Mathematical modelling of the immune response during endometriosis lesion onset

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Abstract

The immune system is hypothesised to contribute to the onset of endometriosis lesions. However, the precise mechanisms underlying its role are not yet known. This paper introduces a novel compartmental mathematical model that describes the predicted interactions between the innate immune system (specifically macrophages and natural killer cells) and endometrial cells occurring within the peritoneal fluid during the early stages of endometriosis lesion onset. Our model suggests that an increased retrograde influx of endometrial cells into peritoneal fluid correlates with heightened M1-type macrophage activation, but does not lead to an increase in disease. We compare the system's response to changes in immune cytotoxicity and ability to detect ectopic endometrial cells. The model indicates that a reduction in cytotoxicity is a key driver of disease. These findings align with the increased immune activation observed clinically. Lastly, our model predicts that an individual can transition to a diseased state following a reduction in immune system cytotoxicity and/or reduced ability to detect ectopic cells. Due to hysteresis, a significant improvement is then required to restore an individual to the disease-free state. This work provides a valuable framework to explore hypotheses of endometriosis lesion onset and assist in understanding of the disease.

Keywords: endometriosis, mathematical biology, computational biology, immune system, macrophages, natural killer cells

1 Background

Endometriosis is a chronic gynaecological condition that affects approximately one in nine women [1]. The disease is characterised by the presence of endometrial-like tissue (tissue similar to the lining of the uterus) growing in lesions outside of the uterus. Endometriosis results in a variety of symptoms, including chronic pain and fertility issues [2, 3].

Despite its high prevalence, the mechanisms behind the onset of endometriosis remain an open question [1, 2]. The prevailing hypothesis is Sampson's theory of retrograde menstruation [2–4]. Under this hypothesis, menstrual debris, which includes endometrial stromal and epithelial cells as well as immune cells, is cleared from the uterus

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via the fallopian tubes, rather than through the cervix and vagina, during menstruation. After exiting the fallopian tubes, endometrial cells and other menstrual debris collect in the peritoneal fluid (4–16mL [5, 6]) within the peritoneal cavity. Endometrial cells are carried by this fluid and attach to internal tissues, invade the tissue, and develop into lesions.

However, studies have found the prevalence of people with retrograde menstruation is greater than the prevalence of endometriosis [7], indicating that the retrograde menstruation theory alone is insufficient to explain disease onset. Supporting this, a recent review has questioned whether previous evidence is sufficient to confirm differences in frequency and volume of retrograde menstruation in endometriosis patients [8]. This leads to two key hypotheses: (A) in endometriosis patients, a normally functioning immune system is overcome by an increased concentration of endometrial cells from menstrual debris, or (B) there exists an additional dysfunction of the immune system in those that experience disease. These hypotheses are still open questions for endometriosis pathophysiology [2].

In support of Hypothesis A, studies have shown a decreased proportion of cells undergoing apoptosis within eutopic endometrium of endometriosis patients [9], indicating an increased number of endometrial cells with the potential to form lesions enter peritoneal fluid. Endometriosis has also been associated with shorter menstrual cycle length, increased menstrual flow duration, and heavy menstrual flow volume [10–12], which all indicate increased influx into the peritoneal cavity. However, other studies have shown no significant difference in clonogenicity or concentrations of endometrial mesenchymal stromal and epithelial stem cells in both peritoneal fluid and menstrual blood between endometriosis patients and controls, although much higher variation was observed in the endometriosis patients [13].

Ectopic endometrial cells in the peritoneal cavity should be cleared by the immune system, preventing lesion formation. Consequently, a dysfunction in the ability of the immune system to detect or clear the cells could be involved in disease onset (Hypothesis B) [14]. Several studies have shown differences in immune cell profiles in the peritoneal fluid of endometriosis patients compared to controls [3, 15–18]. Due to the complexity of the immune system, it is difficult from observation alone to identify which differences are causes of immune dysfunction, a consequence of immune dysfunction, or simply a consequence of the presence of the endometrial cells. Mathematical modelling enables us to understand the relationship between hypothesised immune dysfunctions and emergent immune system profiles, for example viral infections [19] and cancer growth [20].

The early immune response is dominated by the innate immune system. Macrophages and natural killer (NK) cells are two innate immune cell types commonly implicated in the endometriosis literature. Macrophages contribute to the inflammatory environment of the peritoneal fluid, and are involved in the clearance of endometrial cells through detection of apoptotic markers. NK cells help clear endometrial cells via their strong cytotoxic activities.

Traditionally, macrophage activation states have been categorised into two types: M1-type (also known as classically activated macrophages) or M2-type (also known as alternately activated macrophages). This binary classification is a useful simplification of an activation spectrum [21]. M1-type macrophages secrete inflammatory cytokines and likely contribute to maintaining a level of chronic inflammation that is associated with endometriosis [2, 22]. M2-type macrophages are known to promote lesion growth, through upregulation of angiogenic factors and endometrial cell proliferation [2, 23].

Studies exploring macrophage activation in the peritoneal fluid of endometriosis patients consistently find an increased proportion of macrophages expressing markers of activation [15–18]. These studies have shown a significant increase in the proportion of cells expressing M2-type markers [16–18]. Ectopic endometrial stromal cells in co-culture have been shown to induce macrophage secretion of IL-10 and TGF-β [24], which could promote M2-type activation [22, 25]. Increases in macrophages expressing M1-type markers, in peritoneal fluid, have also been observed, but to a smaller (or non-significant) degree compared to M2-type [16–18]. However, observations of increased pro-inflammatory cytokine expression in peritoneal fluid [3, 18, 26], and increased secretion of IL-1 [3, 26] and cytotoxicity of peritoneal macrophages (from peritoneal fluid) in early-stage disease [27] are all associated with an M1-type activation. Cytotoxicity of peritoneal macrophages (from peritoneal fluid) has also been shown to decrease in late-stage (stage III/IV) disease [27]. Together, this suggests M1-type activation may increase in early but not late stage disease, reflecting an evolving disease environment that could explain the high variability in M1-type activation across patients. Observations of decreased peritoneal macrophage cytotoxicity against both eutopic and ectopic endometrial cells compared to peripheral macrophages in endometriosis patients [28] could also indicate immune function changes as a response to persistent exposure to an altered immune environment, rather than a pre-existing dysfunction. A potential contributor to the inflammatory peritoneal environment of endometriosis patients is the increased M1-type activation in eutopic endometrium which has been observed in endometriosis patients [29].

Macrophages mediate the clearance of endometrial cells through the detection of apoptotic markers, which have been shown to be dysregulated in endometriosis [30–32]. Several studies have established that ectopic endometrial

tissue does not display the same cyclic changes in apoptotic markers as eutopic tissue [33, 34], has a decreased level of apoptosis compared to eutopic endometrium in the same patient [35], and increased anti-apoptotic and decreased pro-apoptotic marker expression compared to eutopic endometrium [33, 34, 36]. Decreased estrogen receptor expression is associated with upregulation of anti-apoptotic markers [34]. In endometriosis patients, decreased [37] and out of phase [38] variation in estrogen receptors on ectopic cells have been observed over the menstrual cycle. In eutopic endometrium, a reduction in spontaneous apoptosis has been observed [36]. These observations indicate a reduction in expression of apoptotic markers on these cells, resulting in a decrease in detection and recognition of these cells as ectopic by the immune system (Hypothesis B1).

