A two-variable model of cytokine-mediated inflammation in rhcumatoid arthritis

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1 Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease preferentially affecting the joints and leading, if untreated, to progressive joint damage and disability. Cytokines, a group of small inducible proteins, which act as intercellular messengers, are key regulators of the inflammation that characterizes RA. They can be classified into pro-inflammatory and anti-inflammatory groups. We use a two-variable model for the interactions between pro-inflammatory and anti-inflammatory cytokines, and demonstrates that mathematical modelling may be used to investigate the involvement of cytokines in the disease process. We also use that model to demonstrate bistability and oscillations.

2 The basis for the Model

The synovium consists of a variety of cells including fibroblasts, macrophages and T cells, and each individual cell has a different response pattern. We neglect this variability in cell behaviour and the synovium is modelled as a spatially uniform collection of homogeneous, generic cells. We focus on the cells production of pro-inflammatory and anti-inflammatory cytokine molecules. The binding of pro-inflammatory cytokine molecules to membrane-bound receptors induces the production of both pro-inflammatory and anti-inflammatory cytokines while the binding of anti-inflammatory molecules causes a downregulation in the production of pro-inflammatory molecules.

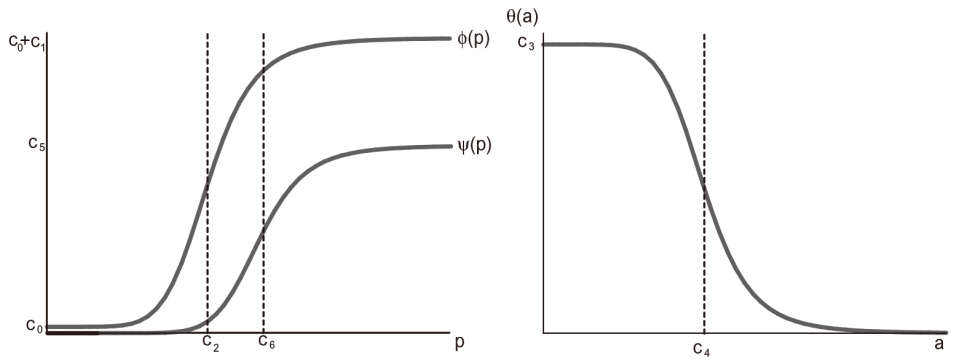

Figure 1: Examples of qualitative forms for the production feedback functions $\Phi(p)$, $\Theta(p)$ and $\Psi(P)$.

We denote the concentration of pro-inflammatory cytokine molecules by p and the concentration of anti-inflammatory cytokine molecules by a. The degradation of a cytokine concentration is assumed to be linear, with rates dp and da. The general form of the equations for the cytokine dynamics is then

$$\frac{dp}{dt} = -d_p p + \Phi(p)\theta(a) \tag{1}$$

$$\frac{da}{dt} = -d_a a + \Psi(P) \tag{2}$$

The product $\Phi(p)$,$\Theta(p)$ models the combined effect of pro-inflammatory and anti-inflammatory stimuli on pro-inflammatory cytokine production, based on the assumption that anti-inflammatory molecules work by inhibiting the synthesis of pro-inflammatory cytokine molecules. $\Phi(p)$ and $\Psi(P)$ are increasing saturating functions of p, so that they represent induced up-regulation with some maximum production rate. Similarly,$\Theta(p)$ represents the down-regulation of p in response to an increase in a and with a decreasing effect from some maximum at $a = 0$. Examples of functions that have these properties are

$$\Phi(p) = c_0 + c_1 \frac{p^{m_1}}{c_2^{m_1} + p^{m_1}} \tag{3}$$

$$\Theta(a) = c_3 \frac{c_4^{m_2}}{c_4^{m_2} + a^{m_2}} \tag{4}$$

$$\Psi(p) = c_5 \frac{p^{m_3}}{c_6^{m_3} + p^{m_3}} \tag{5}$$

where c_0, c_1, c_2, c_3, c_4, c_5 and c_6 are non-negative constant parameters. Since pro-inflammatory production is stimulated by an external stimulus, a background production term c_0 has been included in $\Phi(p)$, anti-inflammatory production is stimulated only by pro-inflammatory cytokine molecules and so no background term is necessary. The coefficients m_1,m_2 and m_3 will all be taken as 2 for the analysis of this system since values greater than 2 show qualitatively similar behaviour and a value of 1 reduces the range of behaviours. This is discussed further in Appendix A .Some sample forms for these feedback functions are shown in Fig. 1. The model equations are non-dimensionalized using

$$p = p * c_2, a = a * c_a, t = t * \frac{1}{d_a} \tag{6}$$

With the asterisks dropped for notational simplicity and setting $m_1 = m_2 = m_3 = 2$, with the equations for Φ, θ and Ψ, become

$$\frac{dp}{dt} = -\gamma p + \frac{1}{1+a^2}(\alpha_1 + \alpha_2 \frac{p^2}{1+p^2}) \tag{7}$$

$$\frac{da}{dt} = -a + \alpha_4 \frac{p^2}{\alpha_3^2 + p^2} \tag{8}$$

where

Table 1: Summary of the dimensionless parameters in the cytokine dynamics model

Parameter	Interpretation
α_1	Background pro-inflammatory production rate
α_2	Magnitude of additional pro-inflammatory cytokine production
α_3	Pro-inflammatory cytokine concentration at which anti-inflammatory production is half maximal
α_4	Magnitude of anti-inflammatory cytokine production
γ	Relative rate of clearance of pro-inflammatory cytokine to anti-inflammatory cytokine

$$\alpha_1 = \frac{c_0 c_3}{c_2 d_a}, \alpha_2 = \frac{c_1 c_3}{c_2 d_a}, \alpha_3 = \frac{c_6}{c_2}, \alpha_4 = \frac{c_5}{c_4 d_a}, \gamma = \frac{d_p}{d_a} \tag{9}$$

The parameter α_1 is the background production rate for pro-inflammatory cytokine so that when $a = p = 0$, pro-inflammatory production occurs at a rate of α_1. The parameter α_2 corresponds to the maximum rate of pro-inflammatory cytokine production over and above the basal rate. α_3 is the concentration of pro-inflammatory cytokines at which anti-inflammatory production is half maximal. α_4 corresponds to the maximum rate of production of anti-inflammatory cytokine. Here, γ is the ratio of the rate of pro-inflammatory and anti-inflammatory decay. The significance of these parameters is summarized for reference in Table 1 and appropriate values for these parameters are discussed in Appendix B.

