

Multiple essential roles for Notch signaling during T cell development and differentiation

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Notch signaling plays multiple roles during early hematopoiesis and T cell development. Notch signaling is essential for the emergence of hematopoietic stem cells (HSCs) during fetal life, but its effects on adult HSCs are controversial. In gain-of-function experiments, activation of Notch signaling in adult HSCs increased their self-renewal potential in vitro and in vivo. However, loss-of-function studies have provided conflicting results as to the role of physiological Notch signaling in HSC maintenance and homeostasis. To address this question, we expressed DN-MAML1, a GFP-tagged pan-inhibitor of Notch signaling, in mouse HSCs. Our data suggest that Notch signaling is dispensable to maintain the stem cell function of adult HSCs, at least when signaling is mediated through the canonical ICN/CSL/MAML pathway. We have also developed methods to inactivate Notch signaling in the fetus at timepoints after fetal HSC establishment. In contrast to adult hematopoiesis, our data suggest that continuous Notch signaling is required for normal development of fetal HSCs during mid-gestation. These data suggest a differential requirement for Notch signaling in fetal and adult HSC homeostasis.

We also used mice that conditionally express DN-MAML1 to investigate the function of Notch signaling during helper T cell differentiation. Recent data implicate Notch signaling in helper T cell development, although the precise function of Notch in this process is controversial as Notch signaling has been implicated in both Th1 and Th2 development. To clarify the role of Notch in Th cell differentiation, we generated mice that conditionally inactivate Notch signaling in mature T cells. Mice that lack Notch signaling in CD4⁺ T cells fail to develop a protective Th2 response against the gastrointestinal helminth *Trichuris muris*. In contrast, they exhibit effective Th1 responses and are able to control *Leishmania major* infection. These data demonstrate that Notch signaling is an essential regulator of type 2 immunity. To investigate the mechanism by which Notch influences Th2 differentiation, we focused on IL-4 and GATA-3. We show that *Gata3* is a direct transcriptional target of Notch that synergizes with Notch signaling to generate optimal Th2 cell responses.

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