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Mathematical Model of the Roles of T Cells in Inflammatory Bowel Disease

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Abstract Gut mucosal homeostasis depends on complex interactions among the microbiota, the intestinal epithelium, and the gut associated immune system. A breakdown in some of these interactions may precipitate inflammation. Inflammatory bowel diseases, Crohn's disease, and ulcerative colitis are chronic inflammatory disorders of the gastrointestinal tract. The initial stages of disease are marked by an abnormally high level of pro-inflammatory helper T cells, Th1. In later stages, Th2 helper cells may dominate while the Th1 response may dampen. The interaction among the T cells includes the regulatory T cells (Treg). The present paper develops a mathematical model by a system of differential equations with terms nonlocal in the space spanned by the concentrations of cytokines that represents the interaction among T cells through a cytokine signaling network. The model demonstrates how the abnormal levels of T cells observed in inflammatory bowel diseases can arise from abnormal regulation of Th1 and Th2 cells by Treg cells.

Keywords Inflammatory bowel disease · T helper cells · Cytokines · Treg control · Mathematical model

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1 Introduction

Crohn's Disease (CD) and Ulcerative Colitis (UC) are chronic intestinal disorders known as idiopathic inflammatory bowel diseases (IBD) (Abraham and Cho 2009). CD affects approximately 1.4 million Americans with a peak onset occurring at 15–30 years of age. The development of CD and UC requires a genetic predisposition, a dysregulated immune response and an environmental trigger. Candidate genes include those that regulate innate immunity, adaptive immunity, and epithelial barrier function.

The classical immune paradigm of Inflammatory bowel diseases identified Crohn's disease as a Th1 mediated process while ulcerative colitis as a Th2 type of inflammation (Bamias et al. 2011; Matsuoka et al. 2004). The primary function of Th1 cells is protection from intracellular pathogens. Th2 cells confer protection against extracellular pathogens. Th1 differentiation is under the control of transcription factor T-bet. Antigen recognition by APCs (antigen presenting cells) results in production of Th1 polarizing cytokines IL-12 and IFNγ, which activate the signal transducer and activator STAT4 and STAT1, respectively (Matsuoka et al. 2004). T-bet expression in naive T cells suppresses Th2 development and upregulates IFNγ production thus reinforcing the Th1 phenotype. The Th2 response appears to be the default polarization of naive T cells, under the control of transcription factor Gata3 (Zhu et al. 2012). IL-4, IL-5 and IL-13 are signature cytokines of the Th2 response.

Th1 inflammatory response in patients with IBD, especially in early phase, leads to production of high levels of TNF-α (Kugathasan et al. 2007). Neutralization of TNF-α in patients with Crohn's disease and ulcerative colitis can induce mucosal healing in some but not all patients (Rubin et al. 2012). The variable treatment outcome reflects the relative Th1/Th2 balance as well as additional control through regulatory T cells (Treg) and Th17 cells (Ishikawa et al. 2012). Similar to Th1 and Th2 cells, transcription factors Foxp3 and RORγ promote development of Treg and Th17 cells, respectively (Eastaff-Leung et al. 2010). Intestinal immune system has the difficult task to maintain both a state of tolerance toward commensal bacteria and to protect the host from pathogens (Sansonetti 2004). Subsets of Treg cells exert an immunosuppressive effect on Th1/Th2 and Th17 effector cells. IL-2 cytokine promotes the growth of both Th1 and Treg cells. Under basal conditions, low levels of IL-2 preferentially activate Treg cells, and thus the net effect is immune tolerance (Germain 2012). Under pathogenic conditions, enhanced IL-2 secretion coupled with high CD25 (IL-2 receptor) expression drives the proliferation of proinflammatory Th1 cells. Increased production of anti-inflammatory cytokines IL-10 and TGF-β accompany resolution of inflammation. The latter cytokine has an important role for the development of Treg cells, and consequently the reinstatement of an immune tolerant state. Impaired TGF-β signaling in patients with inflammatory bowel diseases alters the balance between effector and regulatory T cells and promotes a state of chronic inflammation (Ishikawa et al. 2012).

The complexity of immune processes in disease states such as inflammatory bowel diseases poses significant challenges to data interpretation. In the current study, we aim to apply a systems biology approach by combining a mathematical model with published experimental data. Our main goal was to provide a simplified framework,

limited to the relationships between Th1, Th2, and Treg cells in the initial phase of the inflammatory process. This in turn will become a scaffold for additional regulatory mechanisms that incorporate Th17 and innate immune cells as well as prospective experimental data from patients with IBD. Our mathematical model is based on earlier work by Yates et al. (2004), which considered Th cell differentiation into Th1 and Th2. Here, we extend the model to include Treg cells, and additional cytokines that are involved in IBD.

2 Mathematical Model

2.1 Notations

The model includes the following nondimensional variables:

$T_1(t)$:	Concentration of T-Bet (transcription factor for Th1 development) in a single cell
$T_2(t)$:	Concentration of Gata3 (transcription factor for Th2 development) in a single cell
$T_r(t)$:	Concentration of Foxp3 (transcription factor for Treg development) in a single cell
$I_\gamma(t)$:	Cytokine signal of IFNγ per cell
$I_4(t)$:	Cytokine signal of IL-4 per cell
$I_\beta(t)$:	Cytokine signal of TGF-β per cell
$I_2(t)$:	Cytokine signal of IL-2 per cell
$\Phi(T_1, T_2, T_r, t)$:	Cell population density
$N(t)$:	Total number of the cells

These variables satisfy a system of differential equations with terms non-local in the space spanned by the concentrations of cytokines, based on the network shown in Fig. 1, and the parameters of the system are given in Tables 1 and 2.

