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NC3Rs Maths Study Group 2013

Mathematical modelling of steroid responsiveness in severe asthma and COPD

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1 Background

Over a billion people worldwide suffer from inflammatory lung diseases, of which half are diagnosed with asthma or chronic obstructive pulmonary disease (COPD). About 5% of these exhibit a severe form of disease, characterised notably by a poor or inexistent response to anti-inflammatory corticosteroid treatment [Chu12]. Despite its low prevalence, this condition represents a very serious health issue, as the people affected exhibit the worst respiratory symptoms, and are often at risk of death. Moreover, their treatment represents 50% of the financial burden of inflammatory lung diseases. Therefore, a lot of research effort is dedicated to improving the understanding of severe asthma and COPD, together with the mechanisms of steroid-resistance [AB08]. One important finding is the role played by the kinase enzyme p38 mitogen-activated protein kinase (MAPK) in controlling the anti-inflammatory effects of CS, notably through phosphorylation of the glucocorticosteroid receptor (GR) [Chu11]. Recent studies have shown that peripheral blood mononuclear cells (PBMCs) and lung macrophages from patients with severe asthma exhibit lesser suppression of pro-inflammatory cytokines by CS, linked to augmented activation of p38 MAPK (Fig. 1; [BHK$^+$08, BKH$^+$10]).

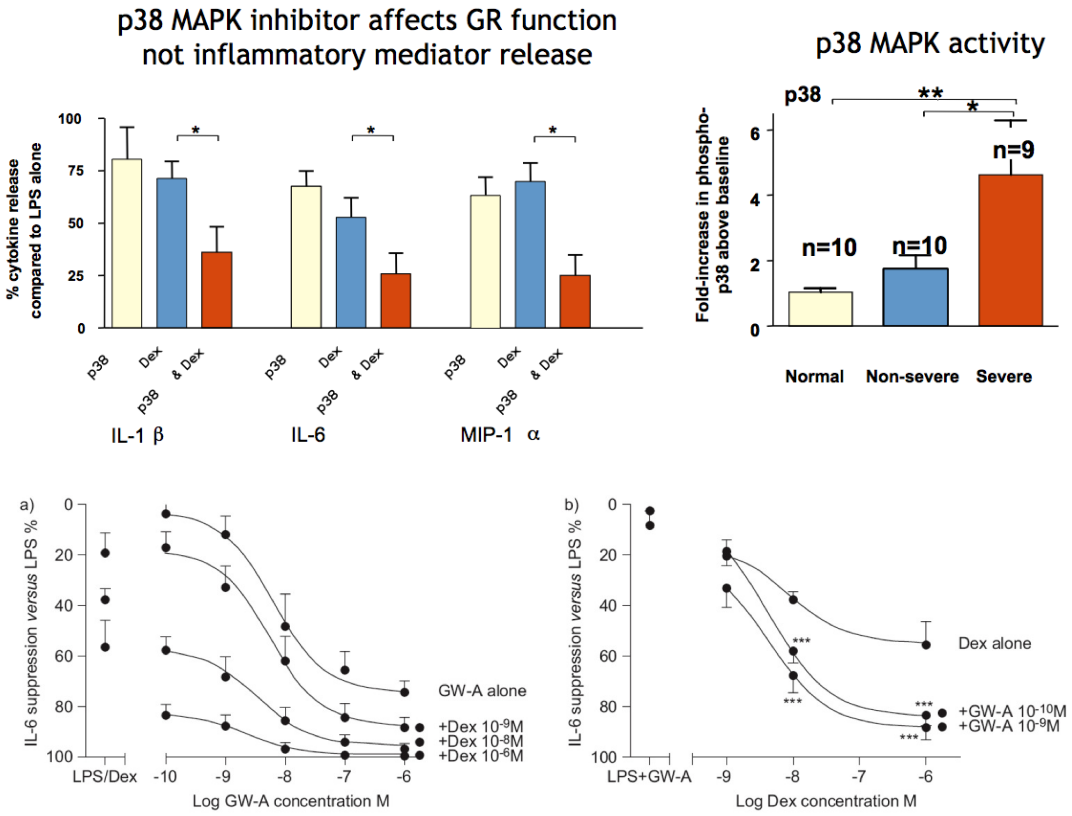

Figure 1. *Top: p38 MAPK sensitivity in BAL macrophages from patients with severe asthma (reproduced from [BHK$^+$08]). Bottom: Inhibition of lipopolysaccharide (LPS)-induced cytokine release from peripheral blood mononuclear cells by the p38 inhibitor GW-A, in the presence of different concentrations of the corticosteroid dexamethasone (Dex), and vice-versa (reproduced from [BKH$^+$10]).*

