Development of Human Lymphoid Cells

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Key Words

human lymphocytes, T cells, B cells, NK cells, DC

Abstract

The lymphocytes, T, B, and NK cells, and a proportion of dendritic cells (DCs) have a common developmental origin. Lymphocytes develop from hematopoietic stem cells via common lymphocyte and various lineage-restricted precursors. This review discusses the current knowledge of human lymphocyte development and the phenotypes and functions of the rare intermediate populations that together form the pathways of development into T, B, and NK cells and DCs. Clearly, development of hematopoietic cells is supported by cytokines. The studies of patients with genetic deficiencies in cytokine receptors that are discussed here have illuminated the importance of cytokines in lymphoid development. Lineage decisions are under control of transcription factors, and studies performed in the past decade have provided insight into transcriptional control of human lymphoid development, the results of which are summarized and discussed in this review.

INTRODUCTION

CLP: common lymphoid precursor

TdT: terminal deoxynucleotidyl transferase

All hematopoietic cells are derived from pluripotent hematopoietic stem cells (HSCs). These cells differentiate into mature hematopoietic cells through various intermediate cell types that are defined by expression of cell surface antigens. Traditionally, investigators have assumed that the first step in hematopoietic development is differentiation of HSCs into myeloid and lymphocyte precursors. Myeloid precursors differentiate into erythroid, megakaryocytic, and granulocytic/monocytic (GM) lineages, whereas lymphoid precursors develop into natural killer (NK), T, and B cells. A wealth of data support this concept. However, a recent study has put this model into question with the identification of a precursor that possesses a combined GM and lymphoid precursor potential but is unable to develop into an erythrocyte or megakaryocyte (1). Most researchers agree that the lymphocyte T, B, and NK cells originate from a common precursor, generally referred to as a common lymphoid precursor (CLP), although some researchers maintain that T and B cells are not derived from a common precursor without myeloid precursor activity but instead that there are T/macrophage and B/macrophage precursors (2). The developmental status of dendritic cells (DCs) is more complicated. The DC lineages consist of several often not clearly defined populations that seem to have a myeloid, lymphoid, or mixed lymphoid/myeloid origin.

Most of our knowledge about lymphoid development stems from studies with mice. The obvious reason for the reliance on mice studies is the possibility of performing in vivo experiments to test precursor activities of certain cell populations. In addition, genetically modified mice have been instrumental not only in illuminating developmental pathways but also in elucidating mechanisms behind certain developmental cellular transitions. By contrast, most information on human hematopoietic development is derived from in vitro studies, although certain genetic abnormalities have greatly contributed to our understanding of some underlying mechanisms of human hematopoietic cell development. Studies on human hematopoiesis seem to be generally consistent with principles outlined in experimental models, but the cell surface phenotypes of human transitional cell populations are often different from those in the mouse. In this review, we discuss the current knowledge regarding human lymphoid cell development, in particular of T, B, and NK cells and DCs. We do not exhaustively review current information from mouse models, as excellent reviews have appeared recently (2a–d, 94, 160), but this information is used as reference to compare human and mouse lymphocyte development.

HUMAN LYMPHOID PRECURSORS

The idea that hematopoiesis progresses by gradually limiting the developmental potential of precursors predicted that precursor cells exist with limited lymphocyte-restricted precursor potential. Indeed, precursor cells that are restricted to lymphoid lineages have been identified in both mice and humans.

That all hematopoietic precursors in humans are present within a population of cells that express CD34 is well established (reviewed in 3), and this marker is useful in elucidating pathways in the development of particular hematopoietic lineages. In searching $CD34^+$ human bone marrow cells for the presence of cells that express lymphocyte-restricted antigens, several research groups have found $CD34^+$ cells expressing the lymphocyte markers terminal deoxynucleotidyl transferase (TdT) and CD10, and they speculate that these cells represent precursors of T and B cells (4, 5). Galy and coworkers (6) obtained experimental support for this idea by demonstrating that $CD34^+CD10^+CD45RA^+$ cells obtained from fetal and adult bone marrow develop into $CD33^+$ and $CD1a^+CD33^+$ DCs and $CD19^+$