In the following sections we will show how bistability and oscillatory behaviour can arise from this model and consider possible interpretations of this behaviour in a biological context.

3 Model analysis

3.1 Nullclines and steady states

To analyse the steady states of this system, we will consider the forms of the nullclines, and consider only the positive quadrant. The nullclines of the system, respectively, are as follows:

$$a = N_1(p) = \frac{\alpha_4 p^2}{\alpha_3 + p^2} \tag{10}$$

$$a = N_2(p) = \sqrt{f(p)} \tag{11}$$

where

$$f(p) = \frac{p^2(\alpha_1 + \alpha_2) + \alpha_1}{\gamma p(1 + p^2)} - 1 \tag{12}$$

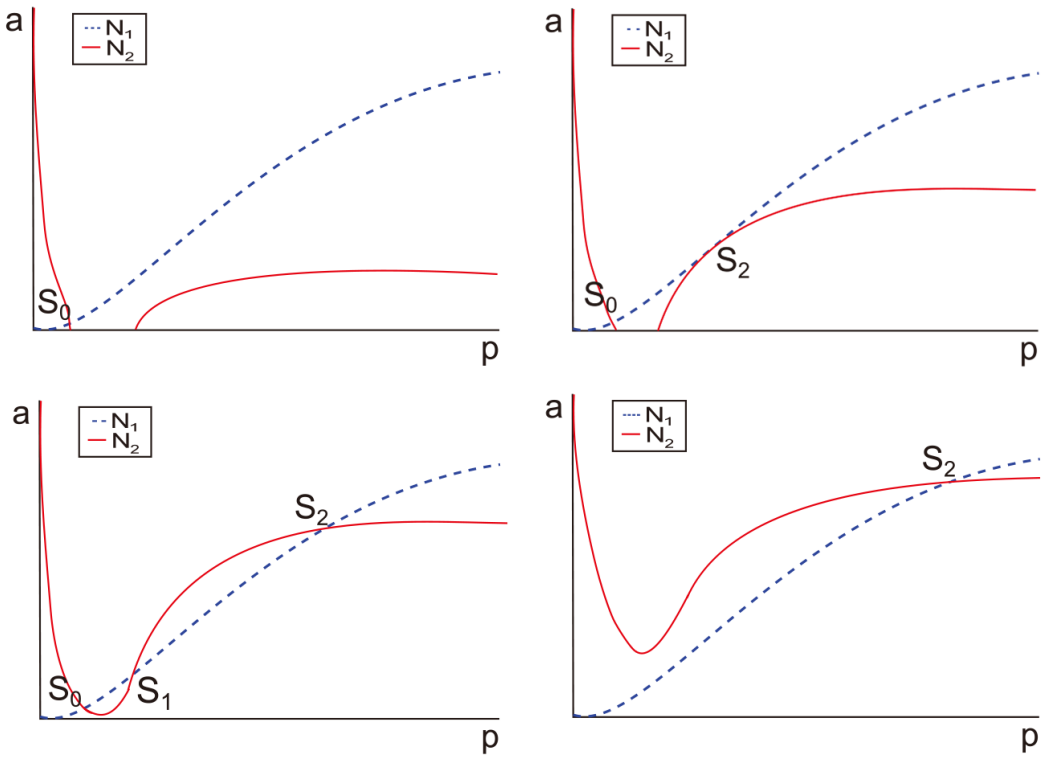

Figure 2: Schematic showing the nullclines of the system (2.3), (2.4) and the different ways they may intersect when $\alpha_2 > 8\alpha_1$. The dashed line represents the a nullcline (da/dt = 0) and the solid line represents the p nullcline (dp/dt = 0).

Figure 2 shows the ways that these nullclines may intersect and hence how the steady states may arise. It is clear from this diagram that there is always at least one steady state and for some parameter values three steady states exist.

Analysis of the turning points of the nullclines tells us that, when $\alpha_2 < 8\alpha_1$ there can only be one steady state, and when $\alpha_2 > 8\alpha_1$ there is either one, two or three steady states (see Fig. 2). The case of two state states only occurs when the nullclines touch but do not cross and as such only exists for an extremely narrow set of parameters. For this reason, throughout this paper, we will only consider the one and three steady state cases. The steady states are denoted S_0, S_1 and S_2. Where they exist, S_0 is stable, S_1 is unstable and S_2 can be either stable or unstable(Appendix C).

3.2 One parameter bifurcation diagrams

Of the five free parameters, the cytokine production rates (α_2 and α_4) are rates that change as part of the immune response and so are likely to change over time in response to injury or therapeutic intervention. If we assume that the rate of clearance is determined by the size and structure of the cytokine and by the chemical environment within the host, it is reasonable to assume that the decay rate parameter γ will remain constant in an individual (or vary over a much longer timescale than that over which cytokine interactions occur). Similarly, we assume that the background production rate and the anti-inflammatory production threshold parameter, α_1 and α_3, respectively, are fixed within an individual. To demonstrate the types of behaviour that can arise from this model, we consider bifurcation diagrams of variations in α_2 for a range of different values of the other parameters. The types of behaviour displayed are summarized in Table 2

and discussed in detail below. All bifurcation plots and simulations in this paper were produced in XPPAUT.

Table 2: Summary of the behaviour types in the cytokine dynamics model

Case	Steady states	Limit cycles
A_1	S_0:stable	–
A_{ii}	S_2:stable	–
A_{iii}	S_0:stable; S_1:unstable;S_2:unstable	–
B	S_2:unstable	L_1:stable
C_i	S_0:stable; S_1:unstable;S_2:stable	–
C_{ii}	S_0:stable; S_1:unstable;S_2:stable	L_2:unstable
D_i	S_0:stable; S_1:unstable;S_2:unstable	L_1:stable
D_{ii}	S_0:stable; S_1:unstable;S_2:unstable	L_1:stable;L_2:unstable