2.2 Transcription Factors

The following equations are used to describe the concentrations of the transcription factors T-Bet (Th1), Gata3 (Th2), and Foxp3 (Treg) in a cell:

$$\frac{dT_1}{dt} = \underbrace{-\mu_1 T_1}_{\text{decay}} + \left(\underbrace{\alpha_1 \frac{T_1^n}{K_1^n + T_1^n}}_{\text{autoactivation}} + \underbrace{\sigma_\gamma \frac{I_\gamma}{\zeta_\gamma + I_\gamma}}_{\text{cytokine signalling}} \right) \underbrace{\left(\frac{1}{1 + T_2/\gamma_2} \right) \left(\frac{1}{1 + T_r/\gamma_3} \right)}_{\text{inhibition}} + \underbrace{\beta_1}_{\text{activation}}, \tag{1}$$

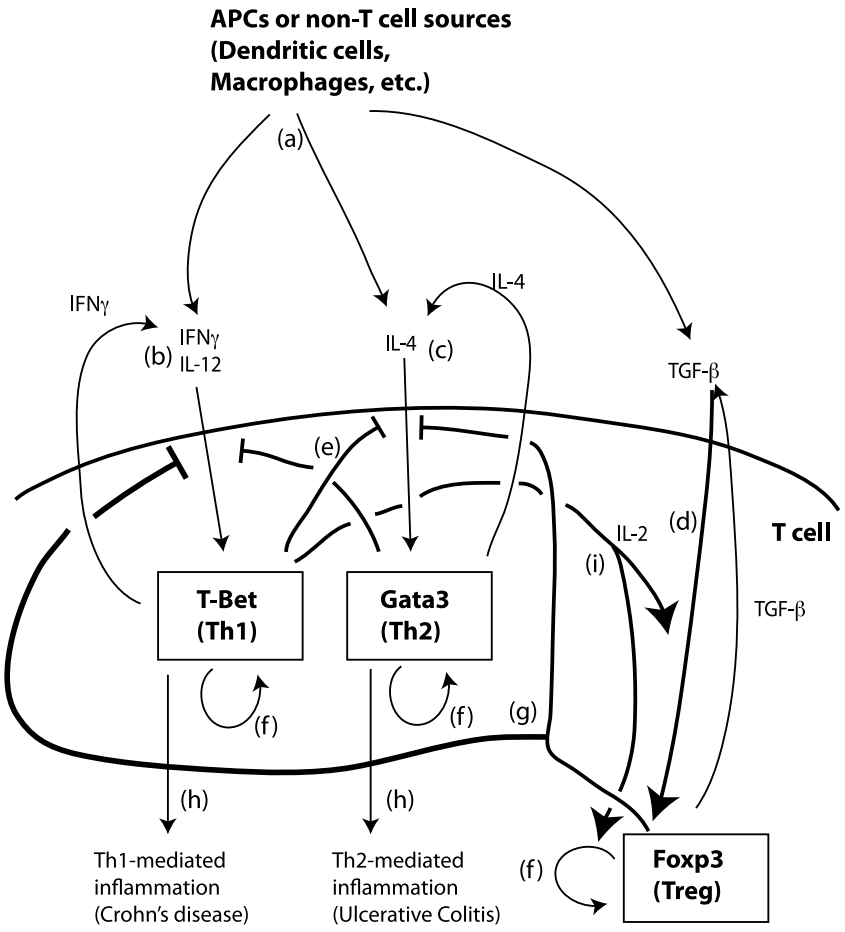

Fig. 1 Schematic diagram of immune system with application to inflammatory bowel disease (IBD). (**a**): Expression of T-Bet (transcription factor for Th1 development), Gata3 (transcription factor for Th2 development) and Foxp3 (transcription factor for Treg development) is initially determined by cytokine signal from APCs or other non-T-cell sources (Yates et al. 2004; Zenewicz et al. 2009). (**b**): IFNγ and IL-12 activates Th1 transcription factor T-Bet (Baumgart and Carding 2007; Yates et al. 2004; Zenewicz et al. 2009) and IFNγ signaling produced by Th1 cells acts as a positive feedback amplification to drive further Th1 differentiation (Maloy and Powrie 2011; Yates et al. 2004). (**c**): IL-4 simulation leads to activation of Th2 transcription factor Gata1 (Baumgart and Carding 2007; Yates et al. 2004; Zenewicz et al. 2009) and Gata3 further drives expression of IL-4, forming a positive feedback loop in Th2 differentiation (Maloy and Powrie 2011; Yates et al. 2004; Zenewicz et al. 2009). (**d**): TGF-β triggers Foxp3 expression (Maloy and Powrie 2011; Zenewicz et al. 2009) and Treg cells express more TGF-β, forming a positive feedback loop in Treg differentiation (Maloy and Powrie 2011; Zenewicz et al. 2009). (**e**): Th1 and Th2 cells suppress each other through pro-inflammatory cytokines they express (Szabo et al. 1997; Yates et al. 2004). (**f**): The autoactivation may occur at the transcriptional level in each transcriptional factor (Yates et al. 2004). (**g**): Treg cells suppress the capacity of Th1 cells, moderately suppress Th2 cells to downregulate the immune system (Stummvoll et al. 2008). (**h**): IBD refers to chronic inflammatory disorders that cover two main clinical forms: Crohn's disease (CD) and ulcerative colitis (UC). The data support that CD is a Th1-mediated inflammatory disease and UC is a Th2-mediated inflammatory disease (Bouma and Strober 2003). (**i**): IL-2 is produced by Th1 cells (Hwang et al. 2005; Osugi et al. 1997) and IL-2 is critical for the development and peripheral expansion of Treg cells (Nelson 2004)

Table 1 Parameters used in the Eqs. (1)–(3)