B cells, although these cells were unable to develop into $CD14^+$ monocytes. Importantly, investigators found that 14% of single-sorted fetal bone marrow $CD34^+CD10^+$ cells produced B cells, NK cells, and DCs. In addition, they showed that a limited number of $CD34^+CD10^+$ cells injected into fragments of fetal thymus were able to develop into T cells, but technical limitations prevented testing T cell development on a clonal level (6). Mouse bone marrow CLP, as described for the first time by Kondo et al. (7), express the IL-7Rα chain. Indeed, two groups reported that the IL-7Rα chain is expressed on a large proportion of human bone marrow $CD34^+CD10^+$ cells, which were CD45RA and CD43 positive but negative for CD24 (8, 9). 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 (8, 9). These characteristics, and the similarity of these cells with those defined by Galy et al. (6), suggested that the $CD10^+$IL-7Rα^+ cells represent CLP. However, the number of NK cells recovered after four weeks culture of these cells with Sys-1 cells, a murine stromal cell line in the presence of stem cell factor (SCF), Flt3-L, IL-7, and IL-2, was much lower than that of B cells (9), and the T cell precursor activity of the $CD34^+CD10^+$IL-7Rα^+ bone marrow precursors was not tested, leaving open the possibility that these IL-7Rα^+ precursor cells were biased to the B cell lineage. This notion is supported by identification of a very similar cell type in cord blood, which was shown to be B cell biased (10) (see below).

Many studies have used CD7 as a marker for lymphoid progenitors because this marker is not expressed or is only weakly expressed on myeloid cells (11). An early study demonstrated that $CD34^+CD7^+$ bone marrow cells contain NK cell precursors, but no information was provided about T cell, B cell, and DC developmental potential (12). More recently, a careful phenotypic and functional analysis of $CD34^+$ bone marrow cells revealed the presence of $CD34^+lin^-$, $CD7^+CD10^-$ (2%), $CD7^-CD10^+$ (10%), and $CD7^+CD10^+$ (0.3%) cells (13). Both the bone marrow $CD7^+$ and $CD10^+$ populations had NK and B cell precursor activities, but the B cell potential of $CD34^+lin^-CD10^+$ was much higher than that of the $CD7^+$ cells. Because of the scarcity of the $CD7^+CD10^+$ populations, the functional activity of those cells was not tested (13). Polymerase chain reaction (PCR) analysis of B cell– and NK cell–specific transcripts of these populations strongly suggested that the $CD34^+lin^-$, $CD7^+CD10^-$ and $CD7^-CD10^+$, are NK and B cell biased, respectively (13). The chemokine receptor CXCR4 is expressed on cells with restricted lymphoid precursor activities, and the $CD34^+CXCR4^+$ cells can be further subdivided into IL-7Rα^+ and IL-7Rα^- cells. Although the developmental potential of $CD34^+CXCR4^+$IL-7Rα^- bone marrow cells has yet to be tested (14), these cells may be precursors of recently identified lymphoid precursors in neonatal cord blood, which were found to be IL-7Rα^- (10, 15). Hao et al. (15) identified $CD34^+CD38^{lo}CD45RA^+CD7^+CD10^+$IL-7R$\alpha^-$ cells in cord blood able to differentiate into NK cells, B cells, and $CD1a^+$ DCs, and although the T cell potential of these cells was not tested, Hao et al. suggested that these were lymphoid precursors. Two other groups confirmed that $CD34^+CD7^+CD45RA^+$ cord blood cells have lymphoid potential but found that CD10 and CD7 tended not to be coexpressed on $CD34^+CD45RA^+$ cells (10, 13). Interestingly, the $CD34^+CD45RA^+CD7^-CD10^+$ population expressed IL-7Rα, whereas IL-7Rα was absent on $CD34^+CD45RA^+CD7^+$ cells (10). Both $CD34^+CD7^+$IL-7Rα^- and $CD34^+CD10^+$IL-7Rα^+ populations had lymphoid potential, but the $CD34^+CD45RA^+CD7^+$ cells preferentially differentiated into T and NK cells, whereas the $CD34^+CD45RA^+CD10^+$ population was biased to develop into B cells (10, 13). Although differentiation assays revealed

