Available online at www.sciencedirect.com
ScienceDirect
Mathematical and Computer Modelling 47 (2008) 714–725
MATHEMATICAL AND COMPUTER MODELLING
www.elsevier.com/locate/mcm

Sensibility of the quorum growth thresholds controlling local immune responses

N.J. Burroughs[a], B.M.P.M. Oliveira[b,c,*], A.A. Pinto[b], H.J.T. Sequeira[b]

[a] *Mathematics Institute, University of Warwick, Coventry CV4 7AL, UK*
[b] *Faculdade de Ciências da Universidade do Porto, Portugal*
[c] *Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Porto, Portugal*

Received 11 January 2007; accepted 21 June 2007

Abstract

The consequences of regulatory T cell (Treg) inhibition of interleukine 2 secretion are examined by mathematical modelling. We demonstrate that cytokine dependent growth exhibits quorum T cell population thresholds that determine whether immune responses develop on activation and whether the immune system returns to a control state. We study the effects in the quorum T cell population thresholds, by the T cell maximum growth rate, by the growth rate ratio between Tregs and T cells, by the value of the secretion rate of cytokines, and by the effectiveness of T cell secretion inhibition by Tregs.

Keywords: Immunology; Tregs; Cytokines; Secretion inhibition; Growth model; Quorum threshold; ODE model

1. Introduction

Regulatory T cells, or Tregs, have emerged over the last decade as a fundamental component of the T cell repertoire, being generated in the thymus under positive selection by self peptides [1]. The Treg repertoire is as diverse as conventional T cells [1] and performs vital immune suppressive functions. Removal of Tregs, e.g. by (cell sorted) adoptive transfer experiments causes a variety of autoimmune disorders in rodents, whilst many autoimmune diseases can be associated with a misregulation of Tregs, e.g. IPEX [2].

Under exposure to their specific antigen, conventional T cells are activated leading to secretion of growth cytokines (predominantly interleukine 2, denoted IL-2), and expression of the interleukine 2 receptor which triggers cytokine driven proliferation. However, in the presence of active Tregs the growth of conventional T cells is inhibited. Part of this growth inhibition is the inhibition of IL-2 secretion by T cells [3,4]. Significantly, addition of IL-2 abrogates inhibition, whilst IL-2 appears to be a key intermediary in the dynamics between Tregs and conventional T cells [5–7]. The process of Treg signalling to conventional T cells is still a matter of debate, evidence exists for both cell:cell mediated inhibition and soluble mediators such as TNFβ and IL-10 [2]. It is likely that multiple methods of regulation are involved. Further, most studies indicate that regulation is not T cell specific, i.e. Tregs inhibit all conventional

* Corresponding address: FCNAUP, R. Dr. Roberto Frias, 4250-465 Porto, Portugal. Tel.: +351 225074320; fax: +351 225074329.
E-mail address: bmpmo@fcna.up.pt (B.M.P.M. Oliveira).

0895-7177/$ - see front matter
doi:10.1016/j.mcm.2007.06.007

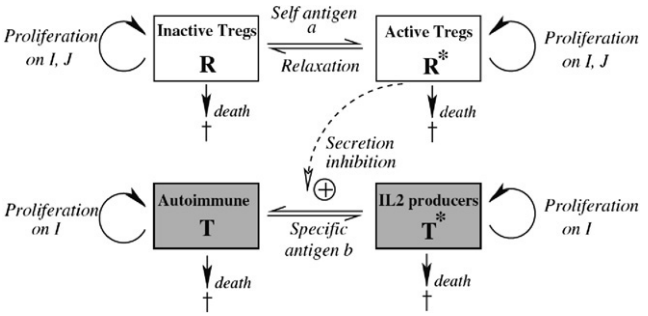

Fig. 1. Model schematic showing growth, death and phenotype transitions of the Treg populations R, R^*, and autoimmune T cell T, T^* populations. Cytokine dynamics are not shown: IL-2 (denoted I) is secreted by activated T cells T^*, adsorbed by all the T cell populations equally, cytokine J is produced from a tissue source and adsorbed by Tregs only.

T cells independent of their antigen specificity [8], although a recent report suggests the contrary [9]. In [10], it has been proposed a dynamical model to explain the delicate balance between appropriate immune activation and immune response suppression being achieved. In this paper, we study the influence in this dynamical model of the T cell maximum growth rate, of the growth rate ratio between regulatory T cells and T cells, of the secretion rate of cytokines, and of the effectiveness of T cell secretion inhibition by Tregs. One of our main interests is to analyse the dependence of the concentration of the regulatory T cells on the concentration of the T cells at equilibria. The dynamics of the model, as discussed in [10], has a bistability region bounded by two thresholds b_L and b_H of antigenic stimulation of T cells. Another main interest is to describe the equilibria manifold in a neighbourhood of the default values for the parameters and variables in Section 4.

2. Theory

There are a number of different (CD4) T cell regulatory phenotypes reported; we use a model of Tregs that is currently identified as $CD25^+$ T cells, although this is not a definitive molecular marker. At a genetic level, these Tregs express Foxp3, a master regulator of the Treg phenotype inducing CD25, CTLA-4 and GITR expressions, all correlating with a suppressive phenotype [2].

The model in [10] uses a population of Tregs (denoted R, R^*) and conventional T cells (T, T^*) with processes shown schematically in Fig. 1. Both populations require antigenic stimulation for activation, Tregs being activated by self antigens. The levels of antigenic stimulation are denoted by a and b for Tregs and conventional T cells respectively. On activation, conventional T cells secrete IL-2 and acquire proliferative capacity in the presence of IL-2 while Tregs proliferate in the presence of IL-2, although less efficiently than normal T cells [3], and they do not secrete IL-2. Activated Tregs suppress IL-2 secretion [3] thereby inhibiting T cell growth. However, if IL-2 is present (CD4) T cells can still proliferate [2,4]. The model in [10] assumes that T cells activated by exposure to their specific antigen have a cytokine secreting state (a normal activated state T^*) and a nonsecreting state (denoted by T) to which they revert to at a constant rate k; thus in the absence of antigen, growth halts. The regulatory T cells can be active (denoted by R^*) or inactive (denoted by R). Activated Tregs R^* also induce a transition in the T cells to the (inhibited) nonsecreting state, this transition rate is assumed to be proportional to the Treg population density. This transition can either be through direct cell:cell contact or be induced by soluble inhibitors [2], both of which give identical mass action kinetics over suitable density ranges. T cells regain secretion status on coreceptor stimulation (CD28, Thornton et al., 2004), which we assume to correlate with antigen exposure through an increased conjugate formation rate. Thus in the presence of costimulation and Tregs, the T cell population would be a mixture of partially inhibited, and normal T cells. Note that exogenous IL-2 does not reverse the suppressed phenotype, i.e. secretion status is not reacquired on cell proliferation [7].

