Lymphoid development; a dynamic interplay of timing and dosing
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CHAPTER 1

Lymphoid development; a dynamic interplay of timing and dosing

A step-wise approach to cell fate
Every form of new life begins as a single cell. This is an omnipotent cell, harboring all genetic information required to develop to a full organism of varying complexity. When full differentiation has been established, animals consist of an assembly of different cell types, each with a unique palette of phenotype and function. Throughout the development of cells to fully differentiated cells, both during fetal and adult life, cell fate decision are continuously taking place. We find this process distinctly reflected in the development of the immune system and its maintenance throughout life. T cells, B cells and natural killer (NK) cells, together with a proportion of dendritic cells (DC), form the lymphocyte population. Mature lymphocytes, which act with other leucocytes to establish our immune defense, originate from a common multipotent progenitor (i.e. a cell with restricted developmental choices as compared to omnipotent cells) which undergoes successive steps of increasingly divergent differentiation. This differentiation ‘tree’ is a simultaneous acquisition and elimination race of functions. The end result is an extensively diverse but coherent population of cells active in host defense.

The outcome of lineage decisions is regulated by endogenous factors, such as transcription factors, but also environmental factors, like cytokines, antigens and cell-cell interactions. These elements influence not only the genetic programming, but can affect cell survival and proliferation as well, i.e. major parameters which additionally determine the accumulation and functional potency of developing cells. This thesis focuses on the factors involved in the processes driving lymphoid lineage decisions and the subsequent maintenance of the generated populations, with a specific interest for T lymphocytes.

Multilineage hematopoiesis
All blood cells arise from hematopoietic stem cells (HSC) which reside in the fetal liver or the adult bone marrow, are multipotent and have self-renewal ability. Previous work suggested an early separation of common lymphoid progenitors (CLP), which are lymphoid-cell-lineage-restricted progenitors, and common myeloid progenitors (CMP), which are myeloid-cell- and erythroid-cell-lineage-restricted progenitors (1). This model suggested that all B and T cells arise from a CLP stage of development and hypothesized that the thymus is settled strictly by lymphoid-restricted hematopoietic progenitors. Recent studies have instead established the existence of lymphoid–myeloid progenitors (LMP) which possess both
lymphoid and myeloid lineage potentials but lack erythroid potential (2, 3). Myeloid and lymphoid potentials are present at the clonal level in early thymic progenitors, confirming that progenitors settling the thymus include lymphoid–myeloid progenitors. A schematic representation of the current model of hematopoiesis is depicted in figure 1.

**Hematopoietic stem cell decisions towards lymphoid differentiation**

**Phenotype of early progenitors**

Cell surface markers are used to identify and purify hematopoietic cells. All HSC in humans are found to be present within a population of cells which is characterized as Lin-CD34+CD38-. Nevertheless, this population is heterogeneous and also contains cells which lack long-term reconstitution ability (4, 5), raising the possibility that both HSC and MPP exist in this cell pool. Recently, the earliest HSC have been suggested to be Lin-CD34+CD38-CD90+, and their MPP progeny CD90- (4). The phenotype of lymphoid-restricted progenitors from cord blood and fetal and adult bone marrow is not yet firmly established. However, there is consensus that CD7+ and/or CD10+CD34+CD45RA+ precursors are biased to, but probably not fully committed to, lymphoid lineages at the cost of myeloid potential (reviewed in (6)). In the myelo-erythroid pathway, CMP, GMP and MEP subsets are CD34+ and negative for the early lymphoid markers CD10, CD7 or IL-7Rα. These myelo-erythroid progenitors can be isolated according to the expression of CD45RA and IL-3Rα; CMP are CD45RA-IL-3Rαlo, GMP are CD45RA+IL-3Rαlo, and MEP are CD45RA-IL-3Rα- (7).

**Transcription factors involved in differentiation of early progenitors**

During the progression from the HSC to lineage committed cells, the progenitors pass through a number of clearly defined checkpoints. Several transcriptional networks with dominant regulatory roles in various lineages have been identified. To the extent that they have been studied evolutionarily, the roles of many of these essential regulators appear to be highly conserved among vertebrates, for instance GATA transcription factors for erythroid and megakaryocytic lineage specification (8) and PU.1 and CCAAT enhancer binding protein α (C/EBPα) for granulocytic and monocytic lineage differentiation (9).