pDC: plasmacytoid dendritic cell

TSP: thymus seeding progenitor

ETP: early thymic progenitor

that both $CD34^+CD45RA^+CD7^+$ and $CD34^+CD45RA^+CD10^+$ populations are biased to lymphoid lineages and completely lack the ability to develop into erythrocytes and megakaryocytes, they possess some GM precursor potential (10). It is not clear whether all cells within the $CD34^+CD45RA^+CD7^+$ population are multipotent or whether this population contains both GM and lymphoid precursors. More recently, Chicha et al. (16) reported that either $CD34^+CD38^+CD10^+$ or $CD34^+CD38^+CD7^+$ cord blood precursors can develop into B cells and plasmacytoid DCs (pDCs), but that they fail to develop into GM cells. This study did not show whether the $CD34^+CD7^+$ cord blood cell precursors coexpress CD10 or not, and these authors did not use anti-CD45RA in separating their precursors (16), making it difficult to compare their results with those of others (10, 15).

The phenotype of lymphoid-restricted progenitors from cord blood and fetal and adult bone marrow is not yet firmly established. An important reason for this lies in the use by different groups of dissimilar antibodies against the same differentiation antigen or the same antibodies tagged with different fluorochromes. For example, anti-CD7-FITC (fluorescein isothiocyanate) used by Haddad et al. (10) is much less sensitive than anti-CD7-PE (phyco erythrin) or Tricolor, and the use of CD7-FITC misses cells that do express CD7 but at lower levels (11). Another reason the phenotype has not been firmly established is the controversy concerning expression of CD38, which was absent on lymphoid precursors in one study (17) but expressed in two others (10, 16); this difference could be due to the use of anti-CD38 antibodies with different affinities. With these caveats in mind, there is consensus that CD7 and CD10 define $CD34^+CD45RA^+$ precursors biased to, but probably not fully committed to, lymphoid lineages (**Figure 1**). In addition, within the $CD34^+CD45RA^+$ population both in bone marrow and cord blood, there are $CD7^+CD10^+$, $CD7^+CD10^-$, and $CD7^-CD10^+$ cells, although the existence and function of the very rare $CD7^+CD10^+$ cells have yet to be confirmed. It is tempting to speculate that $CD34^+lin^-CD45RA^+CD7^+CD10^+$ are the precursors of the $CD7^+CD10^-IL\text{-}7R\alpha^-$ T/NK cell–biased and $CD7^-CD10^+IL\text{-}7R\alpha^+$ B cell–biased populations (10, 13), but this has to be proven by using various antibody preparations combined with functional, preferentially clonal assays.

DEVELOPMENT OF T CELLS

Human Thymus Seeding Progenitor (TSP) and Early Thymic Progenitor (ETP)

The transient developmental potential of thymic precursors demands that a continuous source of thymus seeding progenitors (TSP) can enter the thymus. Because the thymus remains active probably through an advanced age, TSP should be present in adult blood as well. As discussed in a previous section, $CD34^+CD45RA^+CD7^+$ cells that have T, B, and NK and some GM precursor activities have been found in cord blood (10, 15). These cells resemble $CD34^+CD38^{lo}$ ETP with T cell, NK cell, and DC precursor activities (18) (**Figure 2**). An analysis of the T cell receptor (TCR) rearrangement status confirms that the $CD34^+CD38^{lo}$ cells form the most immature population in the thymus (19), and it is tempting to speculate that $CD34^+CD38^{lo}$ thymocytes, which mostly coexpress CD10, are the direct progeny of the $CD34^+CD45RA^+CD7^+CD10^+CD38^{lo}$ cord blood cells (15).