Based on these experimental results and prior works on the p38 MAPK pathway

[HHC08] and the GR signalling cascade (e.g., [AC01]), a detailed model of the cross-talk between the p38 and GR pathways has been proposed (Fig. 2, [HYAG12]). However, the corresponding mathematical model has not been validated by quantitative data because it is too complicated to be parametrised by the limited number of measurable downstream targets of the pathway. Hence, the participants of the study group have aimed at developing a reduced mathematical model of steroid-resistance, in which the parameters could be related more directly to experimental data.

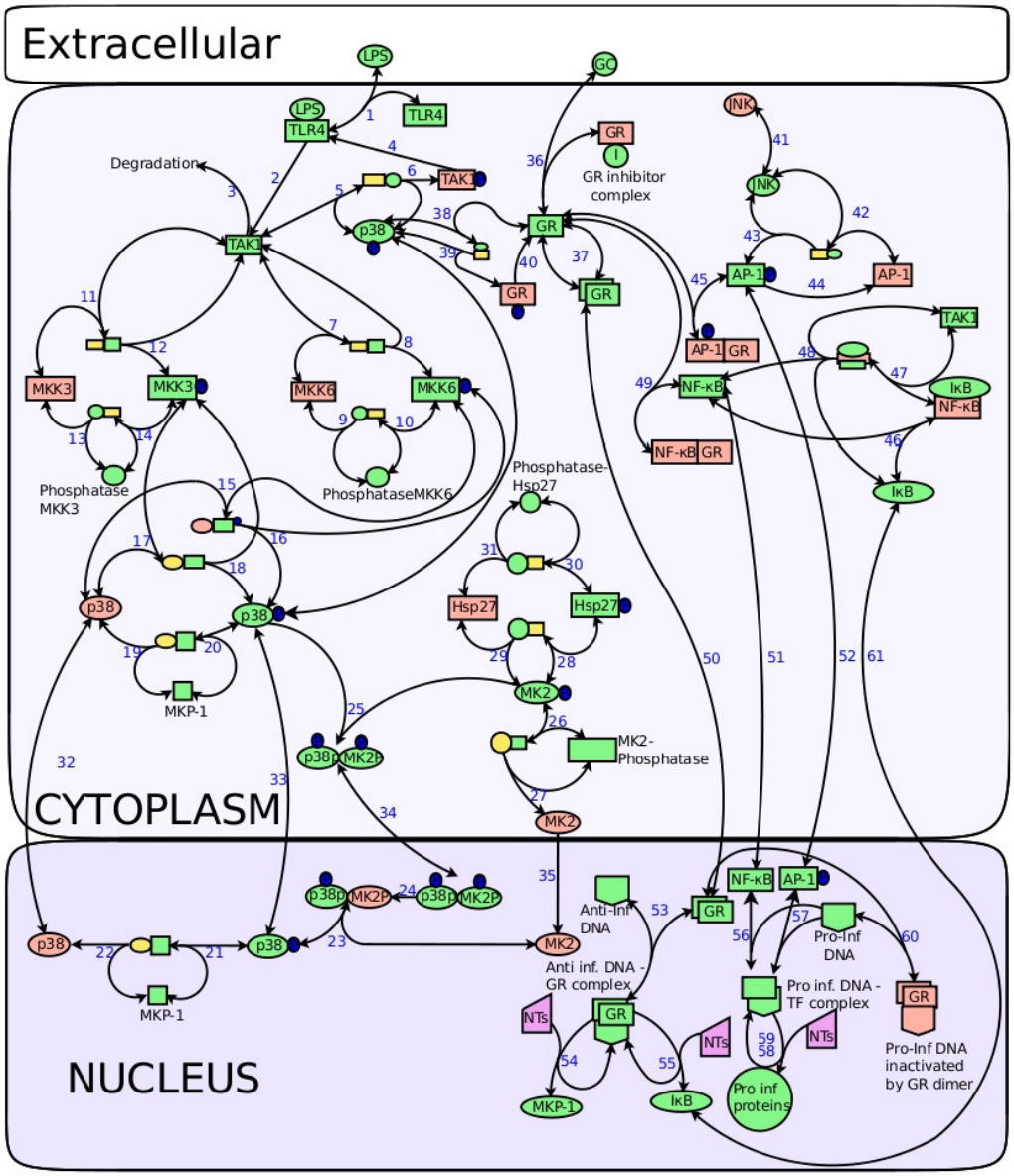

Figure 2. *The p38 MAPK and GR signalling pathways and their crosstalk via TGFβ kinase-1 (TAK1), MAPK phosphatase-1 (MKP-1), and phosho-p38 [HYAG12].*

