( \frac{nf^*}{hd^* + 1} \right) (1 - p_{Cox2}^{m}(t)) - k_{-7} p_{Cox2}^{m}(t) \]  
(7)

\[ \frac{dp_{NFkB}^{m}(t)}{dt} = k_5 \left( \frac{nf^*}{hd^* + 1} \right) (1 - p_{NFkB}^{m}(t)) - k_{-15} p_{NFkB}^{m}(t) \]  
(8)

Supplementary Material S2 for the mathematical deduction of these equations. For Eqs. (5)–(7), the variable \( p_{i}^{m}(t) \), with \( i \in \{IL-6, IFN\beta, Cox2, NFkB\} \), is the probability that the gene \( i \) is expressed at time \( t \). \( hf \) is the amount of HDCA2 in nucleus, and \( k_2, k_{-2}, k_3, k_{-3}, k_7, k_{-7}, k_{15}, k_{-15} \) are rate constants (Table 1).

We assume that free cytoplasmic HDAC2 is produced according to the rate equation:

\[ \frac{d(hd)}{dt} = \frac{r}{\eta(nsp5 + 1)} - D_2 \cdot hd \]  
(9)

where \( hd \) is the amount of free cytoplasmic HDAC2 that is produced at a constant rate \( r \). \( D_2 \) is the rate of transport of free HDAC2 into the nucleus. In this equation, \( nsp5 \) is the amount of Nsp5 in the infected cell that sequester HDCA2 in cytoplasm (El-Baba & Herbein, 2020), and \( \eta \) is a constant (Table 1). Free cytoplasmic HDAC2 enters the nucleus according to the equation:

\[ \frac{d(hd^*)}{dt} = D_2 \left( \frac{V_{Cox2}}{V_n} \right) hd - k_{deg2} hd^* \]  
(10)

where \( hd^* \) is the amount of HDAC2 in the nucleus, and \( k_{deg2} \) is a rate constant (Table 1).

Nsp5 is a protein with only one link in the virus interactome (Gordon et al., 2020; Diaz, 2020a; Hekman et al., 2020), which is an input to the circuit of Figure 2. However, the real kinetic mechanism of production of this viral protein is unknown, thus we assume a plausible simple kinetic mechanism that leads Nsp5 to have a maximum constant concentration of 20 nM. We set the parameters \( \phi \) and \( k_{18} \) to the values shown in Table 1.

\[ \frac{d(nsp5)}{dt} = \phi - k_{18} nsp5 \]  
(11)

where \( \phi \) is the constant rate of production of Nsp5, and \( k_{18} \) is a rate constant (Table 1). The effect of the variation of parameter \( \phi \) on the dynamics of the circuit of Figure 2 is analyzed in Supplementary Material S3.

During the early stage of infection by SARS-CoV-2, the rate of variation of the concentration of IL-6 outside the lung cells depends on the rate of production of IL-6 by monocytes, leukocytes and other cells under stimulation by Toll-like Receptors (TLRs) [denoted by \( \alpha \) (t)] (Jafarzadeha et al., 2020; Magro, 2020), on the rate of production by lung cells due to nuclear NF \( \kappa B \) transcriptional activity enhanced by Nsp5 (Figure 3A), and on the rate of degradation of IL-6 by different mechanisms. Finally, we assume that the concentration of IKK activated by IL-6 is proportional to the external concentration of the cytokine (Eq. (2)).

\[ \frac{d(i6l)}{dt} = \alpha(t) + V_{max} p_{IL6}^{m}(t) - k_{i6l}6 \cdot ikb - k_{i6l6} \]  
(12)

where \( V_{max} \) is the maximum rate of translation of IL6, \( p_{IL6}^{m}(t) \) is the probability that the IL6 gene is activated at time \( t \), and \( k_5 \) and \( k_6 \) are rate constants (Table 1).