The presence of multipotential precursors in the human thymus indicates that T cell commitment takes place within this organ in line with data in the mouse (20). Recently, however, investigators argued that the murine thymus is seeded not only by multipotent precursors but also by precursor cells that are lineage restricted (21, 22). If that is also the case in humans, such cells should be present in cord

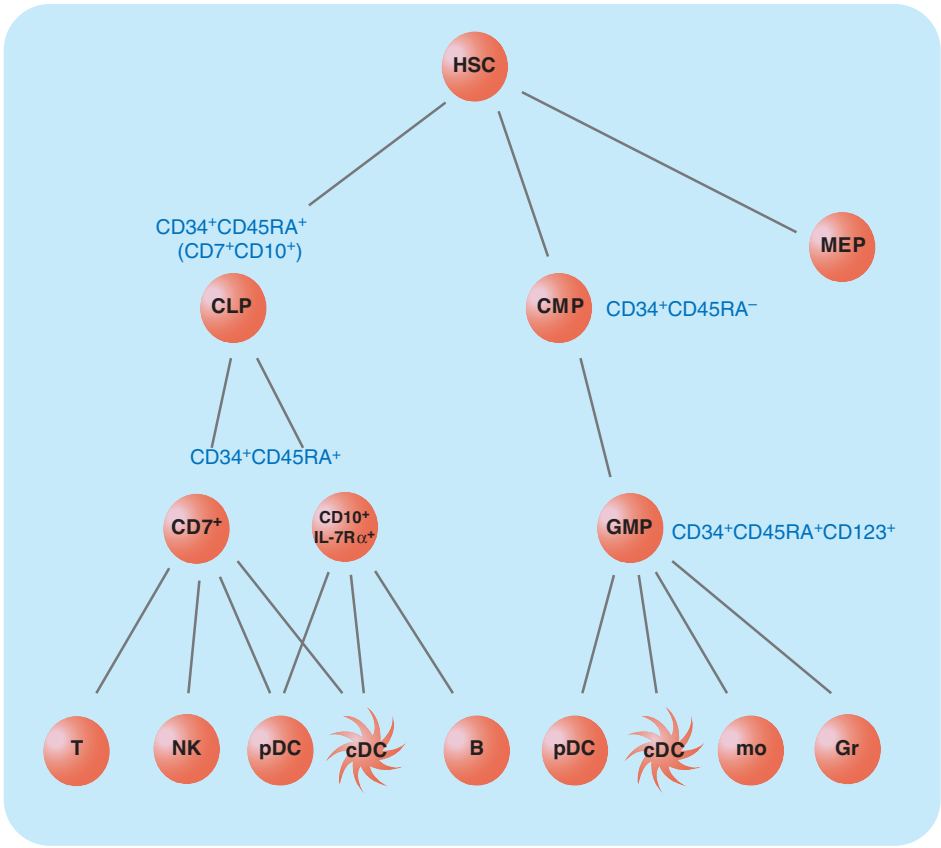


Figure 1

Model for development of CLP and GMP. CLP develop into pDCs and cDCs, but whether they derive from precursors that are $CD7^+$, $CD10^+$, or $CD7^+CD10^+$ is not completely clear (Reference 16) (MEP, megakaryocyte/erythrocyte progenitor).

blood. One study failed to find evidence for the presence of TCR rearrangements in cord blood $CD34^+$ cells (23), but another study reported the presence of complete TCRδ and partial TCRβ (Dβ-Jβ) rearrangements in $CD34^+CD7^+$ cord blood precursors (24). This issue should therefore be readdressed by single-cell PCR analysis of the recently identified rare $CD34^+CD45RA^+CD7^+$ cord blood population (10, 15). T cell–restricted precursors were convincingly identified in human bone marrow (25), but whether these cells can migrate to the thymus is unclear. $CD34^+CD19^+$ B cell precursors are also present in the human thymus (26). Recently, it was reported that although $CD34^+lin^-$ cord blood cells could develop into B cells in a fetal thymic organ culture (FTOC) in the presence of Notch inhibitors (26a), $CD34^+CD1a^-$ thymocytes failed to do so, suggesting that the latter cells lack B cell precursor potential. Thus, the thymic B lineage cells (26) may be derived from $CD34^+CD10^+$IL-$7R\alpha^+$ cord blood progenitors that express a B cell transcript signature and are developmentally biased to the B cell lineage (10).

