Discovery and Assessment of New Target Sites for Anti-HIV Therapies

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1 Introduction

Human immunodeficiency virus (HIV) infects cells by endocytosis and takes over parts of the cell's reaction pathways in order to reproduce itself and spread the infection. One such pathway taken over by HIV becomes the inflammatory pathway which uses Nuclear Factor κB (NF-κB) as the principal transcription factor. Therefore, knocking out the NF-κB pathway would prevent HIV from reproducing itself. In this report, our goal is to produce a simple model for this pathway with which we can identify potential targets for anti-HIV therapies and test out various hypotheses.

In the nucleus of the cell, NF-κB runs along a strand of DNA and produces an NF-κB inhibitor (IκB). IκB is transported from the nucleus into the cytoplasm where it forms a complex with cytoplasmic NF-κB. The complex is degraded by IκB kinase (IκK), and, in the process, the IκB component of the complex is destroyed. IκK exists in various forms within the cytoplasm and is converted from its inactive form to its active form in the presence of Tumour Necrosis Factor (TNF). Thus, a possible strategy for treating HIV would be to decrease the amount of IκK in the cytoplasm, which would reduce the degradation of NF-κB–IκB complexes. Since the NF-κB cannot enter the nucleus while it is in one of these complexes, it cannot participate in the HIV reproduction cycle.

Previous models for the inflammatory pathway have been proposed. Hoffmann *et al.* [1] modelled this pathway using 24 ordinary differential equations (ODEs) and 49 parameters.

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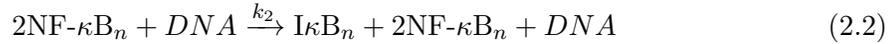
The model explicitly tracked three forms of IκB (α, β, and ϵ), showing that IκBα provided the main negative feedback to turn off the NF-κB response, while the other two forms played stabilizing roles over longer times. This model was amended by Lipniacki *et al.* [4] who, among other things, focused only on the α form of IκB; their model had 15 ODEs and 30 parameters. Kearns *et al.* [2] expanded the model of [1] with an additional description of the transcription of IκBβ and IκBϵ. All of these models contain large numbers of variables and parameters. In contrast to these large models, Krishna *et al.* [3] investigated a minimal model with 3 ODEs and 5 (dimensionless) parameters and showed that it produced spiky oscillations.

Following these lines of research, in Section 2, we present a very simple model with four coupled first-order ODEs and see what happens if we treat IκK concentration as a parameter that can be controlled (by some unspecified means). In Section 3, we augment this model to account for activation and deactivation of IκK, which is controlled (again, by some unspecified means) by TNF.

2 A simple first model

In this section, we start by formulating a simple model for the NF-κB pathway. A cartoon of the pathway that we consider is shown in Figure 1. We suppose that cytoplasmic NF-κB is transferred in and out of the nucleus at rates k_1 and k_{-1}, respectively. Inside the nucleus, two NF-κB molecules work as catalysts for producing transcripts from genes in the DNA to produce IκB in the nucleus at a rate k_2, without using up any of the NF-κB. IκB is transported out of the nucleus into the cytoplasm at a rate k_3, where it reacts with the NF-κB at a rate k_4 to form a stable complex. In this complex form, NF-κB cannot enter the nucleus. However, in the presence of IκK, the complex is broken down, the IκB component is destroyed, and NF-κB is released at rate k_5.

Thus we have the following chemical reactions (note that these are not conventional chemical reaction equations because some chemical species are not conserved):

$$\text{NF-}\kappa\text{B}_c \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{NF-}\kappa\text{B}_n \tag{2.1}$$

$$2\text{NF-}\kappa\text{B}_n + DNA \xrightarrow{k_2} \text{I}\kappa\text{B}_n + 2\text{NF-}\kappa\text{B}_n + DNA \tag{2.2}$$

$$\text{I}\kappa\text{B}_n \xrightarrow{k_3} \text{I}\kappa\text{B}_c \tag{2.3}$$

$$\text{I}\kappa\text{B}_c + \text{NF-}\kappa\text{B}_c \xrightarrow{k_4} \text{NF-}\kappa\text{B–I}\kappa\text{B}_c \tag{2.4}$$

$$\text{NF-}\kappa\text{B–I}\kappa\text{B}_c + \text{I}\kappa\text{K}_c \xrightarrow{k_5} \text{NF-}\kappa\text{B}_c + \text{I}\kappa\text{K}_c \tag{2.5}$$