3.2.1 Monostable and bistable behavior

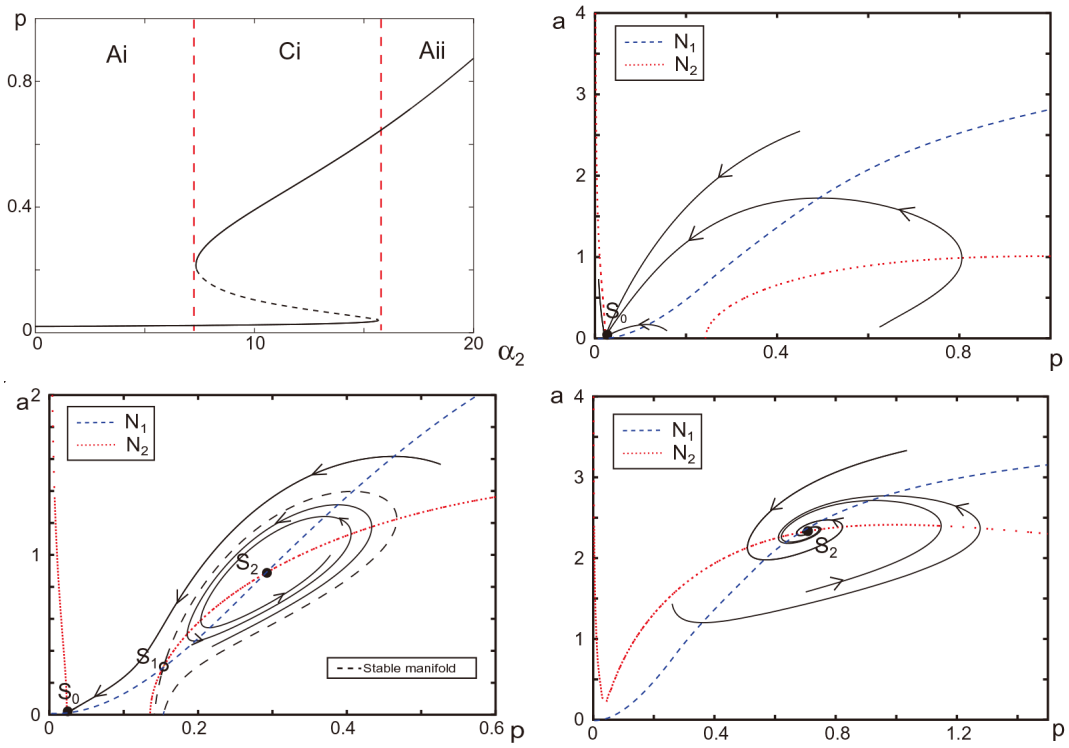

Figure 3: Monostable and bistable behaviour in the model (2.32.4) for the interaction between pro- and anti-inflammatory cytokines($\alpha_1 = 0.025$,$\alpha_3 = 0.5$, $\alpha_4 = 3.5$ and $\gamma = 1.25$). (a) The bifurcation plot of p against α_2. The solid lines represent stable branches while the dashed lines represent unstable branches. The vertical dashed lines signify the thresholds between different behaviour types. (b) The phase plane plot of Case A_i, a single healthy steady state ($\alpha_2 = 5$). (c) The phase plane plot of Case C_i, two stable steady states (S_0 and S_2) and one unstable steady state (S_1) ($\alpha_2 = 8$). (d) The phase plane plot of Case A_{ii}, a single unhealthy steady state with ($\alpha_2 = 17$).

3.2.2 Monostable and bistable behavior with oscillations

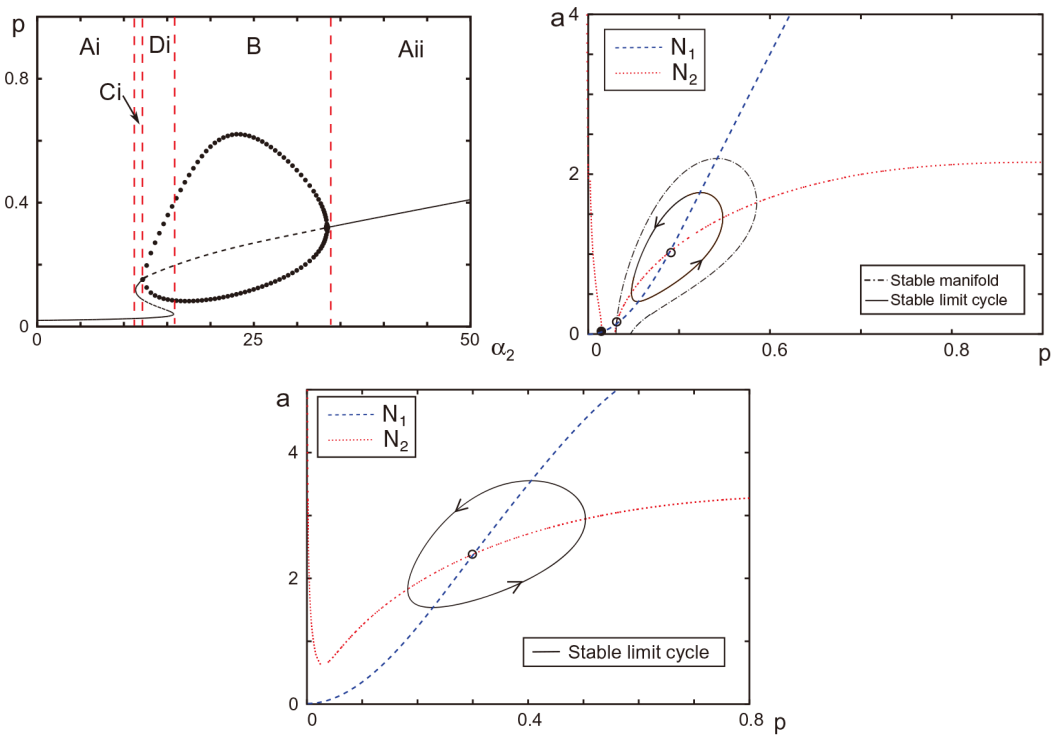

Figure 4: Monostable and bistable behaviour with oscillations in the model (2.32.4) for the interaction between pro- and antiinflammatory cytokines ($\alpha_1 = 0.025, \alpha_3 = 0.5, \alpha_4 = 9$ and $\gamma = 1.25$). (a) The bifurcation plot of p against α_2. The solid lines represent stable branches, whereas the dashed lines represent unstable branches. The vertical dashed lines signify the thresholds between different behaviour types. (b) The phase plane plot of Case D_i, one stable steady state (S_0), two unstable steady states and a stable limit cycle around S_2 ($\alpha_2 = 15$). (c) The phase plane plot of Case B, one unstable steady state (S_2) surrounded by a globally stable limit cycle ($\alpha_2 = 30$). Cases A_i, C_i and A_{ii} are shown in Fig. 3.