2 Model formulation

Following the suggestion of the problem presenters, we focused on the main steps of the NF-κB inflammation pathway and its regulation by corticosteroids (CS) and p38 MAPK. The key steps are the following (Fig. 3). When cytosolic NF-κB is activated by an inflammatory stimulus, such as TNF-α or LPS, it translocates into the nucleus and binds to DNA, which induces the production of inflammatory mediators. In steroid-naive people, this inflammatory pathway is heavily down-regulated by CS treatment. CS bind to gluco-corticoids receptors (GR) in the cytosol, which allow them to enter the nucleus and bind to NF-κB. Since GR-bound NF-κB is less efficient in inducing gene expression, this reduces substantially the production of inflammation. However, inflammatory stimulation also induces the activation of p38 MAPK. This kinase phosphorylates GR, which reduces its ability to down-regulate DNA-bound NF-κB. In this work, our hypothesis is that this p38 MAPK-GR crosstalk is responsible for steroid-resistance in people with severe asthma and COPD.

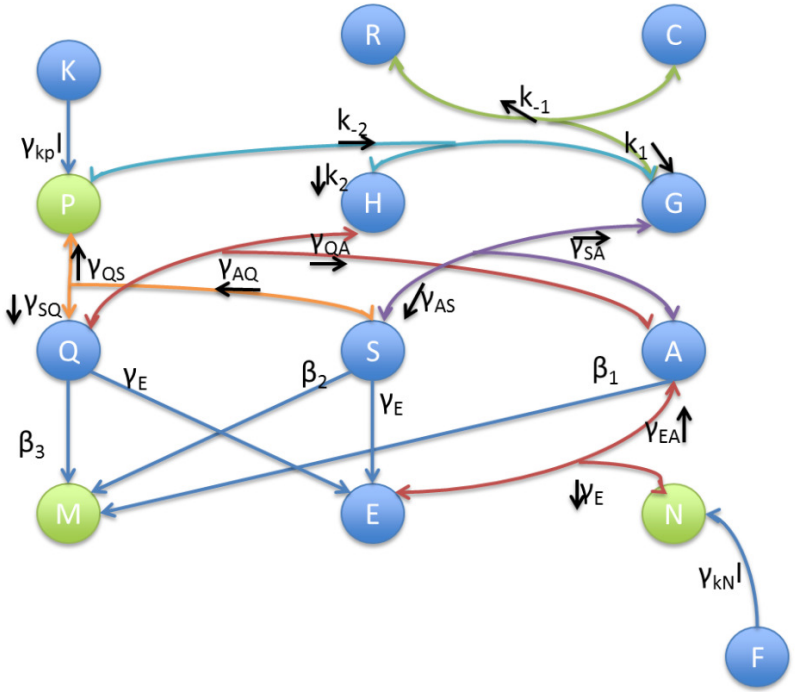

Figure 3. *The core signalling pathway established during the study group. See Table 1 for the meaning of the quantities and Table 2 for the list of reactions.*

The simplified pathway schematised in Fig. 3 is modelled by mass-action kinetics, according to the reaction scheme in Table 2 (see Table 1 for the description of the species).

The following assumptions were used to establish the reactions:

- GR rarely bind to free-NF-κB in the cytosol
- When NF-κB unbinds from DNA, it is degraded in the process, together with the GR possibly bound to it;

Abbreviation	Quantity
I	input inflammation
F	inactive NF-κB
N	active NF-κB
C	cortico-steroid CS
R	inactive GR
G	active GR
K	inactive p38 MAPK
P	active p38 MAPK
H	phosphorylated G
E	empty DNA binding site
A	N-bound DNA
S	N-G-bound DNA
Q	N-H bound DNA
M	output inflammation