The mechanisms regulating the transition from multipotent progenitors (MMP) to lymphoid progenitors in the bone marrow is not well understood, however some crucial requirements have been identified. Recent results suggest that Wnt4 signaling affect these developmental stages, via a non-canonical (β-catenin-independent) pathway (10). In addition, the transcription factor Ikaros has been implicated in generating functional early lymphoid
progenitors such as LMP and CLP. This developmental specification and commitment is driven by the joint action of important factors such as recombination-activating gene (Rag)1 and Rag2, as well as the cytokines interleukin (IL)-7 and FMS-like tyrosine kinase (Flt)3 (11-13). The 5’ regulatory region of the genes encoding Rag proteins and the receptors of the aforementioned cytokines are demonstrated to contain functional Ikaros binding sites (14, 15) matching the observations in Ikaros-null mice in which hematopoietic progenitors lacked expression of these essential genes (16, 17). Consistent with this, Ikaros-null mice, or mice that express a dominant-negative form of Ikaros, lack all B cells, NK cells and T cells (18), demonstrating the importance of Ikaros as an initiator of the lymphoid program.

Like Ikaros, PU.1 is expressed in a variety of hematopoietic precursors, however at different levels: high in myeloid progenitors cells, intermediate in B cell precursors and undetectable in pre-T cells, which is a reflection of the dosage-dependent role of this factor in lineage specification. High expression of PU.1 in PU.1+/- fetal liver progenitors enhances the generation of macrophages, whereas low levels of expression induce B cell formation. PU.1-null mice die perinatally, lack B, T, NK or myeloid cells in the fetal liver, and have dramatically reduced numbers of LMP. The resemblance in lymphoid defects between Ikaros-null and PU.1-null mice suggest that they have non-redundant functions in the same or parallel pathways (19).

Members of the ‘E protein’ family are similarly essential for generation of early lymphoid progenitors. The E proteins form a class of transcription factors that consist of a helix-loop-helix (HLH) dimerization motif, containing two transactivation domains, and a basic DNA binding domain that binds to conserved E-box motifs in the genome. Members of the E-protein family include E2-2, HEB, and the E12 and E47 splice variant products of the E2A gene. They can form homodimers or heterodimers, and can function as transcription activators or repressors through the recruitment of distinct co-activator or co-repressor complexes. E proteins can also dimerize with any of the four inhibitor of DNA binding (Id) proteins (Id1–Id4). These are HLH proteins that lack a basic region and therefore prevent E proteins from binding to DNA. E and Id proteins are widely expressed throughout the hematopoietic system and are known to have crucial roles in cell fate decisions, differentiation and proliferation in other tissues (reviewed in (20)). E2A transcription factors up-regulate the expression of a range of lymphoid-associated genes, while suppressing non-lymphoid genes (21).
Cytokines involved in differentiation of early progenitors

Several cytokines have been identified that contribute to HSC expansion, self-renewal, and maintenance, such as the c-Kit–ligand stem cell factor (SCF) and Flt3L (22-26), thrombopoietin (TPO) (27, 28) and IL-6 (29, 30). Some cytokines are also implicated in lineage outcome. TPO, the ligand for Mpl, was found to be the long-elusive cytokine responsible for the regulation of megakaryocyte proliferation and differentiation (31). In vitro, TPO increases human megakaryocyte proliferation and differentiation (32, 33). In striking contrast to mouse hematopoiesis, where Flt3 is expressed in CLPs but not GMPs and is associated with up-regulation of the expression of a range of lymphoid-associated genes (34, 35), Flt3 is expressed in human GMP as well as in human CLP at a high level and does not mark human lymphoid-primed progenitors (36).

IL-7 has long been proposed to be a major factor in lymphoid development. In the classical model of hematopoietic differentiation, based on the early and exclusive separation of the CLP and CMP, the CLP could be identified by the expression of the IL-7Rα (37). This model has been revised, but nevertheless, IL-7Rα expression is still strongly linked to lymphoid development. Two groups reported that the IL-7Rα chain is expressed on a large proportion of human bone marrow Lin−CD34+CD38+ cells, which were CD45RA and CD10 positive (7, 38). These cells expressed transcripts characteristic for the B cell lineage, such as Pax-5 and Igβ, and the T cell–associated transcripts GATA3 and pTα, and were able to differentiate into B cells and NK cells (7, 38).

