Two feedback loops control Th1 differentiation

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***Short Abstract* — In this work we describe the sequence of regulatory events 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 show that instruction for Th1 differentiation is a two-step process. An IFN-γ dependent positive feedback loop initiates T-bet expression, while a subsequently activated IL-12 dependent loop maintains expression in the late phase of differentiation when T-bet acts to imprint the Th1 phenotype.**

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].

Differentiation along the Th1 pathway requires two input signals: the cognate antigen acting as a mitogen and the Th1 inducing cytokine Interleukin-12 (IL-12). After processing by a complex transcriptional network, involving up-regulation of the IL-12 receptor and the transcription factor T-bet, the cells respond with production of Interferon-γ (IFN-γ) [2]. Through this differentiation process they acquire the ability to re-express IFN-γ upon subsequent antigenic stimulation, independently of IL-12. The mechanisms underlying IFN-γ memory remains poorly understood.

II. Results

In order to observe the physiologic transition from the naïve to the differentiated state, we study freshly isolated lymphocytes from mice during their differentiation in cell culture. 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. Through comparison of these measurements with predictions of our ODE model, we uncovered two previously unknown regulatory interactions completing the network structure.

A. Sustained T-bet expression requires IL-12 signaling

We find that T-bet expression is controlled by two sequentially acting signals. While T-bet expression in the first two days is regulated by a previously known positive feedback loop formed through mutual induction of T-bet and IFN-γ, expression in the late phase requires IL-12 signaling and is independent of IFN-γ. As T-bet is a known inducer of the IL-12 receptor, we have uncovered a second positive feedback loop of T-bet regulation.

B. The IFN-γ/T-bet feedback loop starts differentiation

The two feedback loops controlling T-bet expression act strictly sequential. While the IFN-γ/T-bet loop requires concomitant antigen stimulation, the T-bet/IL-12R loop is inhibited in the presence of antigenic signals through repression of the IL-12 receptor. We find that the IFN-γ dependent feedback loop is required to start up T-bet expression, which can then be maintained by the IL-12 dependent loop.

C. IFN-γ memory is imprinted by the T-bet/IL-12R loop

To understand how input signals determine the outcome of differentiation, we measure the fraction of cells that would re-express IFN-γ upon subsequent stimulation. The expression level of T-bet in the late IL-12 dependent phase, not in the early IFN-γ dependent phase, is highly predictive for IFN-γ re-expression. Therefore, maintenance of T-bet expression by IL-12 is critical for induction of IFN-γ memory.

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 find 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.

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