The simulation of Th1-Th2 cell differentiation by system dynamic model and stochastic processes

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Abstract

In this article, we use system dynamical model (base on a set of nonlinear differential equations) and stochastic process model (Master equations of elementary reaction) to simulate the T helper cell differentiation (including Th1-Th2-Th17-Treg), especially the Th1-Th2 differentiations signaling network. We draw a conclusion that the critical trigger factor of naive T helper cells differentiation is the concentration of differentiation induced cytokines. We also compare the result of these two models as well as discussed the advantage and disadvantage of them.

Introduction

Mathematical modeling is a widely used method to simulate the intracellular transduction networks of different cells. For the differentiation of cells of immune system, many scholars have made achievements by different mathematical methods [1,2,3,4,5], system dynamic model is the most popular method in the field of computational biology, especially for the simulation of cell differentiation behavior[6,7]. Hofer et.al simulate transcriptional pathway of Th2 cell by a mathematical model, he points out that the GATA-3 is a critical gene in differentiation of in Th2 lymphocytes [8]. Bergmann et.al construct a system dynamic model to simulate the immune response of T helper cells [9]. Gilchrist et.al also build this model to define a key factor (NF-κB) in transcription network of T lymphocyte [7]. Mendoza introduce a Boolean network system model to describe the whole intracellular gene network transduction pathway, taking into account of IFN-gamma, IL-4, IL-12, IL-18, IFN-beta, IL-4R, IL-12R, IL-18R, STAT-1, STAT-6, STAT-4, IRAK, SOCS-1, GATA-3 and other gene loci, and calculate the stable steady states of the Th cell differentiation network[10]. Wang et.al build a novel time lag system dynamic model to simulate the differentiation and re-differentiation behavior of

Th1-Th2 lymphocytes and they draw a very important conclusion that the differentiation potential disappeared when the model derived to equilibrium points, it is the critical point of the differentiation process. In the field of differ there is another. [11] We are going to follow the pattern shown by this article to calculate the critical points of T helper cell differentiation in a bigger transduction network which including Th1, Th2, T17, and T reg cells.

There is another important paper which highly influenced our research. A stochastic model made by Siminovitch et.al, simulating the proliferation of stem cells, show us a new way to explain the small amount of abnormal differentiation cells which could not be describe by the model made by system biology models. [12] Stochastic process model is used to represent the evolution of some random system over time. There are several (often infinitely many) directions in which the process may evolve. Scientists use a collection of random variables to analysis the intraocular transduction networks and simulate the behavior of different cells [12,13,14,15,16].

Although there are many scientists use different mathematical models to simulate T helper cell differentiation process, there is no researcher used stochastic process model to simulate T helper cell differentiation process before. For the first time, we use both system biology model and stochastic process model to simulate the T helper cell differentiation, compared the results of these two models, and analysis the advantage and disadvantage of these models.
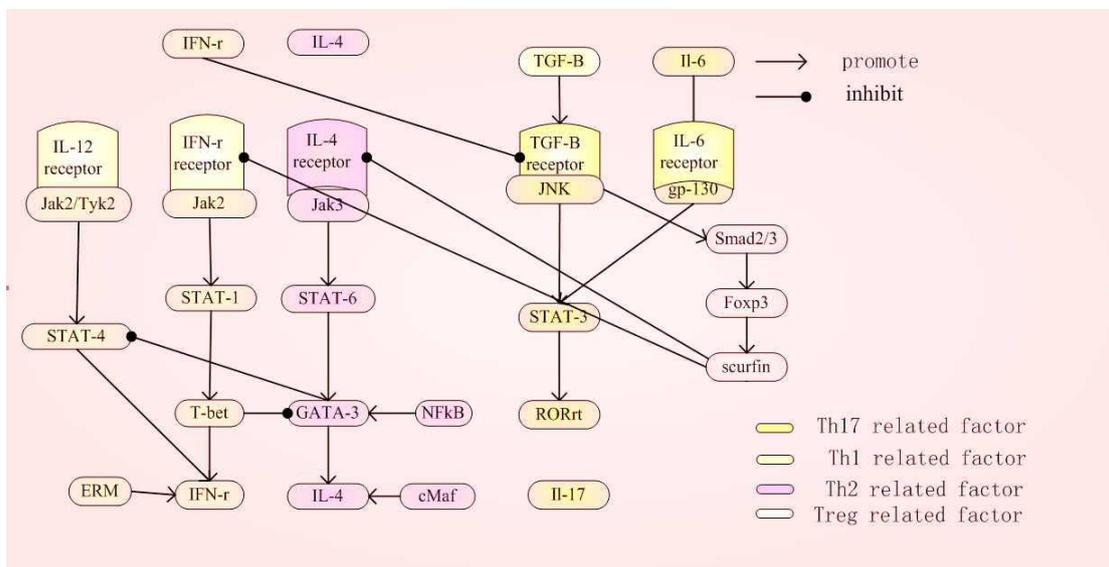

Figure 1. Transduction Pathway of Th1-Th2-T17-Treg

Mathematic model

System dynamic model:
System dynamic model is a very important method in simulation of intracellular network, In the first part of our paper, we follow the method of Wang et.al to simulate

the intracellular network of Th cells and discuss the behavior of cell differentiation.[10]

We assume that gene could be transcribed many times in one time iteration. While inhibit factor is located in a certain gene loci, this gene is unable to be transcribed. For example, the influence of GATA-3 to T-bet gene, can be changed by the concentration of STAT-1. While the concentration of STAT-1is low, GATA-3 tend to combine to T-bet gene loci, and when the concentration of STAT-1is high, GATA-3 have less influence to T-bet gene. We assume the total number of receptors keep consistent and the intracellular factor concentration of STAT family protein cannot be influenced by others factors.