The main transcription factors and cytokines involved in early hematopoiesis are shown in figure 1.

T cell differentiation and lineage commitment

Phenotype of T cell progenitors

Adult HSC reside in the bone marrow, which is also the site where they differentiate into the different blood cell types, with the notable exception of T lymphocytes. The colonization of the thymus by hematopoietic progenitors and their step-wise exposure to the thymic microenvironment is required for proper T cell differentiation and maturation. Different T cell progenitor populations appear to enter the thymus during embryonic development versus adult life. Recent work has identified CD34+CD45RA+CD7+ cells in fetal human BM as a potential thymic colonizing cell; this progenitor gives rise to the first T cells during embryonic development (39). These cells are first detected in the fetal BM at gestation.
weeks 8–9, accumulate through the second trimester, but decrease substantially by the end of gestation. Functionally, these cells were found to have NK, B, and T potential at the
population level. Another lymphoid-restricted progenitor population persists in adults. This population, identified phenotypically as Lin\(^-\)CD34\(^+\)CD10\(^+\)CD24\(^-\), displayed very low myeloid potential but could generate B, T and NK cells and co-expressed RAG1, terminal deoxynucleotide transferase, PAX5, IL-7R\(\alpha\) and CD3\(\varepsilon\) (40). At this point, the relative contributions towards human T-cell development from each of the progenitors remain unknown. Most recently, Lin\(^-\)CD34\(^+\)CD7\(^-\) cells were isolated from human thymi and shown to possess erythroid, myeloid, and lymphoid potential in clonal assays (41). These latter results indicate that CD7\(^-\) multipotent progenitors may contribute to human thymopoiesis, as it appears they do in mice (42, 43). Together these findings suggest that several progenitor populations may contribute to T lymphopoiesis in humans, similar to what is found in mice (44).

Once in the thymus, alternative lineage cell fates are constrained and the multipotent progenitors become committed to the T-cell lineage. An analysis of the T cell receptor (TCR) rearrangement status indicates that the CD34\(^+\)CD38\(^{lo}\) cells form the most immature population in the thymus (45). Initially, the majority of early thymic progenitors (ETP) retain developmental potentials for NK and dendritic cell (DC) lineages in clonal assays. Rare ETP within the Flt3\(^+\) CCR9\(^+\) subset can also give rise to B cells, however the vast majority of ETP lack B potential (reviewed in (46)). Recently, it has also been shown that at the clonal level ETP possess myeloid potential (42, 43). Downstream of ETP, some mouse CD4\(^-\)CD8\(^-\) double-negative (DN)2 cells (CD44\(^+\)CD25\(^+\)) can still give rise to NK, DC and myeloid cells, but most of the DN2 cells are committed to the T cell lineage (42, 43, 47, 48). The T cell fate is further consolidated at the DN3 stage (CD44\(^-\)CD25\(^+\)), where developmental potential for alternative lineages is completely lost. Human equivalents to mouse DN2 and DN3 thymocytes are CD34\(^+\)CD1a\(^-\) and CD34\(^+\)CD1a\(^+\) cells, respectively (49, 50). The demonstration of non-T lineage potentials in ETP implies the requirement of active mechanisms within the thymus to promote the T cell fate while inhibiting the development of alternative lineages.

**Transcription factors involved in T cell development**

The main driving force that initiates T cell development is the Notch signaling pathway, which is required to stay active throughout the early stages of T cell differentiation in order to maintain T lineage commitment. In the thymic microenvironment ligands of the Delta family are expressed which trigger Notch receptors and activate their signaling pathway. Notch activation results in the cleavage of the Notch receptor, thereby releasing Notch
intracellular domain (N-ICD), the active form of the receptor that translocates to the nucleus (51). N1-ICD, i.e. the activated intracellular form of Notch1, suppresses transcription factors that direct towards alternative fates than the T cell lineage, whereas withdrawal of Notch1 signals in ETP reversely leads to the development of other cell lineages (9, 52). In the nucleus, N-ICD interacts with the transcriptional repressor CSL (CBF1 Suppressor of Hairless/LAG-1) and this process results in the recruitment of co-activator molecules such as proteins of the Mastermind-like family, resulting in the formation of a transcription activation complex. Direct target genes include hairy/enhancer of split homologue (HES)-1, Deltex1, CD25, pTα, and e-myc (reviewed in (53)). As a result of the Notch induced T lineage specification process, a wide variety of genes is activated, including the T cell receptor (TCR) complex signaling chain CD3ε (54) and the transcription factors GATA-3 and T cell factor (TCF)-1 (55). The latter two are part of a transcriptional network that also includes MYB, RUNX1-CBFβ, E proteins, and Ikaros; members which cooperate with Notch in a tightly regulated manner throughout the ETP, DN2 and DN3 stages (56).