where the subscripts c and n indicate whether the species is in the cytoplasm or in the nucleus, respectively. Initially, we included the reverse reaction in (2.2) with rate k_{-2}, but decided to set $k_{-2} = 0$ because $k_{-2} \ll k_2$. We assume that each reaction progresses according to the law of mass action. We denote the concentration of NF-κB by A, the concentration of IκB by B, the concentration of NF-κB–IκB complex by C, and the concentration of IκK by D. For simplicity, we suppose that D is constant. The evolution
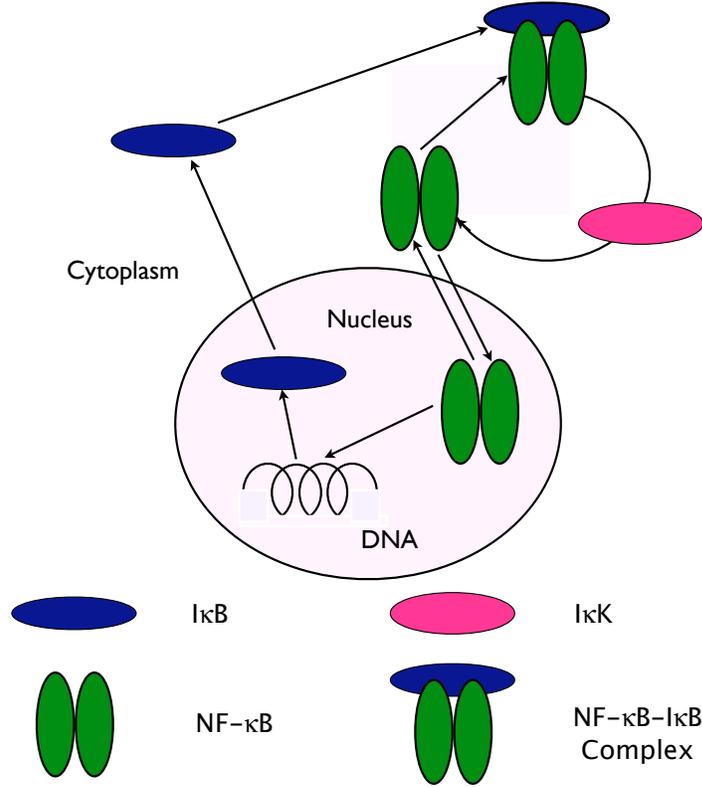
Figure 1 Cartoon of a simple version of the NF-κB pathway

equations for the concentrations of the chemical species are

$$\frac{dA_c}{dt} = k_{-1}A_n - k_1A_c - k_4A_cB_c + k_5DC_c, \tag{2.6}$$

$$\frac{dA_n}{dt} = -k_{-1}A_n + k_1A_c, \tag{2.7}$$

$$\frac{dB_c}{dt} = k_3B_n - k_4A_cB_c, \tag{2.8}$$

$$\frac{dB_n}{dt} = k_2A_n^2 - k_3B_n, \tag{2.9}$$

$$\frac{dC_c}{dt} = k_4A_cB_c - k_5DC_c. \tag{2.10}$$

Adding (2.6), (2.7), and (2.10), we find that

$$\frac{d(A_c + A_n + C_c)}{dt} = 0, \tag{2.11}$$

i.e., the total amount of NF-κB is conserved, which corresponds to biochemical reality over the timescale of events we consider (a few days). Denoting the total amount of NF-κB by

A^*, we can remove A_n from the system, to leave us with

$$\frac{dA_c}{dt} = k_{-1}A^* - (k_{-1} + k_1 + k_4 B_c)A_c - (k_{-1} - k_5 D)C_c, \tag{2.12}$$

$$\frac{dB_c}{dt} = k_3 B_n - k_4 A_c B_c, \tag{2.13}$$

$$\frac{dB_n}{dt} = k_2(A^* - A_c - C_c)^2 - k_3 B_n, \tag{2.14}$$

$$\frac{dC_c}{dt} = k_4 A_c B_c - k_5 D C_c. \tag{2.15}$$

Although we have eliminated A_n from the system, it is convenient to express the steady state in terms of A_n $(= A^* - A_c - C_c)$ as follows:

$$A_c = \frac{k_{-1}}{k_1}A_n, \quad B_c = \frac{k_1 k_2}{k_{-1}k_4}A_n, \quad B_n = \frac{k_2}{k_3}A_n^2, \quad C_c = \frac{k_2}{k_5 D}A_n^2. \tag{2.16}$$