$[A]_T$ is the concentration of intracellular factor A produced by T helper cell, $[A]_C$ is the concentration of extracellular factor A produced by other cells except T helper cell

$$[A] = [A]_C + [A]_T$$

1．IFN-gamma transcription process
(1)STAT-4a active process：

$$[IL\text{-}12] = [IL\text{-}12]_C$$

$[IL\text{-}12]_C$ is the concentration of extracellular factor IL-2 produced by other cells except T helper cell.

$$\frac{d[STAT\text{-}4a]}{dt} = k_{IIS4}[IL\text{-}12] \times [IL\text{-}12re] \times [STAT\text{-}4u] - r_{S4}[STAT\text{-}4a]$$

STAT-1a active process：

$$[IFN\text{-}\gamma] = [IFN\text{-}\gamma]_T + [IFN\text{-}\gamma]_C$$

$[IFN\text{-}\gamma]_T$ is the concentration of IFN-gamma produced by a T-helper cell,

$[IFN\text{-}\gamma]_C$ is the concentration of extracellular factor IFN-gamma produced by other cells except T helper cell.

$$\frac{d[STAT\text{-}1a]}{dt} = k_{FS1}[IFN\text{-}\gamma] \times [IFN\text{-}\gamma re] \times [STAT\text{-}1u] - r_{S1}[STAT\text{-}1a]$$

(2)T-bet gene transcription process
The increase of activated STAT-1 concentration has a positive impact to the length of transcription time of T-bet gene. When T-bet gene remodeling is near complete, the transcription of T-bet gene is faster, when the STAT-1 and T-bet gene fully combined.

$$\frac{d[T\text{-}bet]}{dt} = \frac{k_{S1v} \times [STAT\text{-}1a]}{k_{S1m} + [STAT\text{-}1a]} \times \frac{k_{1G3}}{k_{1G3} + [GATA\text{-}3]} - r_{Tb}[T\text{-}bet]$$

(3)IFN-gamma gene transcription process:

$$\frac{d[IFN\text{-}\gamma]_T}{dt} = \frac{k_{TFv} \times [T\text{-}bet]}{k_{TFm} + [T\text{-}bet]} \frac{k_{S4v} \times [STAT\text{-}4a]}{k_{S4m} + [STAT\text{-}4a]} - r_{IN}[IFN\text{-}\gamma]_T$$

2．IL-4 gene transcription process
(1)STAT-6a active process

$$[IL\text{-}4] = [IL\text{-}4]_T + [IL\text{-}4]_C$$

$[IL\text{-}4]_T$ is the concentration of IL-4 produced by a T-helper cell $[IL\text{-}4]_C$ is the concentration of extracellular factor IL-4 produced by other cells except T helper cell.

$$\frac{d[STAT\text{-}6a]}{dt} = k_{L4S6}[IL\text{-}4] \times [IL\text{-}4re] \times [STAT\text{-}6u] - r_{S6}[STAT\text{-}6a]$$

(2)GATA-3 gene transcription process:

$$\frac{d[GATA\text{-}3]}{dt} = \frac{k_{S6m} \times [STAT\text{-}6a]}{k_{S6v} + [STAT\text{-}6a]} \times \frac{k_{1T}}{k_{1T} + [T\text{-}bet]} - r_{G3}[GATA\text{-}3]$$

(3)IL-4gene transcription process:

$$\frac{d[IL\text{-}4]_T}{dt} = \frac{k_{GL4m} \times [GATA\text{-}3]}{k_{GL4v} + [GATA\text{-}3]} - r_{I4}[IL\text{-}4]_T$$

Stochastic process model (Master equation elementary reaction):
There are several important ways of DNA remodeling: DNA methylation, histone acetylation (increased transcription), histone methylation (reduced transcription). Histone acetylation and methylation adjust gene transcription speed by change gene accessibility. Remodeling is mainly regulated by the transcription factor.

1．The produce of IFN-gamma
This section is about STAT-4 protein activation and nuclear translocation process, assuming the number of intracellular STAT-4 factor and the number of IL-12 receptor keep constantly. IL-12re denote the number of IL-12 receptors (XX cytokine receptors abbreviated as XXre), STAT-4u denote the number of inactivated STAT-4. STAT-4a denotes the number of activated STAT-4:

$$IL\text{-}12 + IL\text{-}12re \underset{k_{-LLl}}{\overset{k_{LLl}}{\rightleftharpoons}} IL\text{-}12IL\text{-}12re$$

$$IL\text{-}12IL\text{-}12re + STAT\text{-}4u \xrightarrow{k_{S4}} IL\text{-}12IL\text{-}121re + STAT\text{-}4a$$
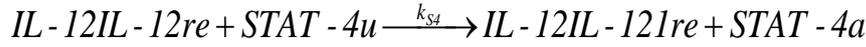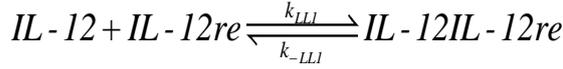
These two equations represent STAT-1 protein activation process and nuclear translocation process, assuming the intracellular STAT-1 receptor protein and the number of IFN-g constant, STAT-1u represents an activated STAT-1, STAT-1a represents the activation of STAT-1. We assume that nuclear pore complex operation is very efficiency. The transcription factor in the nucleus is the same with concentration of this factor outside of the nucleus.

$$IFN\text{-}\gamma + IFN\text{-}\gamma re \underset{k_{-FF}}{\overset{k_{FF}}{\rightleftharpoons}} IFN\text{-}\gamma IFN\text{-}\gamma re$$

$$IFN\text{-}\gamma IFN\text{-}\gamma re + STAT\text{-}1u \xrightarrow{k_{S1}} IFN\text{-}\gamma IFN\text{-}\gamma re + STAT\text{-}1a$$
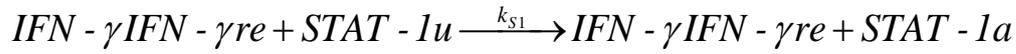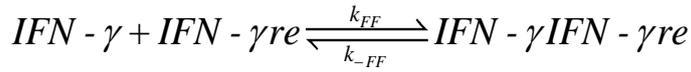
The following equations show the transcription and translation process of T-bet. STAT-1 protein triggers T-bet gene remodeling:

$$STAT\text{-}1a + T\text{-}betgene \underset{k_{-ST}}{\overset{k_{ST}}{\rightleftharpoons}} STAT\text{-}1T\text{-}betgene$$

$$STAT\text{-}1T\text{-}betgene \xrightarrow{k_{1m}} STAT\text{-}1T\text{-}betgene + T\text{-}betmRNA$$

$$T\text{-}betmRNA \xrightarrow{k_{TR}} T\text{-}bet$$
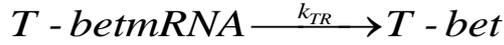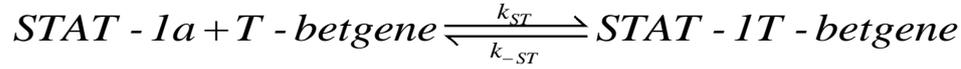
STAT-1inactivite with a certain probability after iteration, it changes into the inactivated STAT-1again and leave nucleus.

