Modeling the Mechanisms of Action Underlying the Plasticity of the CD4+ T cell Differentiation Process

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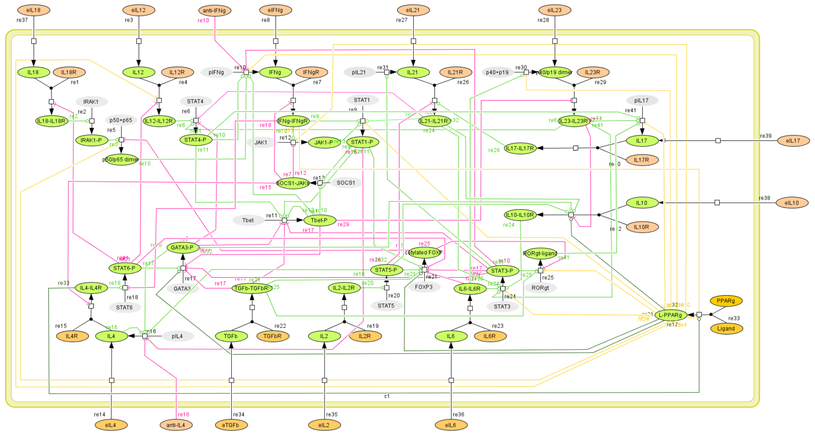
1. Introduction

The CD4+ T cell differentiation process delineates commitment towards effector (Th1, Th2, Th17) or regulatory functions (Treg). This process is controlled by a complex set of intracellular networks, transcription factor and cytokines. However, most CD4+ T cell differentiation studies have used reductionist approaches. Computational approaches have become a powerful tools for multiparametric and network analysis of biological processes and diseases. We present a comprehensive analysis of CD4+ T cell responses using computational modeling approaches in combination with experimental validation.

2. The CD4+ T cell differentiation network model

We describe a network model illustrating intracellular pathways controlling a naïve T cell differentiation into Th1, Th2, Th17 or iTreg phenotypes. The model is comprised of 37 differential equations representing 40 reactions and 81 species **(Figure 1**), including secretion of cytokines, activation of specific transcription factors and regulation by positive/negative feedbacks, inhibitions and activations. Moreover, our network includes the nuclear transcription factor peroxisome proliferator-activated receptor γ (PPARγ) that modulates the Th17/iTreg plasticity.



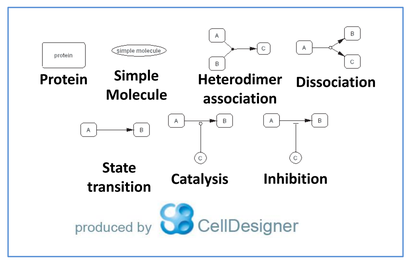


Figure 1. MIEP's CellDesigner-based illustration of the intracellular network model of CD4+ T cell differentiation that reproduces four functional subsets: T helper (Th)1, Th2, Th17 and regulatory T cells.

The COmplex PAthway SImulator (COPASI) software (freely available at www.copasi.org) allow multitask analysis and calculation of steady-states, time-courses and parameter estimations, among other functions. Once the model was calibrated using COPASI, our mathematical model reproduced the behavior of the four phenotypes (**Figure 2**).

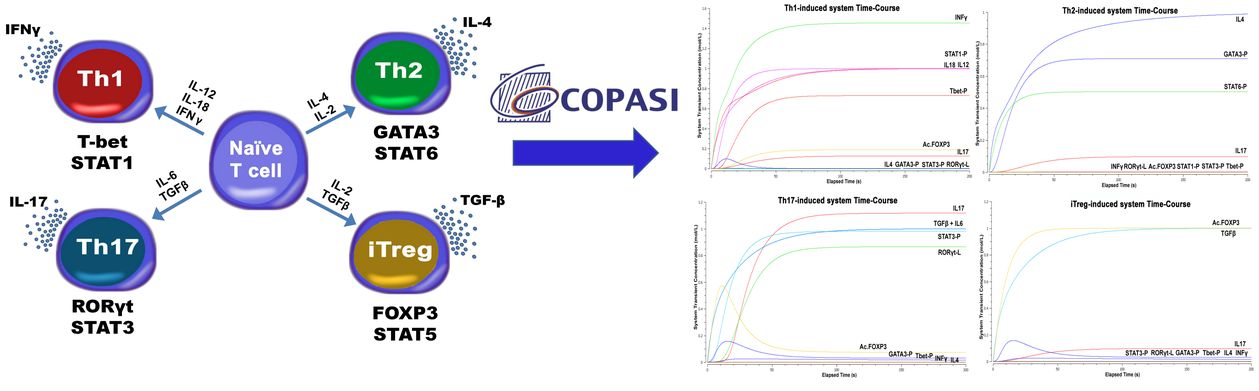


Figure 2. MIEP's CD4+ T cell differentiation model is firmly grounded on experimental observations and reproduces four CD4+ T cell phenotypes upon external stimulation with appropriate cytokine combinations.

3. *In silico* experimentation using the CD4+ T cell network model

The *in silico* experiment was designed to investigate a possible role of PPARγ in modulating the plasticity or elasticity between CD4+ T cell phenotypes. We induced our model towards a Th17 phenotype by adding two units of IL-6, one unit of TGF-β, one unit of anti-IFNγ and one unit of anti-IL4. To determine whether our model was successfully induced towards Th17 we run a time-course as shown in **Figure 3 A**. Following induction of the Th17 phenotype we added 10 units of internal PPARγ into the system. The inductors IL-6 and TGF-β were not turned off to guarantee that the effect measured did not originate in changes in induction of a Th17 phenotype.