The rate of variation of the amount of free IxB in the system is the balance between the rate of translation of gene IxB and the rate of formation of new IxB-NF \( \kappa B \) complexes:

\[ \frac{d(ixb)}{dt} = V_{max} p_{IxB}^{m}(t) - k_{i} ixb \cdot nf \]  
(13)

where \( V_{max} \) is the maximum rate of translation of IxB, and \( p_{IxB}^{m}(t) \) is the probability that the gene IxB is activated at time \( t \) (Table 1).

The rate of variation of the amount of the enzyme Cox2 in the system depends on the rate of translation of the Cox2 gene, and its rate of inhibition by different mechanisms:

\[ \frac{d(cox2)}{dt} = V_{max} p_{Cox2}^{m}(t) - k_{18} cox2 \]  
(14)

where cox2 is the amount of the enzyme in the system, \( V_{max} \) is the maximum rate of variation, \( p_{Cox2}^{m}(t) \) is the probability that the gene Cox2 is activated at time \( t \), \( k_5 \), \( k_9 \) and \( k_{10} \) are rate constants (Table 1).

The rate of variation of the amount of PGE2 in the system depends on the amount of Cox2, on the amount of IL-6, and on its rate of degradation:

\[ \frac{dpge}{dt} = k_{17} cox2 - k_{11} pge2 \cdot ikb - k_{12} pge2 \]  
(15)

where \( k_{17}, k_{12} \) and \( k_{17} \) are rate constants (Table 1).

**RESULTS**

In this section we present in detail and focus on the results that are relevant to understand the role of Nsp5 in the process of acute lung inflammation. The rest of the analyses and results related to the mathematical aspects of the model and its stability analysis are reported in Supplementary Material S3.

We solved the model using the Euler predictor-corrector and Runge-Kutta 4,5 methods with time step of 0.05 s and 720,000 integration steps to simulate a period of 10 h post infection, which is the estimated time for the first bursting of new virions into the extracellular medium (Bar-On et al., 2020; Kumar et al., 2020). We made a Boolean simulation of the circuit to confirm the dynamical behavior predicted by the ODEs model. We used the parameter values shown in Table 1 to generate an arbitrary reference state (ARS) of the system for the initial conditions ikb = 5 pg ml\(^{-1}\), and the rest of the variables equal to 0 at time \( t = 0 \). In this case, the input \( \alpha (t) = 10 \) pg ml\(^{-1}\) s\(^{-1}\) and nsp5 = 0 for \( t \in [0, \infty) \). In this ARS, the translation velocities of all genes are set to 2 pg mL\(^{-1}\) s\(^{-1}\) (Hausser et al., 2019). In the case of the ODEs
model, both numerical methods produce identical outputs indicating no bias in the predicted dynamics of the circuit due to the integration method and that the transitory sharp initial increase observed in the concentration of the proteins of the circuit are due to the transitory initial response of the feedback loops to the input. The solution of the Boolean model confirms that the trajectories of the circuit lead to a stable node or attractor where all the nodes are in on state, except HDAC2 that is in state off, when Nsp5 is in state on.

Figure 4A shows that the output of the model is the transitory activation of NF κB, Cox2, and PGE2, in contrast with a sustained high activation of IL-6. In order to clarify the source of the steady high activation of IL-6, we solved the model with the same initial conditions and $\alpha(t)$ = 0. In this case, the system does not turn on during all the time of simulation (data not shown), indicating that the signal from monocytes and other cells is a necessary condition to start the IL-6 sustained production in lung cells (Figure 4A). Figure 4B shows that when nsp5 ≠ 0, $\alpha(t)$ = 0 and equal gene translation rates, the response of the system is a high sustained level of activation of NF κB, Cox2, and PGE2 with respect to the ARS. In this case, the concentration of IL-6 in the cell was only slightly affected

