

File ID 116408
Filename Summary

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type Dissertation
Title From progenitor cell to immune cell : cytokines and transcription factors in human
 hematopoietic development
Author W. Dontje
Faculty Faculty of Medicine
Year 2008
Pages 188

FULL BIBLIOGRAPHIC DETAILS:

<http://dare.uva.nl/record/284234>

Copyright

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use.

Summary

This thesis focuses on the development of human hematopoietic progenitor cells towards immune cells and the factors that are involved in this process.

Chapter 1 introduces the major signaling pathways, cytokines and immune cells that are studied in the following chapters. The Notch, IL-7 and FLT3 signaling pathways are described and in addition current knowledge about the development of hematopoietic progenitors towards T cells, NK cells and pDC is summarized.

In **chapter 2** we describe the effects of exogenously administered IL-7 on human lymphoid development in the 'Humanized Immune System' (HIS) mouse model. IL-7 is known to regulate T cell differentiation and homeostasis, however we demonstrated that IL-7 has more widespread effects on human lymphoid development. Remarkably, IL-7 did not enhance T cell lymphopoiesis, but only transiently boosted T cell proliferation.

In **chapter 3** we study the role of Notch signaling on human T cell and pDC development *in vitro*. Human CD34⁺CD1a⁻ thymic progenitors express Notch1, which is maintained upon T cell commitment, but down regulated upon development towards the pDC lineage. By coculturing thymic progenitors on OP9 cells expressing the Notch ligand DL1 we demonstrated that DL1-mediated Notch triggering induced development of TCRαβ⁺ and TCRγδ⁺ T cells at the expense of pDC. In contrast, Jagged1-mediated Notch signaling allowed differentiation of both T cells and pDC. The transcription factor GATA-3 is essential for T cell differentiation whereas the Ets family member Spi-B is indispensable for pDC development. We identified DL1/Notch1 signaling to induce expression of GATA-3 and to down regulate Spi-B expression. Together, our findings indicate that the DL1/Notch1 signaling pathway controls the T cell-pDC lineage switch by regulating the balance between the transcription factors GATA-3 and Spi-B.

In **chapter 4** we address the question what the role is of pDC in the thymus where also the T cells develop. We show that activated thymic pDC, which produce IFN-α, impacts on IL-7-induced T cell development. As all thymocytes express the IFN-α receptor β-chain (CD118), they are potentially responsive to type I IFN. By co-culturing human progenitors on OP9-Jagged1 cells, we observed that after stimulation with either virus or CpG, pDC-derived IFN-α impaired the differentiation of autologous progenitors into T cells. This effect was reversible as addition of neutralizing antibodies against type I IFN restored T cell differentiation, indicating that T cell development is hindered by endogenous IFN production. We demonstrated that IFN-α interfered with IL-7-mediated T cell differentiation by delaying the transition from the CD4⁻CD8⁻ DN to the CD4⁺CD8⁺ DP stage. Moreover, IFN-α inhibited TCRβ rearrangement, expression of CD1a and TCR components, and proliferation of the progenitors.

Chapter 5 describes the interplay of Id2 and IL-15 in NK cell development. T cell development was blocked by ectopic expression of Id2 in human thymic CD34⁺CD1a⁻ progenitor cells, although this did not enhance NK cells differentiation when cocultured on OP9 cells, compared to control GFP transduced progenitors. Instead we observed that Id2 expanded the number of early CD1a⁻CD5⁺ thymocytes that upon addition of IL-15 differentiated into NK cells. Forced expression of the bHLH factor HEB, but not E2-2, E12 or E47, prevented the accumulation of Id2⁺CD1a⁻CD5⁺ thymic progenitor cells. However, forced expression of HEB is not sufficient to overcome the Id2-induced block in T cell differentiation, suggesting that other E proteins are required for proper T cell

development as well. Together, our findings imply that Id2 exhibits two functions regarding T/NK cell lineage determination. First, Id2 represses E protein activity, thereby impairing T cell development. Secondly, Id2 specifically expands thymic CD1a⁻CD5⁺ early thymocytes that develop into NK cells in response of IL-15.

In **chapter 6** we demonstrated a role for Notch4 in the development of IL-7Rα⁺ NK cells. We determined that a subset of NK cells in the human thymus expressed IL-7Rα. These cells are capable of recognizing target cells and to produce IFN-γ. Since IL-7 and Notch both are essential for T cell development we addressed whether Notch signalling may be involved in the development of IL-7Rα⁺ NK cells. Both Notch1 and Notch4 are expressed at higher levels on IL-7Rα⁺ NK cells compared to IL-7Rα⁻ NK cells when freshly isolated from the human thymus. Ectopic expression of the intracellular domain of Notch1 (icNotch1) in human thymic CD34⁺CD1a⁻ progenitor cells generated T cells as expected, while overexpression of icNotch4 induced the development of IL-7Rα⁺CD56⁺ cells, which strongly depended on IL-7, but not IL-15. While phenotypically these cells closely resembled freshly isolated IL-7Rα⁺ thymic NK cells, the Notch4 induced IL-7Rα⁺CD56⁺ cells appeared functionally immature. By using a 4HT-regulatable icNotch4-ER construct, we observed that release of the constitutive active nature of icNotch4 allows for functional differentiation of the NK cells. Together our findings provide insight in the molecular mechanism that induces the development of this novel subset of NK cells.

In **chapter 7** we investigated the effect of mutations in FLT3 on the development of pDC. Development of pDC strongly depends on FLT3 signaling. Mutations in FLT3 leading to internal tandem duplications (FLT3-ITD) result in aberrant Signal Transducer and Activator of Transcription (Stat)5 signaling and are found in 25-30% of patients diagnosed with acute myeloid leukemia (AML). We analyzed peripheral blood of AML patients and determined that the proportion of pDC is strongly reduced in AML patients with FLT3-ITD, compared to AML patients that only expressed wild type (wt)FLT3. To further investigate the relationship between the presence of FLT3-ITD and the reduction in pDC, we cocultured human CD34⁺ cord blood (CB) derived progenitors ectopically expressing wtFLT3 and FLT3-ITD on OP9 cells. Progenitors expressing only wtFLT3 developed into pDC, whereas cells transduced with FLT3-ITD were blocked in their potential to develop into pDC. We determined that, in contrast to FLT3-ITD, wtFLT3 does not activate Stat5 in human CD34⁺ CB derived progenitors. Overexpression of a constitutive active (ca) form of Stat5 (caStat5) in CD34⁺ progenitors mimicked the effect of mutant FLT3-ITD on pDC development by autonomously blocking pDC development.

Finally, in **chapter 8** the work described in the former chapters is discussed in light of current knowledge. We speculate about the developmental origin and potential functions of IL-7Rα⁺ NK cells. The use of Humanized Immune System (HIS) mice may provide insight in these unresolved issues. Furthermore, mechanisms to regulate lineage decisions by Notch signaling are presented, which may involve lineage specific Notch signaling or regulation by Notch signal strength. Additionally, the effects of FLT3-ITD mutations on pDC differentiation and heterogeneity of the pDC population are discussed. Together, the work described in this thesis increases our knowledge about the regulation and requirements for human hematopoietic development.