Natural killer (NK) cells are categorised into two types: $CD56^{bright}$ (also known as $CD56^{bright}CD16^{-}$) and $CD56^{dim}$ (also known as $CD56^{dim}CD16^{+}$). $CD56^{bright}$ cells have high cytokine production, namely IFN-γ and TNF-α, while $CD56^{dim}$ cells have higher cytotoxicity [14]. In the endometrium, the majority of NK cells are $CD56^{bright}$ [2]. However, the NK cell profiles in peritoneal fluid during normal function are not fully described. There is mixed evidence on the activation of NK cells in peritoneal fluid of endometriosis patients. After activation by monocytes, NK cells release IFN-γ and TNF-α, the latter of which is consistently raised in endometriosis patients [14]. However, the activity of NK cells has been shown to be decreased in endometriosis patients compared to controls [39]. Peritoneal fluid from endometriosis patients has been shown to reduce cytotoxicity in NK cells *in vitro* [39, 40]. Some studies show IFN-γ induces apoptosis in eutopic but not ectopic endometrial cells [14]. This leads to the hypothesis of reduced clearance of the ectopic endometrial cells by NK cells (Hypothesis B2).

In this paper, we describe a novel compartmental mathematical model of the immune response to endometrial cells in peritoneal fluid. Our model focuses on the early-stages of disease and immune response, and consequently we only consider innate immune cells. We model the effects of NK cells and macrophages, as these are the cells most commonly hypothesised to be involved in endometriosis onset, as detailed above. We focus on dysfunctions of the immune system, rather than abnormalities or disease promoting actions of the ectopic endometrial cells. Using this model, we investigate two questions around endometriosis onset:

1. How does the peritoneal fluid immune cell profile change when the amount of retrograde influx is varied, and can increased influx explain observed immune cell profiles in endometriosis patients and disease onset?

2. Which immune system disorder: reduced detection or reduced clearance of endometrial cells by the immune system, is most associated with disease and is consistent with the observed immune cell profiles?

2 Methods

In this section we describe the model assumptions governing the interaction between the macrophages, NK cells, and endometrial cells, and define three emergent attachment states which dictate the disease-free and diseased states.

2.1 Model

We model the interaction between macrophages (M), natural killer cells (K), and endometrial (stromal and epithelial) cells (E) in peritoneal fluid. Each cell type has the following activation states:

- Macrophages are **resting** (M_0), **M1-type** (M_1) or **M2-type** (M_2).
- Natural killer cells are **resting** (K_0) or **activated** (K_A).
- Endometrial cells are **eutopic** (E_0), **in fluid** (E_F) or **attached** (E_A).

We consider attached endometrial cells to be early lesion cells. A diagram of the system model is given in Fig. 1(a) and explanations of each of the cell state transitions and cell interactions is given below.

2.1.1 Macrophages

We denote macrophage as resting, M_0, which activate to either a pro-inflammatory M1-type, M_1, or anti-inflammatory M2-type, M_2.

Macrophage turnover: macrophage influx occurs from: a) recruitment from the blood stream; or b) resting macrophage proliferation. The recruitment process is upregulated by the presence of activated macrophages (M_1 and M_2), and is independent of the activation state. Macrophage cell loss occurs via natural cell turnover from all states.

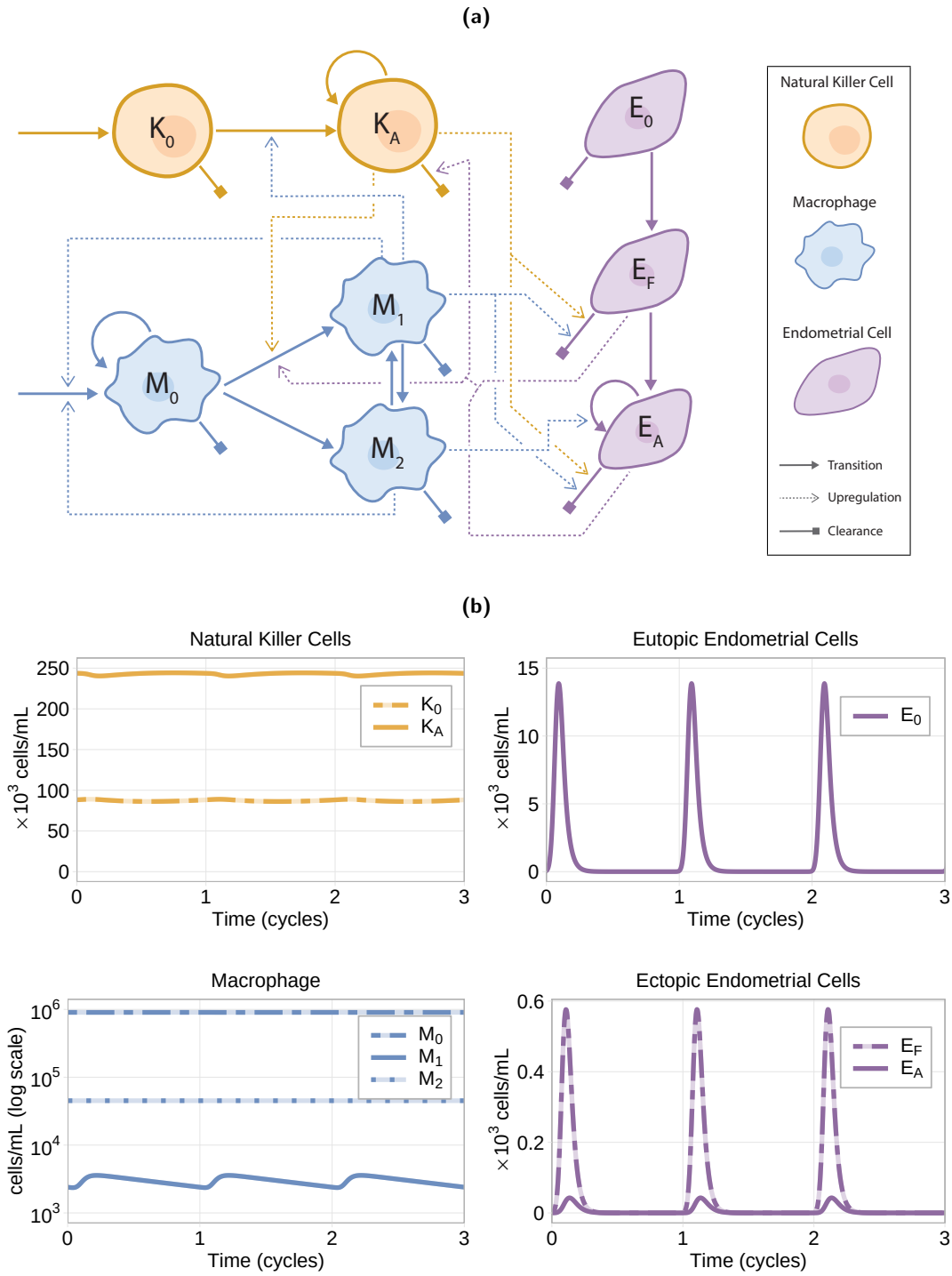

Figure 1: (a) Model diagram with resting (K_0) and activated (K_A) natural killer cells (orange); resting (M_0), M1-type (M_1), and M2-type (M_2) macrophages (blue); and eutopic (E_0), in fluid (E_F), and attached (E_A) endometrial cells (purple). Arrowed solid lines represent cell influx, transition, and proliferation; square arrows represent the cell clearance; and dashed lines represent upregulation of a process. (b) Endometrial and immune cell dynamics over three menstrual cycles (on average 28 days) using the default parameter values (see Supplementary S2). The peak in the eutopic endometrial cells, E_0 (top right of (b)), is the menstrual phase of the cycle and these peaks are reflected in the ectopic endometrial cells, E_F and E_A (bottom right of (b)). Similar oscillations can also be seen in the immune system cells (left column of (b)). We see all endometrial cells are cleared before the end of each cycle.