Regulatory T cells are assumed to be in homeostasis, thus Treg density is controlled through some type of (nonlinear) competition. The model in [10] uses a generic mechanism that utilises a cytokine (denoted J), analogous to interleukine 7 which is known to homeostatically regulate memory T cells [11]. It is assumed in the model (see [10]) that the cytokine is secreted by the local tissues, thereby sustaining a local population of Tregs activated by a probably tissue specific profile of self antigens. Tregs compete for this cytokine by adsorption and thus population homeostasis

is achieved. It is also assumed that conventional T cells cannot utilise this cytokine whilst both inactive and active Tregs proliferate under cytokine J; this can be relaxed, conventional T cells only being required to be less efficient at utilising J for survival and thus being out competed under homeostatic conditions. We also include a growth limitation mechanism; we use a (quadratic) Fas-FasL death mechanism [12], that is assumed to act on all T cells equally. Results will be similar with any saturation mechanism. Finally, we include an influx of (auto) immune T cells into the tissue (T_{input} in cells per ml per day), which can represent T cell circulation or naive T cell input from the thymus. We note that T cell population numbers are stochastic. In the model it is assumed that there is a constant influx of autoimmune T cells. This should probably be interpreted as an influx of a diversity of self reacting T cells from the thymus and circulation instead of a specific T cell population. Under a stochastic flux model the system behaves as the $T_{\text{input}} = 0$ model between inputs, and so T cell populations eventually die. Thus only if the input frequency is large enough, is a nonzero population maintained. During an infection, there will also be feedback on the flux from the lymph nodes. This is transient, only lasting as long as the infection, and would not affect the stability of the immune state.

The model proposed in [10] consists of a set of ordinary differential equations employed to study the dynamics, with a compartment for each T cell population (inactive Tregs R, active Tregs R^*, nonsecreting T cells T, secreting activated T cells T^*), interleukine 2 density I and the homeostatic Treg cytokine J,

$$\begin{aligned}
\frac{\mathrm{d}R}{\mathrm{d}t} &= (\epsilon\rho(I+J) - \beta(R+R^*+T+T^*) - \hat{d})R + \hat{k}(R^* - aR),\\
\frac{\mathrm{d}R^*}{\mathrm{d}t} &= (\epsilon\rho(I+J) - \beta(R+R^*+T+T^*) - \hat{d})R^* - \hat{k}(R^* - aR),\\
\frac{\mathrm{d}J}{\mathrm{d}t} &= \hat{\sigma}(S - (\hat{\alpha}(R+R^*) + \hat{\delta})J),\\
\frac{\mathrm{d}T}{\mathrm{d}t} &= (\rho I - \beta(R+R^*+T+T^*) - d)T + k(T^* - bT + \gamma R^*T^*) + T_{\text{input}},\\
\frac{\mathrm{d}T^*}{\mathrm{d}t} &= (\rho I - \beta(R+R^*+T+T^*) - d)T^* - k(T^* - bT + \gamma R^*T^*),\\
\frac{\mathrm{d}I}{\mathrm{d}t} &= \sigma(T^* - (\alpha(R+R^*+T+T^*) + \delta)I).
\end{aligned} \tag{1}$$

Parameters are defined in Table 1. Our model has components that have been used in previous models, for instance cytokine dependent growth [18,19], cytokine kinetics [20], Fas-FasL mediated death [21], and positive feedback of T cells on Tregs [22,23], in our model this is explicitly though IL-2.

3. Analysis of the model

We describe the equilibria manifold in a neighbourhood of the default values for the parameters and variables that will allow us to present our results in the next section. We exhibit an explicit formula for the concentration of the Tregs $y(x) = R + R^*$ as a function of the concentration of the T cells $x = T + T^*$. This explicit formula for the concentration of the Tregs $y(x) = R + R^*$ is fundamental to describe the equilibria manifold by an explicit formula of the level of the antigenicity stimulation $b(x, y(x))$ depending only upon the concentration $x = T + T^*$ of the secreting and nonsecreting T cells, and of the parameter values of the model.

We consider the equilibria of the ODE model presented in [10]:

$$\begin{cases}
(\epsilon\rho(I+J) - \beta(R+R^*+T+T^*) - \hat{d})R + \hat{k}(R^* - aR) = 0\\
(\epsilon\rho(I+J) - \beta(R+R^*+T+T^*) - \hat{d})R^* - \hat{k}(R^* - aR) = 0\\
\hat{\sigma}(S - (\hat{\alpha}(R+R^*) + \hat{\delta})J) = 0\\
(\rho I - \beta(R+R^*+T+T^*) - d)T + k(T^* - bT + \gamma R^*T^*) + T_{\text{input}} = 0\\
(\rho I - \beta(R+R^*+T+T^*) - d)T^* - k(T^* - bT + \gamma R^*T^*) = 0\\
\sigma(T^* - (\alpha(R+R^*+T+T^*) + \delta)I) = 0.
\end{cases}$$

For the simplicity of the analysis, we assume that $\hat{d} = d$ and $\hat{k} = k$.

The concentration of T cells varies between a minimum value corresponding to the *homeostasis concentration of T cells* T_{hom}, i.e. when there is no antigenic stimulation ($b = 0$), and a maximum value, that we call the *capacity of*

Table 1
Model parameters

Parameter	Symbol	Range	Value
T cell T, T^*			
T cell Maximum growth rate[a]	ρ/α	<6 day^{-1}	4 day^{-1}
Death rate of T cells	$d = \hat{d}$	0.1–0.01 day^{-1} [13]	0.1 day^{-1}
Capacity of T cells[b]	$\rho/(\alpha\beta)$	10^6–10^7 cells/ml [14]	10^7 cells/ml
Input rate	T_{input}	0–10^4 cells/ml/day	0, 100 cells/ml per day
Secretion reversion (constant)[c]	k	h–days	0.1 h^{-1}
Antigen stimulation level	bk	$0.001–200 \times a\hat{k}$	Bifurcation parameter
Tregs R, R^*			
Growth rate ratio T_{reg}:T	ϵ	<1	0.6
Homeostatic capacity R_{hom}	$(\epsilon\rho S/\hat{d} - \hat{\delta})/\hat{\alpha}$	10–10^5 cells/ml	10^4 cells/ml
Relaxation rate	$\hat{k}$	hrs–days	0.1 h^{-1}
Death rate ratio T_{reg}:T	$\hat{d}/d$		1
T_{reg} antigen stimulation level	$a\hat{k}$	0–10 per day	1 per day
Secretion inhibition[d]	γ	$0.1–100 \times R_{\text{hom}}^{-1}$	$10\ R_{\text{hom}}^{-1}$
Cytokines			
Max. cytokine concentration[e]	$1/\alpha$	100–500 pM	200 pM
IL-2 secretion rate	σ	[f] 0.07, 2 fgrms h^{-1} [15]	10^6 molecs s^{-1} cell^{-1}
Relative adsorbance J to IL-2	$\hat{\sigma}\hat{\alpha}/\sigma\alpha$	<1	0.1
Relative secretion rate of J	$\hat{\sigma}/\sigma$	<1	0.01
Cytokine decay rate	$\sigma\delta = \hat{\sigma}\hat{\delta}$	hrs–days	1.5 h^{-1} [16]

[a] Minimum duration of SG_2M phase $\alpha\rho^{-1} \approx 3$ h.
[b] Maximum T cell density for severe infections (based on LCMV).
[c] This is in absence of Tregs.
[d] This is in terms of the homeostatic Treg level R_{hom} which we set to 10^4 cells per ml.
[e] This is taken as 20 times the receptor affinity (10 pM [17]).
[f] Naive and memory cells respectively. This corresponds to 3000–10^5 molecules per h, IL-2 mass 15–18 kDa.

T cells T_{cap}, which is obtained for high levels of antigenic stimulation ($b = +\infty$). Using Lemma 2, the values T_{hom} and T_{cap} are implicitly determined as zeros of a polynomial. In particular, for the default values of the parameters, these values are given by $T_{\text{hom}} = 9.6 \times 10^2$ and $T_{\text{cap}} = 9.7 \times 10^6$.