$$STAT\text{-}1a \xrightarrow{r_{s1}} STAT\text{-}1u$$
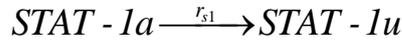
T-bet mRNA and T-bet degrade in a certain probability:

$$T\text{-}betmRNA \xrightarrow{r_T} \phi$$

$$T\text{-}bet \xrightarrow{r_{Tm}} \phi$$
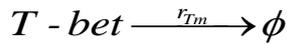
The transcription of IFN-gamma can be describe as: there are two kind of proteins can directly combined to the IFN-gamma gene loci, these two factors will cause different degrees of genetic remodeling, T-bet and STAT-4 can be combined to IFN-gamma gene loci at the same time. There is a synergistic effect of gene remodeling when both T-bet and STAT-4 factor combined with gene loci at the same time:

$$T\text{-}bet + IFN\text{-}\gamma gene \underset{k_{-TF}}{\overset{k_{TF}}{\rightleftharpoons}} T\text{-}betIFN\text{-}\gamma gene$$

$$STAT\text{-}4a + IFN\text{-}\gamma gene \underset{k_{-S4F}}{\overset{k_{S4F}}{\rightleftharpoons}} STAT\text{-}4aIFN\text{-}\gamma gene$$

$$T\text{-}bet + STAT\text{-}4aIFN\text{-}\gamma gene \underset{k_{-TS4F}}{\overset{k_{TS4F}}{\rightleftharpoons}} T\text{-}betSTAT\text{-}4IFN\text{-}\gamma gene$$

$$STAT\text{-}4a + T\text{-}betIFN\text{-}\gamma gene \underset{k_{-S4TF}}{\overset{k_{S4TF}}{\rightleftharpoons}} T\text{-}betSTAT\text{-}4IFN\text{-}\gamma gene$$
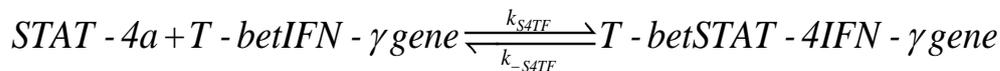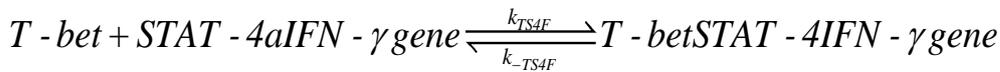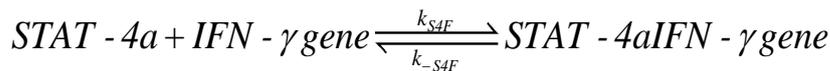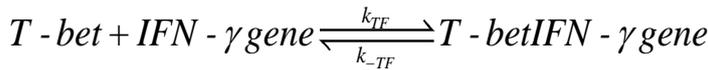
STAT-4 inactivates with a certain probability after iteration it changes into the

inactivated STAT-4 again and leave nucleus.

$$STAT\text{-}4a \xrightarrow{r_{S4}} STAT\text{-}4u$$
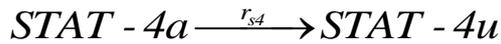
IFN-g transcription speed is different when combining with different transcription factors (different remodeling level infers to different transcription speeds):

$$T\text{-}betIFN\text{-}\gamma gene \xrightarrow{k_{TFm}} T\text{-}betIFN\text{-}\gamma gene + IFN\text{-}\gamma mRNA$$

$$STAT\text{-}4IFN\text{-}\gamma gene \xrightarrow{k_{S4Fm}} STAT\text{-}4IFN\text{-}\gamma gene + IFN\text{-}\gamma mRNA$$

$$T\text{-}betSTAT\text{-}4IFN\text{-}\gamma gene \xrightarrow{k_{TS4Fm}} T\text{-}betSTAT\text{-}4IFN\text{-}\gamma gene + IFN\text{-}\gamma mRNA$$
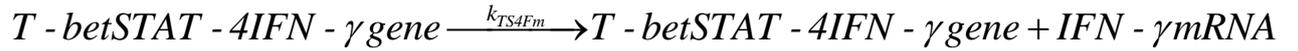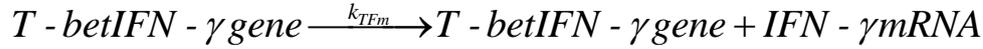
INF-gamma mRNA enters the ribosome, trigger the translation process, and produce INF-gamma:

$$IFN\text{-}\gamma mRNA \xrightarrow{k_{TR}} IFN\text{-}\gamma$$
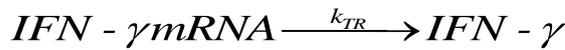
IFN-gamma and its mRNA inactivates with a certain probability after iteration
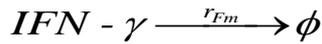
$$IFN\text{-}\gamma mRNA \xrightarrow{r_F} \phi$$

$$IFN\text{-}\gamma \xrightarrow{r_{Fm}} \phi$$

Although the binding site of theT-bet and GATA-3 still remain unknown, theyhave a negative impact on each other on the transcription process (Wang etal). We assume that the gene could not bind with other transcription factor after binding with a transcription factor. The symbol Tg denote the T-bet gene.

$$GATA\text{-}3 + T\text{-}betgene \underset{k_{-GT}}{\overset{k_{GT}}{\rightleftharpoons}} GATA\text{-}3T\text{-}betgene$$
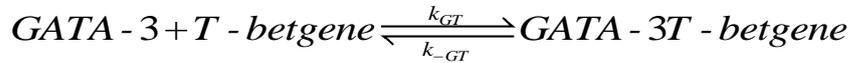
2．The production of IL-4

This section is about STAT-6 protein activation and nuclear translocation process, assuming the number of intracellular STAT-6 factor and the number of IL-4 receptor keep constantly. IL-4re denote the number of IL-4 receptors (XX cytokine receptors abbreviated as XXre), STAT-4u denote the number of inactivated STAT-6. STAT-6a denotes the number of activated STAT-6

$$IL\text{-}4 + IL\text{-}4re \underset{k_{-LL4}}{\overset{k_{LL4}}{\rightleftharpoons}} IL\text{-}4IL\text{-}4re$$

$$IL\text{-}4IL\text{-}4re + STAT\text{-}6u \xrightarrow{k_{S6}} IL\text{-}4IL\text{-}4re + STAT\text{-}6a$$
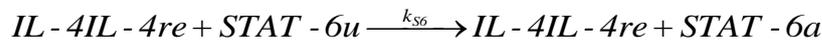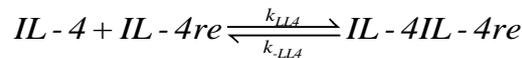
The following equations show the transcription and translation process of GATA-3. STAT-6 protein triggers GATA-3 gene remodeling:

$$STAT\text{-}6a + GATA\text{-}3gene \underset{k_{-SG}}{\overset{k_{SG}}{\rightleftharpoons}} STAT\text{-}6GATA\text{-}3gene$$