Activation of macrophages: activated macrophages can be in an M_1 or an M_2 state. We assume this occurs through both activation from a resting state and re-polarisation between the two activated states. We also assume that the cells activate to the inflammatory M_1 state when they detect the presence of the endometrial cells. This process is upregulated by activated NK cells, via the cytokine IFN-γ [14, 22], as detailed below.

Activated macrophage actions: M1-type macrophages produce pro-inflammatory cytokines, such as interleukins IL-12 and IL-1 [21]. These are known to activate and stimulate production of NK cytokines. We therefore assume M_1 cells upregulate activation of NK cells. We model M1-type cells as anti-lesion cells that assist in clearance of ectopic endometrial cells, and M2-type cells as pro-lesion cells that promote the growth of lesions once endometrial cells have attached to the peritoneal tissue [16].

2.1.2 Natural killer cells

NK cells exist in one of two states: resting NK cells, denoted by K_0, and activated NK cells, denoted by K_A. We model both NK dim and bright cells simply as activated NK cells with moderate cytokine production and cytotoxicity.

Resting NK cells: Resting NK cell turnover is through a balance of steady recruitment from the blood stream and loss through natural cell turnover and activation. We assume K_0 cells have insignificant cytokine production and cytotixicity compared to their activated form, K_A.

Activation of NK cells: NK cell activation occurs through pro-inflammatory cytokines, such as interleukin IL-12, and interferons IFN-α and IFN-γ [14, 41, 42]. We assume the main producers of these are macrophages, particularly IL-12 which is produced at high levels by M1-type macrophages [21]. Consequently we assume that M_1 cells upregulate activation of K_A cells.

Activated NK cell action: Activated NK cells are assumed to be responsible for cytokine release, cell clearance, and NK proliferation. This is modelled as upregulation of M1-type polarisation (via IFN-α [14, 22]), clearance of endometrial cells, and proliferation of activated NK (via IL-2 [41]).

Activated NK cell clearance: Activated NK cells have a natural turnover rate similar to resting NK cells. Additionally, activated NK cells are assumed to become exhausted following the execution of endometrial cell clearance, after which they no longer contribute to cells clearance. To accommodate this, we model K_A to have a higher cell loss rate than K_0.

2.1.3 Endometrial cells

We define the endometrial cells to be in three states: eutopic (within the uterus, E_0), in (peritoneal) fluid (E_F), and attached (to the peritoneum and/or external surface of the reproductive system/gut, E_A). E_A cells are early lesion cells which then progress to endometriosis. Transitions between the three cell states are strictly sequential (i.e. an attached endometrial cell will never detach).

Eutopic endometrial cells: these are endometrial stromal and epithelial cells located on the internal surface of the uterine cavity. The functionalis layer of the endometrium sheds during menstruation, with stromal and epithelial cells found in menstrual fluid. These cells are cleared from the uterine cavity via 'normal' menstruation (cleared via cervix) or enter the peritoneal cavity via retrograde menstruation, where they become fluid cells, E_F.

Fluid and attached cell turnover: when cells enter the peritoneal cavity via retrograde menstruation, they are first collected within the peritoneal fluid, and hence we denote them to be in the 'fluid' cell state. The fluid cells attach to the peritoneum, from which they can form a lesion. Both fluid and attached cells are cleared via natural cell turnover and clearance initiated by the immune system. Attached cells proliferate to form lesions, and this proliferation is upregulated by the presence of M_2 cells.

Endometrial cell action: fluid and attached endometrial cells are detected by macrophages, and their presence results in activation of resting macrophages to an M_1 state. We limit the scope of this study to a system in which the endometrial cells themselves have no dysfunction or effect on immune cell function.

2.2 Model Equations

Using the assumptions described above, we construct a system of ordinary differential equations, given in Supplementary S1. Upregulation terms follow either mass-action or Hill kinetics, and proliferation terms are modelled as logistic growth. The shape of the cyclic function for eutopic (E_0) endometrial cell influx, $\mu_E(t)$ (Supplementary Eq. S9), is fit to normalised menstrual cycle volume data [43]. A plot of the eutopic endometrial cell dynamics is given in Fig. 1(b) and details on the choice of function and parameters can be found in Supplementary S3.

2.2.1 Constant influx surrogate model

Taking $\bar{\mu}_E$ as the average influx over the cycle, we define a surrogate model for the system in which we assume a constant influx into E_0, i.e.:

$$\mu_E(t) = \bar{\mu}_E \ . \tag{1}$$

This results in a sufficient match between the constant influx and the cyclic influx systems, in terms of both average quantities and emergent behaviour. The surrogate model provides two advantages to the cyclic influx model: 1) it is computationally more efficient, and 2) it allows for clearer analysis of some aspects of the emergent system dynamics.

2.2.2 Parameters and initial conditions

Unless otherwise specified, the parameters for the model are given in Supplementary S2, and the response of the system for these parameter values is shown in Fig. 1(b). Where possible, we use parameters from previous within-host immune dynamics models, or estimate them using *in-vitro, in-vivo*, or clinical data. Details on the parameterisation are given in Supplementary S3.

Since information regarding peritoneal cell populations is not available, the initial conditions of all state variables are chosen to align with model steady state concentrations and observations from control patients in peritoneal fluid composition studies [17, 39].

2.3 Characterisation of disease dynamics

2.3.1 Attachment and disease definitions

We use the value of E_A at the end of an endometrial influx cycle (28 day cycle) to determine if the response results in a diseased state. A low/no disease system is defined as $E_A(t = 28k) > \varepsilon$, $k \in \mathbb{Z}_{\geq 0}$, and $t > \tau$, where τ is the initial transient time for the system to reach a cyclic state, for some $\varepsilon \in \mathbb{R}_{\geq 0}$.

In addition to classifying responses as high disease or low/no disease, we consider different response states during the menstrual cycle. We classify system dynamics into three attachment states: low attachment (low/no disease): $E_A(t) \leq \varepsilon \quad \forall t > \tau$; transient attachment (low/no disease): $\hat{E}_A > \varepsilon,\ E_A(t = 28k) \leq \varepsilon \quad \forall k \in \mathbb{Z}, t > \tau$; and sustained attachment (diseased): $E_A(t = 28k) > \varepsilon \quad \forall t > \tau$, where $\hat{E}_A$ is the maximum E_A value over the menstrual cycle, and $E_A(t = 28k)$ the number of attached cells at the end of the menstrual cycle after initial transient time τ days. The term low/no disease is used as the nature of the modelling paradigm does not permit $E_A = 0$, for $t > 0$ days. Examples of each of these attachment states is given in Fig. 2.