In Lemma 1, we present the relation between the concentration of T cells and the concentration of the Tregs, for values of the concentration of T cells between T_{hom} and T_{cap}.

Let Y_1, Y_2, Y_3 be the following polynomials

$$Y_1(x) = -\hat{\alpha}C(x) - \beta\hat{\delta}B(x)$$
$$Y_2(x) = 2\hat{\alpha}\beta B(x)$$
$$Y_3(x) = Y_1^2(x) - 2(\delta C(x) - \epsilon\rho S x)Y_2(x),$$

where $B(x) = (1-\epsilon)x$ and $C(x) = \epsilon T_{\text{input}} + dB(x) + \beta B(x)x$.

Lemma 1. *The concentration of Tregs $y = R + R^*$ is given by the Treg curve*

$$y(x) = \frac{Y_1(x) + \sqrt{Y_3(x)}}{Y_2(x)}, \tag{2}$$

where $x = T + T^*$ is the total concentration of T cells.

The proof of Lemma 1 is presented in [24].

In Lemma 2, we obtain the level of the antigenic stimulation $b(x, y(x))$ from the concentration x of the T cells, using the auxiliary Treg curve $y(x)$. Let $b(x, y)$ be the *antigen function* given by

$$b(x, y) = \frac{\varphi(x, y)(kx(1 + \gamma Ay) + T_{\text{input}})}{k(1 - \epsilon)\rho x^3(\hat{\alpha}y + \hat{\delta}) - kx\varphi(x, y)}, \tag{3}$$

where $\varphi(x, y) = (\epsilon\rho Sx - T_{\text{input}}(\hat{\alpha}y + \hat{\delta}))(\alpha(x + y) + \delta)$.

Lemma 2. *Let $b(x, y)$ be the antigen function, and let $y(x)$ be as in Lemma 1. The level of the antigenic stimulation is given by $b(x, y(x))$ when the system is at equilibrium (stable or unstable).*

Conversely, given an antigenic stimulation level b, the concentration x of T cells is given, implicitly, as a zero of a twelfth order polynomial that can be explicitly constructed. The proof of Lemma 2 is presented in [24].

Let $\tilde{b}(x)$ be the *antigen function in the absence of Tregs* given by

$$\tilde{b}(x) = \frac{(\alpha x + \delta)(kx + T_{\text{input}})(\beta x^2 + dx - T_{\text{input}})}{kx((\alpha x + \delta)(-\beta x^2 - dx + T_{\text{input}}) + \rho x^2)}\,. \tag{4}$$

Lemma 3. *Let us consider the simplified model with the concentration of Tregs equal to zero (i.e. $y = 0$). The level of the antigenic stimulation is given by $\tilde{b}(x)$, when the system is at equilibrium (stable or unstable).*

Conversely, given an antigenic stimulation level $\tilde{b}$, the concentration x of T cells is a zero of a fourth order polynomial that can be explicitly constructed. The proof of Lemma 3 is presented in [24].

4. Results

It is likely that the levels of antigen stimulation present both fluctuations and slow variation over time, e.g. during puberty. Thus, if antigenic stimulation rises above the threshold b_H (see figures in [10]), control is lost and autoimmunity arises. Note that even if the antigen stimulation level b falls to the original value, at which control was originally achieved, control may not be reacquired, and is only attained if stimulation falls below the second threshold b_L (see figures in [10]). This phenomena, termed hysteresis, is common in many physical and biological systems.

Our system displays a control state and an immune state. This biphasic behaviour is a consequence of the IL-2 driven dynamics where the IL-2 concentration must be high enough such that the growth rate exceeds the death rate; this requires a sufficiently high density of secreting T cells. Thus even in the absence of Tregs, T cells can display this behaviour (in fact in the blue line in Fig. 3B the parameters are such that the bifurcation points b_L, b_H are lost and hysteresis is no longer observed although a sharp transition with b remains). The presence of Tregs increases the thresholds b_L, b_H, Fig. 3B, thereby enhancing the control state. The hysteresis is unfold for low values of the secretion rate of cytokine J (parameter S), for low values of the growth rate ratio between Tregs and T cells (parameter ϵ), for low growth rates (parameter ρ), and for high thymic inputs (parameter T_{input}). The unfold of the hysteresis, for some of these parameters, occurs out of the biological region. We find that the parameters that control the distance between b_L and b_H are the effectiveness of T cells secretion inhibition by Tregs (parameter γ), the cytokine degradation rate (parameter δ), the reversion rate to an inactive state (parameter k) and the antigen stimulation of Tregs (parameter a). The IL-2 secretion rate by T cells (parameter σ) has no effect on the steady states when we keep the ratio $\hat{\sigma}/\sigma$ constant.

The growth rate ratio Treg:T cells (parameter ϵ) unfolds the hysteresis for $\epsilon \approx 0.105$ due to Treg insufficient growth (see Figs. 2 and 3). Higher response of Tregs to the IL-2 (higher values of the parameter ϵ) increases the antigenic stimulation thresholds b_L and b_H (see Fig. 4A). When ϵ gets close to the value 1 the thresholds b_L and b_H tend to infinity. The concentration $x(b_L)$ of T cells increases with ϵ and the concentration $x(b_H)$ of T cells has a minima for $\epsilon \approx 0.3$ (see Fig. 4B). The concentrations $y(b_L)$ and $y(b_H)$ of Tregs increase with ϵ (see Fig. 4C). For values of $\epsilon \lesssim 0.5$ the concentration of T cells (black lines in Fig. 3B), in the presence of Tregs ($y > 0$) intercepts the concentration of T cells (blue lines in Fig. 3B) for the simplified model without Tregs ($y = 0$).

A cusp is found for low growth rates $\rho \approx 0.0025$, corresponding to a maximum T cells growth rate of 0.5/day (see Figs. 5 and 6). The antigenic stimulation threshold b_L decreases with ρ and the threshold b_H has a minima