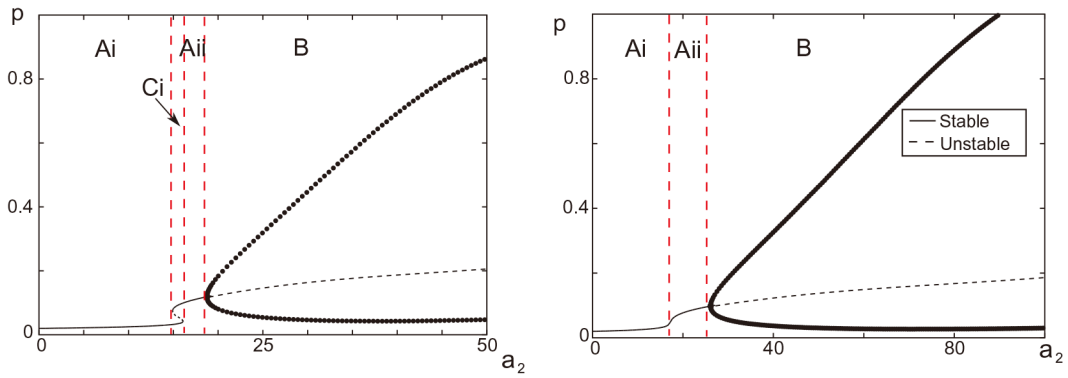

Figure 5: Bifurcation plots for different values of α_4 showing how cases Ci and Di are lost compared with Fig. 4(a) ($\alpha_1 = 0.025, \alpha_3 = 0.5$ and $\gamma = 1.25$) The solid lines represent stable branches, whereas the dashed lines represent unstable branches. The vertical dashed lines signify the thresholds between different behaviour types. (a) $\alpha_4 = 18$, first Hopf bifurcation moves to right of the second fold and case Di (b) $\alpha_4 = 30$, folds coalesce and all bistability is lost.

3.2.3 Monostable and bistable behavior with homoclinic bifurcations

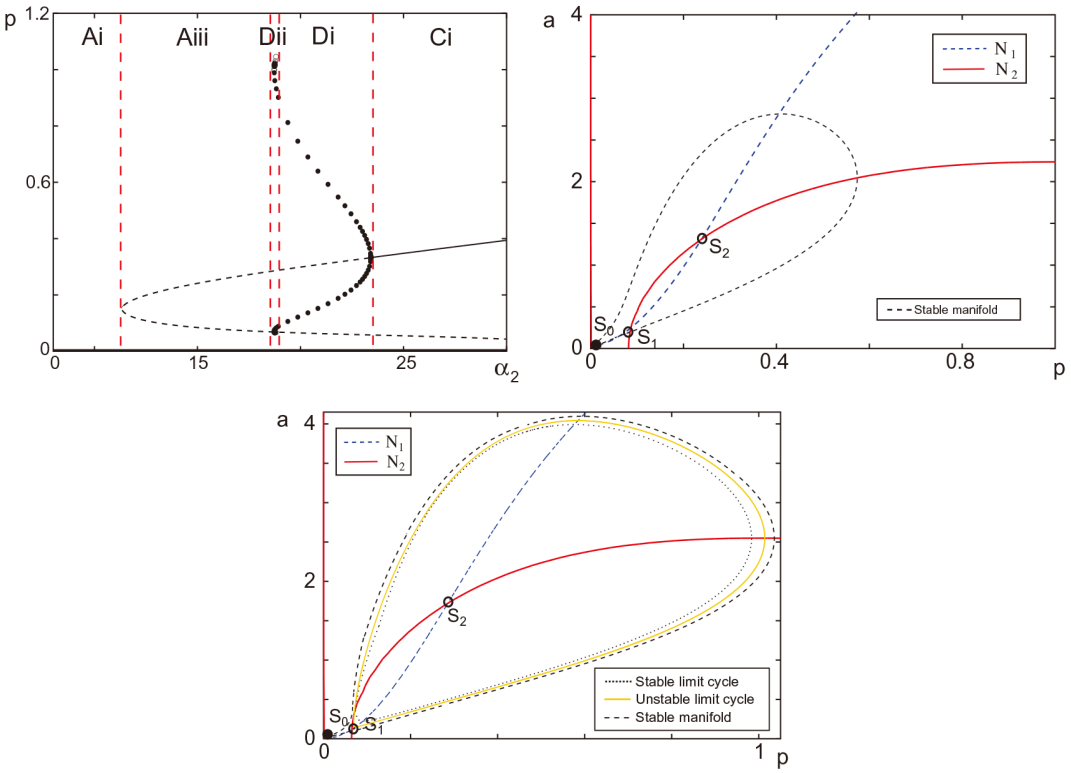

Figure 6: Monostable and bistable behaviour with homoclinic bifurcations in the model (2.32.4) for the interaction between pro- and anti-inflammatory cytokines, showing the new behaviours A_{iii} and D_{ii}. (a) The bifurcation plot of α_2 against p. The solid lines represent stable branches, whereas the dashed lines represent unstable branches. The vertical dashed lines signify the thresholds between different behaviour types. (b) The phase plane plot of Case A_{iii}, a stable steady state (S_0) and two unstable steady states ($\alpha_2 = 15$). (c) The phase plane plot of Case D_{ii}, a stable steady state (S_0), two unstable steady states, a stable limit cycle and an unstable limit cycle ($\alpha_2 = 18.73$). Cases A_i, C_i and D_i are shown in Fig. 4. ($\alpha_1 = 0, \alpha_3 = 0.5, \alpha_4 = 7$ and $\gamma = 1.25$).