**FIGURE 4** Effect of Nsp5 on IL-6 production. (A) Arbitrary reference state (ARS) of the circuit of Figure 2. In absence of the viral protein Nsp5, the response to an external input $\alpha(t)$ = 10 pg ml$^{-1}$s$^{-1}$ is a transitory activation of NF κB, Cox2, and PGE2. In this case, IL-6 shows a steady high concentration. In this case, all the gene translation rates are set to 2 pg ml$^{-1}$s$^{-1}$. (B) In presence of Nsp5 the circuit shows steady higher concentrations of NF κB, Cox2, and PGE2 (red line) with respect to ARS (black line). IL-6 steady concentration (black line) is unaffected by the presence of the viral protein (red line). In this panel, $\alpha(t)$ = 10 pg ml$^{-1}$s$^{-1}$, and all the gene translation rates are set to 2 pg ml$^{-1}$s$^{-1}$. (C) When NF κB and κB translation rates are $V_{\text{max}}^{\text{NF}} = 10$ pg mL$^{-1}$s$^{-1}$ and $V_{\text{max}}^{\kappa B} = 15$ pg mL$^{-1}$s$^{-1}$, respectively, and Cox2 and IL-6 translation rates are 2 nM s$^{-1}$ the circuit shows a weak sustained activation of NF κB, Cox2, and PGE2 (black line) in response to the external input $\alpha(t)$ = 10 pg ml$^{-1}$s$^{-1}$ and in absence of Nsp5. In this case, IL-6 steady concentration does not change (black line). In presence of Nsp5 the circuit shows steady higher concentrations of NF κB, Cox2, and PGE2 (red line), although the concentration of IL-6 is lower than is absence of the virus. (D) In the over stimulated immune state (OSIS), the presence of Nsp5 produces an enhanced production of NF κB, Cox 2, PGE2 and IL-5 (red lines). This particular state is obtained when $\alpha(t)$ = 10 pg ml$^{-1}$s$^{-1}$, $V_{\text{max}}^{\text{NF}} = 10$ pg mL$^{-1}$s$^{-1}$, $V_{\text{max}}^{\kappa B} = 15$ pg mL$^{-1}$s$^{-1}$, $V_{\text{max}}^{\text{Cox}} = 2$ pg mL$^{-1}$s$^{-1}$, and $V_{\text{max}}^{\text{IL}} = 20$ pg mL$^{-1}$s$^{-1}$.
by the virus. Figures from 4A to 4D show the first 5 minutes of simulation during which the circuit of Figure 2 reaches its steady state. This result indicates that the presence of the virus induce a sustained and persistent production of NF κB, Cox2, and PGE2 in the circuit of Figure 2.

Translation rates of the set of genes of the model rarely have the same value, even when their activation is promoted by the same transcription factor (NF κB in this case). As shown in Table 1, the results of Figures 4A,B are obtained when the rates of translation are the same for all genes. However, in real cells this is not the case, and Cox2, IL-6, NF κB and IκB are translated at different rates. In Homo sapiens the estimated average rate of translation ($V_{\text{max}}$) of a gene is about $10^4$ proteins per hour (Hauser et al., 2019). However, this average value can be increased according to the state of activity of the cell. In the present work, we arbitrary chose the value of $V_{\text{max}} = 2\text{ pg mL}^{-1}\text{s}^{-1}$ for all genes in the ARF (Table 1). However, we made parameter variation to know how different values of this set of parameters affect the qualitative behavior of the model (Supplementary Material S1). Figure 4C shows that when in the system $V_{\text{max}}^{nf} = 10\text{ pg mL}^{-1}\text{s}^{-1}$, $V_{\text{max}}^{nf} = 15\text{ pg mL}^{-1}\text{s}^{-1}$, $V_{\text{max}}^{Cox2} = 2\text{ pg mL}^{-1}\text{s}^{-1}$, and $V_{\text{max}}^{IL6} = 15\text{ pg mL}^{-1}\text{s}^{-1}$, $\alpha(t) = 10\text{ pg mL}^{-1}\text{s}^{-1}$ and nsp5 = 0, the circuit tends to a steady state in which NF κB, Cox2, and PGE2 exhibit a constant low concentration during the time of simulation, while IL-6 steady concentration is unaffected with respect to the value shown in Figure 4A. When nsp5 ≠ 0, the steady concentration of NF κB, and Cox2, are increased ~10 fold with respect to their maximum concentration in the ARS, while PGE2 concentration is increased ~4 fold; Nsp5 produces a ~4 fold decrease in IL-6 steady concentration with respect to the reference concentration shown in Figure 4A.