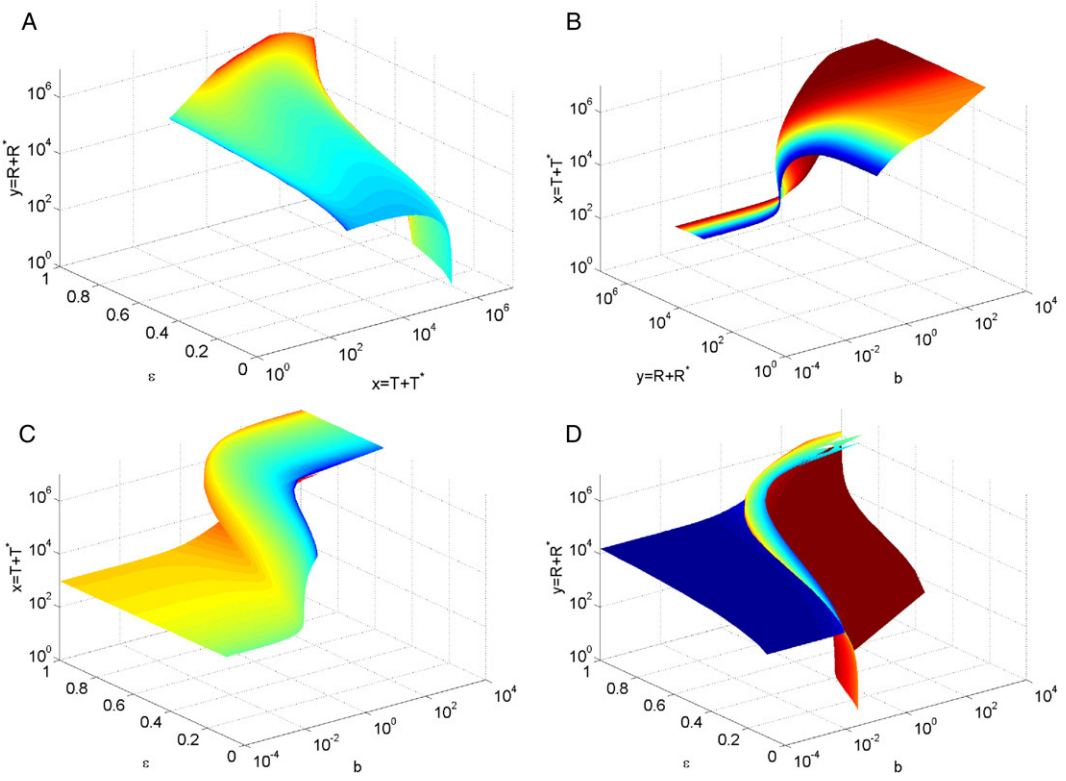

Fig. 2. The hysteresis of the equilibria manifold for values of the growth rate ratio Treg:T cells, parameter $\epsilon \in [0.1, 0.99]$. The hysteresis unfolds for low values of the parameter ϵ. A: Horizontal axis: ϵ; "away axis": $x = T + T^*$; vertical axis: $y = R + R^*$. Low values of b are bluish and higher values are reddish. B: Horizontal axis: $y = R + R^*$; "away axis": b; vertical axis: $x = T + T^*$. Low values of the parameter ϵ are bluish and higher values are reddish. C: Horizontal axis: ϵ; "away axis": b; vertical axis: $x = T + T^*$. Low values of $y = R + R^*$ are bluish and higher values are reddish. D: Horizontal axis: ϵ; "away axis": b; vertical axis: $y = R + R^*$. Low values of $x = T + T^*$ are bluish and higher values are reddish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

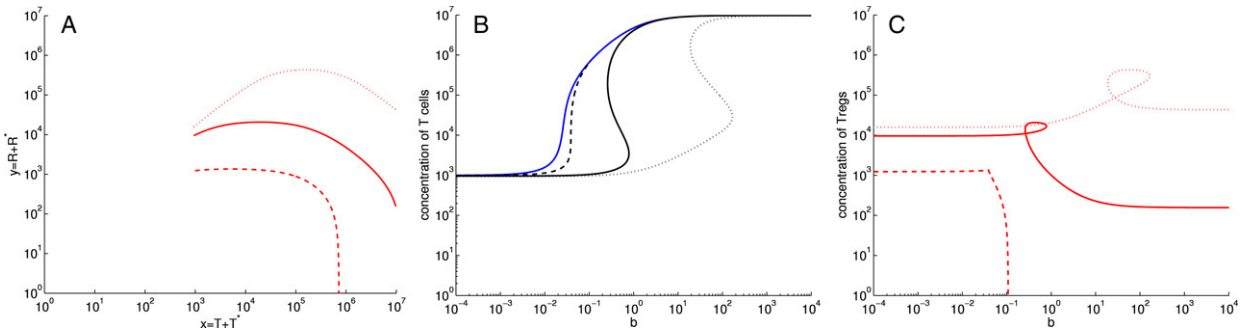

Fig. 3. Cross sections of the equilibria manifold, for the following values of the growth rate ratio Treg:T cells: $\epsilon = 0.1$ (dashed lines); $\epsilon = 0.6$ (bold lines); and $\epsilon = 0.99$ (dotted lines). The other parameters are at their default values. A: The horizontal axis is the total concentration $x = T + T^*$ of T cells, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. B: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $x = T + T^*$ of T cells. Black lines: concentration of T cells when Tregs are present ($y = R + R^* > 0$). Blue line: concentration of T cells in the simplified model without Tregs ($y = R + R^* = 0$). We note that the dashed black line and the blue bold line intercept at a point $b \approx 0.11$. C: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for $\rho \approx 0.005$. The ratio b_H/b_L increases with the parameter ρ (see Fig. 7A). The concentration $x(b_L)$ of T cells increases and the concentration $x(b_H)$ of T cells decreases with ρ (see Fig. 7B). The concentrations $y(b_L)$ and $y(b_H)$ of Tregs increase with ρ (see Fig. 7C).

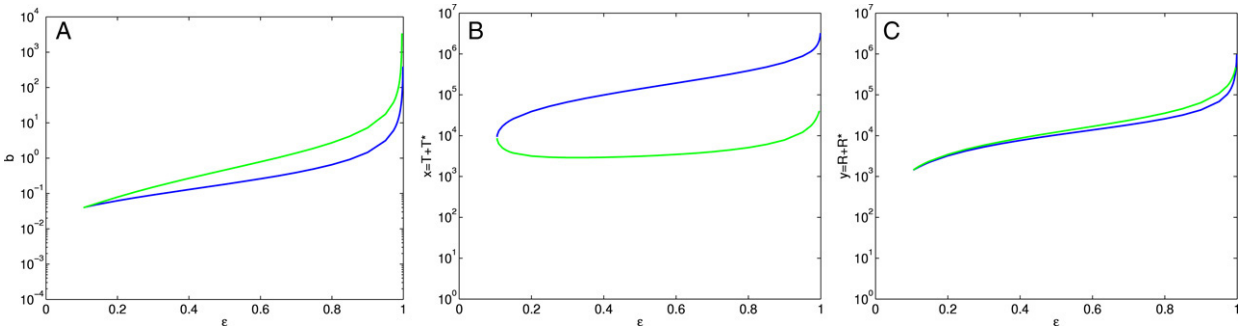

Fig. 4. Dependence of the thresholds on the growth rate ratio Treg:T cells, parameter $\epsilon \in [0.105, 0.99]$ for the model with Tregs present. A: The thresholds of the antigenic stimulation b_L (blue) and b_H (green) increase with ϵ. The cusp b_C occurs at $\epsilon \approx 0.105$, unfolding the hysteresis. When ϵ converges to 1 the thresholds b_L and b_H tend to $+\infty$. B: The concentration $x(b_L)$ (blue) of T cells increases with ϵ and the concentration $x(b_H)$ (green) of T cells has a minima for $\epsilon \approx 0.3$. C: The concentrations $y(b_L)$ (blue) and $y(b_H)$ (green) of Tregs increase with ϵ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

