Switching gene-regulatory loops in Th1 differentiation

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***Short Abstract* — In this work we describe a new regulatory principle underlying the differentiation of Th1 lymphocytes. We performed quantitative kinetic measurements to identify new interactions and construct a mathematical model for the expression dynamics of critical genes: (1) the Th1 lineage specifying transcription factor T-bet, (2) the receiver of the Th1 differentiation signal, IL-12 receptor, and (3) the Th1 effector cytokine Interferon-γ (Ifn-γ). Model-driven experiments showed that instruction for Th1 differentiation is a two-step process. Initial T-bet induction through a sensitive Ifn-γ dependent pathway accelerates the response of the slow feedback loop between T-bet and IL-12, active late during differentiation.**

I. Biological Background

T-HELPER (Th) lymphocytes regulate adaptive immune responses. According to the nature of the pathogen, naïve Th lymphocytes can differentiate into either Th1 or Th2 cells. These differentiated cell types are critical for the establishment of immunological memory [1].

Th1 development is regulated by a complex genetic network. Multiple signals are integrated to modulate expression of the master regulator T-bet [2]. The differentiation process is started by the antigen and the cytokine IL-12 and is amplified by an autocrine Ifn-γ-mediated feedback loop. We have identified an unknown IL-12 dependent pathway of T-bet induction as the major mechanism of imprinting the Th1 phenotype.

II. Results

In order to observe the physiologic transition from the naïve to the differentiated state, we study freshly isolated lymphocytes from mice. The differentiation process is started in cell culture, allowing for tight control over experimental conditions. To deduce the structure of the underlying gene-regulatory network we systematically perturb network components by genetic and biochemical means. Using quantitative RT-PCR we measure the altered expression kinetics of key genes in the perturbed system. The discovery of new interactions completing the network structure and the use of quantitative data enabled us to build an ODE-model of the gene-regulatory network underlying Th1 differentiation.

A. Sequential activation of subnetworks

We find that during different times of differentiation, distinct parts of the network are functioning. We could divide the process into two major phases. During the first phase, T-bet is up-regulated through Ifn-γ and, after entry into the cell cycle, feeds back into Ifn-γ production. In the last phase, triggered by antigen removal, T-bet expression can be maintained through (T-bet sensitized) IL-12 signaling.

B. The loop switch

The core of the model is T-bet's transcriptional regulation. It is induced by two positive feedback loops acting sequentially. In the first phase, a fast autocrine Ifn-γ dependent mechanism is active; in the second phase a slow IL-12 controlled pathway takes over that is feedback-enhanced through induction of the IL-12 receptor by T-bet. The loop switch is controlled by the antigen as it is permissive for the first loop, but repressive for the latter.

C. Phenotype maintenance

The model can explain well the expression kinetics observed during the differentiation process under various experimental conditions. But the network cannot maintain a self-sustained differentiated state upon cytokine withdrawal because it is strongly dependent on extracellular signals.

III. Conclusion

Using quantitative measurements we have developed a mathematical description of the gene-regulatory network underlying Th1 differentiation. A central feature is the sequential coupling of two regulatory loops. We hypothesize that the fast Ifn-γ dependent loop, active in the early phase, sets a favorable initial condition for the slow IL-12 dependent loop. This loop takes over maintenance of T-bet expression in the late phase, imprinting the phenotype through enhanced IL-12 signaling and T-bet expression. To test this prediction experimentally, we want to investigate if the differentiation defect of Ifn-γ receptor deficient cells can be overcome by ectopic expression of the IL-12 receptor. As the model does not show a stable differentiated state, we conclude that the phenotype is not imprinted through changes in gene expression, but rather in chromatin structure.

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