Table 1. *Reacting species in the model.*

$$* \overset{\beta_F}{\to} F \overset{\delta_F}{\to} \emptyset$$

$$F + I \underset{\delta_N}{\overset{\gamma_{FN}}{\rightleftharpoons}} N$$

$$E + N \overset{\gamma_{EA}}{\to} A \overset{\delta_D}{\to} E$$

$$* \overset{\beta_R}{\to} R \overset{\delta_R}{\to} \emptyset$$

$$R + C \underset{\delta_G}{\overset{\gamma_{RG}}{\rightleftharpoons}} G$$

$$A + G \overset{\gamma_{AS}}{\to} S \overset{\delta_D}{\to} E$$

$$* \overset{\beta_K}{\to} K \overset{\delta_K}{\to} \emptyset$$

$$K + I \underset{\delta_P}{\overset{\gamma_{KP}}{\rightleftharpoons}} P$$

$$G + P \underset{\delta_H}{\overset{\gamma_G H}{\rightleftharpoons}} H$$

$$A + H \overset{\gamma_{AQ}}{\to} Q \overset{\delta_D}{\to} E$$

$$S + P \overset{\gamma_{SQ}}{\to} Q$$

$$A \overset{\beta_1}{\to} A + M$$

$$S \overset{\beta_2}{\to} S + M$$

$$Q \overset{\beta_3}{\to} Q + M$$

Table 2. *Reactions corresponding to Figure 3.*

- the rate of NF-κB unbinding from DNA is the same for all 3 forms of DNA-bound NF-κB ($\gamma_{AE} = \gamma_{SE} = \gamma_{QE} = \delta_D$);
- the probablity of GR unbinding from DNA-bound NF-κB is unlikely, as well as its dephospholrylation on NF-κB (note that this implies that A,S, and Q can only switch back to E);
- $\beta_1 > \beta_3 > \beta_2$;
- the inflammation output does not feedback on the input; in other words, we are modelling a cell culture experiment where the marker chosen to quantify inflammation can be chosen distinct from the inflammatory stimulus;
- the amount of input inflammation and steroids are constant during the experiment.

In addition, we assume that the total amount of DNA binding sites, D_0, is constant. This leads to the following set of ODEs:

$$
\begin{aligned}
\frac{dF}{dt} &= \beta_F - \delta_F F - \gamma_{FN} FI + \delta_N N \\
\frac{dN}{dt} &= \gamma_{FN} FI - \delta_N N - \gamma_{EA} EN \\
\frac{dR}{dt} &= \beta_R - \delta_R R - \gamma_{RG} RC + \delta_G G \\
\frac{dG}{dt} &= \gamma_{RG} RC - \delta_G G - \gamma_{GH} GP - \gamma_{AS} AG + \delta_H H \\
\frac{dK}{dt} &= \beta_K - \delta_K K - \gamma_{KP} KI + \delta_P P \\
\frac{dP}{dt} &= \gamma_{KP} KI - \delta_P P - \gamma_{GH} GP - \gamma_{SQ} SP + \delta_H H \\
\frac{dH}{dt} &= -\gamma_{AQ} AH - \delta_H H + \gamma_{GH} GP \\
\frac{dE}{dt} &= -\gamma_{EA} EN + \delta_D (A + S + Q) \\
\frac{dA}{dt} &= \gamma_{EA} EN - \gamma_{AS} AG - \gamma_{AQ} AH - \delta_D A \\
\frac{dS}{dt} &= \gamma_{AS} AG - \gamma_{SQ} SP - \delta_D S \\
D_0 &= E + A + S + Q \\
\frac{dM}{dt} &= \beta_1 A + \beta_2 S + \beta_3 Q - \delta_M M
\end{aligned}
\tag{1}
$$

The terms in red are neglected with the rationale that the number of proteins (N, G, P, H) is much larger than the number of DNA binding sites (E, A, S, Q), so that the amount of free proteins which disappear via DNA binding is negligible compared to the amount lost/gained through other processes. The terms in blue can be neglected if we assume that N and P do not switch back to resp. F and K but are degraded directly. Including the terms in green (as we did during the study group) implies that we do not make this assumption for G and H: they decay into resp. R and G before being degraded.