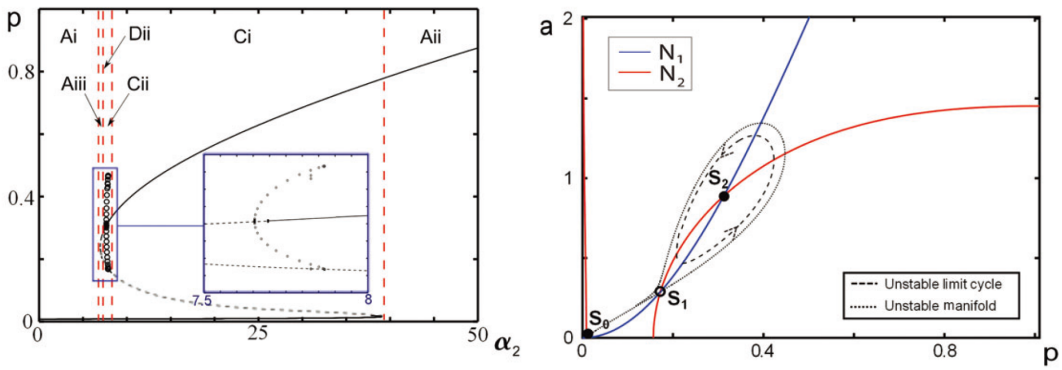

Figure 7: Bistable behaviour with a homoclinic bifurcation in the model (2.32.4) for the interaction between pro and antiinflammatory cytokines. (a) Bifurcation plot showing α_2 plotted against p. The inset shows how Case C_{ii} arises through a supercritical Hopf bifurcation where the branch of limit cycles turns and becomes unstable almost immediately after bifurcation. The solid lines represent stable branches whilst the dashed lines represent unstable branches. The vertical dashed lines signify the thresholds between different behaviour types. (b) Phase plane plot showing case C_{ii}, two stable steady states (S_0 and S_2), an unstable state(S_1) and an unstable limit cycle around S_2 ($\alpha_2 = 7.75$). Cases A_i, A_{ii}, A_{iii} and C_i are shown in Figs 4 and 6. ($\alpha_1 = 0.01, \alpha_3 = 1, \alpha_4 = 10$ and $\gamma = 1.25$).

3.2.4 Bistable behavior with a homoclinic bifurcation

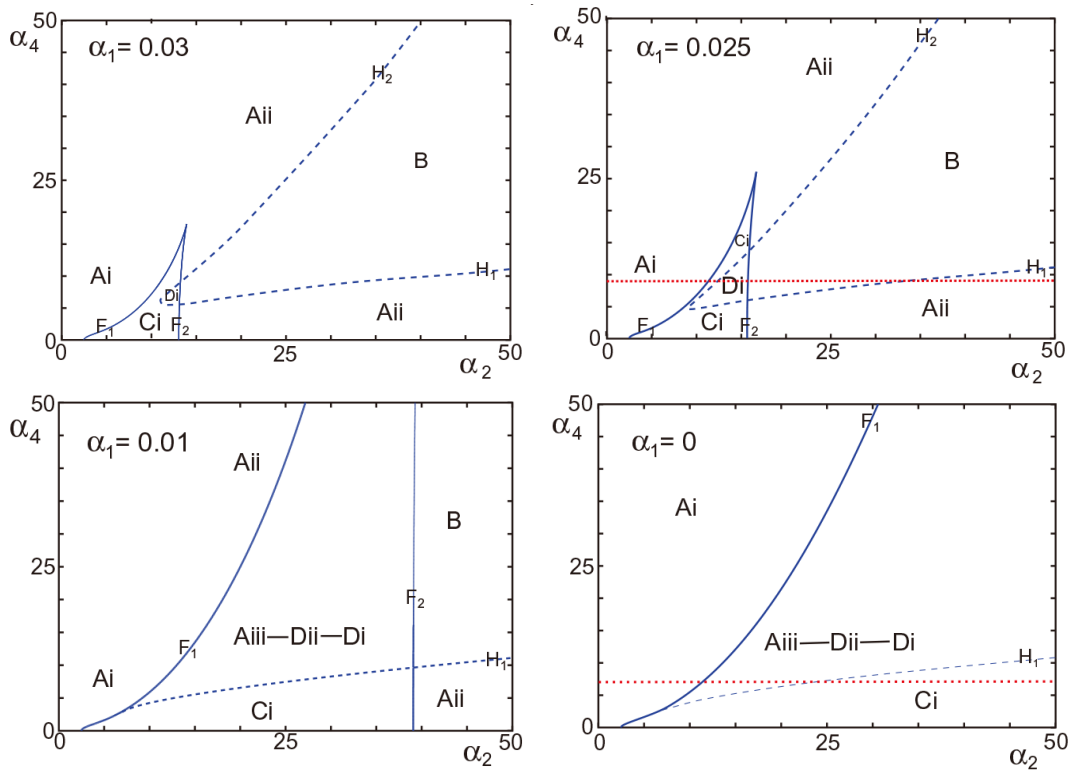

Figure 8: Parameter space plots in $\alpha_2\alpha_4$ ($\alpha_3 = 0.5$ and $\gamma = 1.25$) showing the fold (F1 and F2) and Hopf (H1 and H2) bifurcations and types of the phase space for decreasing values ofα_1.(a) Cases A_i, A_{ii}, B, Ci and D_i are shown. (b) Cases A_i, A_{ii}, B, Ci and D_i are shown. The horizontal dashed line represents a slice through the parameter space at $\alpha_4 = 9$, consistent with the bifurcation plot in Fig. 4 ($\alpha_1 = 0.025$). (c) Cases A_i, A_{ii}, A_{iii}, B, Ci, D_i and D_{ii} are shown., (d) Cases A_i, A_{ii}, A_{iii}, Ci, D_i and D_{ii} are shown. The horizontal dashed line represents a slice through the parameter space at $\alpha_4 = 7$, consistent with the bifurcation plot in Fig. 6 ($\alpha_1 = 0$).

So far we have considered only variations in the pro-inflammatory cytokine production rate α_2. It is likely that the anti-inflammatory cytokine production rate α_4 is also important in determining disease activity since anti-inflammatory cytokines will mitigate the pro-inflammatory cytokine response. Hence, in the next section we will look at the $\alpha_2\alpha_4$ parameter space for different values of the other three parameters.