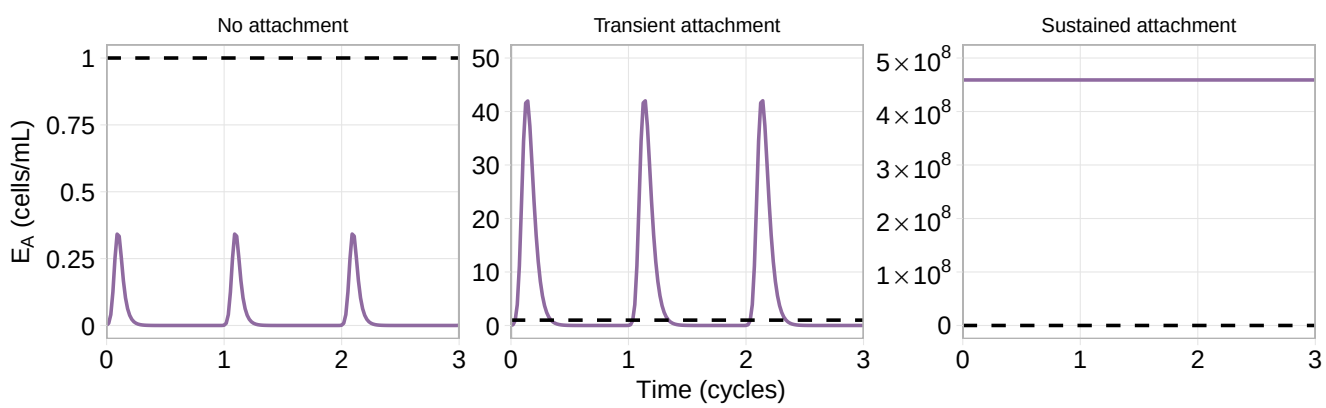

Figure 2: Examples of the three emergent attachment state dynamics observed, assuming a threshold of $\epsilon = 1$ cell/mL (indicated by the black dashed line in the plots). The left two plots (no attachment and transient) are hypothesised to be the low/no disease states, and the right plot (sustained) is hypothesised to be the diseased state.

2.3.2 Parameters and response metrics of interest

To investigate the questions of interest around endometriosis lesion onset, we examine the model's response to the variation of three parameters: the proportion of endometrial cells cleared retrograde (ρ_0), macrophage detection

rate of endometrial cells (β), and the clearance rate of the endometrial cells by the immune cells (ω). The primary system responses we are interested in are: the number of attached endometrial cells (E_A) at the end of the cycle, as an indicator for the level of disease; and the level of macrophage activation, measured as the proportion of total macrophages in each activation state M_1 and M_2, denoted by $\tilde{M}_1$ and $\tilde{M}_2$ respectively, as a comparison to immune profiles observed in endometriosis patients. We use proportions for macrophage activation to allow for simple comparison with studies on peritoneal fluid cell compositions, which report the proportions of macrophages expressing specific markers.

2.4 Code availability

The code to generate these results is openly available at `https://github.com/clairemiller/math_modelling_immune_endo`. The ODE system was simulated in Matlab R2024b [44], and the bifurcation analysis was performed using the software AUTO-07p [45].

3 Results

3.1 Low attachment analysis

We perform an eigenvalue analysis to identify the stability of each of the steady states. By requiring the disease-free steady state to be stable, we find that the following inequality must be satisfied:

$$\underbrace{\eta_E \frac{M_2}{C_{M_2} + M_2}}_{E_A \text{ proliferation}} < \underbrace{\omega \left[\gamma K_A + (1-\gamma) M_1\right]}_{E_A \text{ clearance}} + \underbrace{\delta_E}_{E_A \text{ turnover}} . \tag{2}$$

The full analysis is given in Supplementary S4. The biological interpretation of this disease-free state is that the proliferation rate of the attached endometrial cells, upregulated by M_2, must be smaller than the clearance rate of endometrial cells—both cell clearance due to the immune response and natural turnover.

This analysis only provides information for when the system is in the disease-free state, and does not provide insight into how a transition to disease may occur.

3.2 Hypotheses of disease onset

3.2.1 Retrograde influx

We are interested in understanding whether the observed immune profile of peritoneal fluid from endometriosis patients could emerge due to the presence of the endometrial cells with no immune dysfunction, and if increased endometrial presence is associated with disease (Hypothesis A). To investigate this, we explore the response of the system as the level of retrograde influx into the peritoneal fluid increases. This is done by increasing ρ_0, the proportion of menstrual debris that clears retrograde from the uterus.

The system response to variations in the retrograde influx is plotted in Fig. 3. This figure shows the dynamics of the attached endometrial cells and activated immune cells. High levels of retrograde influx is associated with an increase in the proportion of macrophages activated to M1-type ($\tilde{M}_1$, Fig. 3(c)) and the concentration of activated NK cells (K_A, Fig. 3(a)), but there is no significant change to the proportion of macrophage activated to M2-type ($\tilde{M}_2$, Fig. 3(d)). The increase in $\tilde{M}_1$, and consequently K_A, is attributed to the upregulation of M_1 in response to the presence of E_F and E_A, as described in Supplementary S1. We also observe that the relaxation time of M_1 and K_A is longer than the menstrual cycle length, resulting in a sustained state of inflammation. This result aligns with the description of endometriosis as a chronic inflammatory disease [2, 22].

We see that a higher retrograde influx results in an increase in the peak level of endometrial cell attachment Fig. 3(e). Contrary to expectations, low influx leads to higher attachment levels at the end of the cycle (see $\rho_0 = 0.005$, dotted line in Fig. 3(e)). This is because an increased influx of endometrial cells causes an increased activation of M_1 cells, and consequently K_A cells, as noted above. As a result, immune clearance of the endometrial cells is increased. At low levels of ρ_0, there is a decrease in the activation of M_1 cells, and consequently a decrease in the immune clearance of endometrial cells. The proliferation rate of the attached endometrial cells is comparable between the different influx levels, as increasing ρ_0 has an insignificant effect on the M_2 cell levels. Consequently, the low influx systems maintain a low level of sustained attachment while high influx systems exhibit only transient attachment.

Therefore, we see that our model predicts an increase in retrograde influx is associated with a small, sustained increase in inflammation, but is not associated with increased disease, opposing Hypothesis A.