$$STAT\text{-}6GATA\text{-}3gene \xrightarrow{k_{6m}} STAT\text{-}6GATA\text{-}3gene + GATA\text{-}3mRNA$$

$$GATA\text{-}3mRNA \xrightarrow{k_{GR}} GATA\text{-}3$$

$$STAT\text{-}6a \xrightarrow{r_{s6}} STAT\text{-}6u$$
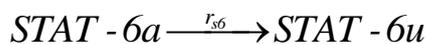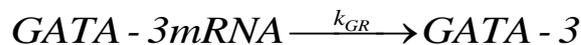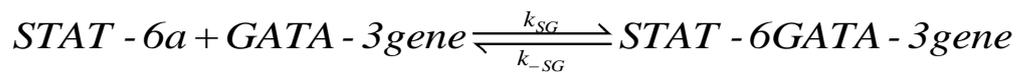
STAT-6 inactivates with a certain probability after iteration it changes into the inactivated STAT-6 again and leave nucleus.

$$GATA\text{-}3mRNA \xrightarrow{\ r_G\ } \phi$$

$$GATA\text{-}3 \xrightarrow{\ r_{Gm}\ } \phi$$
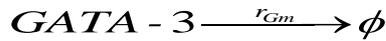
The transcription of IL-4, GATA-3triger the IL-4 remodeling of IL-4 gene

$$GATA\text{-}3 + IL\text{-}4gene \underset{k_{-GL}}{\overset{k_{GL}}{\rightleftharpoons}} GATA\text{-}3IL\text{-}4gene$$

$$GATA\text{-}3IL\text{-}4gene \xrightarrow{\ k_{GLm}\ } GATA\text{-}3IL\text{-}4gene + IL\text{-}4mRNA$$
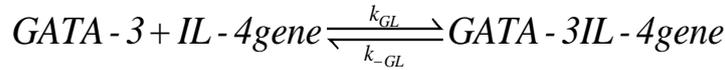
IL-4 mRNA enters the ribosome, trigger the translation process, and produce IL-4:

$$IL\text{-}4mRNA \xrightarrow{\ k_{LR}\ } IL\text{-}4$$

IL-4 and the mRNA of IL-4 degrade in a certain probability:

$$IL\text{-}4mRNA \xrightarrow{\ r_L\ } \phi$$

$$IL\text{-}4 \xrightarrow{\ r_{Lm}\ } \phi$$

Although the binding site of the T-bet and GATA-3 still remain unknown, they have a negative impact on each other on the transcription process (Wang etal). We assume that the gene could not bind with other transcription factor after binding with a transcription factor. The Gg denote the GATA-3 gene.

$$T\text{-}bet + GATA\text{-}3gene \underset{k_{-TG}}{\overset{k_{TG}}{\rightleftharpoons}} T\text{-}betGATA\text{-}3gene \ \text{d}$$
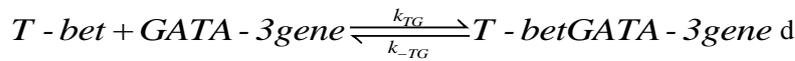
Results and discussion

System dynamic model

We assume that f_{Th1} represent the influence of Th1 induced cytokine to naive T helper cell as well as f_{Th2} represent the influence of Th2 induced cytokine to naive T helper cell. k_{I2}, k_{IF} and k_{I4} denote the influence of certain cytokines to the differentiation process.

$$f_{Th1} = k_{I2}[IL\text{-}12] + k_{IF}[IFN\text{-}\gamma]$$

$$f_{Th2} = k_{I4}[IL\text{-}4]$$

$$\Rightarrow f_{Th1} = k_{I2}[IL\text{-}12]_C + k_{IF}[IFN\text{-}\gamma]_C + k_{I2}[IL\text{-}12]_T + k_{IF}[IFN\text{-}\gamma]_T$$

$$f_{Th2} = k_{I4}[IL\text{-}4]_C + k_{I4}[IL\text{-}4]_T$$

Because of the concentration of IFN-gamma and IL-4 include the amount of the

cytokine outside of the cell, so we choice the critical transcription factor GATA-3 and T-bet to show the differentiation process in the following section.

System dynamic model:

In this model, $[IL\text{-}12]_C$, $[IFN\text{-}\gamma]_C$, $[IL\text{-}4]_C$ is consistent numbers while the value

of $[IL\text{-}12]_T$, $[IFN\text{-}\gamma]_T$, $[IL\text{-}4]_T$ can be changed by time or degree of differentiation.

1. The simulation of un-differentiation cell

There are a small amount of cytokines in extracellular environment such as IL-12, IFN-gamma, IL-4 secreted by dendritic cells, monocytes and macrophages when the Th0 cells remain undifferentiated. In this stage, Th0 cells expressing low level IL-4 and IFN-gamma.

2. The simulation of differentiated cell (Th1)

If the concentration of Th1 cytokines satisfy the inequality $f_{Th1} > f_{Th2}$, the IL-12，the

Th1 cytokines such as IFN-gamma and IL-12 have a stronger impact than Th1 cytokines, the Th0 cells will increase the expression level of IFN-gamma, form a positive looping, Th0 cell stimulated by Th1 cytokine will differentiate toward Th1 stage.
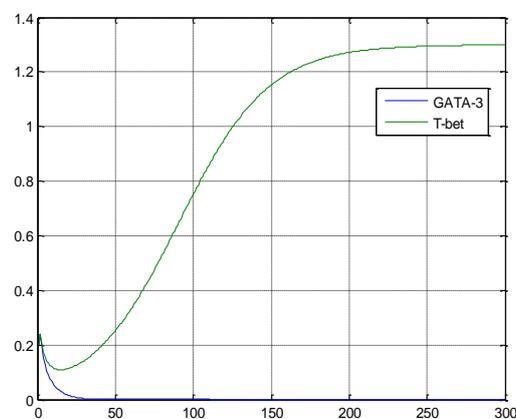

Figure 2. Differentiation process of Th1 cell by system dynamic model

In the differentiation process, if put the half differentiated Th1 cell on the Th1-inducing environment, or Th1, Th1 cytokines in the local concentration of sudden changes, there will be two possible cases :

(1) If the differentiation level of T helper cell is higher than a certain threshold, the concentration of Th1 cytokines still satisfy the inequality $f_{Th1} > f_{Th2}$, the direction of differentiation will not change.