Especially E-proteins seem to fulfill critical non-redundant functions next to Notch signaling. In particular, CCR9 and Notch1 expressions in early lymphoid progenitors are regulated by E2A, underlining its role in the initiation of T cell development by generating progenitors endowed with thymic homing molecules (21, 57). When imposing a block on all E protein activity by enforced expression of Id3 in human post-natal ETP, T cell development is completely abolished (58). However, single E2A, HEB or E2-2 deficient mice only exhibit partially impaired T cell development (59-61). As E-box binding complexes in T cells contain high amounts of both E2A and HEB proteins, it is thought that the block in T-cell development is partly rescued by HEB and/or E2-2 compensating for the loss of E2A (20). So far, only a few E2A-protein-dependent genes have been identified in T-cell progenitors. In vitro studies of E2A-/- hematopoietic cell lines revealed that E2A proteins cooperate to activate a subset of Notch1 targets, including genes encoding HES1 (which is crucial for T-cell progenitor expansion) and pTα (a known direct target of other E proteins such as HEB). Taken together, these observations indicate that E-protein and Notch1 activity and regulation are intertwined (reviewed in (20)). Despite these findings, there is still much to be determined about the function of E proteins during early T-cell development, particular in processes such as commitment to the T cell fate and αβ lineage decision.

**Cytokines involved in T cell development**

Further interactions with the thymic environment drive the maturation of T cell precursors
into T lymphocytes and make sure that these mature T cells can seed and function in the peripheral lymphoid organs (see chapter 2 of this thesis). During the completion of the differentiation program of thymocytes, only the cytokine IL-7 is reported to be indispensable (reviewed in (6)), which is illustrated by IL-7Rα-deficient patients displaying a profound T cell deficiency (63). Ligation of IL-7 to its receptor leads to activation of downstream signaling cascades, such as STAT molecules, PI3 kinase and Src kinase pathways (64). IL-7 has an important role in mediating survival and proliferation of human T cell precursors, largely as a result of IL-7-induced PI3K activation (62). Whether IL-7 is important in T cell differentiation as well, remains a matter of debate (6).

**B cell differentiation and lineage commitment**

*Phenotype of B cell progenitors*

Early human B cell development occurs in the bone marrow as HSC develop via various B lineage-restricted precursors into immature B cells, which leave the BM and enter the periphery. The previously identified and defined Lin<sup>−</sup>CD34<sup>−</sup>CD10<sup>+</sup> human CLP population, with restricted B, T, NK, and dendritic cell potential (66), was later shown to be biased toward B lineage development and the population was termed early B cells (67, 68). The following stages of differentiation can be identified by the expression of CD markers and the rearrangement status of Ig H and L chains. The current consensus is that B lineage-committed cells pass through the CD34<sup>+</sup>CD10<sup>+</sup>CD19<sup>+</sup>CLP early B cell stage after which they develop via CD34<sup>−</sup>CD19<sup>−</sup>CD10<sup>−</sup> pro-B cells, CD34<sup>−</sup>CD19<sup>−</sup> large pre-B I and II cells, and CD34<sup>−</sup>CD19<sup>−</sup> small pre-B II cells into CD34<sup>−</sup>CD19<sup>−</sup>sIgM<sup>+</sup> immature B cells (6, 65).