If all of the rate constants are positive, then A_c and B_c are positive only if A_n is positive. In terms of A_n, the constraint $A^* = A_n + A_c + C_c$ becomes

$$A^* = \left(1 + \frac{k_{-1}}{k_1}\right)A_n + \frac{k_2}{k_5 D}A_n^2. \tag{2.17}$$

For any given positive values of A^*, D, and the k_i's, it is evident that equation (2.17) has a unique nonnegative solution A_n. Therefore, our system has a unique physical (i.e., nonnegative) solution for all positive parameter values.

We now nondimensionalise the system (2.12)–(2.15) by setting

$$A_c = \frac{k_{-1}}{k_3}A^* A_c', \quad B_{c,n} = \frac{k_2 A^{*2}}{k_3}B_{c,n}', \quad C_c = A^* C_c', \quad t = \frac{1}{k_3}t'. \tag{2.18}$$

The resulting equations (dropping the primes) are

$$\frac{dA_c}{dt} = 1 - (a_{-1} + a_1 + a_4 B_c)A_c - (1 - a_5)C_c, \tag{2.19}$$

$$\frac{dB_c}{dt} = B_n - \frac{a_4}{a_2}A_c B_c, \tag{2.20}$$

$$\frac{dB_n}{dt} = (1 - a_{-1}A_c - C_c)^2 - B_n, \tag{2.21}$$

$$\frac{dC_c}{dt} = a_{-1}(a_4 A_c B_c - a_5 C_c), \tag{2.22}$$

where $a_{-1} = k_{-1}/k_3$, $a_1 = k_1/k_3$, $a_2 = k_2 A^*/k_{-1}$, $a_4 = k_2 k_4 A^{*2}/k_3^2$, and $a_5 = k_5 D/k_{-1}$. Note that the rate constants, k_4 and k_5 have dimensions $\mu M^{-1}s^{-1}$, whereas the remaining rate constants have dimension s^{-1}.

We now introduce the parameter values given in Table 1 into the above equations and solve them numerically. The results (with D, the IκK concentration, set to 1 μM) are shown in Figure 2. We see that the system settles down to its steady state within three days, but we observe that there is an initial transient followed by a slow decay.

In Figure 3, we consider the effect of doubling the amount of IκK and the effect of removing all the IκK from the system. Note that in the latter case, the equations (2.16) and (2.17) are no longer valid because of the zero divisor, $D \equiv 0$. We see that doubling the amount of IκK results in an increase in the steady state amount of IκB both in the nucleus and in the cytoplasm, with a small increase in the amount of cytoplasmic NF-κB. This is

Parameter	Value	Units	Source
A^*	0.1	μM	[2]
k_{-1}	8×10^{-5}	s^{-1}	[2]
k_1	0.09	s^{-1}	[2]
k_{-2}	0	s^{-1}	[2]
k_2	1.6×10^{-2}	s^{-1}	[1]
k_3	2×10^{-4}	s^{-1}	[2]
k_4	0.5	μM^{-1}s^{-1}	[2]
k_5	1.13×10^{-4}	μM^{-1}s^{-1}	S. Qazi

