70

Canonical Wnt signalling is required for thymus organogenesis

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The thymus achieves two interrelated functions essential for the adaptive immune system; the life-long generation of new T cells and the selection of a repertoire of T cells tolerant to self-antigens but reactive to foreign peptides. Thymus organogenesis is initiated in the mouse at embryonic day 10.5 when endodermal cells of the third pharyngeal pouch become committed to a thymus fate. The formation of an epithelial primordium and the subsequent differentiation of thymic epithelial cells (TEC) into distinct subpopulations constitute the necessary prerequisite for the formation of a thymic microenvironment proficient to support T cell development. However, the precise molecular signals that dictate TEC fate commitment and differentiation remain largely unknown. Wnt signaling has, however, been implicated in thymus organogenesis Here we demonstrate that thymus organogenesis is differentially affected depending on the timing of the loss in canonical Wnt signalling. Deletion of canonical Wnt signalling during developmental stages prior to the formation of a thymus anlage causes thymic agenesis. In contrast, loss of canonical Wnt signalling at a later stage does not preclude the formation of a regularly structured and composed thymic microenvironment but leads to thymic hypoplasia despite normal T cell development. Taken together, these results demonstrate an essential role for canonical Wnt signaling during early stages of thymus organogenesis whereas activation through the same pathway determines thymus cellularity during later stages and organ maintenance of the thymus.

71

Mature Aire+ mTEC development is controlled by antigen-specific TCR-MHC class II mediated interactions with autoreactive CD4+ thymocytes

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Medullary thymic epithelial cells (mTEC) are specialized for inducing central immunological tolerance to self-antigens. To accomplish this, mTEC must adopt a mature phenotype characterized by expression of the autoimmune regulator Aire, which activates the transcription of numerous genes encoding tissue-restricted self-antigens. The mechanisms that direct mTEC maturation are poorly understood. We have discovered that the number of mature mTEC is controlled in the postnatal thymus by direct physical interactions between CD4+ thymocytes bearing autoantigen-specific TCR and mTEC displaying the cognate self-peptide/MHC class II complexes. The competence of CD4+ thymocytes for this process is imparted by their expression of CD40L, which delivers an essential signal upon binding to CD40L on mTEC. This crosstalk between CD4+ thymocytes and mTEC defines a novel checkpoint pivotal for thymic stromal and lymphoid development because it generates a mature mTEC population competent for ensuring central T cell tolerance.

72

Role of Notch1 and Notch2 receptors in CD4+ T helper differentiation

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Notch proteins are evolutionary conserved receptors involved in cell differentiation and development processes, especially in binary cell fate decisions. The role of the Notch pathway in T helper (Th) differentiation remains controversial. In order to characterize the role of Notch1 and Notch2 in CD4+ Th differentiation, we used mice with a T cell-specific ablation of the Notch1 (N1-/-), Notch2 (N2-/-) or both Notch1 and Notch2 (N1-/-N2-/-) and infected them with Leishmania mexicana, which induces IL-4-dependent cutaneous leishmaniasis and Th2 differentiation when injected in the back rump. Evolution of lesions and of the immune response was compared to that of their control littermates (+/+). Lesion development as well as parasite number within the lesions did not differ significantly within the infected groups, however, parasite metastasis was observed only in control groups but not in the Notch-/- groups. Fourteen weeks after infection with L. mexicana, control+/+ mice developed significant levels of L. mexicana-specific IgG1 and IgE antibodies indicating of the development of a Th2 immune response. In contrast, infection of N1-/- mice resulted in a decreased production of parasite-specific IgG1 and IgE in the serum, with corresponding increase in Th1-specific IgG2a antibodies. This decrease in Th2 antibodies with the corresponding increase in IgG2a antibodies was even more drastic when CD4+ T cells lacked both Notch1 and Notch2 (N1-/-N2-/- mice). In contrast, L. mexicana-infected N2-/- mice had similar levels of IgG1 and IgE as controls littermates but the secretion of IL-4 and IL-10 by CD4+ T cells was significantly enhanced while the secretion of IFN-g was decreased, suggesting a role of Notch2 in Th1 differentiation. Taken together, these results reveal that the IL-4 produced by CD4+ T cells is not essential for lesion development following infection with L. mexicana, but is involved in parasite metastasis. In this model of infection, Notch1 contributes to Th2 differentiation while Notch2 appears to be involved in Th1 response and both Notch1 and Notch2 seems to regulate each other.

73

T-cell development occurs in the combined absence of the canonical Wnt signal-transducers beta- and gamma-catenin

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The canonical Wnt signaling pathway plays key roles in stem cell maintenance, expansion of committed progenitor cells and control of lineage decisions in a variety of tissues including the hematopoietic system. Beta-catenin is the central molecule in the canonical Wnt signaling pathway. Beta catenin transmits Wnt signals into the nucleus, where it acts as transcriptional co-activator by binding to members of the LEF/TCF (Lymphoid enhancer factor/T cell factor) family of transcription factors. The related molecule gamma catenin (plakoglobin) can fulfill similar functions to activate target genes upon Wnt signaling.
Gene ablation of TCF-1 impaired T cell development. TCF-1 deficiency resulted in incomplete blocks within the CD4-8- thymocyte compartment and at the transition from immature single positive to the CD4+8+ stage. Strikingly, a genetic complementation approach showed that the N-terminal domain of TCF-1 was essential to rescue thymocyte development in TCF-1 deficient mice. This domain in TCF-1 includes a beta catenin binding site. We show that this domain also contains a gamma catenin interaction site, suggesting that catenin binding to TCF-1 is critical for T cell development. However, results obtained by individual gene ablations of the two known signal transmitters beta- and gamma catenin did not recapitulate the phenotype of TCF-1-deficient mice.
Based on this discrepancy we analyzed thymopoiesis in the combined absence of both, beta- and gamma catenin. Unexpectedly, we find that T cell development occurs normally in the combined absence of the two known Wnt signal transmitters beta- and gamma catenin. We performed reporter assays to address whether canonical Wnt signaling was not needed for thymocyte development or whether such signals were transduced in the absence of beta- and gamma catenin. We find that beta- and gamma catenin-deficient thymocytes and peripheral T cells retain a significant capacity to transduce canonical Wnt signals. These data suggest a novel mechanism of TCF-1/Wnt signal transduction in the hematopoietic system.

74

Interleukin-7-driven networking in normal and ectopic lymphoid organ development

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Antimicrobial adaptive immune responses are generated in secondary lymphoid organs such as spleen, lymph nodes (LNs) and Peyer's patches (PPs). In addition, inducible ectopic lymphoid tissues form in inflammatory lesions of chronic infections, autoimmune diseases, allergic reactions and chronic graft rejection. These so-called "tertiary lymphoid tissues" can function as inductive sites for adaptive immune responses against self and non-self antigens. During embryogenesis,