$$f_{Th1} > f_{Th2} \Rightarrow k_{I2}\left[IL\text{-}12\right]_C + k_{IF}\left[IFN\text{-}\gamma\right]_C + k_{IF}\left[IFN\text{-}\gamma\right]_T > k_{I4}\left[IL\text{-}4\right]_C + k_{I4}\left[IL\text{-}4\right]_T$$

$$\Rightarrow \quad k_{IF}\left[IFN\text{-}\gamma\right]_T - k_{I4}\left[IL\text{-}4\right]_T > k_{I4}\left[IL\text{-}4\right]_C - k_{I2}\left[IL\text{-}12\right]_C - k_{IF}\left[IFN\text{-}\gamma\right]_C$$
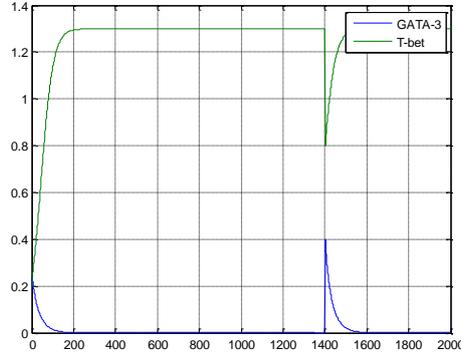
Figure 3. Th2 induced cytokines do not change the differentiation of Th1 cell in late phase by system dynamic model

(2) If the differentiation level of T helper cell is lower than a certain threshold, the concentration of Th1 cytokines do not satisfy the inequality $f_{Th1} < f_{Th2}$, the direction of differentiation will change.

$$k_{IF}\left[IFN\text{-}\gamma\right]_T - k_{I4}\left[IL\text{-}4\right]_T < k_{I4}\left[IL\text{-}4\right]_C - k_{I2}\left[IL\text{-}12\right]_C - k_{IF}\left[IFN\text{-}\gamma\right]_C$$
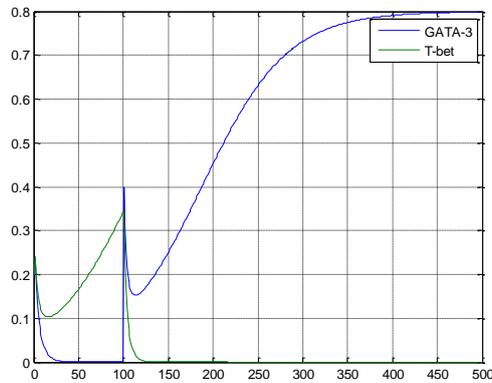

Figure 4. Th2 induced cytokines trigger the re-differentiation of Th1 cell in early phase by system dynamic model

In different differentiation stage, add equal amount of Th2 cytokines have different effects on half differentiation Th1 cell.

3. The simulation of differentiated cell (Th2)

If the concentration of Th2 cytokines satisfies the inequality $f_{Th1} > f_{Th2}$, Th2 cytokines

such as IL-4 have a stronger impact than Th1 cytokines, the Th0 cells will increase the expression level of IFN-gamma, form a positive looping, Th0 cell stimulated by Th1 cytokine will differentiate toward Th2 stage.
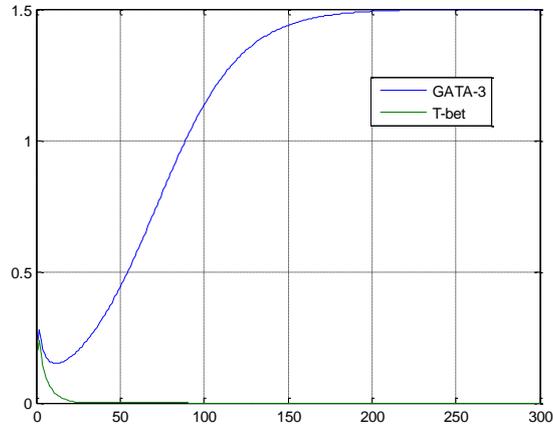

Figure 5. Differentiation process of Th2 cell by system dynamic model

In the differentiation process, if put the half differentiated Th2 cell on the Th1-inducing environment, or Th1, Th2 cytokines in the local concentration of sudden changes, there will be two possible cases :

(1) If the differentiation level of T helper cell is higher than a certain threshold, the concentration of Th2 cytokines still satisfy the inequality $f_{Th2} > f_{Th1}$, the direction of differentiation will not change.

$$k_{I4}\left[IL\text{-}4\right]_T - k_{IF}\left[IFN\text{-}\gamma\right]_T > k_{I2}\left[IL\text{-}12\right]_C + k_{IF}\left[IFN\text{-}\gamma\right]_C - k_{I4}\left[IL\text{-}4\right]_C$$
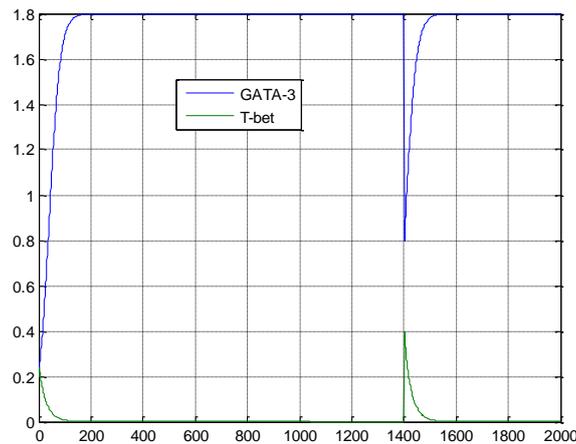

Figure 6. Th1 induced cytokines do not change the differentiation of Th2 cell in late phase by system dynamic model

(2) If the differentiation level of T helper cell is lower than a certain threshold, the

concentration of Th2 cytokines do not satisfy the inequality $f_{Th1} < f_{Th2}$, the direction of differentiation will change.

$$k_{I4}\left[IL\text{-}4\right]_T - k_{IF}\left[IFN\text{-}\gamma\right]_T < k_{I2}\left[IL\text{-}12\right]_C + k_{IF}\left[IFN\text{-}\gamma\right]_C - k_{I4}\left[IL\text{-}4\right]_C$$
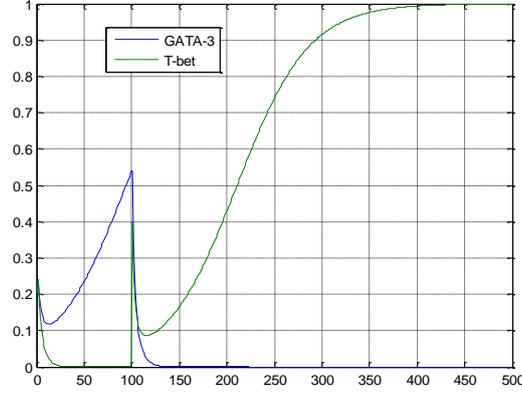

Figure 7. Th1 induced cytokines trigger the re-differentiation of Th2 cell in early phase by system dynamic model

In different differentiation stage, add equal amount of Th1 cytokines have different effects on half differentiation Th2 cell.