In summary, at least a proportion of the precursors that seed the human thymus are multipotential. Whether or not some of the precursors that migrate into the thymus are lineage restricted before entrance is unclear.

Cellular Stages in the Development of ETP into $CD4^+CD8^+$ Double-Positive Immature T Cells

Over the past few decades, the various transitional stages of T cell development in the human thymus have been characterized

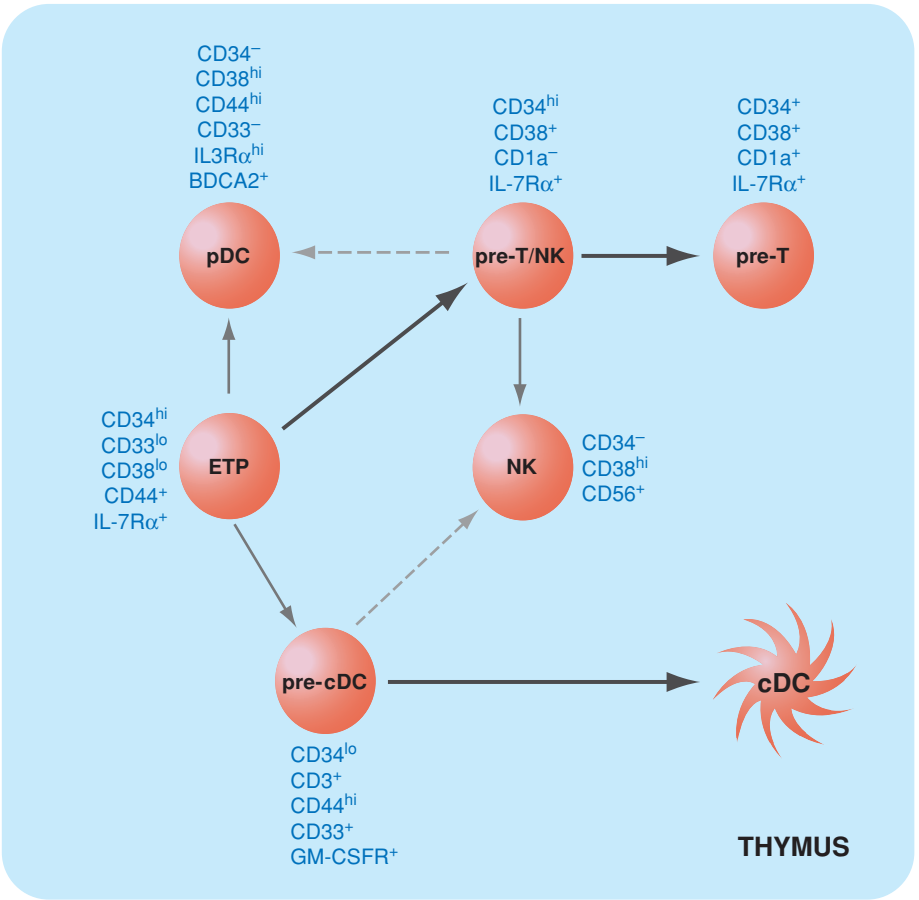


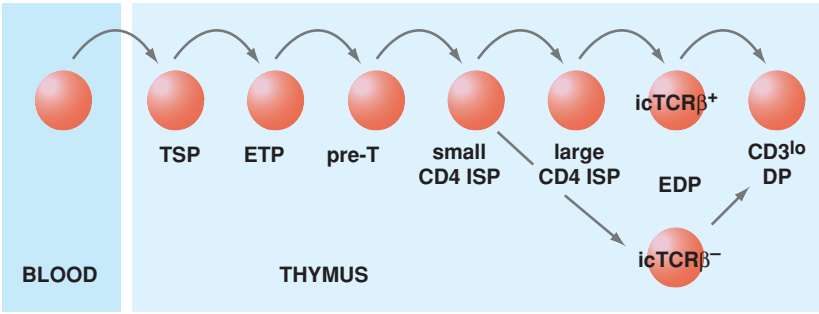
Figure 2

Model for the earliest stages of development in the thymus. Evidence from in vitro experiments suggests that NK cells can develop not only from T/NK precursors but also from NK/DC myeloid precursors. On the basis of expression of pTα, CD2, CD5, and CD7, thymic pDCs may develop from ETP via a lymphoid pathway, but it is possible that the $CD34^+CD44^{hi}$ myeloid precursors can also develop into pDCs in the human thymus.

regarding phenotype and status of the TCR gene rearrangements (reviewed in 27, 28) (**Figure 3**). As discussed in the previous section, ETP are enclosed within the $CD34^+CD1a^-$ population. The downstream $CD34^+CD1a^+$ population is committed to the T cell lineage because they are unable to develop into non-T cells (27, 29–31). A recent study has shown that the subsequent CD4 immature single-positive (CD4 ISP) (32) population can be divided into two subgroups: a $CD4^+CD1a^+$ ($CD4\ ISP^{lo}$) and a $CD4^{hi}CD1a^{hi}$ ($CD4\ ISP^{hi}$) population (S. Ligthart, Y. Yasuda, H. Spits, and B. Blom, manuscript submitted). Downstream of the CD4 ISP subset are $CD4^+CD8\alpha^+\beta^-$ (early double-positive) and $CD4^+CD8\alpha^+\beta^+$ populations (29, 32), the latter cells being the precursors of double-positive $TCR\alpha\beta^+$ cells.