3.3 Two-parameter bifurcation diagrams

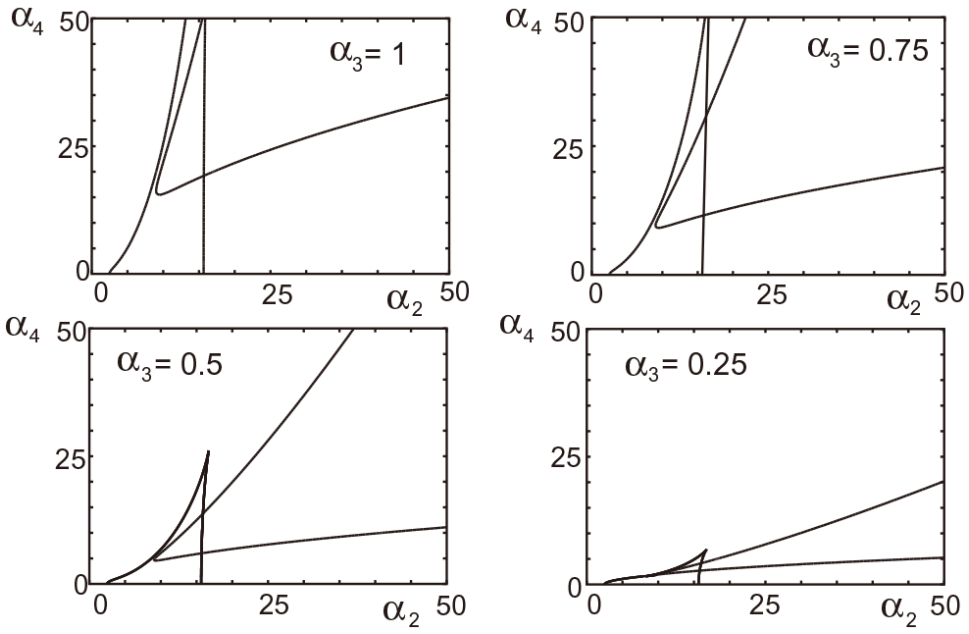

Figure 9: Figure showing the dependence on threshold parameter α_3 of the location of fold and Hopf bifurcations in the $\alpha_2\alpha_4$ parameter space for parameter values ($\alpha_1 = 0.025$, $\gamma = 1.25$).

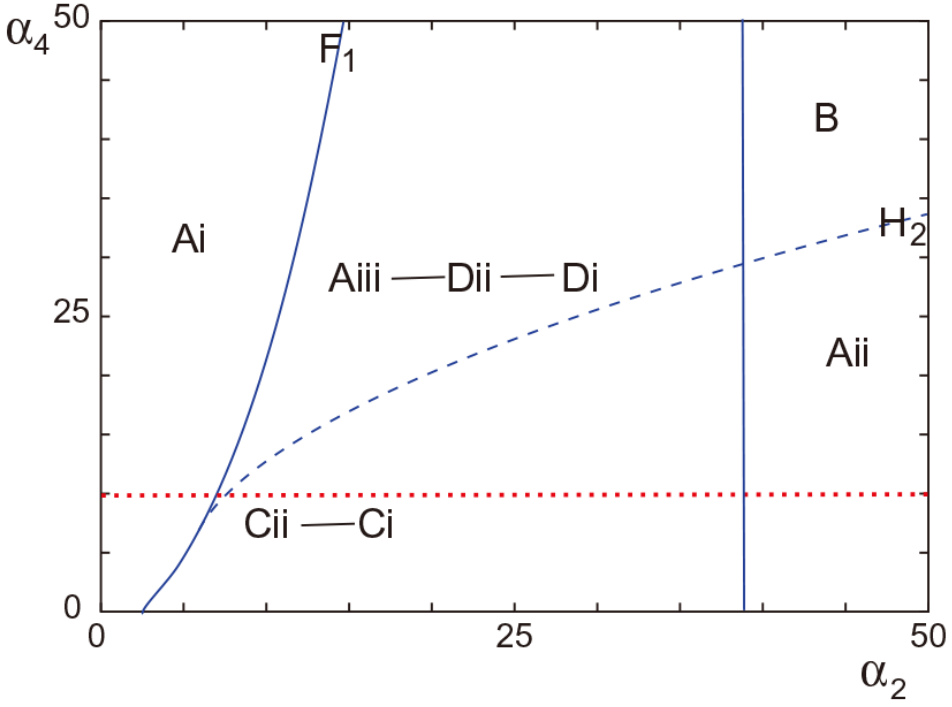

Figure 10: The $\alpha_2\alpha_4$ parameter space showing all cases. The horizontal dashed line represents a slice through the parameter space at $\alpha_4 = 10$, consistent with the bifurcation plot in Fig. 7 ($\alpha_1 = 0.01, \alpha_3 = 1$ and $\gamma = 1.25$)

Figure 9 shows $\alpha_2\alpha_4$ parameter space diagrams for a range of values of α_3. As α_3 increases, the cusp at which the fold bifurcations meet and are destroyed occurs for a higher value of anti-inflammatory production parameter α_4. The proinflammatory production parameter α_2 at the cusp varies little with α_3. One consequence of this effect is that if the threshold α_3 is large, then the range of states which can exhibit health and disease is increased. When α_3 is small, most conditions lead to the case where there is a single state with pro- and anti-inflammatory concentrations varying according to α_2. Figure 10 shows a two-parameter bifurcation diagram for a large value of α_3 but a smaller value of α_1. Here, all the possible behaviours are observed through variations in α_2 and α_4.

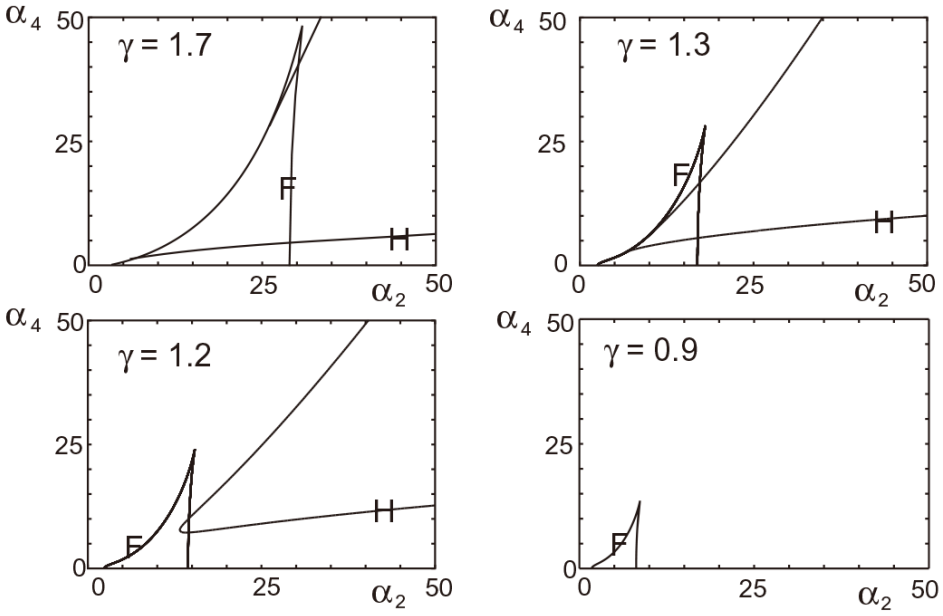

Figure 11: Diagrams showing the positions of the fold (F) and Hopf (H) bifurcations in the $\alpha_2\alpha_4$ parameter space for decreasing values of γ and the parameters ($\alpha_1 = 0.025$ and $\alpha_3 = 0.5$).