Table 1 Parameter values for system (2.12)–(2.15). The value for k_5 was provided by S. Qazi (problem presenter).
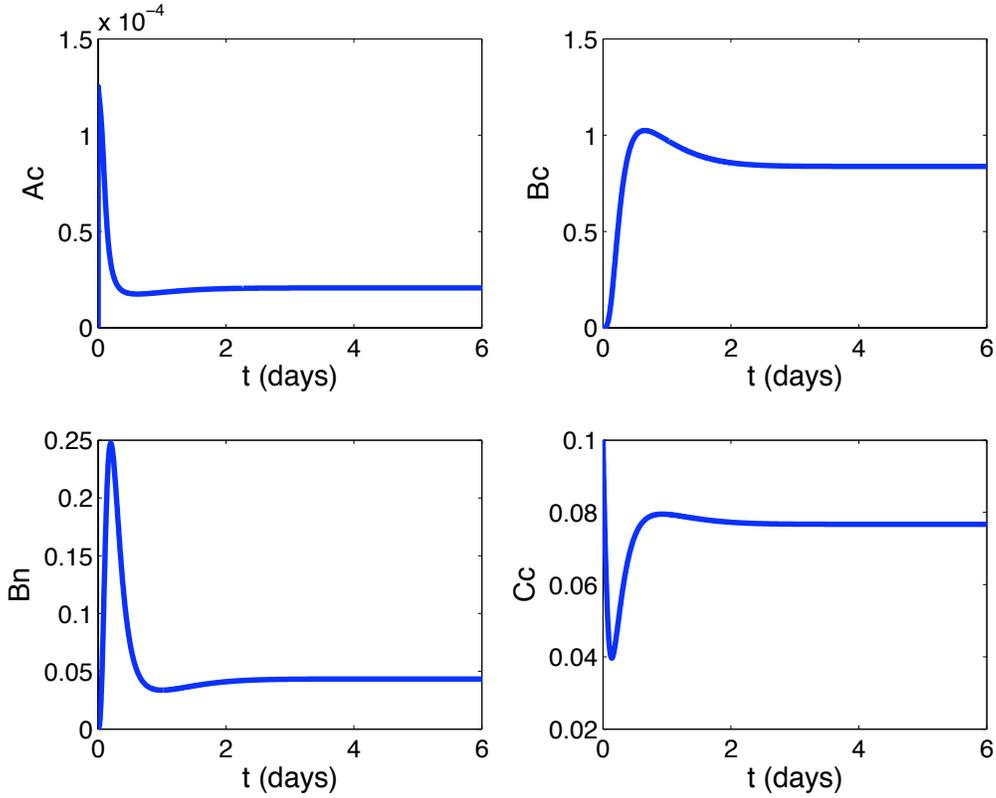
Figure 2 Numerical solution of the system (2.12)–(2.15), using the parameters from Table 1. The system reaches a steady state within ≈ 3 days.

coupled with a small decrease in the amount of the complex NF-κB. Removing all of the IκK results in the complete removal of IκB from the nucleus, because in the absence of IκK, all of the NF-κB is bound in the complex in the cytoplasm, and hence the transcription in the nucleus cannot be activated.
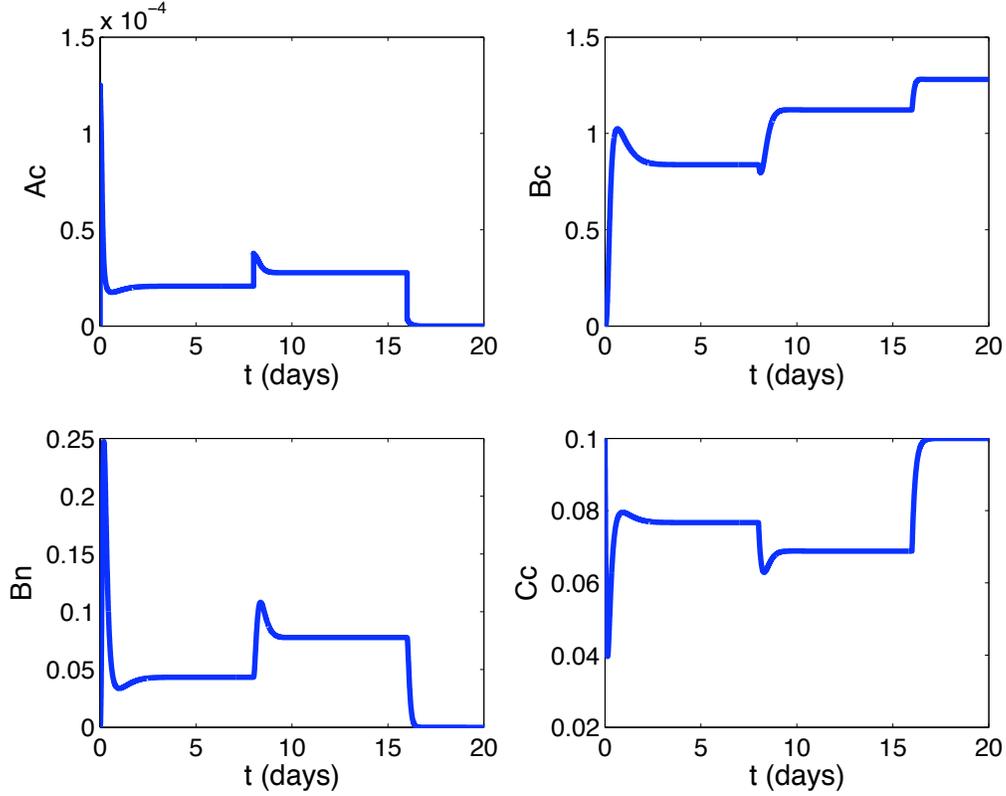
Figure 3 Numerical solution of the system (2.12)–(2.15), using parameters from Table 1. The amount of IκK is doubled at day 8 and is removed at day 16.