Parameter	Value	Definition	References
Transcription factor			
μ_1	35 week^{-1}	degradation rate of T-bet	Grogan et al. (2001); Lighvani et al. (2001); Yates et al. (2004)
μ_2	35 week^{-1}	degradation rate of Gata3	Grogan et al. (2001); Lighvani et al. (2001); Yates et al. (2004)
μ_r	232.89 week^{-1}	degradation rate of Foxp3	Lee et al. (2008)
α_1	35 week^{-1}	maximum rate of autoactivation of T-bet	Grogan et al. (2001); Lighvani et al. (2001); Yates et al. (2004)
α_2	35 week^{-1}	maximum rate of autoactivation of Gata3	Grogan et al. (2001); Lighvani et al. (2001); Yates et al. (2004)
α_r	232.89 week^{-1}	maximum rate of autoactivation of Foxp3	Lee et al. (2008); Yates et al. (2004)
σ_γ	35 week^{-1}	maximum rate of INFγ signalling	Grogan et al. (2001); Lighvani et al. (2001); Yates et al. (2004)
σ_4	35 week^{-1}	maximum rate of IL-4 signalling	Grogan et al. (2001); Lighvani et al. (2001); Yates et al. (2004)
σ_β	232.89 week^{-1}	maximum rate of TGF-β signalling	Lee et al. (2008); Yates et al. (2004)
β_1	0.35 week^{-1}	basal activation rate of T-bet	Yates et al. (2004)
β_2	0.35 week^{-1}	basal activation rate of Gata3	Yates et al. (2004)
β_r	0.35 week^{-1}	basal activation rate of Foxp3	Yates et al. (2004)
K_1	1	half-saturation constant for T-bet autoactivation	Yates et al. (2004)
K_2	1	half-saturation constant for Gata3 autoactivation	Yates et al. (2004)
K_r	1	half-saturation constant for Foxp3 autoactivation	Yates et al. (2004)
ζ_γ	1	half-saturation constant for IFNγ signalling	Yates et al. (2004)
ζ_4	1	half-saturation constant for IL-4 signalling	Yates et al. (2004)
ζ_β	1	half-saturation constant for TGF-β signalling	Yates et al. (2004)
γ_1	6	half-saturation constant for T-bet inhibition	this work
γ_2	6	half-saturation constant for Gata3 inhibition	this work
γ_3	2	half-saturation constant for Foxp3 inhibition of T-bet	this work
γ_4	4	half-saturation constant for Foxp3 inhibition of Gata3	this work
n	4	Hill coefficient for autoactivation rate	Yates et al. (2004)
η	0.5	half-saturation constant for IL-2 in Treg cell activation	this work

Table 2 Parameters used in the Eqs. (8)–(12)

Parameter	Value	Definition	References
Cytokines			
ν_1	29.12 week^{-1}	activation rate of IFNγ	this work
ν_2	349.37 week^{-1}	activation rate of IL-4	this work
ν_r	349.37 week^{-1}	activation rate of TGF-β	this work
ν_3	537.46 week^{-1}	activation rate of IL-2	this work
δ_1	29.12 week^{-1}	degradation rate of IFNγ	Grassegger and Hopf (2004)
δ_2	349.37 week^{-1}	degradation rate of IL-4	Conlon et al. (1989–1990)
δ_r	349.37 week^{-1}	degradation rate of TGF-β	Kaminskam et al. (2005)
δ_3	537.46 week^{-1}	degradation rate of IL-2	Rao et al. (2004)
ρ_1/N	0–10	initial synthesis rate of IFNγ	variable
ρ_2/N	0–10	initial synthesis rate of IL-4	variable
ρ_r/N	0–10	initial synthesis rate of TGF-β	variable

$$\frac{dT_2}{dt} = \underbrace{-\mu_2 T_2}_{\text{decay}} + \left(\underbrace{\alpha_2 \frac{T_2^n}{K_2^n + T_2^n}}_{\text{autoactivation}} + \underbrace{\sigma_4 \frac{I_4}{\zeta_4 + I_4}}_{\text{cytokine signalling}} \right) \underbrace{\left(\frac{1}{1 + T_1/\gamma_1} \right) \left(\frac{1}{1 + T_r/\gamma_4} \right)}_{\text{inhibition}} + \underbrace{\beta_2}_{\text{activation}}, \tag{2}$$

$$\frac{dT_r}{dt} = \underbrace{-\mu_r T_r}_{\text{decay}} + \left(\underbrace{\alpha_r \frac{T_r^n}{K_r^n + T_r^n}}_{\text{autoactivation}} + \underbrace{\sigma_\beta \frac{I_\beta}{\zeta_\beta + I_\beta}}_{\text{cytokine signalling}} \right) \underbrace{\frac{I_2}{\eta + I_2}}_{\text{activation by IL-2}} + \underbrace{\beta_r}_{\text{activation}}. \tag{3}$$

Equations (1)–(2) for T-Bet and Gata3 are based on the paper of Yates et al. (2004). We assume that T_r satisfies an equation with similar autoactivation and cytokine signaling, which are activated by IL-2 which is produced by Th1 cells (Hwang et al. 2005; Osugi et al. 1997).

The parameters μ_1, μ_2, μ_r are the decay rate of T-bet, Gata3 and Foxp3, respectively. The autoactivation rate of transcription factor T_i is assumed to be given by the Hill-type function

$$\alpha_i \frac{T_i^n}{K_i^n + T_i^n}. \tag{4}$$

Indeed, autoactivation is a multistep process including at least three independent steps of transcription, translation, and binding to the regulatory sequence of the gene (Hofer et al. 2002). Later we take the Hill exponent $n = 4$; this choice can explain the bistability behavior of Th cell differentiation (Yates et al. 2004).

The activation rate by cytokine signal is

$$\sigma_i \frac{I_i}{\zeta_i + I_i}, \tag{5}$$

where ζ_i represents the level of signaling at which the activation rate reaches its half maximum. In Eqs. (1)–(2), the cross-inhibition between T-Bet and Gata3 and the inhibition of T-bet and Gata3 activations from Foxp3 are represented by the term

$$\frac{1}{1 + T_i/\gamma_i}. \tag{6}$$

Th1 cells are critical in promoting and maintaining the concentration of Treg cells through IL-2 so, in Eq. (3), we model this activation by the term

$$\frac{I_2}{\eta + I_2}, \tag{7}$$

where η represents the level of IL-2 at which the promotion rate is at half maximum.

The last terms of Eqs. (1)–(3) incorporate both the basal activation rate of the transcription factors.