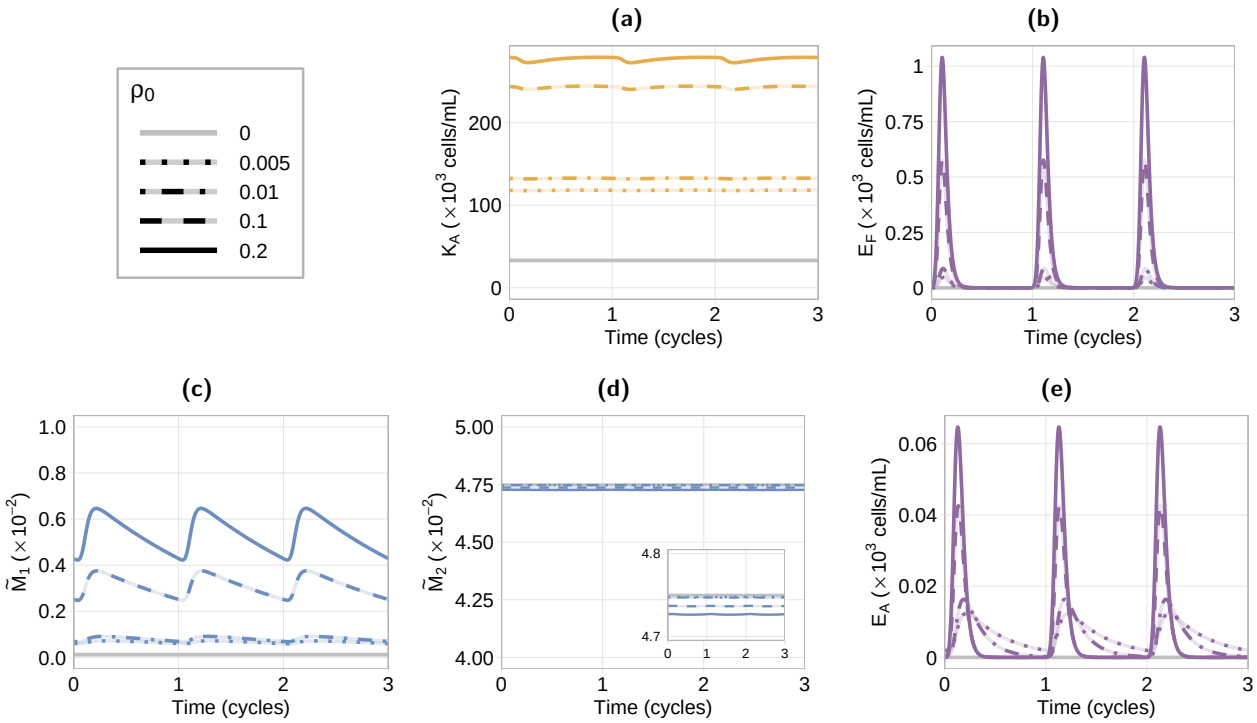

Figure 3: Timeseries of the response for varying endometrial retrograde influx (ρ_0) shown for three menstrual cycles. We see large peaks in the endometrial cells, both in fluid (E_F, (b)) and attached (E_A, (e)) with the menstrual phase of the cycle. Oscillations are also seen in the proportion of macrophage activated to M_1 type, (c), and NK cell activation, (a). Increased influx causes an increase in the proportion of macrophage activated to M_1 and activated NK cells, but does not have a significant effect on the proportion of macrophages activated to M_2, (d). Results show high retrograde influx is associated with a small sustained increase in inflammation but not sustained attachment.

3.2.2 Immune dysfunction

There are several hypotheses regarding immune system dysfunctions in endometriosis pathogenesis. Two immune dysfunctions we explore are altered detection (Hypothesis B1, model parameter β_1) and clearance (Hypothesis B2, model parameter ω) of the endometrial cells. We consider a reduction in clearance rate by both activated immune cell types, M_1 and K_A, noting that NK cells are assumed to be the main clearance cell in the model. Detection dysfunction only applies to macrophages as these are the only cells that detect the endometrial cells.

Five example responses for moderate retrograde influx are shown in Fig. 4(a). Examples 1 and 5 in Fig. 4(a) show a healthy and diseased response respectively. In the healthy response, both E_A and E_F are cleared by the end of the cycle, while in the diseased response, only E_F is cleared, and E_A has a high level of sustained attachment.

The endometrial cell response to variations in both immune detection and clearance ability is shown in Fig. 4(b) for three levels of retrograde influx (low, moderate, and high). These plots show the end of cycle (i.e. cycle day 28) values for endometrial attachment (E_A). We see that, for all ρ_0 levels, E_A transitions from a low attachment state (low/no disease, light purple) to a high attachment state (high disease, dark purple) with decreasing clearance ability. Over a wide range of detection rates, β_1, a reduction in clearance rate, ω, will result in the disease transition. However, for reductions in the detection rate, β_1, this transition only occurs across a narrow range of ω values. This shows the disease transition is dominated by the immune clearance rate, ω.

The dashed lines in Fig. 4(b) (and Fig. 5(a), Fig. 5(b)) partition the regions governed by the inequality in Eq. (2). This partition is calculated using the surrogate model (see Section 2.2.1). Responses in the right region are predicted to exhibit low attachment behaviour, and those to the left, high attachment behaviour. The alignment between this partition and the disease transition in the numerical results indicates that this relationship is a suitable indicator of disease. The region of disagreement showing high E_A but predicted to be in low-disease region is a