*Transcription factors in B cell development*

B-cell commitment is, like T cell commitment, characterized by the extinction of alternative lineage potentials. Thus, whereas mouse CD45R<sup>+</sup>B220<sup>+</sup>CD19<sup>−</sup> B cell precursors (the intermediate stage between the CLP and CD19<sup>+</sup> cells) still have T and NK potentials, CD19<sup>+</sup> pro-B cells are fully restricted to the B cell lineage (69). E2A and Pax5 mediate B cell commitment, as pro-B cells deficient in either gene express non-B lineage genes, enabling them to give rise to T-lineage cells, macrophages, granulocytes and erythrocytes when injected into lethally irradiated mice (70, 71). The actual specification of B cell development involves the expression of the pre-B cell receptor components VpreB, λ5 and mb-1 and the initiation of D–J rearrangements at the immunoglobulin heavy chain (IgH) locus. The transcription factors E2A and early B cell factor (EBF) are essential for these processes, partly through the transcriptional activation of the B cell lineage specific activator
Pax5 gene. However, conditional deletion of E2A in pre-B cells does not cause a dramatic decrease in the expression of its putative target genes VpreB, λ5, mb-1, B29 and IgH (72), suggesting that E2A, like PU.1, is required for the initiation but not for the maintenance of B cell gene expression. Enforced expression of EBF can activate the B lineage program in the absence of E2A (73) or PU.1 (74), which suggests that EBF is a key factor in B cell specification. These observations raise the possibility that the primary function of E2A (and of PU.1) in B cell development is to initiate EBF up-regulation. Together, E2A, EBF and Pax5 are involved on transcriptional level in B cell lineage specification.

Another crucial requirement for proper B cell development is the absence of Notch signaling. Even a low level of Notch activity, which would still be insufficient to promote thymic ETP and DN cell proliferation, is sufficient to block the B cell potential of hematopoietic progenitors (47). A high density of Delta-like Notch ligands is unique to the thymus and is not found in the bone marrow microenvironment (75). This difference in Notch ligand density does not fully explain the difference in environmental support, and it was actually shown that bone marrow precursors actively suppress initiation of Notch signaling by expressing the lymphoma-related factor (LRF) (76).

Cytokines in B cell development
In addition, murine B cell specification is reinforced by c-kit – the receptor for stem cell factor (SCF), Flt3, and IL-7R signaling (70, 77). IL-7 plays an essential role, since B cell development is arrested either at the CLP or the CD45R\(^+\)B220\(^-\)CD19\(^-\) stage in IL-7-deficient and CD127/IL-7R\(^\alpha\) chain deficient mice. It appears that IL-7 is involved in the induction of essential transcription factors, as enforced expression of EBF in CD127- or IL-7-deficient progenitors such as HSC or CLP rescues their capacity to differentiate along the B cell lineage (78, 79). In evident contrast to mouse, the essential growth factors required for the generation of normal human B cell precursors remain elusive. A good illustration of interspecies differences is the fact that IL-7R\(^\alpha\) mutated human individuals have normal levels of B cells (63), while in the mouse genetic ablation of IL-7 or CD127 expression leads to a severe reduction in B cell progenitor numbers in the bone marrow and consequently peripheral B cell numbers (80, 81).

NK cell differentiation and lineage commitment

Phenotype of NK cell progenitors
Two major subsets of NK cells exist in humans: one expressing high levels of CD56 and
low or absent CD16 (CD56\(^{hi}\)CD16\(^{−/−}\)), and a second that is CD56\(^{+}\)CD16\(^{hi}\) (82, 83). The functional properties of these populations are different in that CD56\(^{hi}\)CD16\(^{+}\) cells have relatively lower cytolytic activity and produce more cytokines than the CD56\(^{+}\)CD16\(^{hi}\) cells (82, 83). The developmental relationship between these populations has yet to be resolved.

NK cell development in the bone marrow has been extensively studied in the past. It was not until some NK cells in the peripheral lymphoid organs were found to express rearranged TCR\(\gamma\) genes (84), which were found to be absent in NK cells of athymic nude mice (85), that it was suggested that part of NK cells develop in the thymus (84, 85). We now know that NK cell differentiation actually occurs at several distinct anatomical locations, including the bone marrow, liver, thymus, spleen and lymph nodes, and may involve the circulation of NK cells at different stages of maturation between these sites (reviewed in (86)).