This result indicates that a the difference in translation rates between NF κB and IκB (the intensity of the inhibition of NF κB by IκB, Figure 3A) in absence of the virus with respect to their values in ARS induces a low sustained production of NF κB, Cox2, and PGE2 without affecting IL-6 production. However the presence of Nsp5 induces a high production of NF κB and Cox2, and a moderate increase in PGE2 concentration that decreases IL-6 concentration, indicating that a higher rate of IκB translation with respect to NF κB translation can decrease the concentration of the cytokine IL-6 in presence of the virus probably due to a lower concentration of PGE2 in the circuit (Figures 2, 3A).

Figure 4D shows that when $\alpha(t) = 10\text{ pg mL}^{-1}\text{s}^{-1}$, nsp5 = 0, $V_{\text{max}}^{nf} = 10\text{ pg mL}^{-1}\text{s}^{-1}$, $V_{\text{max}}^{nf} = 15\text{ pg mL}^{-1}\text{s}^{-1}$, $V_{\text{max}}^{Cox2} = 2\text{ pg mL}^{-1}\text{s}^{-1}$, and $V_{\text{max}}^{IL6} = 20\text{ pg mL}^{-1}\text{s}^{-1}$, the circuit tends to a steady state in which NF κB, Cox2 and PGE2 exhibit a constant low concentration during the time of simulation, while IL-6 is unaffected with respect to its steady concentration in Figure 4A. However, when nsp5 ≠ 0 the dynamical behavior of the circuit changes because NF κB and Cox2 show the same steady concentration that in Figure 4C, but PGE2 has a higher steady concentration. In this case, IL-6 shows an increase of ~2 fold with respect to the level shown in Figure 4C. Thus, a high rate of IL-6 translation can overcome the effect of an increased rate of IκB translation in presence of the virus, allowing an overproduction of the cytokine.

All these results suggest an important dynamical property of the circuit shown in Figure 2: a high value of the parameter $V_{\text{max}}^{IL6}$ produces an over production of NF κB, Cox2, PGE2, and IL-6 in...
the circuit in presence of Nsp5 with respect to the ARS (Figures 4A,D). We name over stimulated immune state (OSIS) to the state of the system shown in Figure 4D, for which: $V_{\text{fl}}^{\text{max}} = 10$ pg mL$^{-1}$ s$^{-1}$, $V_{\text{pon}}^{\text{max}} = 15$ pg mL$^{-1}$ s$^{-1}$, $V_{\text{Cox2}}^{\text{max}} = 2$ pg mL$^{-1}$ s$^{-1}$, $V_{\text{IL6}}^{\text{max}} = 20$ pg mL$^{-1}$ s$^{-1}$, $\text{nsp5} \neq 0$ and $\alpha (t) = 10$ pg mL$^{-1}$ s$^{-1}$.

Figure 5 shows the response of the circuit to a step function defined as:

$$
\alpha (t) = \begin{cases} 
10 & 0 \leq t < 10 \\
0 & t \geq 10 
\end{cases}
$$

(16)

The latter simulates an external signal of IL-6 initially generated at a rate of 10 pg mL$^{-1}$ s$^{-1}$ during 10 min, and switched off after the 10 min. Figure 5A is the response of the system in ARS to the step signal, in absence and presence of Nsp5. Interestingly, in the absence of Nsp5, the circuit exhibits a low concentration of NF $\kappa$B, Cox2 and PGE2, and a high concentration of IL-6, all which are zero when the external signal is switched off. In presence of Nsp5 the ARS is perturbed by $\alpha (t)$, and reaches a new steady state with high concentrations of NF $\kappa$B, Cox2, and PGE2 that remain even when the external signal is switched off. Furthermore, the high IL-6 concentration reached when the circuit is turned on falls to a lower concentration different from zero when $\alpha (t) = 0$.