We also examined the stability of the fixed point of the system (2.12)–(2.15). We found that the Jacobian matrix evaluated at this point has positive determinant and negative trace for all positive parameter values, but we could not confirm that all eigenvalues had negative real parts in general. Using the values given in Table 1, we evaluated the eigenvalues of the Jacobian numerically for several values of D ranging from 0.001 up to 2.0. In each case, all four eigenvalues had negative real parts, i.e., the fixed point was stable. For the smaller values of D, all four eigenvalues were real; for larger values, two of the eigenvalues were complex.
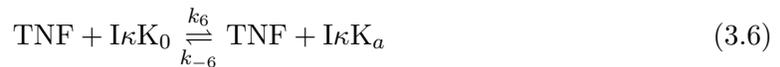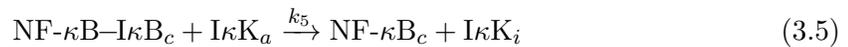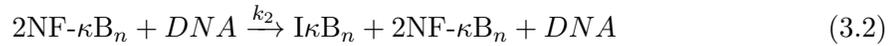
If the variable D, representing IκK, is viewed as a control parameter, then we observe that the interesting limit $D \to 0$ could be interpreted as a singular control problem. However, since IκK is a cytoplasmic molecule, it is not directly controllable from outside the cell. Therefore, this simple model isolates the importance of IκK, but a variable controlling it needs to be incorporated into the model. At this stage, we modify our model so that the concentration of IκK is not constrained to be a constant. This modified model is described in the next section.
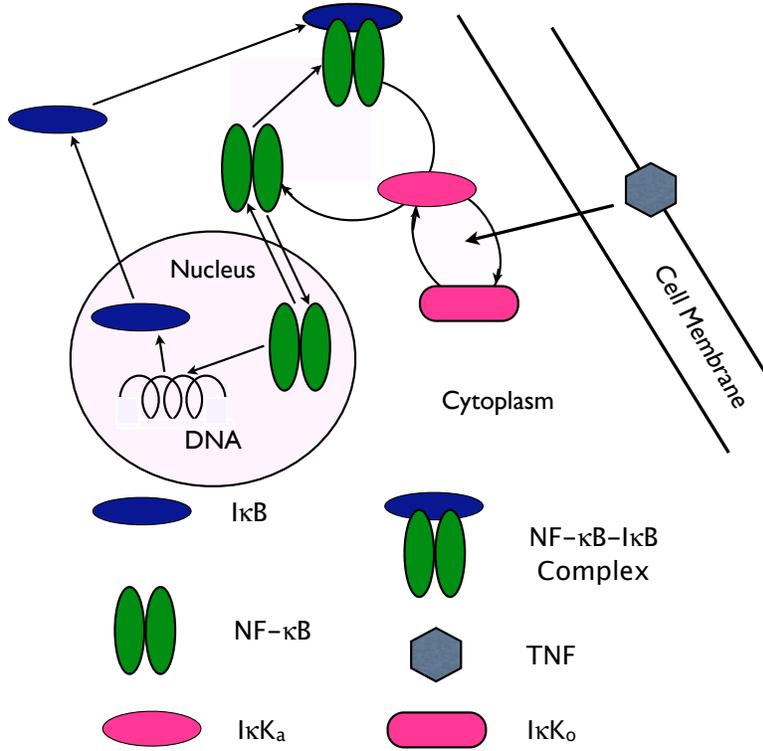
Figure 4 Cartoon showing more complicated version of the NF-κB pathway