Figure 11 shows $\alpha_2\alpha_4$ parameter space diagrams for various values of γ and demonstrates that as γ decreases, the fold and Hopf bifurcations move apart. This means that the parameter region over which there is bistability decreases and the majority of parameter space leads to a single generic stable steady state or a stable limit cycle.

4 Conclusions

The model developed here is a two-variable activatorinhibitor system that simulates the dynamics of two classes of cytokines, pro-inflammatory and antiinflammatory. Five key dimensionless parameters have been identified. We have shown that the model can have either one steady state (S_0 or S_2) or three steady states (S_0, S_1 and S_2). This leads to a range of a phase plane behaviours.

The model shows four types of monostable phase plane behaviour (A_i, A_{ii}, A_{iii} and B). These behaviours may be interpreted as a healthy response (A_i and A_{iii}) due to a low level of p, a disease response due to a high level of p (B) or an unclear response of health/disease due to an intermediate range of p. In addition to monostable behaviour,

the model also shows four types of bistable phase plane behaviour (C_i, C_{ii}, D_i and D_{ii}). These have a stable healthy steady state and a stable disease state which is either a fixed point or a limit cycle. One point to note is that if an individual has a high level of pro-inflammatory production, so that a monostable disease state prevails, increasing the magnitude of anti-inflammatory production, α_4, does not return the system to distinct health, but does reduce the level of p at the fixed point (see Fig. 8(b)). In clinical practice, only cytokine concentrations are changed rather than production rates, and so increasing the magnitude of anti-inflammatory cytokine production would relate only to intrinsic changes in rates at present.

5 Outlook

This model has produced many of the features observed in real cytokine systems, but if the characteristics of this model are to be interpreted in a clinical context, then it is necessary to link the concentration of cytokines to a measurable disease indicator. Ideally, we would like to link the model results to clinical data of cytokine levels over time in individuals with early and late RA. Practical considerations, including the short half-life of cytokines and the difficulty of extracting synovial fluid from the joint, indicate that this type of data is difficult to obtain in humans. It may be possible to collect similar data from animal models or, alternatively, we may be able to use other types of clinical data. The inflammatory marker C-reactive protein (CRP) is routinely used by clinicians as a measure of disease activity in RA. However, the variation between individuals is large and the link between the cytokine level and CRP level or inflammation is as yet unclear. That said, in the majority of cases we identify from our model, the interpretation is very clear. We either have low levels of p indicating health or high levels of p indicating disease. It is only when the levels are intermediate that we are unable to define a clear threshold between health and disease. While there is no precise link between model variables and specific disease markers, the interactions in the model are well established and the predictions are robust to variations in parameter values and functional forms. It would ultimately be desirable to have a model that includes a number of specific cytokines and measurable disease markers to allow a clear link between model behaviour and disease activity. This would give us a better idea of how cytokine levels influence disease manifestations and would provide a clearer definition of health and disease.

Appendix A. Hill coeffients

So far we have taken all the Hill coefficients (m1, m2 and m3) to be 2. In this section, we will justify this choice by examining some other possibilities and considering the effect these would have on the model.

$$\frac{dp}{dt} = -\gamma p + \frac{1}{1+a^{m_2}}(\alpha_1 + \alpha_2 \frac{p^{m_1}}{1+p^{m_1}}) \tag{13}$$

$$\frac{da}{dt} = -a + \alpha_4 \frac{p^{m_3}}{\alpha_3 + p^{m_3}} \tag{14}$$

The Hill coefficients from the functions $\Phi(p)$,$\Psi(P)$ and $\Theta(p)$ also appear in the non-dimensionalized equations in the form of (A.1A.2). There are three main alternatives to

the assumption we have made;firstly that all the coefficients are the same but are some value greater than 2; secondly that all the coefficients are 1 or finally that we have some combination of different coefficients for the different terms in the model.

A.1 Hill coefficients $m_1, m_2, m_3 > 2$

For coefficients greater than 2 the qualitative shape of the Hill function does not change; only its steepness does. This means that, for $m_1, m_2, m_3 > 2$, the nullclines of the system will cross in a similar manner, and we expect qualitatively similar behaviour, with the stability of the steady states and the types of bifurcations unchanged. The only change we would expect is alterations in the values of parameters at which the various bifurcations occur.

A.2 Hill coefficient m1 = m2 = m3 = 1

Since the shape of the Hill function when the coefficient is 1 is different from when it is greater than 1, the behaviour of the model is also likely to change. In this situation, the model equations become

$$\frac{dp}{dt} = -\gamma p + \frac{1}{1+a}(\alpha_1 + \alpha_2 \frac{p}{1+p}) \tag{15}$$

$$\frac{da}{dt} = -a + \alpha_4 \frac{p}{\alpha_3 + p} \tag{16}$$

which gives the nullclines

$$a = N_1(P) = \frac{\alpha_4 p}{\alpha_3 + p} \tag{17}$$

$$a = N_2(p) = \frac{p(\alpha_1 + \alpha_2) + \alpha_1}{\gamma p(1+p)} - 1 \tag{18}$$

As in the original model, N1 is monotonically increasing. However, now N2 is monotonically decreasing in p and hence there can be no more than a single steady state.

A.3 Mixed Hill coefficients

So far we have only considered situations where all three Hill coefficients are 2 or equal to 1, but the coefficients are independent and could have different values. Since values greater than 2 behave the same as a value of 2, we only need to consider combinations of 1 and 2. Also, if we look at the nullcline N1, it is a monotonically increasing function regardless of the value of m3, and so we need only look at two situations: m1 = 2, m2 = m3 = 1 and m1 = 1, m2 = 2, m3 = 1.

In the first case, when m1 = 2, m2 = m3 = 1, N2 becomes

$$a = N_2(p) = \frac{p^2(\alpha_1 + \alpha_2) + \alpha_1}{\gamma p(1+p^2)} - 1 \tag{19}$$

This is the same as f (p) in Appendix C and has two real, positive turning points, meaning that we can have either one or three steady states. This exhibits similar behaviour to the original model except that the steady states tend to occur at larger values of both p and a.