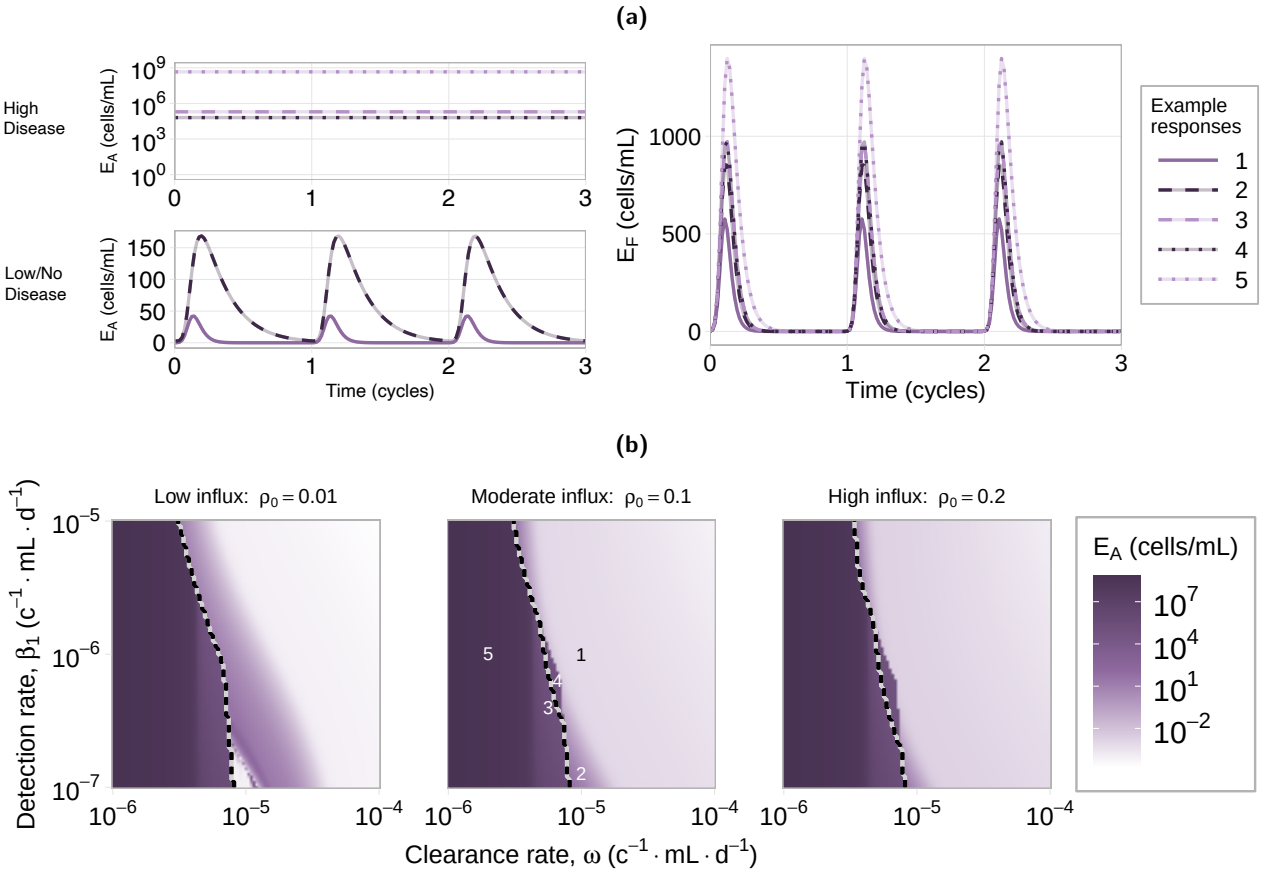

Figure 4: Endometrial attachment and in fluid response to immune dysfunction. (a) Example responses of attached and fluid endometrial cell dynamics at locations indicated on the heatmaps in (b) (moderate influx). (a) top left: the E_A response for the high attachment (high disease) examples. (a) bottom left: the E_A response for the low attachment (low/no disease) examples. (a) right: the level of endometrial cells in fluid, E_F, which we observe are always cleared by the end of each cycle. (b) Heatmap showing the level of attached endometrial cells at the end of a cycle. The dashed line indicates the partition between high and low attachment regions according to the inequality in Eq. (2). Units ($c^{-1} \cdot mL \cdot d^{-1}$) are short for ($cells^{-1} \cdot mL \cdot day^{-1}$). These results indicate that reduced immune clearance ability is a stronger driver of disease compared to reduced detection ability.

region where attached endometrial cell proliferation and removal are approximately equal (see example responses 3 and 4 in Fig. 4(a)).

Figures 5(a) and 5(b) show the macrophage activation response to a reduction in immune clearance and detection rate. In both figures, in the high attachment region, there is a high proportion of M_1 activation and a reduced proportion of M_2 activation as a result of the high endometrial cell presence. This transition can be seen in the Fig. 5(c), with low $\tilde{M}_1$ and high $\tilde{M}_2$ in the two low attachment example responses 1 and 2, and high $\tilde{M}_1$ and low $\tilde{M}_2$ for the highattachment example responses 3–5.

In the low attachment region, the results show an increase in $\tilde{M}_1$ with decreasing clearance ability, while decreasing detection ability results in a decrease in $\tilde{M}_1$. The latter can be seen in example response 2 in Fig. 5(c), which shows a decrease in $\tilde{M}_1$ but no change to $\tilde{M}_2$ compared to example response 1. A decreased detection ability reduces the rate at which macrophages activate into an M_1 state, consequently leading to this reduction in inflammation.

These results show that a reduced immune clearance rate, ω, leads to high disease and high M_1 activation. Neither hypothesis leads to an increase in M_2 activation in our results. Consequently, our model predicts the reduced clearance hypothesis, Hypothesis B2, is most consistent with the observations of increased macrophage activation in endometriosis patients compared to both the reduced detection and increased retrograde hypotheses.

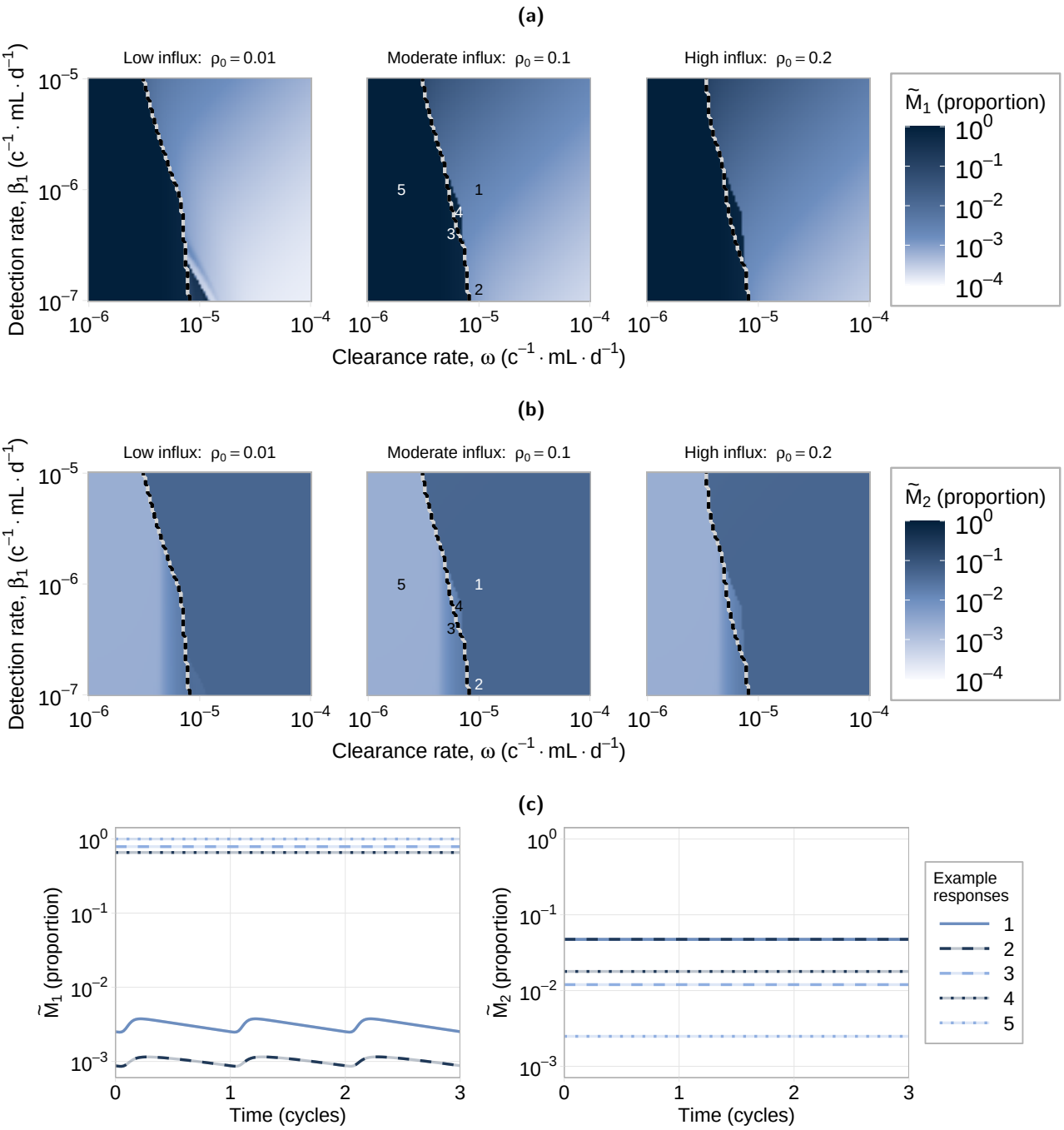

Figure 5: Macrophage activation response to immune dysfunction. (a), (b) Heatmaps showing the proportion of macrophage polarised to M_1 and M_2, respectively, at the end of a cycle. The dashed line indicates the partition between high and low attachment regions according to the inequality in Eq. (2). Units ($c^{-1} \cdot mL \cdot d^{-1}$) are short for ($cells^{-1} \cdot mL \cdot day^{-1}$). (c) Example responses at locations indicated on the heatmaps in (a), (b). (c) left: the proportion of macrophages in an M_1 activation state. (c) right: the proportion of macrophages in an M_2 activation state. Note examples 1 and 2 are overlapping in right plot. The high attachment region is associated with high M_1 activation and low M_2 activation. In the low attachment region, decreasing clearance rate is associated with an increase in M_1 activation while decreasing detection rate is associated with a decrease in M_1 activation. There is no significant effect on M_2 activation in the low attachment region.