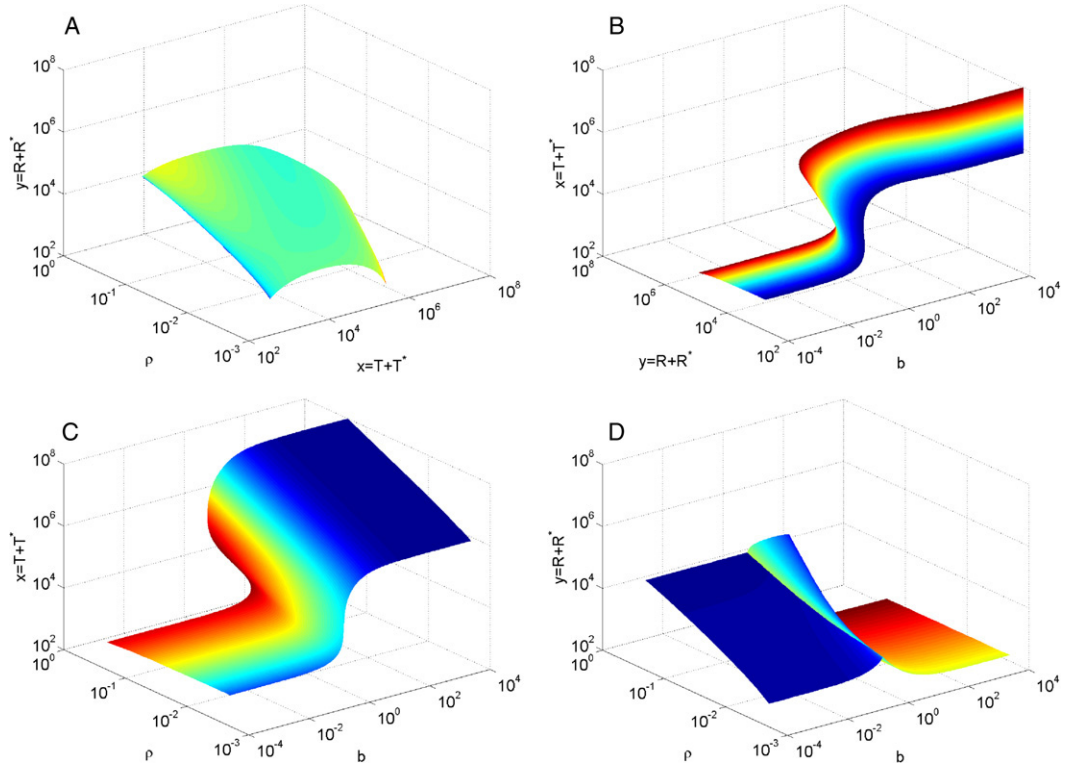

Fig. 5. The hysteresis of the equilibria manifold for values of the growth rate, parameter $\rho \in [0.002, 0.2]$. The hysteresis unfolds for low values of the parameter ρ. A: Horizontal axis: ρ; "away axis": $x = T + T^*$; vertical axis: $y = R + R^*$. Low values of b are bluish and higher values are reddish. B: Horizontal axis: $y = R + R^*$; "away axis": b; vertical axis: $x = T + T^*$. Low values of the parameter ρ are bluish and higher values are reddish. C: Horizontal axis: ρ; "away axis": b; vertical axis: $x = T + T^*$. Low values of $y = R + R^*$ are bluish and higher values are reddish. D: Horizontal axis: ρ; "away axis": b; vertical axis: $y = R + R^*$. Low values of $x = T + T^*$ are bluish and higher values are reddish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The parameter γ (effectiveness of T cells secretion inhibition by Tregs) shifts the hysteresis as expected (see Figs. 11 and 12) but does not unfold the hysteresis. The antigenic stimulation thresholds b_L and b_H increase with the parameter γ, but the ratio ratio b_H/b_L does not change significantly with γ (see Fig. 13A). The concentrations $x(b_L)$ and $x(b_H)$ of T cells increase with γ (see Fig. 13B). The concentration $y(b_L)$ of Tregs decreases with γ and the concentration $y(b_H)$ of Tregs increases with γ (see Fig. 13C).

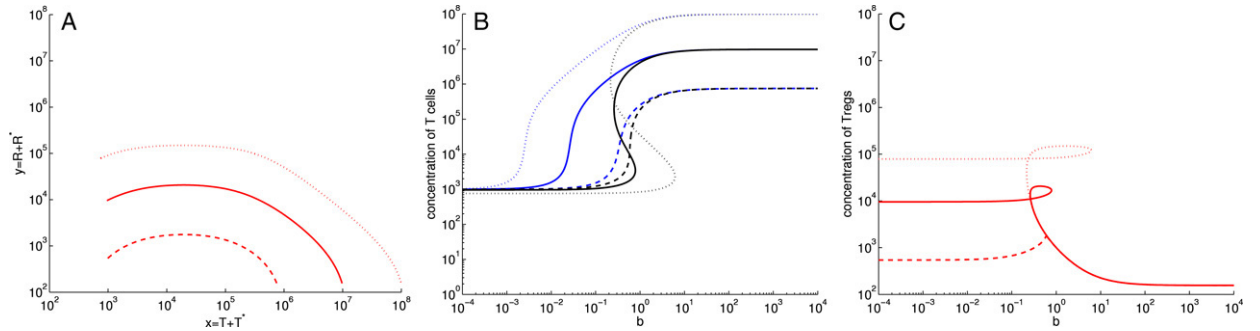

Fig. 6. Cross sections of the equilibria manifold, for the following values of the growth rate: $\rho = 0.002$ (dashed lines); $\rho = 0.02$ (bold lines); and $\rho = 0.2$ (dotted lines). The other parameters are at their default values. A: The horizontal axis is the total concentration $x = T + T^*$ of T cells, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. B: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $x = T + T^*$ of T cells. Black lines: concentration of T cells when Tregs are present ($y = R + R^* > 0$). Blue lines: concentration of T cells in the simplified model without Tregs ($y = R + R^* = 0$). C: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

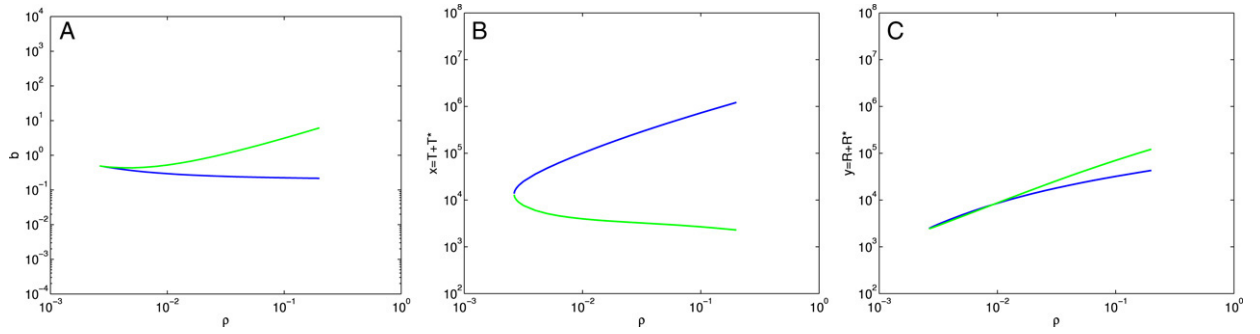

Fig. 7. Dependence of the thresholds on the growth rate, parameter $\rho \in [0.0025, 0.2]$ for the model with Tregs present. A: The threshold of the antigenic stimulation b_L (blue) decreases with ρ and the threshold b_H (green) has a minima for $\rho \approx 0.005$. The cusp b_C occurs at $\rho \approx 0.0025$, corresponding to a maximum T cell growth rate of 0.5/day, unfolding the hysteresis. B: The concentration $x(b_L)$ (blue) of T cells increases and the concentration $x(b_H)$ (green) decreases with ρ. C: The concentrations $y(b_L)$ (blue) of T cells and $y(b_H)$ (green) of Tregs increase with ρ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The parameter σ has no effect on the steady states when we keep the ratio $\hat{\sigma}/\sigma$ constant (see Figs. 8 and 9), nor has any effect on the thresholds b_L and b_H (see Fig. 10), since the antigen stimulation b of the T cells does not depend on this parameter.