3 Second model: Tracking the concentration of IκK

Focussing on the IκK part of the reaction network, we ask how the concentration of IκK can vary. As noted in the introduction, IκK exists in various forms. The active form of IκK (denoted IκK$_a$) is responsible for breaking down the NF-κB–IκB complex, but becomes inactive during the process (turning into IκK$_i$). The active form is produced in the presence of TNF from its neutral form IκK$_0$, which is always present. A revised cartoon is shown in Figure 4. The reaction scheme now is given by

$$\text{NF-}\kappa\text{B}_c \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{NF-}\kappa\text{B}_n \tag{3.1}$$

$$2\text{NF-}\kappa\text{B}_n + DNA \xrightarrow{k_2} \text{I}\kappa\text{B}_n + 2\text{NF-}\kappa\text{B}_n + DNA \tag{3.2}$$

$$\text{I}\kappa\text{B}_n \xrightarrow{k_3} \text{I}\kappa\text{B}_c \tag{3.3}$$

$$\text{I}\kappa\text{B}_c + \text{NF-}\kappa\text{B}_c \xrightarrow{k_4} \text{NF-}\kappa\text{B–I}\kappa\text{B}_c \tag{3.4}$$

$$\text{NF-}\kappa\text{B–I}\kappa\text{B}_c + \text{I}\kappa\text{K}_a \xrightarrow{k_5} \text{NF-}\kappa\text{B}_c + \text{I}\kappa\text{K}_i \tag{3.5}$$

$$\text{TNF} + \text{I}\kappa\text{K}_0 \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \text{TNF} + \text{I}\kappa\text{K}_a \tag{3.6}$$

We suppose that $I\kappa K_0$ is produced at the constant rate k_p and degrades at the rate ℓ_0, and that $I\kappa K_i$ degrades at the rate ℓ_i. It is known that TNF, which influences the rates in the reaction (3.6), is produced outside the cell in response to the HIV infection. Denoting the concentrations of each of the $I\kappa K$ types as D_0, D_a, and D_i, and the constant concentration of TNF as T, we have the following system of equations:

$$\frac{dA_c}{dt} = k_{-1}A_n - k_1 A_c - k_4 A_c B_c + k_5 C_c D_a, \tag{3.7}$$

$$\frac{dA_n}{dt} = k_1 A_c - k_{-1}A_n, \tag{3.8}$$

$$\frac{dB_c}{dt} = k_3 B_n - k_4 A_c B_c, \tag{3.9}$$

$$\frac{dB_n}{dt} = k_2 A_n^2 - k_3 B_n, \tag{3.10}$$

$$\frac{dC_c}{dt} = k_4 A_c B_c - k_5 C_c D_a, \tag{3.11}$$

$$\frac{dD_a}{dt} = k_6(T)D_0 - (k_{-6}(T) + k_5 C_c)D_a, \tag{3.12}$$

$$\frac{dD_0}{dt} = k_p + k_{-6}(T)D_a - (k_6(T) + \ell_0)D_0, \tag{3.13}$$

$$\frac{dD_i}{dt} = k_5 C_c D_a - \ell_i D_i. \tag{3.14}$$

Note that D_i appears only in equation (3.14), so we can solve for D_i explicitly in terms of the other functions. In particular, if the system (3.7)–(3.13) converges to a stable fixed point, then D_i converges to its equilibrium value $k_5 C_c D_0/\ell_i$. For this reason, we omit D_i from further analysis.

We substitute for $A_n = A^* - A_c - C_c$ and nondimensionalise the concentrations and time in the same way as before, using, in addition, the nondimensionalisations

$$D_a = \frac{k_p}{k_{-6}}D_a', \quad D_0 = \frac{k_p}{k_3}D_0'. \tag{3.15}$$

The resulting equations (dropping the primes) are

$$\frac{dA_c}{dt} = 1 - (a_{-1} + a_1 + a_4 B_c)A_c + (a_5^* D_a - 1)C_c, \tag{3.16}$$

$$\frac{dB_c}{dt} = B_n - \frac{a_4}{a_2}A_c B_c, \tag{3.17}$$

$$\frac{dB_n}{dt} = (1 - a_{-1}A_c - C_c)^2 - B_n, \tag{3.18}$$

$$\frac{dC_c}{dt} = a_{-1}(a_4 B_c A_c - a_5^* C_c D_a), \tag{3.19}$$

$$\frac{dD_a}{dt} = a_6(T)a_{-6}D_0 - (a_{-6} + a_7 C_c)D_a, \tag{3.20}$$

$$\frac{dD_0}{dt} = 1 - (a_6 + \ell_0^*)D_0 + D_a, \tag{3.21}$$

where $a_5^* = k_5 k_p/k_{-1}k_{-6}$, $a_{-6} = k_{-6}/k_3$, $a_6 = k_6/k_3$, $a_7 = k_5 A^*/k_3$, and $\ell_0^* = \ell_0/k_3$, and $a_p = k_p A^*/k_{-6}$.