In order to reduce the effect of Nsp5 on the circuit of Figure 2, we tested the effect of Nimesulide on NF $\kappa$B production. Nimesulide, a Cox2 inhibitor, acts inhibiting the synthesis of Cox2 and lipoxygenase enzyme and their products (Suleyman et al., 2008). We modified Eq. 14 to introduce an inhibition term that decreases the rate of production of Cox2 in presence of Nimesulide:

$$
d_{\text{Cox2}} = \frac{V_{\text{Cox2}}^{\text{max}} p_{\text{Cox2}}^{\text{on}} (t)}{\text{nim} + 1} - k_{19}\text{Cox2}
$$

(17)

where $\text{nim}$ is the amount of Nimesulide at time $t$, whose rate of variation of its concentration in the cell is:

$$
\frac{d (\text{nim})}{dt} = r_2 - k_{19}p_{\text{nim}}
$$

(18)

where $r_2 = 40$ pg mL$^{-1}$ s$^{-1}$ and $k_{19} = 3$ s$^{-1}$. Solution of the modified model (Eq. (17)) is presented in Figure 6. In Figure 6A, Nimesulide is applied to the circuit in presence of Nsp5, and with all gene translation rates set to 2 pg mL$^{-1}$ s$^{-1}$. In this case, Nimesulide has no effect on NF $\kappa$B, a slightly effect on IL-6, and a significant decrease in Cox2 and PGE2 concentrations to values near to zero in presence of 14 pg mL$^{-1}$ of the drug. The same effect is observed when the circuit is in OSIS (Figure 6B). Thus, Nimesulide is not an effective anti-inflammatory agent in the context of the dynamic circuit modeled here, and does not impact the concentration of IL-6 in the presence of Nsp5, even at high concentrations.

Dexamethasone (DX), is a potent synthetic glucocorticoid that inhibits NF $\kappa$B transcription, and is another anti-inflammatory drug that is being used to ameliorate acute inflammatory states due to SARS-CoV-2 infection. In order to test the effect of DX on the inflammatory process in the circuit of Figure 2, we modified Eq. 8 to introduce an inhibition term that decreases the probability of activation of NF $\kappa$B:

$$
\frac{dp_{\text{NFkB}}^{\text{on}} (t)}{dt} = k_{15} \left( \frac{n f}{(h d + 1) + (DX + 1)} \right) \left( 1 - p_{\text{NFkB}}^{\text{on}} (t) \right) - k_{15} p_{\text{NFkB}}^{\text{on}} (t)
$$

(19)

where $DX$ is the amount of DX at time $t$, whose rate of variation of its concentration in the cell is:

$$
\frac{d (DX)}{dt} = r_3 - k_{20} DX
$$

(20)

where $r_3 = 30$ pg mL$^{-1}$ s$^{-1}$ and $k_{20} = 3$ s$^{-1}$.
Figure 7 shows the effect of DX on the circuit. Figure 7A shows that when DX is applied to the circuit in presence of Nsp5, and when all genes have the same translation rate of 2 pg ml$^{-1}$ s$^{-1}$, the drug eliminates NF$\kappa$B, Cox2, and PGE2 from the circuit, but has a null effect on the concentration of IL-6. However, when the circuit is in OSIS (Figure 7B), DX has little effect on the immune response in presence of Nsp5. Figure 7C shows that when all translation rates are the same (2 pg ml$^{-1}$ s$^{-1}$), 14 pg ml$^{-1}$ of DX decreases the concentration of NF$\kappa$B, Cox2, and PGE2 to zero, but has no effect on IL-6 (black line). Figure 7D shows that when the external signal $\alpha$ (t) is the step function of Eq. 16 of main text and is applied to the circuit in presence of Nsp5 (red line), with all gene translation rates equal to 2 pg ml$^{-1}$ s$^{-1}$, 14 pg ml$^{-1}$ of DX decreases the concentration of NF$\kappa$B, Cox2, and PGE2 to zero (black line). Hence, our results suggest that DX is an effective anti-inflammatory drug during mild inflammatory states, but is less effective during critical acute lung inflammation due to SARS-CoV-2 (Horby et al., 2021). This dynamical behavior of the circuit of Figure 2 is unaffected by higher doses of DX. When DX and Nimesulide are applied together (with 35 pg ml$^{-1}$ of DX and 40 pg ml$^{-1}$ of Nimesulide) to the circuit in OSIS, in which $\alpha$ (t) is the function of Eq. 16, there is also a limited anti-inflammatory effect (Figure 8A).