5. Discussion

In this paper we examined a mechanism of Treg control of immune responses through the regulation of cytokine dependent T cell proliferation. In the model proposed in [10] Tregs have two effects on cytokine levels; firstly they directly inhibit cytokine secretion and secondly they adsorb (and thus compete for) proliferative cytokines. Both of these have an impact on T cell growth. However, secretion inhibition is shown to act as a growth modulator through adjustment of a quorum threshold associated with cytokine growth dynamics. The second effect was minimal in our simulations because the Treg population was always a minor population; however increasing the homeostatic level of Tregs would increase the impact of this competition and reduce immune response growth rates, thereby extending immune response times.

The threshold mechanism discussed here is extremely robust to model details, being effectively a model of activation and escape. Proliferation driven by a secreted cytokine is naturally population size (quorum) dependent [18]; ‘quorum sensing’ imposes a population consensus on immune responses which are only initiated if a sufficiently high number of T cells are locally activated. A locally maintained population of Tregs raises this quorum threshold in the

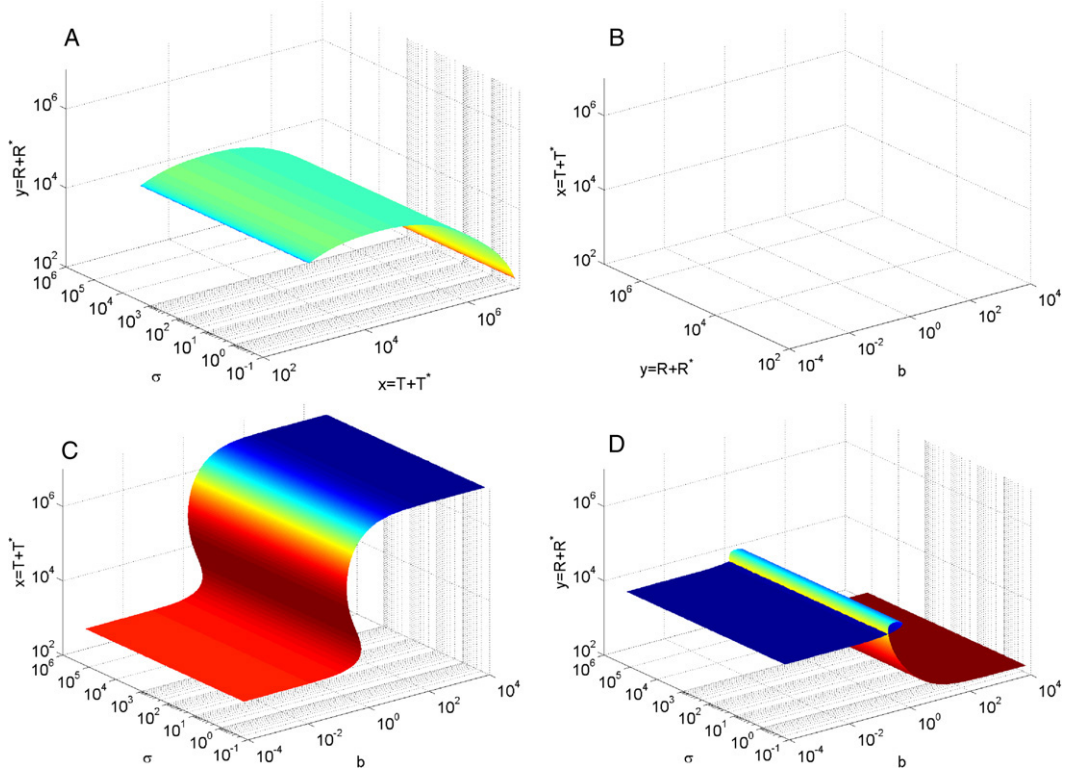

Fig. 8. The hysteresis of the equilibria manifold for values of the secretion rate of cytokines parameter $\sigma \in [0.144, 144000]$. A: Horizontal axis: σ; "away axis": $x = T + T^*$; vertical axis: $y = R + R^*$. Low values of b are bluish and higher values are reddish. B: Horizontal axis: $y = R + R^*$; "away axis": b; vertical axis: $x = T + T^*$. Low values of the parameter σ are bluish and higher values are reddish. C: Horizontal axis: σ; "away axis": b; vertical axis: $x = T + T^*$. Low values of $y = R + R^*$ are bluish and higher values are reddish. D: Horizontal axis: σ; "away axis": b; vertical axis: $y = R + R^*$. Low values of $x = T + T^*$ are bluish and higher values are reddish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

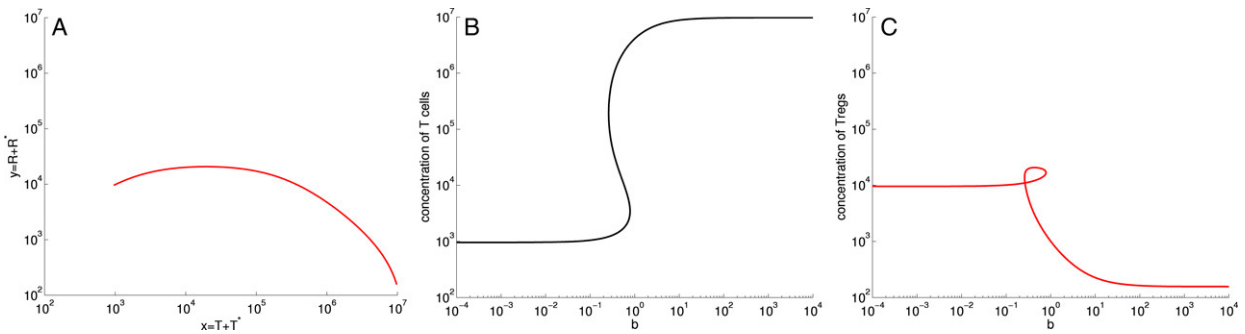

Fig. 9. Cross sections of the equilibria manifold, for the value of the secretion rate of cytokines $\sigma = 144$ (bold lines). These lines are the same for all values of σ. The other parameters are at their default values. A: The horizontal axis is the total concentration $x = T + T^*$ of T cells, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. B: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $x = T + T^*$ of T cells. C: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs.

local tissue. Thus, immune responses are inhibited unless the number of activated T cells is sufficiently large to escape Treg control; such escape is dependent on the higher efficiency with which conventional T cells (responders) can utilise IL-2 compared to Tregs. This quorum mechanism originates from IL-2 driven growth and is independent of whether growth is continually stimulated through antigen exposure, as modelled here, or in a programmed proliferation phase which is still cytokine dependent [25].