CD4 ISP: CD4 immature single-positive cell

During the early stages in T cell development, the TCR loci undergo rearrangement in a sequence $TCR\delta > \gamma > \beta > \alpha$ (19, 33, 34); however, there is disagreement with respect to the cell type in which rearrangement of particular loci occurs, probably owing to differences in sensitivity of the methods used to analyze TCR rearrangements. For example, by using a PCR-based and GeneScanning analysis, some productive TCRβ V-DJ rearrangements were found in the $CD34^+CD1a^+$ cells, although the sensitivity of this assay raises the possibility that a small contamination was responsible for this signal (19). A less-sensitive southern blot analysis detected the first TCRβ V-DJ rearrangements in the CD4 ISP cells (33).



CD34	+	+	+	+	-	-	-
CD1a	-	-	+	+	+	+	+
icTCRβ	-	-	-	-	+	+/-	+
icCD3ε	?	+/-	+	+	+	+	+
CD2	-	+/-	+	+	+	+	+
CD5	-	+/-	+	+	+	+	+
CD7	+	+	+	+	+	+	+
CD4	-	-	-	+	+	+	+
CD8α	-	-	-	-	-	+	+
CD8β	-	-	-	-	-	-	+
CD45RA	+	+	-	-	-	-	-
CD45RO	-	-	-	-	+	+/-	+
IL-7Rα	-	+	+	+	+	+	+
DJβ	-	+	+	+	+	+	+
V-DJβ	-	-	-	+	+	+	+
V-Jα	-	-	-	-	-	-	+

Figure 3

Intermediate stages in development of early thymocyte subsets into double-positive cells. The TCR rearrangement status of the various subpopulations are based on data presented in References 33 and 34. However, it should be noted that a recent study (19) positions the onset of these rearrangements at earlier stages. This is discussed in the text. (Abbreviation: EDP, early double-positive.)