3.3 Disease transition

We now consider the case, for Hypothesis B, where the immune clearance and detection have a gradual decline in function, rather than a pre-existing dysfunction. This can be explored through a bifurcation analysis in ω and β_1. Mathematically, the observed transition from low to high endometrial attachment in Fig. 4(b) with decreased immune function is indicative of bistable system dynamics.

We perform a bifurcation analysis, using the surrogate model. Figure 6(a) shows the biologically observable states, from this analysis, of the attached endometrial cells for varying clearance (left) and detection (right) rate. We reclassify solutions containing limit cycles (using the median value of E_A^*, shown as a dashed line in Fig. 6(a), where E_A^* is a solution containing a limit cycle) as their period of oscillation exceeds the expected timescale of disease onset in the presence of the innate immune response only, beyond which the adaptive immune response is known to play a role [2]. Complete bifurcation diagrams can be found in Supplementary S5.

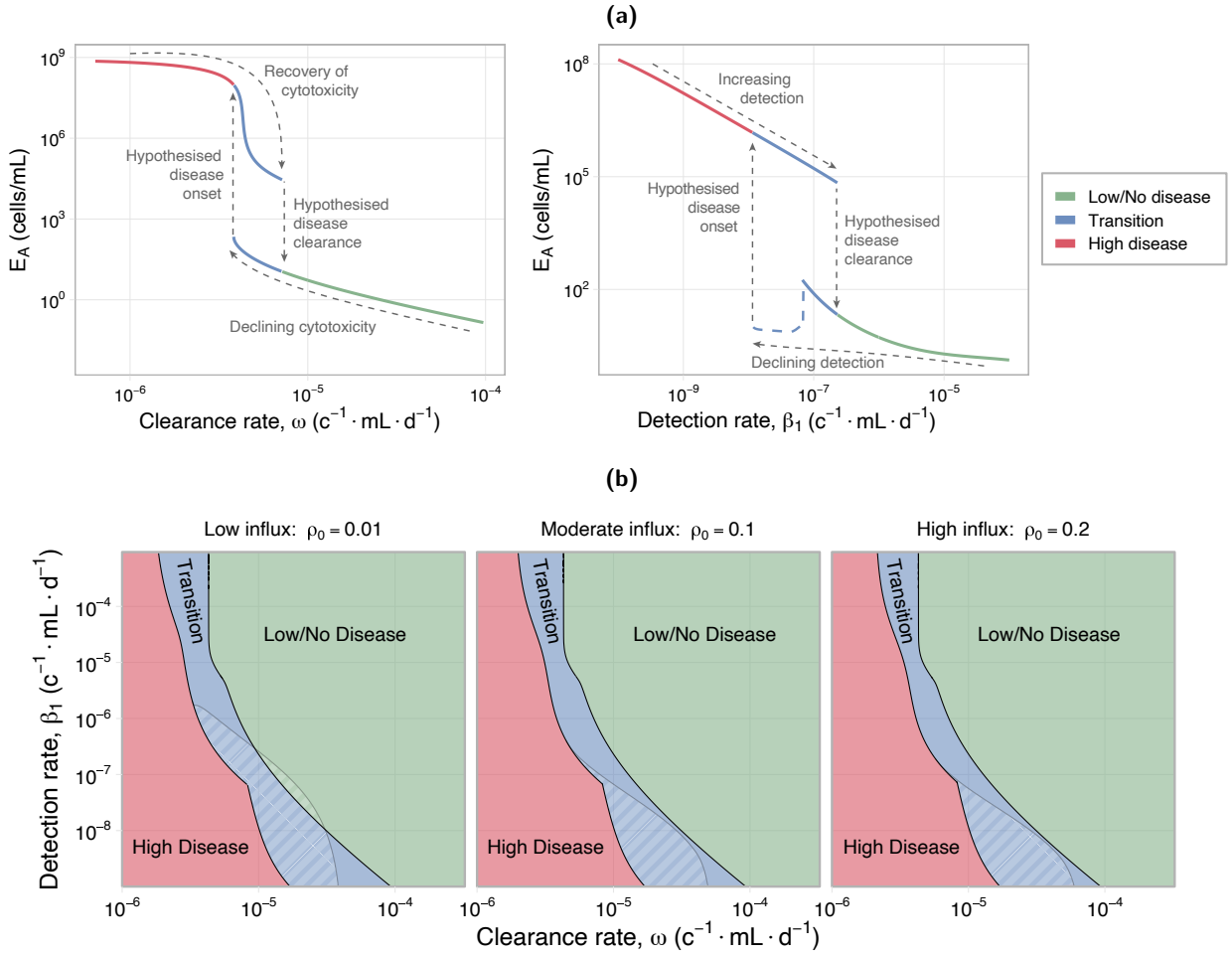

Figure 6: Disease transition plots for clearance and detection rate: (a) clearance (left) and detection (right) rate independently. The dashed blue line (right plot) indicates the solutions reclassified from a limit cycle—showing the median value of E_A^*. (b) Co-dimensional bifurcation in clearance (ω) and detection (β_1) rate. Hatched region shows the reclassified limit cycle solutions. The complete bifurcation diagrams are given in Supplementary S5. Fixed values for (a) are $\beta_1 = 10^{-6}$ cells^{-1} · mL · day^{-1}, $\omega = 10^{-5}$ cells^{-1} · mL · day^{-1}, and $\rho_0 = 0.1$. Units (c^{-1} · mL · d^{-1}) are short for (cells^{-1} · mL · day^{-1}). A patient can start in the disease-free region (green), and then transition through the bistable region (blue), still in low/no disease, but then switch into the high disease state either due to a decline in immune function, or due to additional insult to the system. Disease clearance from this state requires significant improvement to immune function.

For both clearance and detection rate, we observe a bistable regime where the system exhibits hysteresis, which we term the transition region, shown in blue on Fig. 6(a). Biologically, this indicates that a healthy individual

can start in a low/no disease state (green); upon an initial decline in immune function, they enter the transition region, remaining in the low disease state (bottom blue); with further immune function decline, or other changes to the endometrial or immune cell states, the individual will switch into a high disease state (red, or top blue, respectively), which we hypothesis to be disease onset. The individual is unable to recover from this diseased state with subsequent improvement in immune function (top blue), unless the improvement achieves the hypothesised disease clearance indicated on the figure.

We perform a co-dimensional bifurcation analysis, using the surrogate model, to look at both clearance and detection rate simultaneously. A full co-dimensional bifurcation diagram is provided in Supplementary S5.3.The biologically observable states from this analysis are shown in Fig. 6(b) for three levels of retrograde influx (low, moderate, and high), with solutions containing limit cycles reclassified as described above (hatched region in Fig. 6(b)). Regardless of the level of retrograde influx, we observe similar behaviours – changing the influx level simply shifts the transition region in parameter space. This reinforces our finding that increased retrograde influx is neither associated with increased disease, nor a driving factor of system dynamics. Notably, a transition region is always present between the low/no and high disease states. As detailed previously, this indicates that a switch into high disease (disease onset) is not recoverable without significant improvements in immune system function (disease clearance).