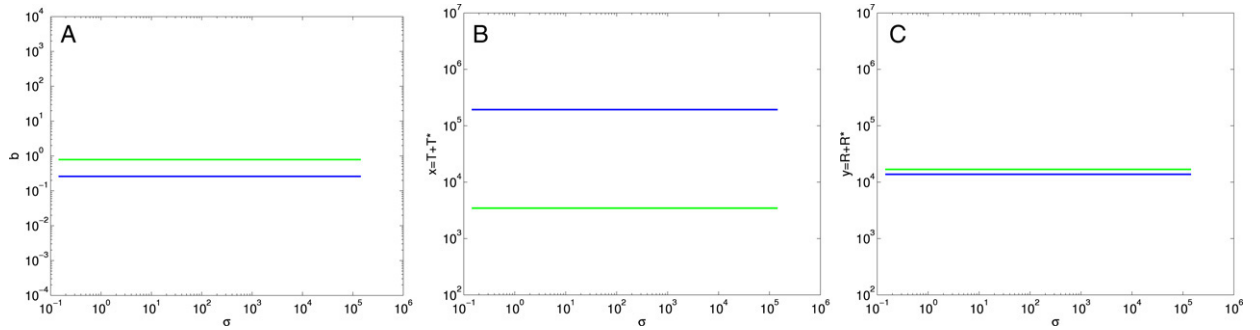

Fig. 10. Dependence of the thresholds on the secretion rate of cytokines parameter $\sigma \in [0.144, 144000]$ for the model with Tregs present. A: The thresholds of the antigenic stimulation b_L (blue) and b_H (green) do not change with σ. B: The concentrations $x(b_L)$ (blue) and $x(b_H)$ (green) of T cells do not change with σ. C: The concentrations $y(b_L)$ (blue) and $y(b_H)$ (green) of Tregs do not change with σ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

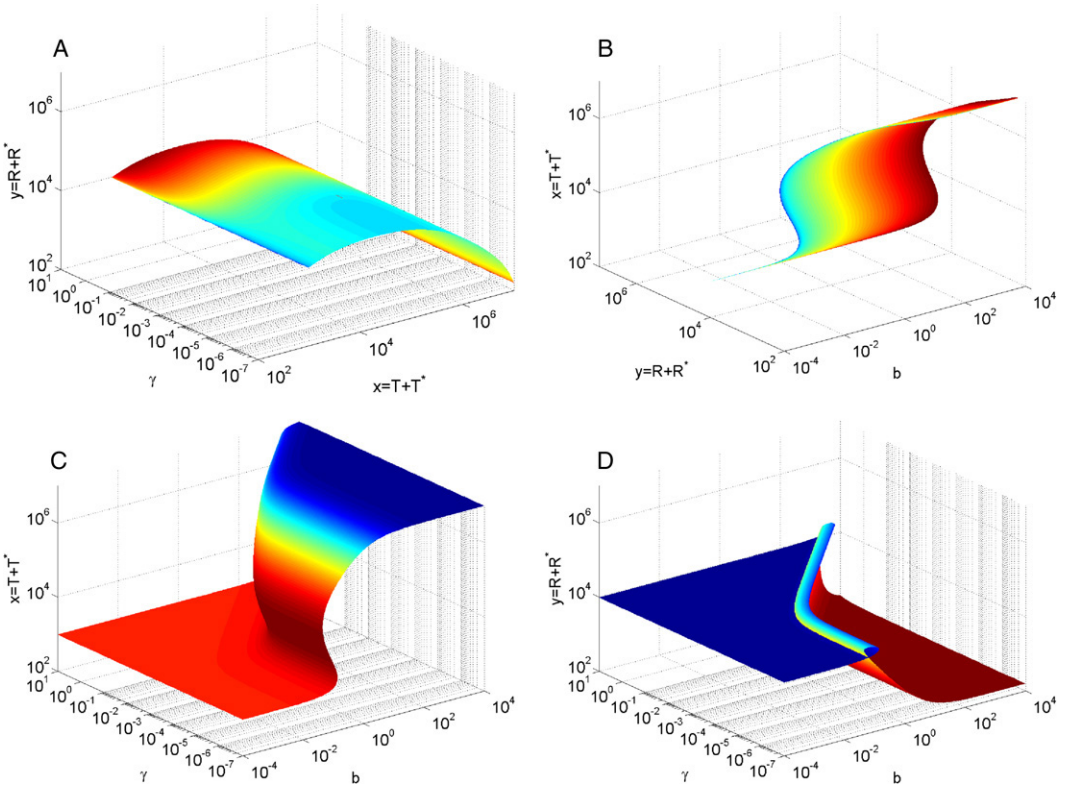

Fig. 11. The hysteresis of the equilibria manifold for values of the effectiveness of T cell secretion inhibition by Tregs, parameter $\gamma \in [10^{-7}, 10^{1}]$. A: Horizontal axis: γ; "away axis": $x = T + T^*$; vertical axis: $y = R + R^*$. Low values of b are bluish and higher values are reddish. B: Horizontal axis: $y = R + R^*$; "away axis": b; vertical axis: $x = T + T^*$. Low values of the parameter γ are bluish and higher values are reddish. C: Horizontal axis: γ; "away axis": b; vertical axis: $x = T + T^*$. Low values of $y = R + R^*$ are bluish and higher values are reddish. D: Horizontal axis: γ; "away axis": b; vertical axis: $y = R + R^*$. Low values of $x = T + T^*$ are bluish and higher values are reddish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Our model also demonstrates bistability; this is in fact a common feature of all the current Treg models [22,26]. The bistability region is bounded by the thresholds b_L and b_H of antigenic stimulation of T cells between which the immune response model has biphasic behaviour. We find that the hysteresis unfolds for low values of the secretion rate of cytokine J (parameter S), for low values of the growth rate ratio between Tregs and T cells (parameter ϵ), for low growth rates (parameter ρ), and for high thymic inputs (parameter T_{input}). Hence, modifications of the values

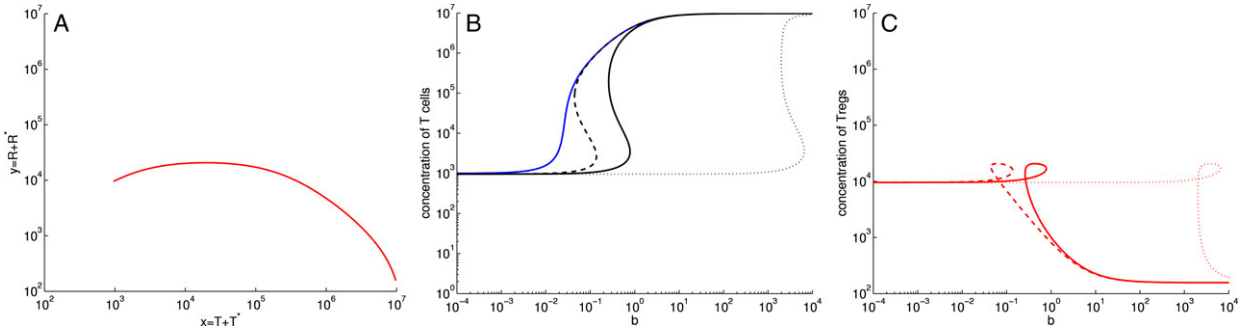

Fig. 12. Cross sections of the equilibria manifold, for the following values of the effectiveness of T cell secretion inhibition by Tregs: $\gamma = 10^{-7}$ (dashed lines); $\gamma = 10^{-3}$ (bold lines); and $\gamma = 10^{1}$ (dotted lines). The other parameters are at their default values. A: The horizontal axis is the total concentration $x = T + T^*$ of T cells, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. The dashed line and the dotted line are superimposed to the bold line for the scale considered. B: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $x = T + T^*$ of T cells. Black lines: concentration of T cells when Tregs are present ($y = R + R^* > 0$). Blue line: concentration of T cells in the simplified model without Tregs ($y = R + R^* = 0$). C: The horizontal axis is the antigenic stimulation level b, and the vertical axis is the total concentration $y(x) = R + R^*$ of Tregs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