3 Results

3.1 Steady-state analysis

With the assumption that the terms in red in Eq. 1 are negligible, the equations decouple substantially. Keeping the terms in blue and green, the steady-state for $\{F, N\}$ is given by

$$
F = \frac{\beta_F}{\delta_F}, \quad N = \frac{\gamma_{FN}}{\delta_N} IF = \frac{\beta_F \gamma_{FN}}{\delta_F \delta_N} I = \alpha_N I, \tag{2}
$$

while that for $\{R, G, K, P, H\}$ is given by

$$
\begin{aligned}
&R = \frac{\beta_R}{\delta_R}, \quad G = \frac{\beta_R \gamma_{RG}}{\delta_R \delta_G} C, \quad K = \frac{\beta_K}{\delta_K}, \quad P = \frac{\beta_K \gamma_{KP}}{\delta_K \delta_P} I, \\
&H = \frac{\gamma_{GH}}{\delta_H} GP = \alpha_H \alpha_G \alpha_P \, CI
\end{aligned}
\tag{3}
$$

where

$$\alpha_N = \frac{\beta_F \gamma_{FN}}{\delta_F \delta_N}, \quad \alpha_H = \frac{\gamma_{GH}}{\delta_H}, \quad \alpha_G = \frac{\beta_R \gamma_{RG}}{\delta_R \delta_G}, \quad \alpha_P = \frac{\beta_K \gamma_{KP}}{\delta_K \delta_P}. \tag{4}$$

On the other hand, if we assume that the terms in blue are negligible (and, simultaneously, that δ_N and δ_P no longer represent switching rates to F and K but direct degradation rates of N and P), we have instead:

$$F = \frac{\beta_F}{\delta_F + \gamma_{FN} I}, \quad N = \frac{\beta_F \gamma_{FN} I}{\delta_N (\delta_F + \gamma_{FN} I)}, \tag{5}$$

and

$$\begin{aligned} &R = \frac{\beta_R}{\delta_R}, \quad G = \frac{\beta_R \gamma_{RG}}{\delta_R \delta_G} C, \quad K = \frac{\beta_K}{\delta_K + \gamma_{KP} I}, \quad P = \frac{\beta_K \gamma_{KP} I}{\delta_P (\delta_K + \gamma_{KP} I)}, \\ &H = \frac{\gamma_{GH}}{\delta_H} GP = \dots \end{aligned} \tag{6}$$

It is interesting to note that (5) reduces to (2) if $\frac{\gamma_{FN}}{\delta_F} I \ll 1$ and $\delta_N = \delta_F$ (same degradation rate in the active and inactive forms). These two assumptions in turn imply that $N \ll F$, which is supported by experimental data [Gho06]. Similarly, (6) reduces to (3) provided $\frac{\gamma_{KP}}{\delta_K} I \ll 1$ and $\delta_P = \delta_K$, in which case $P \ll K$.

Simultaneously (and independently of the protein-degradation mechanism assumed above), we have

$$\begin{aligned} &E = \frac{\delta_D}{\delta_D + \gamma_{EA} N} D_0 = \dots \\ &A = \frac{\gamma_{EA} N E}{\delta_D + \gamma_{AS} G + \gamma_{AQ} H} = \frac{\gamma_{EA}}{\delta_D + \gamma_{AS} G + \gamma_{AQ} H} \frac{\delta_D N}{\delta_D + \gamma_{EA} N} D_0 = \dots \\ &S = \frac{\gamma_{AS} G}{\delta_D + \gamma_{SQ} P} A = \dots \\ &Q = D_0 - E - A - S = \dots \\ &M = \frac{\beta_1 A + \beta_2 S + \beta_3 Q}{\delta_M} = \dots \end{aligned} \tag{7}$$

Hence the model exhibits a unique steady-state.

Using (2)-(4), we obtain

$$\begin{aligned} &A = \frac{\gamma_{EA}}{\delta_D + \gamma_{AS} \alpha_G C + \gamma_{AQ} \alpha_H \alpha_G \alpha_P C I} \frac{\delta_D \alpha_N I}{\delta_D + \gamma_{EA} \alpha_N I} D_0 \\ &S = \frac{\gamma_{AS} \alpha_G C}{\delta_D + \gamma_{SQ} \alpha_P I} A \\ &Q = D_0 - E - A - S = \dots \\ &M = \frac{\beta_1 A + \beta_2 S + \beta_3 Q}{\delta_M} = \dots \end{aligned} \tag{8}$$