2.3 Cytokines

The dynamics of I_γ, I_4, I_β, and I_2 are given by

$$\frac{dI_\gamma}{dt} = \underbrace{\nu_1 \frac{\rho_1(t) + \int T_1 \Phi dT_1 dT_2 dT_r}{N}}_{\text{production}} - \underbrace{\delta_1 I_\gamma}_{\text{decay}}, \tag{8}$$

$$\frac{dI_4}{dt} = \underbrace{\nu_2 \frac{\rho_2(t) + \int T_2 \Phi dT_1 dT_2 dT_r}{N}}_{\text{production}} - \underbrace{\delta_2 I_4}_{\text{decay}}, \tag{9}$$

$$\frac{dI_\beta}{dt} = \underbrace{\nu_r \frac{\rho_r(t) + \int T_r \Phi dT_1 dT_2 dT_r}{N}}_{\text{production}} - \underbrace{\delta_r I_\beta}_{\text{decay}}, \tag{10}$$

$$\frac{dI_2}{dt} = \underbrace{\nu_3 \frac{\int T_1 \Phi dT_1 dT_2 dT_r}{N}}_{\text{production}} - \underbrace{\delta_3 I_\beta}_{\text{decay}}, \tag{11}$$

where δ_1, δ_2, δ_r, and δ_3 are the decay rates of IFN-γ, IL-4, TGF-β, and IL-2, respectively; the first two equation are based on Yates et al. (2004).

The T-Bet, Gata3, and Foxp3 cell population density $\Phi(T_1, T_2, T_r, t)$ is defined such that the number of cells expressing T-Bet, Gata3, and Foxp3 in the range $[T_1, T_1 + \delta T_1]$, $[T_2, T_2 + \delta T_2]$ and $[T_r, T_r + \delta T_r]$ at the time t is $\Phi(T_1, T_2, T_r, t)\delta T_1 \times \delta T_2 \delta T_r$ for sufficiently small δT_i. Hence, for example, the term $\int T_1 \Phi dT_1 dT_2 dT_r$

represents the total expression of transcription factor T-Bet provided by the entire cell population and N, the total number of the cells, is equal to $\int \Phi dT_1 dT_2 dT_r$.

The inflammatory process is initially induced by the non-T cell stimulations of the cytokines: ρ_1 (IFN-γ), ρ_2 (IL-4) and ρ_r (TGF-β). The production rates of cytokines IFN-γ, IL-4 and TGF-β are given by

$$\nu_i \frac{\rho_i(t) + \int T_i \Phi dT_1 dT_2 dT_r}{N}.$$

The cytokine production have two different sources: non-T cell sources with a rate $\nu_i \rho_i(t)/N$ and T cell sources with a rate $\nu_i \int T_i \Phi dT_1 dT_2 dT_r/N$. They are normalized by the total number of cells. We assume that IL-2 is only produced by Th1 cells, so the production rate of IL-2 is given by

$$\nu_3 \frac{\int T_1 \Phi dT_1 dT_2 dT_r}{N}.$$

2.4 Cell Population Density

The T cell density satisfies the following conservation of mass equation:

$$\frac{\partial \Phi}{\partial t} + \frac{(\partial f_1 \Phi)}{\partial T_1} + \frac{(\partial f_2 \Phi)}{\partial T_2} + \frac{(\partial f_r \Phi)}{\partial T_r} = g\Phi, \tag{12}$$

where $f_1 = \frac{dT_1}{dt}$, $f_2 = \frac{dT_2}{dt}$ and $f_r = \frac{dT_r}{dt}$. The parameter g is the rate at which cells divide. Here, we take $g = 0$, and thus the total number of cells N is a constant.

3 Parameter Estimation

The degradation rate of T-bet and Gata3 is equal to 5 day^{-1} = 35 week^{-1} corresponding to a lifetime 4–5 hours (Grogan et al. 2001; Lighvani et al. 2001; Yates et al. 2004). The half life of Foxp3 is less than 30 minutes (Lee et al. 2008); thus we take a degradation rate of Foxp3 of $\mu_r = \ln(2)/(1/336)$ week$^{-1} \approx 232.89$ week^{-1}. There is no experimental data from which we can infer the values of the other parameters in Eqs. (1)–(3) directly. However, these parameters also appeared in Yates et al. (2004) where it was shown that the model provides the bistability behavior of Th cell differentiation; we shall therefore take the values of the other parameters in Eqs. (1)–(3) the same as in Yates et al. (2004).

The half life of IFNγ is around 4 hours (Grassegger and Hopf 2004); thus we take a degradation rate of IFNγ of $\delta_1 = \ln(2)/(1/42)$ week$^{-1} \approx 29.12$ week^{-1}. The half lives of IL-4 and TGF-β are less than 20 minutes (Conlon et al. 1989–1990; Kaminskam et al. 2005); thus we take $\delta_2 = \delta_r = \ln(2)/(1/504)$ week$^{-1} \approx 349.37$ week^{-1}. The half life of IL-2 is approximately 13 minutes (Rao et al. 2004); thus we take $\delta_3 = \ln(2)/(1/775.385)$ week$^{-1} \approx 537.46$ week^{-1}.

Since our model equations are written in dimensionless form, we can take ν_i equal to δ_i. For the half-saturation constants of the inhibitions among T cells, we assume that the inhibition of Treg cells is larger than the cross-inhibition between Th1 and

Th2 cells so we shall take γ_3 and γ_4 to be smaller than γ_1 and γ_2, as in Gross et al. (2010); Th2 cells are less susceptible than Th1 cells to the inhibition by Treg cells (Cosmi et al. 2004) so γ_3 should be less than γ_4. Accordingly, we take $\gamma_1 = 6$, $\gamma_2 = 6$, $\gamma_3 = 2$, and $\gamma_4 = 4$. The half-saturation constant η for IL-2 activation is taken to be 0.5. In our nondimensional system, the choice of N can be taken to be any number; for computational convenience, we take N =30,000 cells, as in Yates et al. (2004).