In contrast, the predicted limited effect of Nimesulide and DX on reverting the OSIS, the molecular docking of Nsp5 with a hypothetical drug like Saquinavir (Xu et al., 2020), is able to completely eliminate inflammation. We tested this hypothesis modifying Nsp5 production in Eq. (11) with an inhibitory term:

\[
\frac{d(nsp5)}{dt} = \frac{\phi}{\text{drug} + 1} - k_{18nsp5}
\]  

(21)

where drug is the amount of the hypothetical drug that can remove Nsp5 from the circuit, whose concentration at time t is given by:
The complexity of the immune response to infections implies a complex set of different kinds of cells that involve complex regulatory networks and molecules. Together, they underlie a complex set of different kinds of cells that involve complex feedback loops shown in Supplementary Material S1 (Nayak et al., 2018).

In most of the ODEs-based biological models so far studied, there is limited knowledge of the real values of the parameters, and this leads to uncertainty concerning the extent to which the model explains the real process under study. In this case, the validation of the model relied on experimental data. Nonetheless, with the exception of some particular cases (Hodgkin and Huxley, 1952), qualitative models have to be proposed and quantitative validations remain ahead (Eftimie et al., 2016). This challenge increases with the dimensionality of the model. Thus, is necessary to choose some particular properties of the network under study (inflammation network in this case) that incorporate key dynamical features of the whole system under study, and that can also aid to reduce the number of parameters of the model to the minimum possible.

In this sense, we limited the scope of this work to the study of the inflammation process of epithelial lung cells during SARS-CoV-2 infection. The epithelial lung cells form a sub-network that have two highly connected nodes: the internal signal integrator NFκB, and the external signal integrator IL-6 (Figures 2, 3). Both integrators trigger the activation of two important intermediate processes: Cox2 and PGE2 synthesis. Thus, we propose, as a first approximation, that the dynamical behavior of this these four nodes of the circuit of Figure 2 reflect with certain accuracy the dynamical properties of the complex inflammation sub-network of lung cells. Thus, the dynamics of IL-6, NFκB, Cox2 and PGE2 nodes is described by a model that consist of 12 nonlinear coupled ordinary differential equations (Table 1), that we assume represents the central dynamical core involved in the lung cells inflammation process in response to SARS-CoV-2 infection. Such core includes three coupled feedback loops shown in Figure 3. We used this low-dimensionality model to explore the effect of Nsp5 in the biological systems such as the one treated here.
SARS-CoV-2 main protease Nsp5 has a role as an epigenetic regulator of host DNA expression in lung cells; and thus its physical association with HADC2 (Gordon et al., 2020; Kumar et al., 2020) avoids the movement of the deacetylase from the cytoplasm to the nucleus, increasing the probability of the binding of NFκB to the promoter site of its target genes (Eqs. 5–8) (El Baba and Herbein, 2020). NFκB is a central integrator of the signals that initiate the inflammation process (Figure 1), which include IL-6 secreted by monocytes, leucocytes, macrophages, among other cells, together with signals from TLR4 and TLR7 that suppress the IkB-NFκB complex in a MyD88 independent manner (Vidya et al., 2017). NFκB enhanced transcriptional activity includes the transcription of IL-6, Cox2, IkB and NFκB itself that are key genes in the process of inflammation (Figure 3).