Stochastic process model (Master equation elementary reaction)：

Because there are a large number of NK cells, dendritic cells, monocytes and macrophages in vivo environment, all of these cells secrete small amounts of IFN-gamma, IL-2 or IL-4 which can affect differentiation of Th0 cells. We assumed that the Th1, Th2 differentiation-inducing cytokines produced by non-T cells in physiological conditions approximate normal distribution:

$$\left[IL\text{-}12\right]_C \sim N(\left[IL\text{-}12\right]_{Ca}, \sigma_{IL\text{-}12}{}^2)\,, \%95\ \text{Confidence interval}\left[\left[IL\text{-}12\right]_{Cmin}, \left[IL\text{-}12\right]_{Cmax}\right]$$

$$\left[IFN\text{-}\gamma\right]_C \sim N(\left[IFN\text{-}\gamma\right]_{Ca}, \sigma_{IFN\text{-}\gamma}{}^2)$$

$\%95$ Confidence interval is $\left[\left[IFN\text{-}\gamma\right]_{Cmin}, \left[IFN\text{-}\gamma\right]_{Cmax}\right]$

$$\left[IL\text{-}4\right]_C \sim N(\left[IL\text{-}4\right]_{Ca}, \sigma_{IL\text{-}4}{}^2)\,, \%95\ \text{Confidence interval}\ \left[\left[IL\text{-}4\right]_{Cmin}, \left[IL\text{-}4\right]_{Cmax}\right]$$

$\left[IL\text{-}12\right]_C$, $\left[IFN\text{-}\gamma\right]_C$, $\left[IL\text{-}4\right]_C$ is random variable, different from system biology model

Theoretically speaking, re-differentiation can happen at any stage of differentiation, but in this discussion, the model assumption simplified to the extracellular cytokine concentration fluctuation range at the 95% confidence interval. This means the

secretion of other cell differentiation inducing factor satisfied the following requirements:

$$\left[IL\text{-}12\right]_{Cmin} < \left[IL\text{-}12\right]_{C} < \left[IL\text{-}12\right]_{Cmax}$$

$$\left[IFN\text{-}\gamma\right]_{Cmin} < \left[IFN\text{-}\gamma\right]_{C} < \left[IFN\text{-}\gamma\right]_{Cmax}$$

$$\left[IL\text{-}4\right]_{Cmin} < \left[IL\text{-}4\right]_{C} < \left[IL\text{-}4\right]_{Cmax}$$

1. Undifferentiated status

In the induce of extracellular cytokines, the concentration of Th cell cytokine temporarily satisfy the following inequality $f_{Th1} > f_{Th2}$, in this Th0 cell, the expression of Th1 cytokine will slightly higher than normal Th0 cell, but the differentiation is not completed at this point. The concentration of cytokines may fluctuate in the physiological environment. If the concentration of cytokine changes into $f_{Th1} < f_{Th2}$ the differentiation direction will change.

Similarly, when the concentration of Th cell cytokine temporarily satisfy the following inequality $f_{Th1} < f_{Th2}$, in this Th0 cell, the expression of Th2 cytokine will slightly higher than normal Th0 cell, but the differentiation is not completed at this point. The concentration of cytokines may fluctuate in the physiological environment. If the concentration of cytokine changes into $f_{Th1} > f_{Th2}$ the differentiation direction will change.
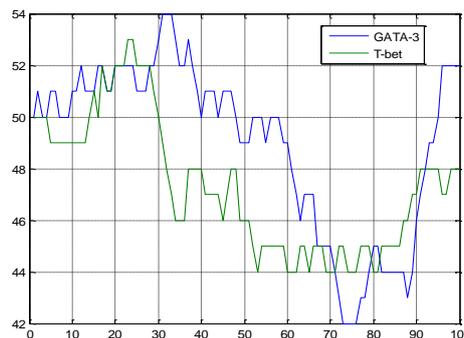

Figure 8. Th0 cells by stochastic process model

2. Th1differentiated status

When the concentration of cytokines meets the following requirement, the influence caused by fluctuation of concentration is smaller than the self-secretion of the Th1 cells. The fluctuation of concentration of extracellular environment cytokine cannot

change the direction of Th cell differentiation, at this point, differentiation is complete.

$$k_{IF}\left[IFN\text{-}\gamma\right]_{T} - k_{I4}\left[IL\text{-}4\right]_{T} > k_{I4}\left[IL\text{-}4\right]_{Cmax} - k_{I2}\left[IL\text{-}12\right]_{Cmin} - k_{IF}\left[IFN\text{-}\gamma\right]_{Cmin}$$

When the concentration cytokines (IFN-gamma, IL-4) satisfied the following requirement,

$$k_{IF}\left[IFN\text{-}\gamma\right]_{T} - k_{I4}\left[IL\text{-}4\right]_{T} = k_{I4}\left[IL\text{-}4\right]_{Cmax} - k_{I2}\left[IL\text{-}12\right]_{Cmin} - k_{IF}\left[IFN\text{-}\gamma\right]_{Cmin}$$

is the critical point of differentiation. Differentiation of Th1:
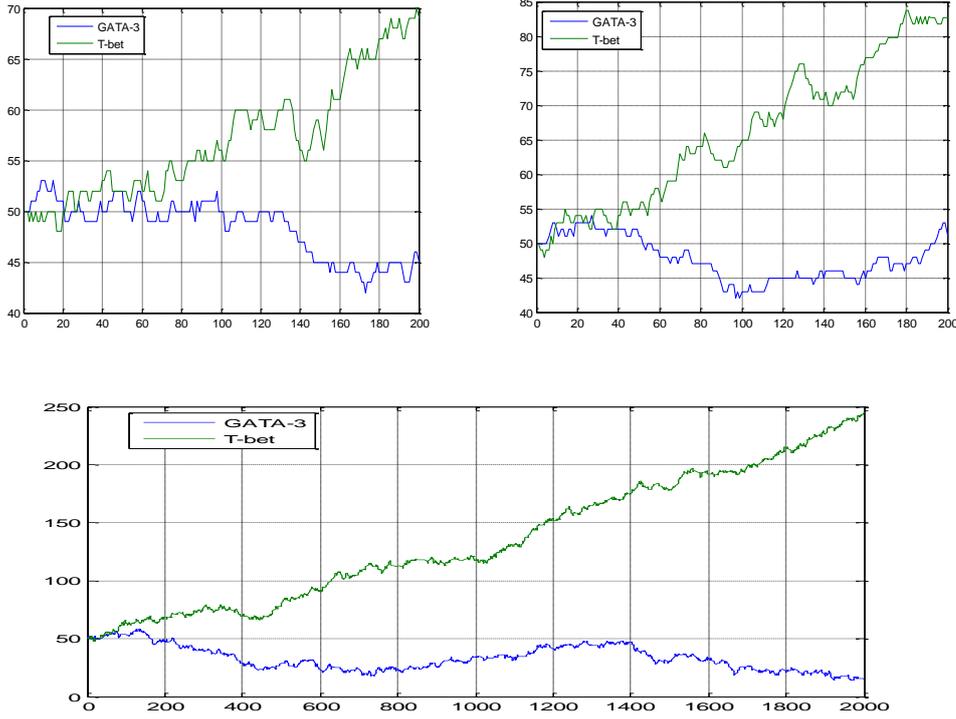
Figure 9. Th1 differentiation by stochastic process model