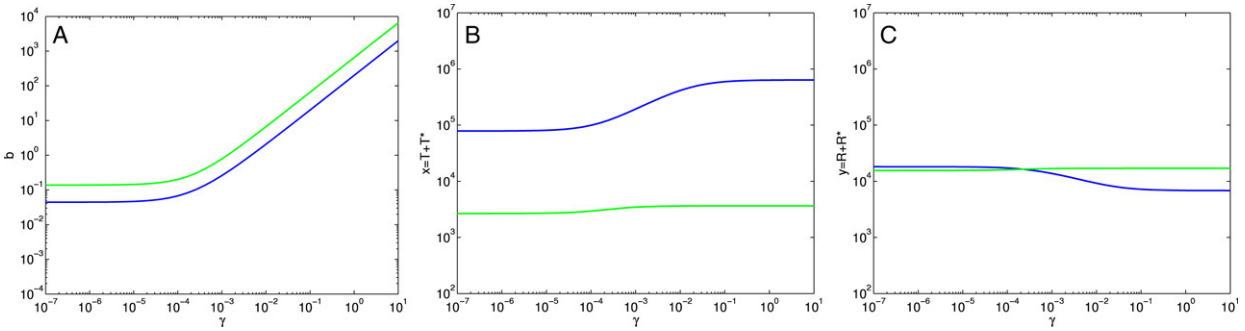

Fig. 13. Dependence of the thresholds on the effectiveness of T cell secretion inhibition by Tregs, parameter $\gamma \in [10^{-7}, 10^{1}]$ for the model with Tregs present. A: The thresholds of the antigenic stimulation b_L (blue) and b_H (green) increase with γ. B: The concentrations $x(b_L)$ (blue) and $x(b_H)$ (green) of T cells increase with γ. C: The concentration $y(b_L)$ (blue) of Tregs decreases with γ and the concentration $y(b_H)$ (green) of Tregs increases with γ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of the above parameter provoke significant changes in the values of the thresholds b_L and b_H. Alterations in these parameters can develop autoimmune diseases.

Acknowledgements

We would like to thank Jorge Zubelli for all the encouragement and very helpful comments. BMPMO, AAP and HJTS were partially supported under CMUP, CMat Uminho, POCTI, POSI and PRODEP III of ESF-EU, FCT and Ministério CTES.

References

[1] C.-S. Hsieh, Y. Liang, A.J. Tyznik, S.G. Self, D. Liggitt, A.Y. Rudensky, Recognition of the peripheral self by naturally arising CD25$^+$CD4$^+$ T cell receptors, Immunity 21 (2004) 267–277.

[2] S. Sakaguchi, Naturally arising CD4$^+$ regulatory T cells for immunological self-tolerance and negative control of immune responses, Annual Review of Immunology 22 (2004) 531–562.

[3] A.M. Thornton, E.M. Shevach, CD4$^+$CD25$^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukine 2 production, The Journal of Experimental Medicine 188 (2) (1998) 287–296.

[4] E.M. Shevach, R.S. McHugh, C.A. Piccirillo, A.M. Thornton, Control of T-cell activation by CD4(+)CD25(+) suppressor T cells, Immunological Reviews 182 (2001) 58–67.

[5] G.C. Furtado, M.A. Curotto de Lafaille, N. Kutchukhidze, J.J. Lafaille, IInterleukin 2 signaling is required for $CD4^+$ regulatory T cell function, The Journal of Experimental Medicine 196 (6) (2002) 851–857.
[6] M. de la Rosa, S. Rutz, H. Dorninger, A. Scheffold, Interleukin-2 is essential for $CD4^+CD25^+$ regulatory T cells function, European Journal of Immunology 34 (9) (2004) 2480–2488.
[7] A.M. Thornton, E.E. Donovan, C.A. Piccirillo, E.M. Shevach, Cutting edge: IL-2 is critically required for the in vitro activation of CD4(+)CD25(+) T cell suppressor function, The Journal of Immunology 172 (2004) 6519–6523.
[8] A.M. Thornton, E.M. Shevach, Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific, The Journal of Immunology 164 (2000) 183–190.
[9] C. Tanchot, F. Vasseur, C. Pontoux, C. Garcia, A. Sarukhan, Immune regulation by self-reactive T cells is antigen specific, The Journal of Immunology 172 (2004) 4285–4291.
[10] N.J. Burroughs, B.M.P.M. Oliveira, A.A. Pinto, Regulatory T cell adjustment of quorum growth thresholds and the control of local immune responses, Journal of Theoretical Biology 241 (2006) 134–141.
[11] K.S. Schluns, W.C. Kieper, S.C. Jameson, L. Lefrancois, Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*, Nature Immunology 1 (2000) 426–432.
[12] S. Nagata, Fas ligand-induced apoptosis, Annual Review of Genetics 33 (1999) 29–55.
[13] C. Michie, A. McLean, C. Alcock, P. Beverley, Life-span of human lymphocyte subsets defined by CD45 isoforms, Nature 360 (1992) 264–265.
[14] D. Moskophidis, M. Battegay, M. van den Broek, E. Laine, U. Hoffmann-Rohrer, R. Zinkernagel, Role of virus and host variables in virus persistence or immunopathological disease caused by a noncytolytic virus, The Journal of General Virology 76 (1995) 381.
[15] H. Veiga-Fernandes, U. Walter, C. Bourgeois, A. McLean, B. Rocha, Response of naive and memory $CD8^+$ T cells to antigen stimulation *in vivo*, Nature Immunology 1 (2000) 47–53.
[16] P.M. Anderson, M.A. Sorenson, Effects of route and formulation on clinical pharmacokinetics of interleukine-2, Clinical Pharmacokinetics 27 (1994) 19–31.
[17] J.W. Lowenthal, W.C. Greene, Contrasting interleukine 2 binding properties of the alpha (p55) and beta (p70) protein subunits of the human high-affinity interleukine 2 receptor, The Journal of Experimental Medicine 166 (1987) 1156–1161.
[18] R.J. de Boer, P. Hogeweg, Immunological discrimination between self and non-self by precursor depletion and memory accumulation, Journal of Theoretical Biology 124 (1987) 343.
[19] A.R. McLean, Modelling T cell memory, Journal of Theoretical Biology 170 (1994) 63–74.
[20] C. Utzny, N.J. Burroughs, Perturbation theory analysis of competition in a heterogeneous population, Physica D 175 (2003) 109–126.
[21] R.E. Callard, J. Stark, A.J. Yates, Fratricide: A mechanism for T memory-cell homeostasis, Trends in Immunology 24 (2003) 370–375.
[22] K. Leon, R. Perez, A. Lage, J. Carneiro, Modelling T-cell-mediated suppression dependent on interactions in multicellular conjugates, Journal of Theoretical Biology 207 (2000) 231–254.
[23] K. Leon, A. Lage, J. Carneiro, Tolerance and immunity in a mathematical model of T-cell mediated suppression, Journal of Theoretical Biology 225 (2003) 107–126.
[24] N.J. Burroughs, B.M.P.M. Oliveira, A.A. Pinto, H. Sequeira, Stability analysis of a model of local immune responses with regulatory T cells (submitted for publication).
[25] M.J.B. van Stipdonk, E.E. Lemmens, S.P. Schoenberger, Naive ctls require a single brief period of antigenic stimulation for clonal expansion and differentiation, Nature Immunology 2 (2001) 423–429.
[26] C. Chan, R.I. Lechler, A.J.T. George, Tolerance mechanisms and recent progress, Transplantation Proceedings 36 (2004) 561S–569S.