4 Simulations

The numerical approach for the following simulations is based on the work by Yates et al. (2004). We use the method of characteristics to reduce Eq. (12) to a set of $C \times 4$ coupled ordinary differential equations for $(\phi^{(i)}, T_1^{(i)}, T_2^{(i)}, T_r^{(i)})$, $i = 1, \ldots, C$, where C is the number of characteristics at the initial state. We apply a standard fourth-order Runge–Kutta algorithm with a temporal step $\Delta t = 0.01$ to solve $C \times 4$ equations of characteristics with four ordinary differential equations (8)–(11) for cytokines. The initial total population N is around 30,000 cells expressing low levels of T_1, T_2, and T_r normally distributed with variance 0.05 over a cube patch of $10 \times 10 \times 10$ characteristics with a spatial difference $\Delta x = 0.01$ centered on the equilibrium levels of these variables in the absence of stimulation from non-T cell sources (non-T cell stimulations), $\rho_1 = \rho_2 = \rho_r = 0$. All the simulations we shall discuss later are performed in a similar way.

The inflammatory process is induced by the non-T cell stimulations of the cytokines: ρ_1, ρ_2, and ρ_r. We assume that after the initial stimulation, ρ_1 and ρ_2 decline exponentially in time: $\rho_1(t) = \rho_{1\max} e^{-t}$ and $\rho_2(t) = \rho_{2\max} e^{-t}$, where $\rho_{1\max} = \rho_{2\max} = 10N$, as shown in Figs. 2A and B; Since the TGF-β response has been shown to be delayed, we assume that ρ_r stimulation is delayed by 3 days, taking $\rho_r(t) = 0$ for $t < 3$ days and $\rho_r(t) = \rho_{r\max} e^{-t+3 \text{ days}}$ for $t \geq 3$ days, where $\rho_{r\max} = 10N$, as shown in Fig. 2C. The levels of initial stimulations and the initial conditions are the same in all the simulations in Figs. 3–5.

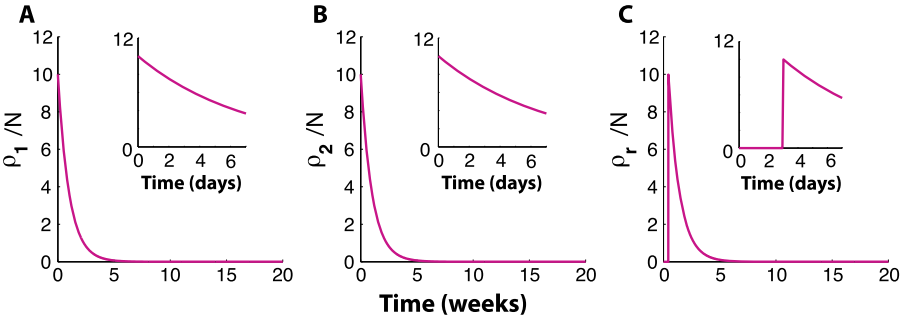

Fig. 2 The non-T cell stimulations of IFN-γ (ρ_1), IL-4 (ρ_2) and TGF-β (ρ_r) as functions of time t. (**A**, **B**) IFN-γ and IL-4 stimulations per cell, ρ_1/N and ρ_2/N, starts at time 0 and the stimulation level decays exponentially with a lifetime 1 week. They can be represented by $\rho_1(t) = \rho_{1\max} e^{-t}$ with $\rho_{1\max} = 10N$ and $\rho_2(t) = \rho_{2\max} e^{-t}$ with $\rho_{2\max} = 10N$ where N is the total number of cells. (**C**) TGF-β stimulation per cell, ρ_r/N, is delayed by 3 days after the stimulations of ρ_1 and ρ_2. We set $\rho_r(t) = 0$ for $t < 3$ days and $\rho_r(t) = \rho_{r\max} e^{-t+3 \text{ days}}$ for $t \geq 3$ days with $\rho_{r\max} = 10N$