3. Th2 differentiated status

When the concentration of cytokines meets the following requirement, the influence caused by fluctuation of concentration is smaller than the self-secretion of the Th2 cells. The fluctuation of concentration of extracellular environment cytokine cannot change the direction of Th cell differentiation, at this point, differentiation is complete.

$$k_{I4}\left[IL\text{-}4\right]_{T} - k_{IF}\left[IFN\text{-}\gamma\right]_{T} > k_{I2}\left[IL\text{-}12\right]_{Cmax} + k_{IF}\left[IFN\text{-}\gamma\right]_{Cmax} - k_{I4}\left[IL\text{-}4\right]_{Cmin}$$

When the concentration cytokines (IFN-gamma, IL-4) satisfied the following requirement,

$$k_{I4}\left[IL\text{-}4\right]_{T} - k_{IF}\left[IFN\text{-}\gamma\right]_{T} = k_{I2}\left[IL\text{-}12\right]_{Cmax} + k_{IF}\left[IFN\text{-}\gamma\right]_{Cmax} - k_{I4}\left[IL\text{-}4\right]_{Cmin}$$

is the critical point of differentiation.
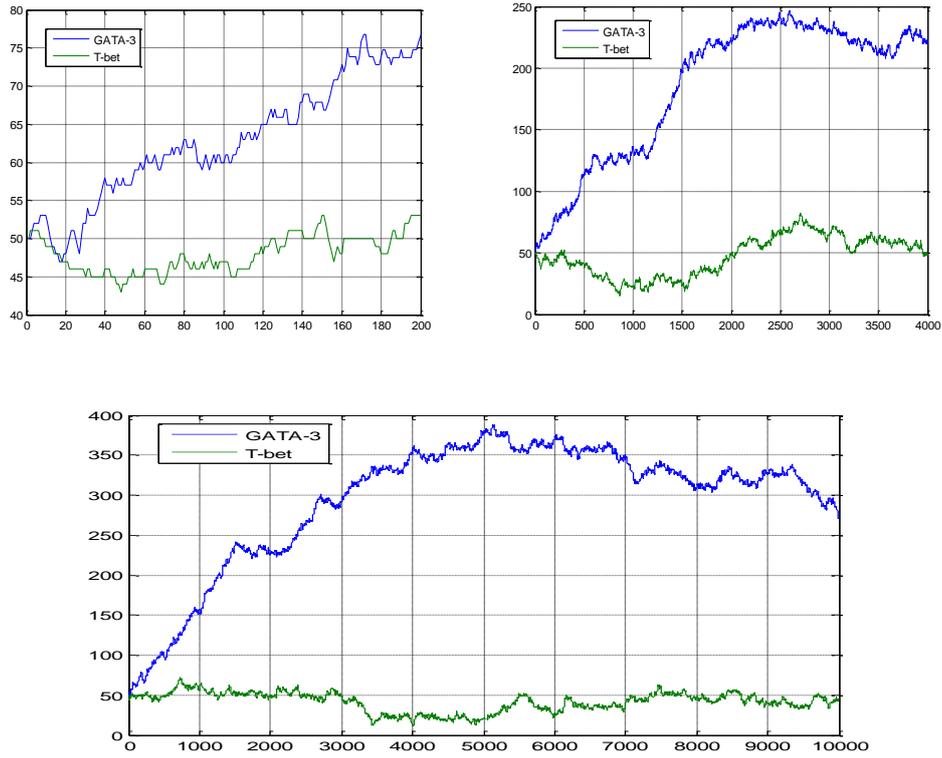
Figure 10. Th2 differentiation by stochastic process model

Extracellular cytokine environment determines the ultimate direction of differentiation, the critical point of Th1 differentiation is requirement (1), while critical point of Th2 differentiation is requirement (2)

$$(1) \, k_{IF} \left[IFN\text{-}\gamma \right]_T - k_{I4} \left[IL\text{-}4 \right]_T = k_{I4} \left[IL\text{-}4 \right]_{Cmax} - k_{I2} \left[IL\text{-}12 \right]_{Cmin} - k_{IF} \left[IFN\text{-}\gamma \right]_{Cmin}$$

$$(2) \, k_{I4} \left[IL\text{-}4 \right]_T - k_{IF} \left[IFN\text{-}\gamma \right]_T = k_{I2} \left[IL\text{-}12 \right]_{Cmax} + k_{IF} \left[IFN\text{-}\gamma \right]_{Cmax} - k_{I4} \left[IL\text{-}4 \right]_{Cmin}$$

IL-12 have a big impact on the differentiation of Th0 cells, if there is not enough IL-12 cytokine exist in the early stage of Th cell differentiation,Th0 cell cannot differentiation normally. However, if there is not enough IL-12 cytokine exist in the late stage of Th cell differentiation, Th0 cell still can differentiate normally. From this model, we can see that Th cell only produce little cytokine, this amount of cytokine can hardly meet the requirement of $f_{Th1} > f_{Th2}$. After the differentiation finished, the secretion amount of IFN-gamma is much larger than the amount of IFN-gamma in the early stage, at this time, we have $\left[IFN\text{-}\gamma \right]_T \gg \left[IL\text{-}12 \right]_C$. Even though reduce the IL-12 concentration will not change the direction of differentiation.

The delay differential equation is capable of stimulating the oscillation during the

early and middle phase of differentiation, but cannot rationalize the phenomenon that a small number of helper T lymphocytes with the feature of polarization switch to a different differentiation path. For example, it is possible for Th0 cells to differentiate into Th2 even in the culture environment of Th1, and vice versa. But according to the equation, Th0 cells can only differentiate into Th1 cells in the culture environment of Th1, unless the culture environment is changed by adding in a particular type of cytokine in polarization phase; when the differentiation is completed, it is still possible to convert the T lymphocytes (Th1 or Th2) to the opposite type (Th2 or Th1), if a certain kind of cytokine is expressed at a high level. It is not the actual case, however.