Parameter	Value	Units	Source
k_{-6}	1.5×10^{-3}	s^{-1}	[4] / S. Qazi
k_p	2.5×10^{-5}	s^{-1}	[4]
ℓ_0	$k_p/10$	s^{-1}	S. Qazi

Table 2 Parameter values for the system (3.7)–(3.13), in addition to those appearing in Table 1. Our definition of k_{-6} is a bit different from that in [4]. The value for ℓ_0 was provided by S. Qazi (problem presenter).

The values for the new parameters are given in Table 2. In our numerical simulations, we chose to fix k_{-6} and to let $k_6(T)$ vary to study the effect of different levels of TNF. Figure 5 shows the results of simulations with each of the fifteen different values $k_6 = q \times 10^{-r}$ where $r \in \{3, 4, 5\}$ and $q \in \{1, 2, 3, 4, 5\}$, and another with $k_6 = 0$. The dark blue horizontal line in each graph represents the solution for $k_6 = 0$. As k_6 decreases to 0 thorough our fifteen nonzero values, the corresponding solutions tend to move towards the $k_6 = 0$ solution. We observe that some of the graphs in Figure 5 qualitatively resemble published work, e.g. in [4], see Figure 3F (NF-κB–IκB complex, our C_c) and Figure 9[A,G] (cytoplasmic IκB, our B_c).

The system (3.7)–(3.13) has a unique rest state for all positive parameter values. To see this, we set the derivatives equal to 0 and express the solution in terms of D_a and the eliminated variable A_n as follows:

$$A_c = \frac{k_{-1}}{k_1} A_n, \quad B_c = \frac{k_1 k_2}{k_{-1} k_4} A_n, \quad B_n = \frac{k_2}{k_3} A_n^2, \quad C_c = \frac{k_2}{k_5} \frac{A_n^2}{D_a}, \qquad (3.22)$$

$$D_a = \frac{k_p k_6 - k_2 (k_6 + \ell_0) A_n^2}{k_{-6} \ell_0}, \quad D_0 = \frac{k_{-6} D_a + k_2 A_n^2}{k_6}. \qquad (3.23)$$

Using $C_c = A^* - A_n - A_c$, the relation $0 = -C_c D_a + k_2 A_n^2 / k_5$ [from the last term in (3.22)] becomes

$$0 = \left[A_n \left(1 + \frac{k_{-1}}{k_1} \right) - A^* \right] \left[\frac{k_p k_6 - k_2 (k_6 + \ell_0) A_n^2}{k_{-6} \ell_0} \right] + \frac{k_2 A_n^2}{k_5} =: H(A_n). \qquad (3.24)$$

Thus A_n is the root of a cubic polynomial H. Let $\alpha = [k_p k_6 / (k_2 (k_6 + \ell_0))]^{1/2}$. Since any physical solution has $D_a \geq 0$, we see from (3.23) that we are only interested in A_n in $[0, \alpha]$. Since $H(0) < 0$, $H(+\infty) = -\infty$, and $H(\alpha) > 0$, it follows that the cubic polynomial H has a unique root in $[0, \alpha]$, which is the unique value of A_n that determines the rest state. In particular, it can be seen that as k_6 decreases to 0, then α decreases to 0 and hence, the equilibrium value of A_n also decreases to 0. Therefore, the equilibrium values of A_c, B_c, B_n, and D_a decrease to 0, while $C_c = A^* - A_c - A_n$ increases to A^* and D_0 converges to k_p/ℓ_0. These qualitative trends are confirmed in Figure 5.