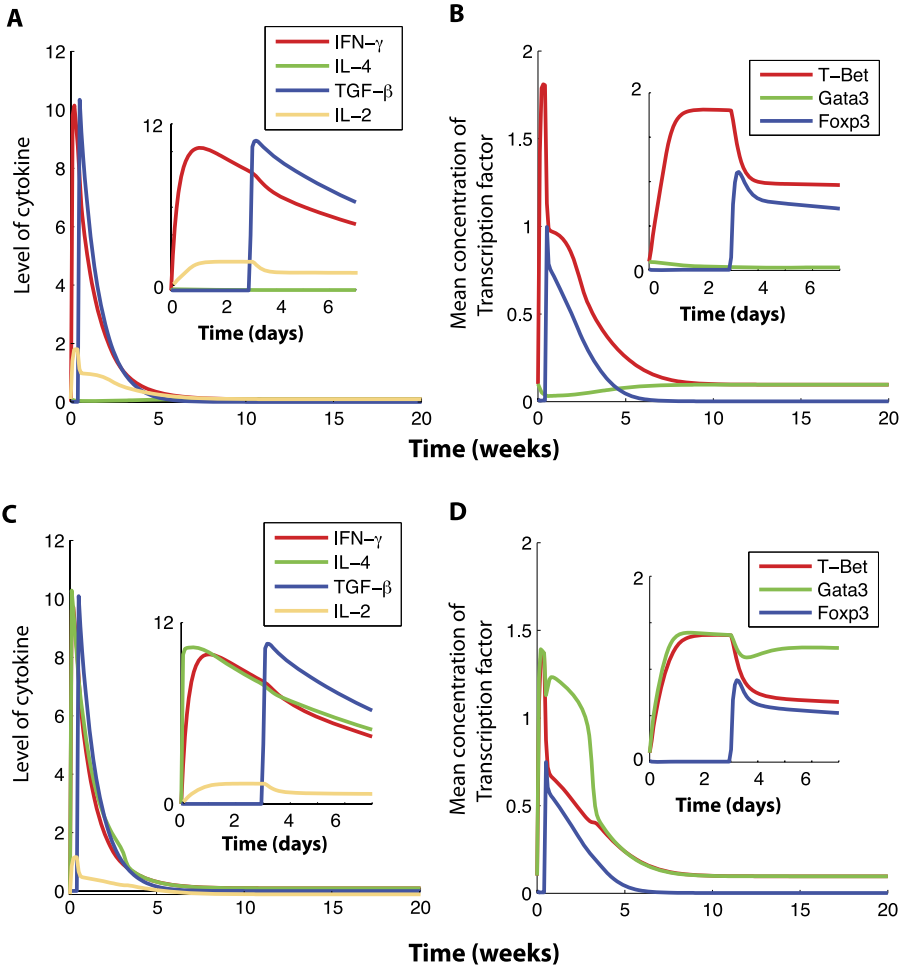

Fig. 3 Normal responses of transcription factors and cytokines. (**A**, **B**) T cells are initially stimulated by only IFN-γ and delayed TGF-β. (**A**) Level of cytokine as a functions of time; the *insert* shows the initial dynamics of cytokines in the first week of the process. (**B**) Level of transcription factor as a function of time; the *insert* shows the initial dynamics of transcription factors in the first week of the process. (**C**, **D**) T cells are initially stimulated by IFN-γ, IL-4 and delayed TGF-β together. (**C**) Level of cytokine as a functions of time; the *insert* shows the initial dynamics of cytokines in the first week of the process. (**D**) Level of transcription factor as a function of time; the *insert* shows the initial dynamics of transcription factors in the first week of the process

In our simulations, we have two types of cytokine stimulation patterns: (1) T cells are initially stimulated by only IFN-γ, followed by delayed TGF-β stimulation, this case corresponds to the stimulation by bacterial infection (Czarniecki and Sonnenfeld 1993); (2) T cells are initially stimulated by IFN-γ and IL-4 together, followed by delayed TGF-β stimulation, this case corresponds to the stimulation by protozoan infection (Robinson et al. 2003).

Figure 3 corresponds to the case of a healthy individual. The simulation in Figs. 3A and B shows the responses of transcription factors and cytokines when the system is stimulated by IFN-γ (ρ_1) and delayed TGF-β (ρ_r). Figure 3A shows the level of cytokine as a functions of time; the insert shows the initial dynamics of cytokines in the first week of the process. Figure 3B shows the level of transcription factor as a function of time; the insert shows the initial dynamics of transcription factors in the first week of the process. In Fig. 3B, T-Bet, transcription factor of Th1 cell, increases significantly in the first few days and goes back to the "normal" level after 10 weeks; Foxp3, transcription factor of Treg cell, has similar time evolution with T-Bet but the initial response is delayed by 3 days; Gata3, transcription factor of Th2 cell, does not change much during the process because there is no non-T cell stimulation by IL-4. The simulation in Figs. 3C and D demonstrates another case when T cells are stimulated by IFN-γ (ρ_1) and IL-4 (ρ_2) and delayed TGF-β (ρ_r) together. In this case, the dynamics of T-Bet and Foxp3 are similar to that in Fig. 3B; Gata3 increases in the first few days and return back to the "normal" level after 10 weeks.

In Figs. 4 and 5, we use our model to explain pathological cases, which are observed in IBD, by modifying some of the parameters, which control the production of the cytokines. Whether the change in these parameters is the actual cause for IBD should be considered as a hypothesis that needs to be verified clinically.

Figure 4 displays two cases of abnormal response when the inhibition of Th1 and Th2 cells by Treg cells is downregulated (by increasing γ_3 and γ_4 by a factor of 2.5). In Fig. 4, we use same combinations of cytokine stimulation used in Fig. 3 but the time evolutions of transcription factors and cytokines are completely different.

The simulation in Figs. 4A and B shows the abnormal responses of transcription factors and cytokines when the system is stimulated by IFN-γ (ρ_1) and delayed TGF-β (ρ_r). Figure 4B shows the level of T-Bet remains high after 20 weeks and a continuous high level of T-Bet will drive a chronic inflammatory responses. The simulation in Figs. 4C and D demonstrates another abnormal response when T cells are stimulated by IFN-γ (ρ_1) and IL-4 (ρ_2) and delayed TGF-β (ρ_r) together. Figure 4D shows the levels of T-Bet and Foxp3 return back to their "normal" level after 10 weeks, but Gata3 level remains high after 20 weeks. This response corresponds to a chronic allergic inflammation. We also get similar results (not shown here) when, instead of reducing the inhibition of Th1 cells and Th2 cells by Treg cells, either the autoactivation or cytokine signaling is upregulated by increasing α_i or σ_i by 50 %.

In the summary for Fig. 4, when the inhibition of Th1 and Th2 cells by Treg cells is downregulated, the non-T cell stimulations shown in Fig. 2 induce a continuous high level response of Th1 cells (T-Bet) or Th2 cells (Gata3).

Figure 5 displays two cases of abnormal response with three kinds of mutations: (1) when the inhibition of Th1 and Th2 cells by Treg cells is upregulated (by decreasing γ_3 and γ_4 from $\gamma_3 = 2$, $\gamma_4 = 4$ to $\gamma_3 = 0.4$, $\gamma_4 = 0.8$); (2) when the delay of TGF-β stimulation is neglected; (3) when the autocatalytic activities of Th1, Th2, and Treg cells are significantly increased (by decreasing the half maximal effective concentrations, K_1, K_2, and K_r, of autoactivations from 1 to 0.3).

The simulation in Figs. 5A and B shows the abnormal responses of transcription factors and cytokines when the system is stimulated by IFN-γ (ρ_1) and delayed TGF-β (ρ_r). Figure 5B shows the levels of T-Bet and Foxp3 arise to some intermediate level, which is significantly lower than the maximum of normal response (see