Several lines of evidence indicate that selection for productive, in-frame TCRβ rearrangements and production of a TCRβ protein, a process referred to as β-selection, can occur in distinct populations of cells that differ in CD4 and CD8 expression. A very low frequency of productive TCRβ V-DJ rearrangements was detected in the $CD34^+CD1a^+CD4^-$ population, but expression of TCRβ protein was not analyzed (19). Nonetheless, these results suggest that a few cells may undergo β-selection before CD4 is expressed. However, not all populations downstream of the $CD34^+CD1a^+CD4^-$ cells are post-β-selection cells because intracytoplasmic (ic) $TCR\beta^-$ populations have been found in the CD4 ISP cells (33; S. Ligthart, Y. Yasuda, H. Spits, and B. Blom, manuscript submitted) and in the $CD4^+CD8\alpha^+\beta^-$ early double-positive cells (33, 35). Ten to twenty

percent of the CD4 ISP are icTCRβ$^+$. In contrast to the TCRβ$^-$ CD4 ISP, the icTCRβ$^+$ CD4 ISP were in cycle; expressed elevated levels of CD1a, CD4, CD28, CD45RO and CD71; and differentiated into TCRαβ$^+$ T cells in a FTOC with faster kinetics than the icTCRβ$^-$ CD4 ISP (S. Ligthart, Y. Yasuda, H. Spits, and B. Blom, manuscript submitted). These data indicate that β-selection can occur within the CD4 ISP population. On the basis of the results of our studies and those of others, we propose that expression of a TCRβ protein and the ensuing β-selection occur within a certain developmental window and are not tightly coupled to regulation of CD4, CD8α, and CD8β expression. Thus, a few cells already undergo β-selection before CD4 is expressed (19). A larger proportion is β-selected after upregulation of CD4 (33; S. Ligthart, Y. Yasuda, H. Spits, and B. Blom, manuscript submitted), and a third group of the pre-T cells upregulate CD4 and CD8α before initiating and completing TCR rearrangements (35, 36).

The Role of Cytokines in Early T Cell Development

The important role of IL-7 in T cell development is well documented. The IL-7 receptor consists of two chains, IL-7Rα and gamma common (γc), which is also part of the receptors for IL-2, IL-4, IL-9, IL-15, and IL-21. Genetic defects in the genes encoding for γc (37, 38), IL-7Rα (39, 40), or the Janus kinase Jak3, a component of the IL-7-induced signal transduction pathway (41, 42), account for the majority of severe combined immune deficiencies (SCID) characterized by strongly reduced numbers of T cells. The most frequent form of SCID is caused by mutations in the γc-encoding gene (reviewed in 43). In these patients, T and NK cells are absent, whereas in contrast to what is observed in γc-deficient mice, B cell development is normal (37, 38, 43). IL-7Rα-deficient patients also display a profound T cell deficiency and have near normal B cell numbers. In contrast to γc-deficient patients, IL-7Rα-deficient patients have normal frequencies of NK cells in the periphery (39, 40). These manifestations argue against an important role of IL-7 in survival and proliferation of lymphoid precursors. Rather, they indicate that human T cell development specifically and critically depends on IL-7.

The precise function of IL-7 in human T cell development is not fully understood. Inhibition of IL-7R signaling by blocking anti-IL-7 and anti-IL-7R antibodies prevents expansion and differentiation of developing T cells in a FTOC (44, 45), indicating an important role of IL-7 in mediating survival and proliferation of human T cell precursors. This effect is mediated by IL-7-induced PI3K activation through one tyrosine residue at position 449 in the cytoplasmic tail of the IL-7Rα chain (45). In the mouse, IL-7 does not appear to be critical for differentiation of TCRαβ cells in the thymus, as near normal distributions of subsets were observed in the thymuses of IL-7Rα-deficient mice (46). There is evidence, however, that IL-7 is important for differentiation of human T cells. First, differentiation of CD34$^+$ precursors in a FTOC in the presence of anti-IL-7R antibody is almost completely blocked at the transition of CD34$^+$CD1a$^+$ cells into CD4 ISP (44). Second, according to one report, thymocytes from γc-deficient infants possess TCRβ D-J but lack V-DJβ rearrangements (47). Although a function of IL-7 in TCRβ rearrangements in human pre-T cells has yet to be confirmed, this finding may explain the complete lack of T cells in many γc- and IL-7Rα-deficient patients (40, 48). On the other hand, IL-7 is required for peripheral T cell homeostasis in humans (49), raising the alternative possibility that the absolute T cell deficiency in γc-deficient patients is caused by a combination of defects in early T cell development and in T cell homeostasis.