In the Master Equation model, when the initial concentrations of $IFN\text{-}\gamma$ and $IL\text{-}4$ are fixed, Th0 cells differentiate into Th1 or Th2 cells with a certain probability which varies in accordance with the concentration difference. The theoretical simulation here will be compared to experimental result (the proportions of Th1 and Th2 cells will be respectively measured at the end of lab culture). The transformation between Th1 and Th2 is less likely to occur when the concentration difference is large.
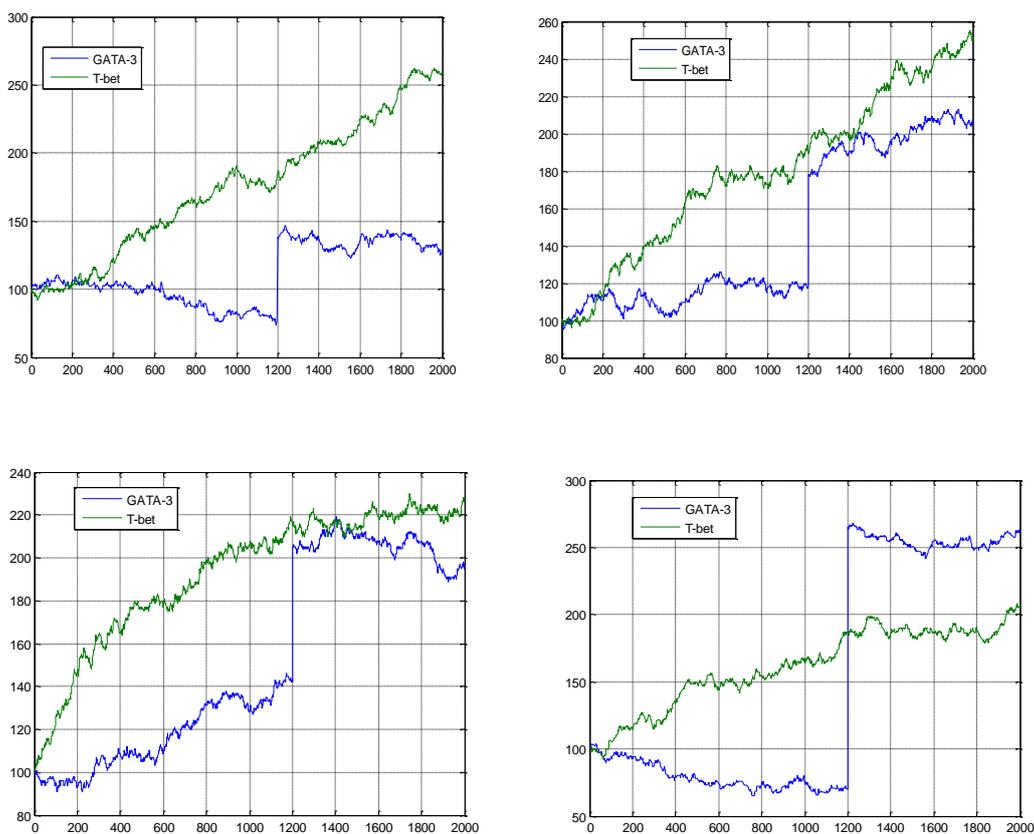

Figure 11. The differentiation and re- differentiation of Th1 by stochastic process model
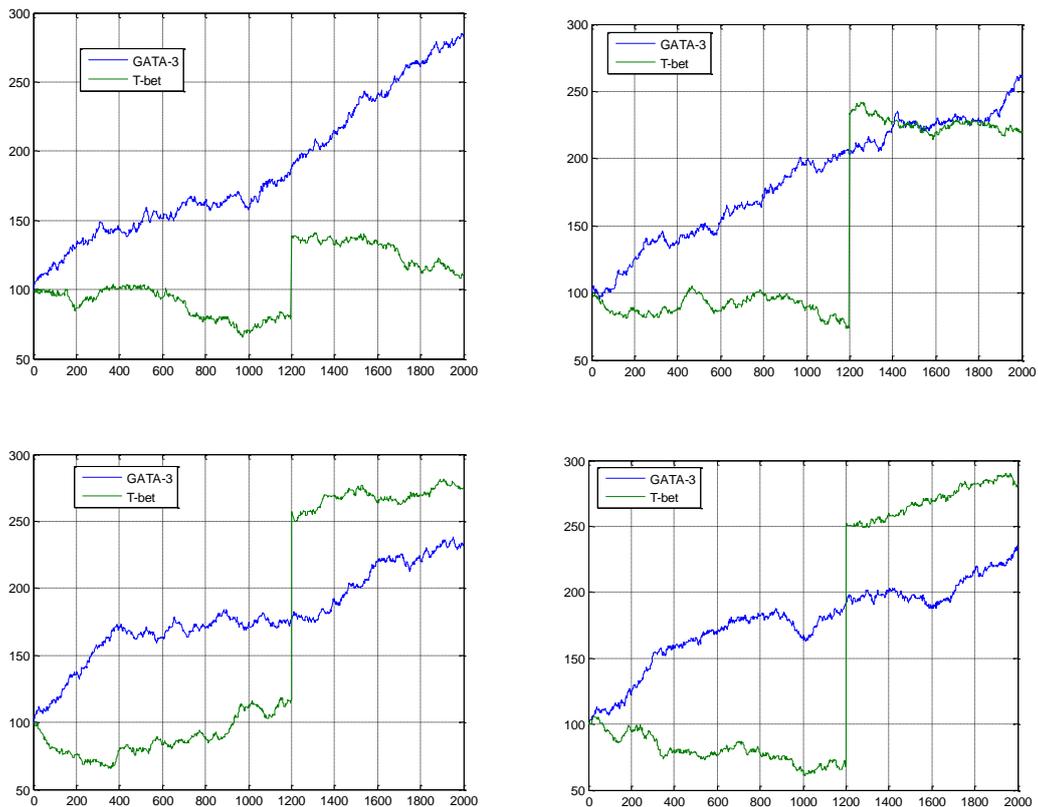
Figure 12. The differentiation and re- differentiation of Th2 by stochastic process model

During the early stage of differentiation, conversion of differentiation direction can be caused by the high-level expression of a certain cytokine, or even by some random factor, whereas the probability of dedifferentiation is extremely slim, unless the expression of the corresponding cytokine is remarkably augmented.

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