In the absence of steroids ($C = 0$), we have

$$M_{(noC)} = \frac{\beta_1}{\delta_M} A = \frac{\beta_1}{\delta_M} \frac{\gamma_{EA} \alpha_N I}{\delta_D + \gamma_{EA} \alpha_N I} D_0 = \frac{\beta_1 D_0}{\delta_M} \frac{I}{\frac{\delta_D}{\gamma_{EA} \alpha_N} + I} \tag{9}$$

which is an increasing, saturating function (sigmoid) of the inflammatory stimulus I, with maximum $M_I = \frac{\beta_1 D_0}{\delta_M}$ and half-activation constant $K_{MI} = \frac{\delta_D \delta_F \delta_N}{\gamma_{EA} \beta_F \gamma_{FN}}$. Hence, for a

given level of input inflammation I, the larger the rates γ_{EA}, β_F, γ_{FN}, and the smaller the degradation rates δ_D, δ_F, δ_N, the larger the output inflammation M will be, as expected from common sense.

In the presence of steroids, but neglecting the inhibitory effect of p38 MAPK on GR (that is, assuming no steroid-resistance), we have

$$M_{C,noP} = \frac{\beta_1 A + \beta_2 S}{\delta_M} = \frac{D_0}{\delta_M} \frac{\gamma_{EA}\alpha_N I}{\delta_D + \gamma_{EA}\alpha_N I} \frac{\beta_1\delta_D + \beta_2\gamma_{AS}\alpha_G C}{\delta_D + \gamma_{AS}\alpha_G C} \tag{10}$$

which is a decreasing sigmoid function of C for $\beta_1 < \beta_2$ since it implies $\partial M/\partial C < 0$. The reduction in inflammation M due to the presence of steroids is given by:

$$\frac{M_{C,noP}}{M_{noC}} = \frac{\delta_D + \frac{\beta_2}{\beta_1}\gamma_{AS}\alpha_G C}{\delta_D + \gamma_{AS}\alpha_G C} \tag{11}$$

As expected, this ratio is always smaller than 1 for $\beta_2 < \beta_1$. It is independent of I and decreases with C.

The expression of M in the general case where p38 MAPK induces resistance to steroids can be computed similarly.

3.2 Timescale analysis

In order to simplify the study of the model dynamics, a timescale analysis was undertaken, relying on the following experimental informations.

- The activation of N, G, and P, once inflammation I and steroids C are applied, are fast processes (occurring over minutes), as is the binding of NF-κB to DNA.
- M takes a few hours to be produced from A, S, or Q.
- The degradation processes occur over slow timescales (days); in particular, the degradation of DNA-bound NF-κB takes about 46h.

Note that experimentally, these rates cannot measured directly - one instead measures "turnover" rates, i.e. the *total* rates of change of the proteins resulting from the competition between the synthesis and the degradation processes (i.e., a slow turnover at time t means that production and degradation almost balance at time t, but this does not especially imply that the degradation rate is small).

Based on the timescale analysis, we concluded that only M, the inflammation output, varies substantially over the timescale of the experiment (half-a-day), since the other processes are either already equilibrated or have not occurred yet. Hence, the dynamics of M following inflammatory (and possibly, steroid) stimulation is a simple exponential relaxation toward a new, elevated equilibrium, which is given by the steady-state computed above.

In order to estimate the parameters entering the equations, the faster and slower timescales have to be considered.

3.3 Temporal dynamics

Let us consider the full time course of A, the amount of DNA-bound NF-κB. Experimentally, upon inflammatory stimulation, it shows a fast increase (over minutes), remains

close to a maximum for a few hours, and then start to decay. In the simplest case, where no steroid is applied and the influence of p38 MAPK is neglected, the dynamics of A obey

$$\begin{aligned}\frac{dF}{dt} &= \beta_F - \delta_F F - \gamma_{FN} F I + \delta_N N \\ \frac{dN}{dt} &= \gamma_{FN} F I - \delta_N N \\ \frac{dA}{dt} &= \gamma_{EA}(D_0 - A)N - \delta_D A\end{aligned} \tag{12}$$

where we have used the fact that $D_0 = E + A$. We assume that initially, $F = F_0$, $N = 0$ and $A = 0$.