Experiments both in mouse and human systems have established that NK cells predominantly descend from a lymphoid precursor (reviewed in (6)). Three stages in NK cell development can roughly be defined: NK lineage commitment, NK receptor repertoire selection, and functional maturation (87, 88). Our knowledge about the early stages of human NK development is still limited. In the thymus, human NK cells develop from a common CD34\(^+\)CD1a\(^−\) NK/T precursor, whereas NK cells developing in extra-thymic locations are described as a direct progeny of CLP such as CD34\(^+\)CD45RA\(^+\)CD7\(^−\)CD10\(^−\) (89) or CD34\(^+\)CD45RA\(^+\)CD7\(^+\)CD10\(^+\) (90) cells. These cells are likely upstream of the recently identified CD34\(^{hi}\)CD45RA\(^+\)α4β7\(^hi\)CD7\(^+\)CD10\(^−\) putative committed NK precursor, which was shown to give rise to CD56\(^{hi}\)CD16\(^{−}\) NK cells in vitro (91). The immature NK cells developing from committed NK cell precursors are defined by expression of CD161 (NKR-P1) (92) and are negative for CD56 or CD16. Immature NK cells can be induced to maturity and upregulate these markers, as well as the activating and inhibitory receptors, CD94/NKG2A and killer inhibitory receptors (KIR), upon culture with stromal cells and cytokines such as IL-15 (93) or Flt3-L (94).

Transcription factors in NK cell development
The early stages of NK development and the nature of the transcription factors specifically involved herein are poorly understood as compared to T and B cell development. Mice deficient for the transcription factors Ikaros, Ets 1, Id2, IRF-1 or IRF-2 have a complete block in NK cell development at various developmental stages (85, 87). Although it was initially assumed that Id2 was important for NK lineage commitment (95, 96), a more recent
and extensive analysis revealed that the number of NK cell precursors was not affected by Id2 deletion, but that the development of these precursors into mature NK cells was blocked (97). In addition, deletion of E2a from Id2−/− mice restores the development of mature NK cells in the bone marrow (97). These observations indicate that the cause of the NK cell deficiency in Id2−/− mice is excess of E protein activity.

Interestingly, in Id2−/− mice, the NK cells in the spleen have a phenotype that is resembling that of thymus-derived NK cells, which express the receptor for IL-7 and have a low expression level of the adhesion receptors CD43 and CD11b (85). This observation raised the possibility that Id2−/− splenic mature NK cells originate from the thymus. However, recent studies indicate that these 'thymic' NK cells may be activated T cells that have acquired an NK-like phenotype, rather than being genuine NK cells (98). The observation that NK precursors develop in Id2−/− mice does not exclude the requirement of Id proteins for specification to the NK cell fate. Id3 expression is increased in Id2−/− NK cell precursors and compensatory actions of Id3 may explain the emergence of NK cell precursors in the bone marrow of Id2−/− mice (97).

**Cytokines in NK cell development**

Various cytokines have been identified that can support development of human NK cells from CD34+ cells in vitro, in particular SCF, Flt3-L, IL-7, IL-2, and IL-15. Data from mouse experiments suggest that SCF and Flt3-L act on early lymphoid precursors and by that means promote NK cell development (87). However, the exact roles of SCF and Flt3-L in human NK cell development remain elusive. IL-15 is proposed to be the most relevant factor for human NK cell development, a finding that is supported by the strongly reduced NK cell numbers in mice deficient for the expression of IL-15 or for components of its receptor, namely IL-15Rα, IL-2Rβ, and IL-2Rγc chains. Severe combined immuno-deficiency (SCID) patients suffering from deficiencies in IL-2Rγc chain expression lack NK cells (99). One SCID patient, who exhibited a lack of NK cells and diminished T cell numbers, was reported to have a severely reduced expression of the IL-2/15Rβ chain and a marked decrease of signaling through this receptor (100). Data in the mouse suggest that IL-15 is not an NK cell differentiation factor but rather maintains the viability and supports the proliferation of developing NK cells, based on the observation that numbers of NK cell precursors are not affected in mice deficient for IL-15 or its receptor components (reviewed in (6)).
pDC cell differentiation and lineage commitment

Phenotype of pDC progenitors

In humans there are two main dendritic cell (DC) subsets: conventional DC (cDC), which are HLA-DR$^+$ and express high levels of CD11c (101), and plasmocytoid dendritic cells (pDC), which are HLA-DR$^+$ and defined by the absence of CD11c expression together with high levels of CD123 (the IL-3Rα chain) and BDCA2 (102). The developmental pathway of pDC is only beginning to be elucidated. It is proposed that the direct progenitor of pDC is contained within the CD34$^+$ compartment of cord blood, fetal liver, and bone marrow. These progenitors are CD45RA$^+$CD4$^+$CD123$^+$, and are referred to as pro-pDC (progenitor of pDC) based on their phenotypic and functional similarities with pDC (103). Although some opposing observations exist (104), it seems credible that human cDC and pDC can both develop from lymphoid- as well as myeloid-restricted precursors, which is consistent with what was observed earlier in mouse models (7, 105-108).