4 Discussion

In this study we develop a novel compartmental mathematical model of the innate immune response to endometrial cell influx from retrograde menstruation, to describe the early stages of endometriosis lesion onset. We use our model to interrogate two key questions around endometriosis onset. Firstly, could increased endometrial cell presence describe altered immune states observed in endometriosis patients and lead to disease (Hypothesis A). Secondly, of two hypothesised disorders: immune system detection and clearance of endometrial cells in menstrual debris (Hypotheses B1 and B2), which is more associated with disease and consistent with clinical observations. This is the first mathematical model that has been developed to interrogate mechanisms of endometriosis lesion onset, highlighting the importance of the immune response.

Our model predicts that an increase in retrograde menstruation, with no immune system dysfunction, is not associated with increased disease, but is associated with a small, sustained increase in inflammation (Fig. 3). We find the key driver of disease onset is a decrease in the clearance rate of endometrial cells by immune cells (Fig. 4), supporting Hypothesis B2. Both decreased clearance rate and high disease are associated with an increase in M1-type macrophage activation, but not M2-type activation (Fig. 5). A decrease in immune detection rate within a specific range of clearance rates will also result in disease onset, indicating that different immune profiles can be indicative of disease. Given an immune profile, we provide a relationship between attached endometrial cell proliferation and removal that is a predictor of disease (Eq. (2)). Through a bifurcation analysis, we show that a transition region exists that dictates the dynamics of disease onset and disease clearance (Fig. 6). This result describes how, following disease onset, a significant recovery of immune function is required to obtain disease clearance. Our analysis supports a hypothesis that an adapting immune system, such as in response to chronic inflammation, may be a driver of disease onset, through the gradual decline of immune function, in particular immune cell cytotoxicity.

A recent review has challenged the hypothesis that frequency and volume of retrograde menstruation is different between endometriosis patients and controls [8]. This is supported by our results, which show no association with increased retrograde influx and disease (Fig. 3). Endometrial stem and progenitor cell concentrations in peritoneal fluid have been observed to be one to two orders of magnitude higher during the menstrual phase, compared to non-menstrual phase, in both control and endometriosis patients [13]. Endometriosis cases had increased variation between patients, with some patients measuring concentrations comparable to control cases, and others measuring significantly higher cell concentrations, which may indicate there is no unique pathway to disease onset. This observation does not support a particular hypothesis based on our results: a significant increase in endometrial cells in fluid can be seen during the menstrual phase for all levels of retrograde influx (Fig. 3), and our bifurcation analysis results show only small changes in endometrial cells in fluid between low/no disease and high disease, particularly in the transition region (Supplementary S5). This example demonstrates that, without high precision on cycle time and additional physiological information, single time point data on peritoneal fluid cell concentrations is insufficient to inform mathematical models or indicate the presence of disease.

Several studies have shown increases in both M1- and M2-type macrophages, as proportions of total macrophage, in endometriosis patients [16, 17, 46]. Our results show an increase in M1-type macrophages is associated with increased retrograde menstruation and decreased immune clearance capabilities; and a decrease in M2-type macrophages

is associated with a decrease in immune clearance capabilities. Our model does not predict an increase in both macrophage activation states under any hypothesis. The lack of increased M2-type activation in our results is due to no M_2 upregulation mechanism. Potential mechanisms could be a dysfunction in the M2-type activation rate in endometriosis patients, or attached endometrial cell signalling. Furthermore, clinical data is only collected after confirmed diagnosis, which is usually several years after disease onset [1, 47]. Consequently, it is not possible to identify whether these observations of increased M2-type cells are consistent with early-stage disease dynamics or emerge as the lesion develops.

Few studies have focused on NK cell concentrations and activation types in peritoneal fluid. A significant increase (approximately four-fold) in the proportion of leukocytes that stained for NHK-1 in Stage I disease has been observed [6], which could indicate an increase in the concentration of activated NK cells. Such an increase is predicted by our model under the increased retrograde menstruation hypothesis, and the decreased cell clearance hypothesis in the low disease state (see Supplementary S5).

To our knowledge, this is the first mechanistic mathematical model developed in the context of endometriosis. Our model combines understanding of macrophage, NK cell, and endometrial cell dynamics. Our model provides a framework that can be used to provide insight into open questions, such as those considered in this paper, around system changes that contribute to disease onset compared to those that emerge due to the disease. It can also be used to better understand the interacting role of different mechanisms hypothesised through *in vitro* models within an *in vivo* environment.

There are several known modelling limitations to this work. We use a deterministic model to describe interactions and transitions, meaning that stochastic effects, such as menstrual cycle variation, low cell numbers, and delays in immune onset, are not accounted for. The cell transitions and interactions are based on understanding of immune interactions from the endometriosis, cancer, and viral literature, as there is limited understanding of interactions between immune cells and endometrial cells. As with many models, there is also little quantitative knowledge of the system, particularly for the early stage of lesion onset. This is in part due to the extensive delays patients experience before they receive a clinical diagnosis for endometriosis [47]. Our model has highlighted the need for several quantitative data requirements. In particular, longitudinal studies of patients, and connected data sets that provide full immune profiles of individuals, ideally alongside menstrual cycle point. In the future we will explore opportunities around parameter estimation using clinical and experimental data. For example, our model would greatly benefit from *in vitro* studies of activation states and clearance rates using co-culture assays, animal models, or organoids to measure apoptosis or immune activation markers over time.

We have focused our investigation on the innate immune system (though it is known that the adaptive system also plays a role [2]), and dysfunctions related to early-stage disease and the pro-inflammatory immune response. We do not include any potential mechanisms for upregulation of M2-type macrophage activation. Further innate immune cells that may play a role in disease, such as neutrophils, mast cells, and dendritic cells [2]. Future work will extend the model to include 1) hormonal effects on immune and endometrial cell behaviours, and 2) influx of immune cells into the peritoneal fluid as a component of the menstrual debris. This will allow us to investigate hypotheses of disease onset relating to progesterone resistance of the endometrial cells [37, 38], hormonal effects on the immune cells [22], and abnormal immune function in eutopic endometrium [29, 48].

There are many unanswered questions regarding disease onset in endometriosis pathophysiology, including contradicting evidence between different studies and uncertainties about mechanisms that drive disease onset versus those that result from the disease. Our study uses mathematical modelling to provide insight into the role of the innate immune system in endometriosis lesion onset. Results show that increased retrograde influx results in small changes to immune profiles but is not associated with disease. Immune dysfunction does lead to disease onset, in particular a dysfunction in immune clearance ability, and recovery from disease requires significant improvements to immune function. Our model is ideally placed to provide better understanding towards the role of different mechanisms hypothesised through *in vitro* models within an *in vivo* context. With further development and robust parameterisation methods, mathematical modelling will facilitate the identification of potential biomarkers and immunotherapies for endometriosis, and improve our understanding of between-patient variation, moving towards the goal of personalised medicine.

CRediT Statement

Claire Miller: Funding acquisition, Conceptualization, Methodology, Software, Investigation, Formal Analysis, Visualization, Writing - original draft, Writing - review & editing. **Domenic Germano:** Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - original draft, Writing - review & editing. **Alicia Chenoweth:** Methodology, Writing - review & editing. **Sarah Holdsworth-Carson:** Writing - review & editing.

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