Results obtained from our model suggest that Nsp5 can effectively transform a weak inflammation response (Long et al., 2020) into a persistent sustained one that has a relative high concentration of the cytokine IL-6 (Figure 4B). In the example of Figure 4B, all genes have the same translation rate, a condition that have low probably of occurrence in the real human immune system in which every gene is subject to different processes of post transcriptional regulation (Hauser et al., 2019). When this restriction is removed, and IL-6 has a high translation rate, Nsp5 boosts the concentration of the four main proteins of the circuit with respect to their respective values in ARS, and the inflammation response becomes strongly persistent with a high IL-6 concentration. Furthermore, in this state that we named OSIS, the dynamics of the circuit of Figure 2 becomes independent of any kind of external signal and never turns off, leading to the deregulation of the production of the cytokine IL-6 (Figure 5B). In this form, Nsp5 changes the qualitative dynamical behavior of the system, in which the trajectories that initially span around the ARS stable node now span around the OSIS stable node (Supplementary Material S3) (Diaz, 2020b).

An important biological consequence of this change in the qualitative dynamics of the circuit is that ACE2 receptor synthesis also becomes persistent and autonomous of any external signal to the lung cells (Figure 2). Furthermore, neither Nimesulide nor Dexamethasone or both can completely eliminate the OSIS suggesting that this dynamical behavior could be the form in which the virus assures the persistence of its reproductive cycle without any perturbation from the natural defenses of the body. This could be also a possible clue about the possible adaptive substitution of N protein used in SARS-CoV for the use of the main protease Nsp5 in SARS-CoV-2 as the switch for the inflammation process, which is necessary for ACE2 sustained production, taking into consideration that SARS-CoV N protein targets Cox2 while SARS-CoV-2 Nsp5 protein targets the master integrator NFκB. If this hypothesis is true, this is the probable cause of the increased pathogenicity of SARS-CoV-2 with respect to SARS-CoV.

It is of interest that the persistence of the OSIS is supported by a high translational rate of IL-6, which in the model must have a value of $V_{\text{IL-6}}^{\max} \geq 7 \text{ pg mL}^{-1} \text{s}^{-1}$. This result pictures a probable hypothetical scenario where the external signal $a(t)$ triggers the initial inflammation response with a low translation rate of IL-6 that can be shut down with DX. As viral infection continues, IL-6, NFκB, and IkB increase their rate of translation ($V_{\text{IL-6}}^{\max}$ value has a little weight in this process) and the dynamical behavior of the circuit of Figure 2 becomes independent of the value of $a(t)$, and self-sustained, i.e., the control of the production of the cytokine IL-6 is translated from the external immune cells to the lung cells, and cannot be completely stopped even with high doses of DX. In this form, in the model, $V_{\text{IL-6}}^{\max}$ is a bifurcation parameter that changes the qualitative dynamical behavior of the circuit of Figure 2 when $V_{\text{IL-6}}^{\max}$ is 10 pg mL$^{-1}$s$^{-1}$ and $V_{\text{IkB}}^{\max}$ is 15 pg mL$^{-1}$s$^{-1}$ are constant values (Supplementary Material S3).

In this extreme situation (OSIS), the unique treatment possible suggested by our model is an inhibitor of Nsp5 like Saquinavir (Xun et al., 2020). In this case, the OSIS is completely shut down, which indirectly implies the down production of ACE2. Saquinavir is an anti-retroviral drug used against the main protease of HIV-1 (Bensussen et al., 2018; Xun et al., 2020), with some undesirable effects like diarrhea, abdominal pain, and nausea. We suggest that this drug could be a plausible treatment against the effects of SARS-CoV-2 in acute lung inflammation, and as a complement to the new vaccine against this coronavirus.

Interferon (IFN) I and III are key secretory factors in the fight against viral infections in lung cells. During the early response to SARS-CoV-2 infection, interferon pathway is blocked by viral proteins like Nsp14, Nsp16, Nsp15, Orf9b, and Nsp3, among others, that interfere with MAVS and TLRs sensing, STATs 1 and 2 activation and IFN synthesis. Experimental results show that this downregulation of IFN activity correlates with an increased severity of COVID19 symptoms (Kim and Shin, 2021). Therefore, we did not include IFN pathway in our model as a primary cause of lung inflammation. However, our model suggest that the initial downregulation of IFN pathway could be partially counteract by the over activation of NFκB caused by Nsp5. The master regulator NFκB targets IFNs genes (Pfeffer, 2011; Kim and Shin, 2021) probably producing a late sustained production of IFN after the initial viral infection adding a component to the cytokine storm.