During the study group, we have assumed that the production/degradation of F are fast processes compared to the others, i.e., $\beta_F - \delta_F F \simeq 0$, which simplies further the equations. However, this assumption, taken together with the other ones made previously, is not compatible with the biphasic evolution of A observed experimentally. Indeed, it implies that

$$\begin{aligned}\frac{dN}{dt} &= \gamma_{FN}(F_0 - N)I - \delta_N N, \\ \frac{dA}{dt} &= \gamma_{EA}(D_0 - A)N - \delta_D A.\end{aligned} \tag{13}$$

The first equation involves N only and can be solved to give

$$N(t) = \frac{\gamma_{FN} I}{\gamma_{FN} I + \delta_N} F_0 \left(1 - e^{-(\gamma_{FN} I + \delta_N)t}\right). \tag{14}$$

N is thus monotonically increasing toward its steady-state. Initially, A is also increasing since the term $\delta_D A$ is zero. For A to maximise and then start decreasing, we need

$$\delta_D A > \gamma_{EA}(D_0 - A)N.$$

This is not possible since, by assumption, the left-hand side no longer increases beyond the maximum where $\delta_D A = \gamma_{EA}(D_0 - A)N$, while the right-hand side keeps increasing as N increases monotonically. Hence, (at least) one of the assumptions made needs to be reconsidered. Lifting the assumption $\beta_F - \delta_F F \simeq 0$ could be sufficient since then, F and N obey a pair of linear ODEs, uncoupled from A, which have for solutions sums of two exponentials. For appropriate parameter values, N may thus exhibit a rising phase followed by a decaying phase. In turn, this could allow A to first increase and then decrease. Whether the constrains on parameter values that allow such a behavior are realistic needs to be investigated.

4 Discussion and Perspectives

We have developed a mathematical model of steroid responsiveness in severe asthma and COPD, focusing on the major steps of the NF-κB inflammation signalling cascade. Namely, we have considered the down-regulation of the NF-κB pathway by steroid receptors (GR) and the influence of p38 MAPK on this regulation. We have computed the steady-state of the model in the absence and in the presence of steroids, and shown that these expressions depend on parameters in a realistic manner. We have also performed

a qualitative timescale analysis in order to simplify the model. This has highlighted the existence of 3 different timescales: that of cellular reactions leading to protein activation (minutes), that of protein transcription (hours), and that of protein degradation (days). In order to use that knowledge to further simplify the model, the assumptions made about relative protein quantities will have to be confirmed (e.g., is there indeed much less active NF-κB than inactive NF-κB, and if so, is it correct to neglect the amount of active-NF-κB lost through DNA binding?). We will also need to non-dimensionalise the equations in order to compare directly the magnitude of the rate parameters (these are currently expressed in different units: β_i, γ_{ij}, and δ_i resp. in M/s, 1/(s M), and 1/s). Experimental references for the parameter values are needed to perform this non-dimensionalisation, some of which could be taken from existing experimental databases (e.g., [MJM$^+$10]), while others will require additional experiments.

In section 3.3, we have had a first look at the implications of the model assumptions on the time course of measurable quantities, in view of the future fitting of the model to experimental data. We have shown that one of the approximations made is incompatible with the behavior reported experimentally. The exact assumption at the origin of this qualitative discrepancy remains to be identified.

It is likely that some of neglected feedback mechanisms (e.g., the inhibition of IKK, responsible NF-κB activation, by NF-κB-bound DNA) will also have to be reintegrated to account for the experimental data. Prior mathematical models of the NF-κB signalling pathway incorporates such feedbacks [FCLA09, WPH$^+$12]. Mathematical models of the p38 MAPK pathway have also been published [HHC08]. The detailed model schematised in Fig. 2 is however the first attempt to account for the crosstalk between the p38 and GR pathways. The modulation of GR by other pathways, such as PI3K and JAK-STAT, could also be the subject of future work. In order to underscore key interacting nodes in the pathway, mathematical tools such as bifurcation analysis and/or parameter sensitivity analysis will be required.

Potential impact on animal use

Animal models of asthma are not predictive particularly with respect to severe asthma. This has resulted in the use of large numbers of animals to test drugs that have proved unsuccessful in man. It is important, therefore, to both improve the animal models used and to have a greater understanding of the mechanisms underlying the pathophysiology of severe asthma and COPD. Mathematical models, combined with human clinical data and 'omics data (available e.g. from UBIOPRED and ECLIPSE), can help to further understand why these diseases do not respond well to conventional anti-inflammatory therapies. Information generated by modelling responses mathematically can be used to inform the development of primary human cell models, which will enable researchers to be more selective in the models of severe asthma and COPD tested. This will in turn facilitate the development of fewer, but more predictive, animal models.

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