For the stability analysis, we checked the Jacobian matrix numerically at the rest state for the same parameter values at which we performed our simulations (Figure 5) (except for the degenerate case $k_6 = 0$). We found that the system was stable in all cases. More precisely, for the fifteen nonzero values of k_6 examined, all six eigenvalues are negative real numbers when $k_6 \leq 2 \times 10^{-4}$ or $k_6 = 5 \times 10^{-3}$; for each of the other values, there were four negative real eigenvalues and two complex eigenvalues with negative real parts.
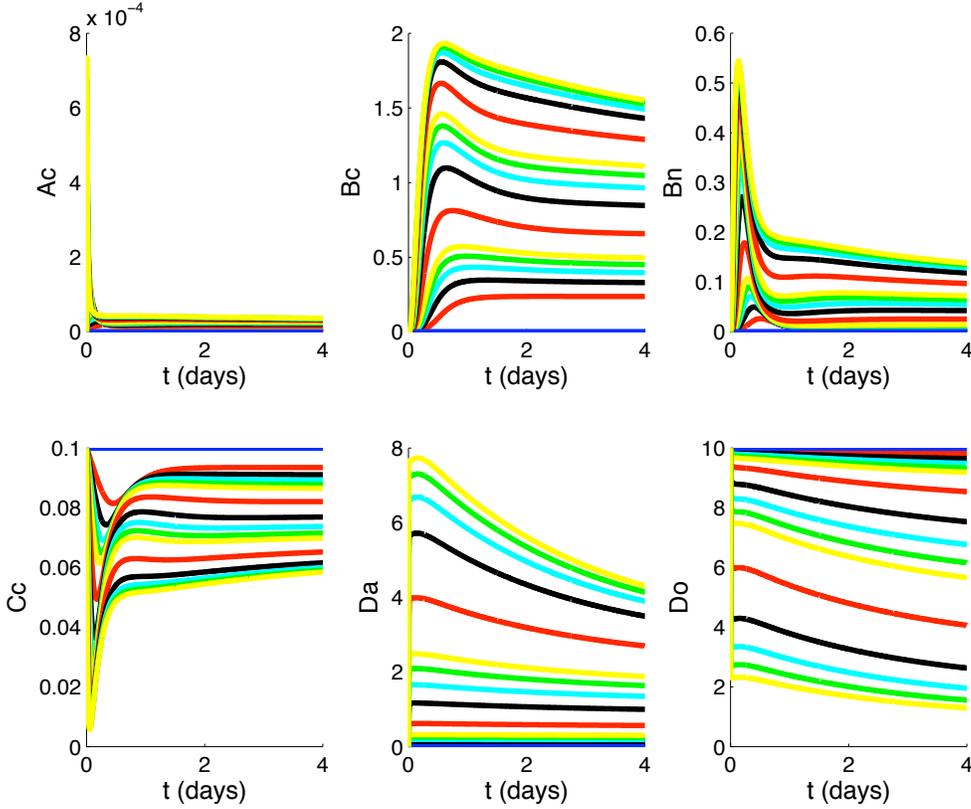
Figure 5 The numerical solutions of system (3.16)–(3.21), using parameter values from Tables 1 and 2 while varying k_6. The dark blue horizontal lines are the solutions for $k_6 = 0$. The other five colours each correspond to solutions for three different values $k_6 = q \times 10^{-3}, q \times 10^{-4}, q \times 10^{-5}$, with $q = 1$ (red), $q = 2$ (black), $q = 3$ (light blue), $q = 4$ (green), and $q = 5$ (yellow). As k_6 decreases to 0, the solutions move monotonically closer to the $k_6 = 0$ solution. Not all curves are visible because of overlap.

4 Discussion and Conclusions

Persistence and evolution of HIV require virus reproduction and spread. In this report, we have focussed on the HIV commandeered inflammatory pathway in cells where NF-κB is the main transcription factor. Thus, one approach to treating HIV is to block this NF-κB pathway, which plays an important role in HIV reproduction by reproducing the virus' DNA in the cell nucleus. We investigated two simplified models of this pathway, based primarily on much larger ODE models that had previously been published. Our simple four variable model pinpoints how the NF-κB pathway can be blocked. Namely, we find that IκK plays a crucial role in controlling the amount of free NF-κB in the cytosolic space that can potentially enter the nucleus to produce more virus DNA. Although it is clear that control of IκK is a potential anti-HIV target, the four variable model does not allow us to address the direct control of these molecules. In our second slightly expanded model, we have been able to capture a reasonable amount of the behaviour observed by others. In

particular, this second model suggested that a new approach to therapy would be to block the extracellular production of TNF, or at least to block TNF's activation of IκK.

A project for future work would be to conduct a more careful sensitivity analysis to account for uncertainties in the parameter values. We also considered the possibility of approaching this as a control problem, say with k_5 and k_6 as control parameters, but it is not yet clear to us what we wish to minimize or what controls (i.e., drugs) are available. Another task could be to investigate the larger models studied earlier and see if they can be systematically reduced to our (second) model by omitting less significant terms.

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