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T CELL DIFFERENTIATION

The T_H1 two step

The cytokines interferon-γ (IFNγ) and interleukin-12 (IL-12) and the transcription factor T-bet are essential for T helper 1 (T_H1) cell differentiation. But how do these three factors cooperate to induce and stabilize the T_H1 cell phenotype? Now, Höfer and colleagues describe a two-step model of positive feedback regulation for T_H1 cell differentiation that is mediated first by a T cell receptor (TCR)–IFNγ–T-bet signalling loop and then by an IL-12–T-bet signalling loop.

Several models have been proposed to explain how T_H1 cell-inducing factors cooperate to mediate T_H1 cell differentiation, but none of these models could explain the observation that IFNγ (which is a potent activator of T-bet expression) cannot induce T_H1 cell differentiation in the absence of IL-12. To address this issue, the authors used a combination of mathematical modelling and kinetics studies to analyse the gene network that governs T_H1 cell differentiation.

Stimulation of naive T cells through the TCR for 48 hours together with treatment with IL-12 and an IL-4-specific blocking antibody for 6 days resulted in two waves of T-bet expression. Stimulation of IFNγ receptor-deficient T cells resulted in a loss of the first wave of T-bet expression, but the second wave was still intact, although T-bet expression was lower and slightly delayed. Inhibition of IL-12 resulted in a loss of the second wave but not the first. So, the first wave of T-bet expression requires IFNγ but IL-12 is dispensable, whereas the second wave is IL-12 dependent but is IFNγ independent.

But how are the two waves of T-bet expression controlled? Early *Ifng* mRNA expression was shown to require signalling through the TCR and was further enhanced by IL-12- and IFNγ-induced T-bet expression. This suggests that the loss of IFNγ expression that occurs after the removal of TCR signalling might result in the decline of T-bet expression between the two phases. However, addition of exogenous IFNγ to the cultures did not prolong the first wave of T-bet expression, indicating that another mechanism that is independent of IFNγ must exist. Further analysis showed that TCR signalling inhibited IFNγ-induced expression of IL-12 receptor β2 (IL-12Rβ2) through the calcineurin–NFAT (nuclear factor of activated T cells) pathway. Loss of TCR signalling resulted in the upregulation of IFNγ-induced IL-12Rβ2 expression and the second wave of T-bet expression mediated by IL-12. Therefore, TCR-induced signalling coordinates the two waves of T-bet expression.

Finally, the authors showed that the late wave of IL-12–IL-12Rβ2-induced T-bet expression is accompanied by the upregulation of the T_H1 cell-associated transcription factors H2.0-like homeobox (HLX), runt-related transcription factor 3 (RUNX3) and active signal transducer and activator of transcription 4 (STAT4) and that it is this phase that is required for the stabilization of the T_H1 cell phenotype.

So, the differentiation of T_H1 cells occurs in two steps: in the early polarizing phase, TCR signalling induces T-bet expression synergistically with IFNγ but represses IL-12Rβ2 expression. Following termination of TCR signalling, IL-12Rβ2 expression is upregulated and IL-12 maintains the second phase of T-bet expression and stabilizes the T_H1 cell phenotype. This two-step process may represent a need for naive T cells to receive pro-inflammatory signals that are sustained beyond the acute antigen-dependent phase of the response before complete commitment to the T_H1 cell lineage can occur.

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ORIGINAL RESEARCH PAPER Schulz, E. G., Mariani, L., Radbruch, A. & Höfer, T. Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-γ and interleukin-12. *Immunity* 30 Apr 2009 (doi:10.1016/j.immuni.2009.03.013)