In a previous work (Diaz, 2020a) the role of the viral protein Orf8 as a hub of the SARS-CoV-2 infection was suggested. This highly connected protein has as main targets those processes related to the vesicular trafficking required for the ensemble of new virions (Figure 1) (Gordon et al., 2020). Thus, the inhibition of the effects of Orf8 in the host cells could block the reproduction cycle of the virus but not the inflammation process because Nsp5 is not directly linked to Orf8 (Gordon et al., 2020). Rapamycin has been suggested as a drug against the effects of Orf8 but produces severe immunosuppressant effects, which, according to the results of our model, will not be of care in cases of severe lung inflammation because Nsp5 uncouple the circuit of Figure 2 from the process of viral replication (Figure 1 and Figure 5B). In this case, a treatment with Rapamycin and Saquinavir can be an alternative for patients with severe lung inflammation. This suggestion needs experimental verification and clinical evaluation.
CONCLUSION

In this work we propose a dynamical model that puts forward a novel hypothetical mechanisms underlying lung cells inflammation process in response to SARS-CoV-2. In this scenario the main protease Nsp5 effectively enhances the inflammatory process, increasing the levels of NFkB, IL-6, Cox2, and PGE2 with respect to the reference state. When the translation rates of NFkB and IκB are increased to a high constant value, and the translation rate of IL-6 is increased above the threshold value of 7 pg ml⁻¹ s⁻¹ the circuit enters in a persistent over stimulated immune state (OSIS) with high levels of the cytokine IL-6. The OSIS never shuts down by itself, and becomes autonomous of the signals from other immune cells like macrophages and lymphocytes. Our model explains why DX or Nimesulide have little effect on the OSIS, and the only means to suppress such acute and sustained inflammation state is by inhibiting the protein Nsp5 with drugs such as Saquinavir.

In this form, the model suggests, in accordance to our hypothesis, that Nsp5 is effectively a key node underlying severe acute lung inflammation during SARS-CoV-2 infection. The persistent production of IL-6 by lung cells during infection can be one of the causes of the cytokine storm observed in critical patients with COVID19. From an evolutionary point of view, the use of Nsp5 as the switch to start inflammation, and the consequent overproduction of the ACE2 receptor, could have driven the increased pathogenicity of SARS-CoV-2 with respect to SARS-CoV.

In the absence of quantitative data on the kinetics of Nsp5 in the host cell that could give us a better overview of the dynamical behaviour of the protein in the model proposed in this work, we highlight the importance of future in vitro and in vivo experimental research. In order to experimentally validate the predictions of our model is necessary to measure the rate of production of Nsp5 during the infection of the host lung cells, and compare it with the rate of production of other viral proteins with immunomodulatory effects as Orf 7b and Orf 8, to know the real magnitude of its contribution to the inflammatory process. Furthermore, the experimental inhibition of Nsp5 could be of aid to eliminate the inhibition of HDAC2, either catalytic or mimetic, in infected cells to confirm experimentally if the predictions of the model are in concordance to the real mechanisms that contribute to the cytokines storm. The implementation of techniques as plasmid induction or transfection for the unique expression of Nsp5 and its effectors in lung epithelial tissue also promises to be a simple option to validate the proposed mathematical model, and in turn it would be an ideal platform to measure the efficacy of Nsp5 inhibitors proposed by bioinformatics and clinics.

The present theoretical work is part of the project “Dynamics of the SARS-CoV-2 network” and is based on the experimental data reported in the literature at the moment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

AB made the stability and sensitivity analysis of the model. JD made the model. EA-B and JD conceived the study, discussed the data, and wrote the paper.

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SUPPLEMENTARY MATERIAL

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