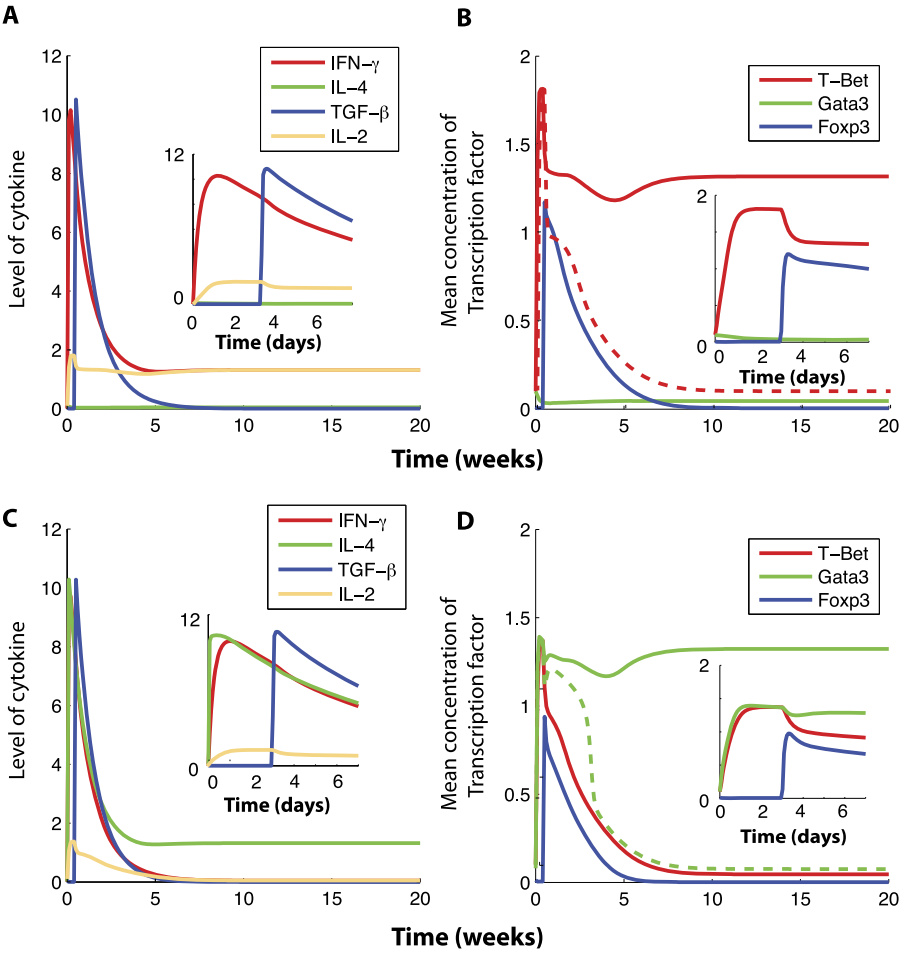

Fig. 4 Abnormal responses of transcription factors and cytokines during inflammation when the inhibition of Th1 and Th2 cells by Treg cells is downregulated (by increasing γ_3 and γ_4 by a factor of 2.5). (**A**, **B**) T cells are initially stimulated by only IFN-γ and delayed TGF-β. (**A**) Level of cytokine as a functions of time; the *insert* shows the initial dynamics of cytokines in the first week of the process. (**B**) Level of transcription factor as a function of time; the *insert* shows the initial dynamics of transcription factors in the first week of the process. The *dashed red line* denotes the normal response of T-Bet in a healthy individual. (**C**, **D**) T cells are initially stimulated by IFN-γ, IL-4 and delayed TGF-β together. (**C**) Level of cytokine as a function of time; the *insert* shows the initial dynamics of cytokines in the first week of the process. (**D**) Level of transcription factor as a function of time; the *insert* shows the initial dynamics of transcription factors in the first week of the process. The *dashed green line* denotes the normal response of Gata3 in a healthy individual

Fig. 3) and remain at that level after 20 weeks. The simulation in Figs. 5C and D demonstrates another abnormal response when T cells are stimulated by IFN-γ (ρ_1) and IL-4 (ρ_2) and delayed TGF-β (ρ_r) together. Figure 5D shows all three transcription factors arise to some intermediate level and remain at that level after 20 weeks. Similar results are observed (not shown here) when, instead of increasing the inhibi-