The results of our simulation demonstrated that when PPARγ was added into the system, there was a phenotype switch from Th17 to iTreg. Specifically, the ten units of PPARγ downregulated RORγt and IL-17 and upregulated the production of FOXP3, thereby causing a phenotype switch (**Figure 3A**).

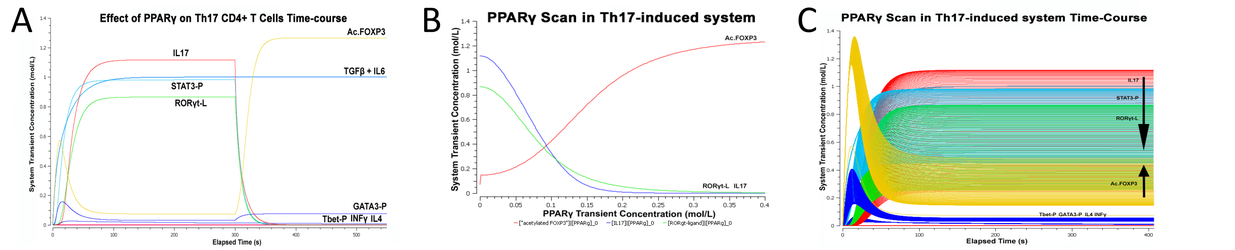


Figure 3. Functional plasticity of T helper (Th) 17 and iTreg phenotypes. (A) COPASI time-course showing the effect of PPARγ on Th17 CD4+ T cells. (B) COPASI's scan-task showing how as we increase the concentration of PPARγ, there is a switch in phenotype denoted by reduction of IL-17 and RORγt and upregulation of FOXP3. (C) PPARγ upregulates FOXP3 and downregulates Th17-related molecules (IL-17, STAT-3 and RORγt) over time illustrated using a combination of time-course plus scan.

To further determine whether the addition of PPARγ into the system resulted in phenotype switch from Th17 to iTreg, we run COPASI's Scan task. The Scan task allows researchers to test the behavior of specific molecules when a concentration of a particular molecule is internally increased. In this case, we tested the behavior of RORγt, FOXP3 and IL-17 with increasing concentrations of PPARγ. The PPARγ scan indicates that as we increase the amount of PPARγ from 0 to 0.4 units the concentrations of FOXP3 increase and those of RORγt and IL-17 decrease in the CD4+ T cell (**Figure 3B**). A third simulation performed to confirm the potential involvement of PPARγ in the Th17 switch to iTreg was a Time-course/Scan combination. This combination provides information on the effect of increasing concentrations of PPARγ over time. To complete this experiment, the system was first induced to Th17 as described before and a Scan was run selecting the display option of Time-course. IL-17, STAT3-P, FOXP3 and RORγt are displayed in the graphic (**Figure 3C**). Each line in each molecule represents an increasing concentration of PPARγ.

4. Experimental validation approaches

Computational modeling is a powerful tool because of its targeted predictions and potential for a low-cost, comprehensive analyses of biological systems. However, every single prediction requires experimental validation.

Our validation studies consisted in differentiate in vitro primary mouse CD4+ sorted spleen T cells into Th17 and treated with increasing concentrations of rosiglitzaone, a PPARγ agonist. Moreover, PPARγ activation increases the expression of FOXP3 in CD4+ T cells cultured in Th17-polarizing conditions, suggesting a role of PPARγ in the plasticity of CD4+ T cells between the Th17 and Treg phenotypes (**Figure 4**). These data support the prediction of our model regarding the modulation of intracellular networks controlling the fate and function of CD4+ T cells by PPARγ.

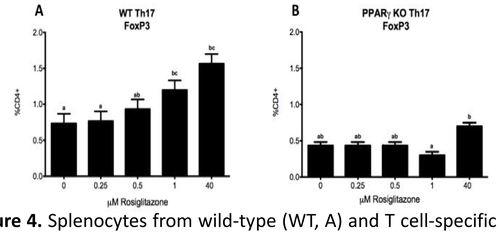


Figure 4. Splenocytes from wild-type (WT, A) and T cell-specific P PAR γ null mice (KO, B) were cultured under T helper (Th)17 differentiating conditions. Cells were treated with 0, 0.25, 0.5, 1 or 40 μM of rosiglitazone. Panel A illustrates the effects of increasing concentrations of rosiglitazone on FOXP3 expression in Th17 cells from WT mice. Panel B illustrates the effect of increasing concentrations of rosiglitazone on FOXP3 expression in Th17 cells from KO mice. Data are represented as mean ± standard error. Points with different letter superscripts are significantly different ($P < 0.05$).

5. Conclusions and future directions

- ✓ PPAR γ regulates the plasticity between Th17 and iTreg CD4+ cells.
- ✓Further studies are ongoing with other transcription factors
- ✓A comprehensive understanding the CD4+ T cell differentiation could possibly uncover novel regulatory mechanisms, intermediate steady-states and model sensitivities of enormous biological significance.
- ✓A high level of integration between the computational and the experimental efforts in MIEP make mathematical and computational modeling a useful tool for examining the dynamics of CD4+ T cell differentiation and novel hypothesis generation

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