Transcription factors in pDC development

E2-2 plays a crucial role in the transcriptional control of pDC development (109, 110). Previous studies had already indicated that the ectopic expression of Id2 in multipotent progenitors in vitro prevented pDC, but not cDC, development (111, 112). Mice with only one functional E2-2 allele have decreased expression of many pDC-associated genes, including lymphoid-associated genes such as terminal deoxynucleotidyl transferase (TdT), CC-chemokine receptor 9 (CCR9) and SpiB, an ETS family transcription factor that is essential for pDC development (109, 113).

Cytokines in pDC development

Most data on the role of cytokines in pDC development is based on mouse studies and these have demonstrated an important role of Flt3L. The generation of pDC from human hematopoietic progenitors was similarly shown to be affected by Flt3L, and further enhanced in the presence of thrombopoietin (TPO) (114).

An overview of the most important identified factors reported in lymphoid development is provided in figure 2.
Figure 2. Model of human lymphoid development. Transcription factors and cytokines involved in lineage commitments are depicted. The earliest lymphoid progenitors (ELP) are phenotypically identified as CD34<sup>hi</sup>CD45RA<sup>hi</sup>CD7<sup>+</sup> (fetal) or Lin<sup>-</sup>CD34<sup>hi</sup>CD10<sup>+</sup>CD24<sup>-</sup> (postnatal). ELP give rise to thymic seeding progenitors (TSP) and common lymphoid progenitor (CLP). The latter have B, NK and T cell differentiation potential. Thymic seeding progenitors (TSP) differentiate mostly to the early T lineage progenitors (ETPs) within the thymus, however they possess limited B, NK, myeloid and dendritic cell (DC) differentiation potentials as well. NK/T progenitors in the thymus are DN2 (mouse)/CD34<sup>+</sup>CD1a<sup>-</sup> (human). DN3 / CD34<sup>+</sup>CD1a<sup>+</sup> precursors are T lineage committed. Cytokines involved in lineage outcome are depicted directly above or at the right of the arrows which denote differentiation, transcription factors are depicted below or at the left of the arrows.

CLP, common lymphoid progenitor; ELP, earliest lymphocyte progenitor; ETP, early T cell progenitor; TSP, thymic seeding progenitor
Outline of this thesis

Within the studies presented in this thesis, factors which are involved in lymphoid lineage decisions and/or the subsequent maintenance of the generated populations are investigated. The chapters 2 and 3 review and discuss technical ways to address questions related to human lymphoid development, lineage decisions and maintenance, both in vitro and in vivo. In chapter 4 we make use of the in vivo model of human lymphoid development (described in chapter 2) to dissect the role of IL-7 signaling at different stages of T cell development and in the maintenance of mature T cells. In chapters 5 and 6 we evaluate the effects of TSLP, a cytokine closely related to IL-7, on early human lymphoid precursors. B cell progenitors are assessed in the first study and T cell progenitors in the latter. In this chapter 6 we specifically address the question whether TSLP is able to substitute for IL-7 in T cell development. In chapter 7 we investigate the effect of early enforced expression of the T cell receptor (TCR) on T cell commitment and specificity. In chapter 8 a TCR and Id2 are simultaneously overexpressed in thymic progenitors. The role of E protein activity in human T cell, and specifically αβ-lineage+ T cell, development is thereafter evaluated.
References


78. Kikuchi, K., A. Y. Lai, C. L. Hsu, and M. Kondo. 2005. IL-7 receptor signaling is necessary
for stage transition in adult B cell development through up-regulation of EBF. The Journal of experimental medicine 201:1197-1203.


93. Miller, J. S., and V. McCullar. 2001. Human natural killer cells with polyclonal lectin and