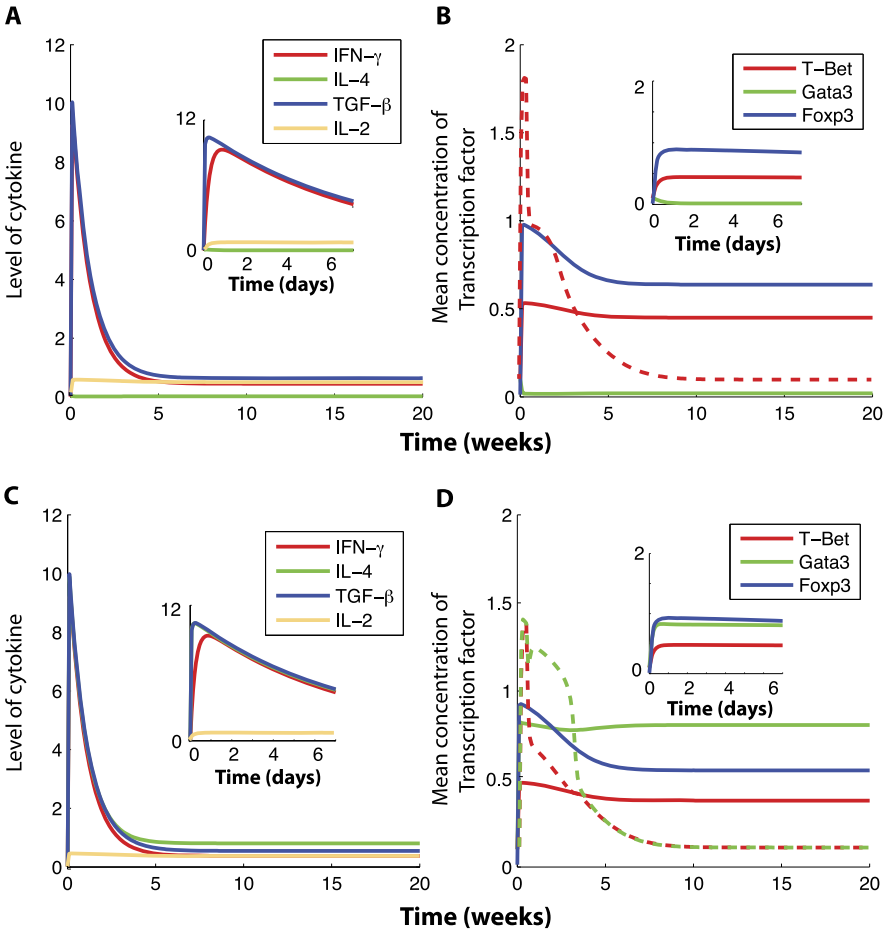

Fig. 5 Abnormal responses of transcription factors and cytokines during inflammation when the inhibition of Th1 and Th2 cells by Treg cells is upregulated (by decreasing γ_3 and γ_4 from $\gamma_3 = 2$, $\gamma_4 = 4$ to $\gamma_3 = 0.4$, $\gamma_4 = 0.8$), and when the delay of TGF-β stimulation is neglected, and furthermore, when the autocatalytic activities of Th1, Th2, and Treg cells are significantly increased (by decreasing the half maximal effective concentrations, K_1, K_2, and K_r, of autoactivations from 1 to 0.3). (**A**, **B**) T cells are initially stimulated by only IFN-γ and delayed TGF-β. (**A**) Level of cytokine as a functions of time; the *insert* shows the initial dynamics of cytokines in the first week of the process. (**B**) Level of transcription factor as a function of time; the *insert* shows the initial dynamics of transcription factors in the first week of the process. The *dashed red line* denotes the normal response of T-Bet in a healthy individual. (**C**, **D**) T cells are initially stimulated by IFN-γ, IL-4 and delayed TGF-β together. (**C**) Level of cytokine as a functions of time; the *insert* shows the initial dynamics of cytokines in the first week of the process. (**D**) Level of transcription factor as a function of time; the *insert* shows the initial dynamics of transcription factors in the first week of the process. The *dashed red* and *green lines* denote the normal responses of T-Bet and Gata3, respectively, in a healthy individual

tion by Treg cells, the inhibition of Th1 cells by Th2 cells is increased (γ_2 is decreased from 6 to 2), but the Th2 cell response in this case is larger than that in Fig. 5B.

In the summary for Fig. 5, when the inhibition of Th1 and Th2 cells by Treg cells is upregulated, and when the delay of TGF-β stimulation is neglected, and further-

more, when the autocatalytic activities of Th1, Th2, and Treg cells are significantly increased, T cell responses arise to some intermediate level and remain at that level for long time.

5 Conclusion and Discussion

Gut mucosal homeostasis depends on complex interactions among the microbiota, the intestinal epithelium, and the gut associated immune system. A breakdown in some of these interactions may precipitate inflammation. Inflammatory bowel diseases, Crohn's disease (CD), and Ulcerative Colitis (UC) are chronic inflammatory disorders of the gastrointestinal tract. The initial stages of disease are marked by an abnormally high level of pro-inflammatory helper T cells, Th1, and Th17. In later stages, Th2 and Th17 helper cells may dominate while the Th1 response may dampen. Th1 and Th17 cells secrete inflammatory cytokines, including IFNγ, IL-12, TNF-α, and IL-17, whereas Th2 cells secrete cytokines like IL-4, IL5 and IL-13 (Bamias et al. 2011; Pak et al. 2012). The interactions among the T cells include the regulatory T cells (Treg), which normally prevent unrestricted proliferation of Th1, Th2, and Th17 cells. Regulatory T cells exert their suppressive effects though cell-cell contact and secretion of anti-inflammatory cytokines IL-10 and TGF-β (Sakaguchi et al. 2009).

A general mathematical model of IBD based on a complex network of cells and genes was introduced by Wendelsdorf et al. (2010). In the present paper, we introduce a different and simpler model which focuses on the balance between Th1, Th2, and regulatory T cells. Our aim is to show that when the relative balance among these cells is altered, homeostasis is disrupted. Consequently, we can have various permutations where either Th1 or Th2 response is dominant with variable degrees of regulatory T cell contribution. Thus, the model is capable of demonstrating the biologically observed levels of Th1 cells in IBD patients. Inflammatory diseases are relapsing-remitting chronic intestinal conditions. The periods of acute flares and remission are quantitatively characterized by different profiles of Th1-Th2-Treg (Kato et al. 2011; Maul et al. 2005). IBD patients with an acute flare have a heightened Th1 response and respond well to the blockade of TNF-α, a Th1 cytokine. A new balance is achieved by effectively lowering the Th1 (TNF-α, IFNγ) and Th2 (IL-13) while the Treg response (IL-10, TGF-β) is increased (Babyatsky et al. 1996; Mitsuyama et al. 2006). Thus, endogenous (secreted) or exogenous (pharmacologic) input of cytokines can lead to various steady states of the host and determine the clinical phenotype. Currently, there are no qualitative or quantitative mathematical models that allow us to predict the effects of immunomodulatory treatments on disease course and activity.

We developed a mathematical model based on differential equations with terms nonlocal in the space spanned by the concentrations of cytokines. Our system includes the interactions among Th1, Th2, and Treg cells and cytokines, which polarize them or which whom they secrete. It is known that these three classes of T cells have inhibitory effect on one another. Our model simulations of an acute inflammatory state demonstrate two abnormal T cell responses:

(1) when the Treg inhibitions are downregulated, the Th1 or Th2 response to cytokines produced by macrophages and dendritic cells (in response to commensal flora) is abnormally high (causing excessive inflammation);
(2) when the Treg inhibitions are upregulated, the Th1 and Th2 cell responses are abnormally low (thus not countering effectively the bacterial infection).

Our model aims to provide a conceptual framework as a basis for future investigations. For simplicity, we have not included Th17 cells (whose pathogenic role is similar to that of Th1 cells) and several inflammatory cytokines in addition to IFNγ. Furthermore, the model accounts for the contributions of macrophages and dendritic cells only tacitly, through some of the signaling molecules they secrete. Nonetheless, our model is broad enough to produce the results established in clinical data. Future work will include the contribution of therapies that block the activity of various endogenous cytokines, and the validity of the simulation model will then be tested through prospective studies that record data on T cell responses before and after medical treatment. This approach will allow us to adjust the model parameters, in order to develop an *in-silico* tool for disease prognosis and treatment decisions.

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