In the second case, when m1 = 1, m2 = 2 and m3 = 1, N2 becomes

$$a = N_2(p) = \sqrt{f(p)} \tag{20}$$

where

$$f(p) = \frac{p(\alpha_1 + \alpha_2) + \alpha_1}{\gamma p(1+p)} - 1 \tag{21}$$

Here f (p) is monotonically decreasing, so that N2 must also be monotonically decreasing in the positive quadrant, and can cross N1 only once, giving exactly one steady state. This situation is similar to the case when all the coefficients are 1 and exhibits similar behaviour. Essentially, m1 must be greater than 1 to give bifurcations and bistability in the model i.e. strong feedback in p is required.

Appendix B. Parameter values

Throughout this work we explore the behaviour of the system by looking at different parameter values and so it is useful to have some idea of the values that would be reasonable. We can gain some insight into this by examining the definitions of the parameters.

We define by $\gamma = dp/da$ the relative rate of clearance and it is the ratio of the pro-inflammatory to anti-inflammatory cytokine degradation rates. If we assume that these degradation rates are likely to be similar, then we would expect γ to be close to 1.

We denote by α_3 the ratio of the EC50 of the anti-inflammatory production to the EC50 of the pro-inflammatory production. Since we require anti-inflammatory molecules to down-regulate the proinflammatory response, we would expect these to have reasonably similar values, and hence we would expect α_3 to be of order 1.

We denote by α_2 the maximum pro-inflammatory cytokine production rate and we would expect this to be of the same order as the maximum anti-inflammatory cytokine production rate α_4. Through numerical analysis and examination of the nullclines, we can see that if α_2 and α_4 are of a greater order of magnitude than the other model parameters, the model only has a single unstable steady state with a stable limit cycle, indicative of disease. Since this is unlikely to be representative of either healthy individuals or RA individuals, we will consider parameter values for α_2 and α_4 of order 1, similar to the other parameters, which allows a greater range of behaviours.

We denote by α_1 the background level of pro-inflammatory cytokine production. To have an effective response to infection and injury, the background level of cytokines must be much smaller than the event stimulated production, and hence α_1 needs to be small, and should be much smaller than α_2.

It has not been possible to obtain more accurate parameter values from the literature, since we are not able to measure these rates in vivo and there is no appropriate in vitro data for RA. However, this work in ongoing and we hope to be able to produce better parameter estimates from experimental data in the future.

Appendix C. Number and stability of steady states

The nullclines of the system (2.4), (2.3), respectively, are as follows:

$$a = N_1(P) = \frac{\alpha_4 p}{\alpha_3 + p} \tag{22}$$

$$a = N_2(p) = \frac{p(\alpha_1 + \alpha_2) + \alpha_1}{\gamma p(1+p)} - 1 \tag{23}$$

Where:

$$f(p) = \frac{p^2(\alpha_1 + \alpha_2) + \alpha_1}{\gamma p(1+p^2)} - 1 \tag{24}$$

We cannot find the steady states of this system analytically but, by looking at the turning points of each nullcline, we can identify how many possible steady states there may be. Here N1(p) is simply an increasing Hill function and hence has no turning points and always goes through the point (p, a) =(0, 0). The number of turning points of N2(p) cannot be found analytically but since we need only consider real positive values of p and a, we can see that the number of turning points of N2(p) will be equal to the number of turning points in f (p).

Differentiating f (p) shows that it has four possible turning points.

$$p = \frac{\sqrt{2}}{2} \frac{\sqrt{(\alpha_1 + \alpha_2)((\alpha_2 - 2\alpha_1) + -\sqrt{\alpha_2^2 - 8\alpha_2\alpha_1})}}{\alpha_1 + \alpha_2} \tag{25}$$

$$p = -\frac{\sqrt{2}}{2} \frac{\sqrt{(\alpha_1 + \alpha_2)((\alpha_2 - 2\alpha_1) + -\sqrt{\alpha_2^2 - 8\alpha_2\alpha_1})}}{\alpha_1 + \alpha_2} \tag{26}$$

We see that p will always be either negative or complex in (C.2), leaving only two possible turning points. If $\alpha_2 < 8\alpha_1$, then both these points will be complex. This means that N2 will be a monotonically decreasing function and will cross N1 only once, giving a single steady state. Otherwise, f (p) and consequently N2 will have two turning points and so will cross N1 three times, giving a maximum of three steady states. It is not possible to find the stability of the steady states analytically, but the Jacobian A, shown below, does give us some information regarding the stability:

$$A = \begin{bmatrix} -\gamma + \frac{2\alpha_2}{(1+a^2)} \frac{p}{(1+p^2)^2} - \gamma - 1 & \frac{-2a}{(1+a^2)^2}(\alpha_1 + \alpha_2 \frac{p^2}{1+p}) \\ 2\alpha_4 \frac{\alpha_3^2 p}{(\alpha_3^2+p^2)^2} & -1 \end{bmatrix}$$

From this the trace and the determinant of the system are, respectively,

$$TraceA = \frac{2\alpha_2}{1+a^2} \frac{p}{(1+p^2)^2} - \gamma - 1 \tag{27}$$

$$DetA = \gamma - \frac{2\alpha_2}{1+a^2} \frac{p}{(1+p^2)^2} + 4\alpha_4 \frac{a}{1+a^2}(\alpha_1 + \alpha_2 \frac{p^2}{1+p^2})(\frac{p}{\alpha_3^2 + p^2} - \frac{p^3}{(\alpha_3^2+p^2)^2}) \tag{28}$$

We can see that when p 1 and a 1, then Trace $A = \gamma 1$ and Det $DetA \approx \gamma$, and so, for a small p and a, S_0 will be stable. At some point, for steady states at larger values of p and a, the steady state loses stability. For very large values of p, Trace $A = \gamma 1$ and $DetA \approx \gamma$; so S_2 is stable for large values of p. The exact thresholds for the loss of stability depend on both the variable and parameter values and cannot be determined

analytically; however, numerical simulation reveals that in the parameter ranges we are interested in, S_0 is always stable, S_1 is always unstable and S_2 can be either stable or unstable.

As an exception, there is one case in which a steady state and stability can be determined analytically: when α_1 is zero. We can see from the nullclines that if α_1 is zero (i.e. there is no background proinflammatory cytokine production), then there is a steady state at (p, a) = (0, 0). Examining the trace and determinant in this case gives Trace $A = \gamma 1$, which will always be negative and $DetA = \gamma$, which will always be positive, meaning that the steady state must be stable.

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