Taken together, the data indicate that IL-7 is indispensable for human T cell development. The data also suggest that IL-7 is more critical for human than for mouse T cell development, which may be attributed to a

role for IL-7 in TCRβ V-DJ rearrangements, but more data are required to confirm this speculation.

Transcription Factors Involved in Human T Cell Development

Recently, it has become clear that Notch receptor ligand interactions play crucial roles in T cell development. Notch receptors control differentiation and proliferation in response to ligands on neighboring cells (50). Interaction of Notch receptors (Notch1, 2, 3, 4) with one of their ligands (DL1, 2, 4, and Jagged-1, -2) results in proteolytical cleavage of its cytoplasmic portion (icNotch), which migrates to the nucleus and interacts with C promoter binding factor (CBF)-1/recombination signal–binding protein (RBP)-J to mediate transcription of target genes. In the absence of icNotch, RBP-J represses transcription by interacting with various corepressors. When icNotch binds to RBP-J, it recruits the coactivator mastermind-like (MAML)-1, which binds to icNotch in the icNotch-CSL-DNA complex, thereby converting the CSL (CBF-1/suppressor of Hairless/Lag1) complex into a transcriptional activator. Radtke et al. (51) were the first to demonstrate that conditional deletion of Notch1 resulted in a switch in development in that, in the thymus, T cell development was inhibited and B cell development stimulated (51). More recently, Jaleco et al. (52) demonstrated that coculture of human cord blood $CD34^+$ cells with the murine stromal cell line S17 expressing the Notch ligand DL1 resulted in generation of $CD7^+$ cells with strong NK cell precursor activity while inhibiting B cell development (52). The cells generated in this system expressed high levels of CD7 and cytoplasmic CD3ε and may represent T/NK precursors (52). These data were confirmed and extended by Zuniga-Pflucker and collaborators (53), who documented that cord blood and bone marrow $CD34^+$ cells cocultured with another DL1-expressing bone marrow stromal cell line, OP9-DL1, IL-7, and Flt3-L, mediated full T cell development (53, 54), similar to murine HSCs (55). Recently, the function of Notch in development of T and non-T cells in a FTOC system (26a) and in the OP9-DL1 system (55a) was investigated using the γ-secretase inhibitor 7 (N-[N-(3,5-difluorophenyl)-L-alanyl]-S-phenylglycinet-butyl ester) (DAPT), which prevents proteolytic cleavage of Notch, thereby inhibiting Notch signaling. As expected, DAPT strongly inhibited T cell development, whereas development of non-T cells was stimulated. As expected, inhibition of TCRαβ development by DAPT was rescued by forced expression of icNotch1 by retrovirus-mediated gene transfer (55a). TCRβ V-DJ but not D-Jβ rearrangements were strongly inhibited in cells that developed from $CD34^+CD1a^-$ thymocytes in a FTOC in the presence of DAPT (26a), as in the mouse (56). This is not, however, the only mechanism of inhibition of T cell development by Notch1 because introduction of TCRαβ-encoding cDNA by retrovirus-mediated gene transfer into $CD34^+CD1a^-$ fails to overcome the requirement of Notch for development of these precursors into T cells (N. Legrand & H. Spits, unpublished observations). These findings indicate that Notch is required for induction and maintenance of T cell specification, as found previously in the mouse (57). We and others also observed that, in the presence of DAPT, thymic $CD34^+CD1a^-$ cells generate more NK cells, monocytic/DCs (26a), and pDCs (55a), strongly suggesting that Notch drives lineage decisions in thymus of humans, similar to what occurs in the mouse thymus (58).

Two research groups have documented that ectopic expression of icNotch1 in $CD34^+$ cells affected T cell development in an in vitro FTOC (59, 60). icNotch1 favored development of human TCRγδ above TCRαβ cells when expressed in precursor cells before the TCRβ selection checkpoint, suggesting that a

γc: gamma common chain of the IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptor

DL1: Delta-like 1

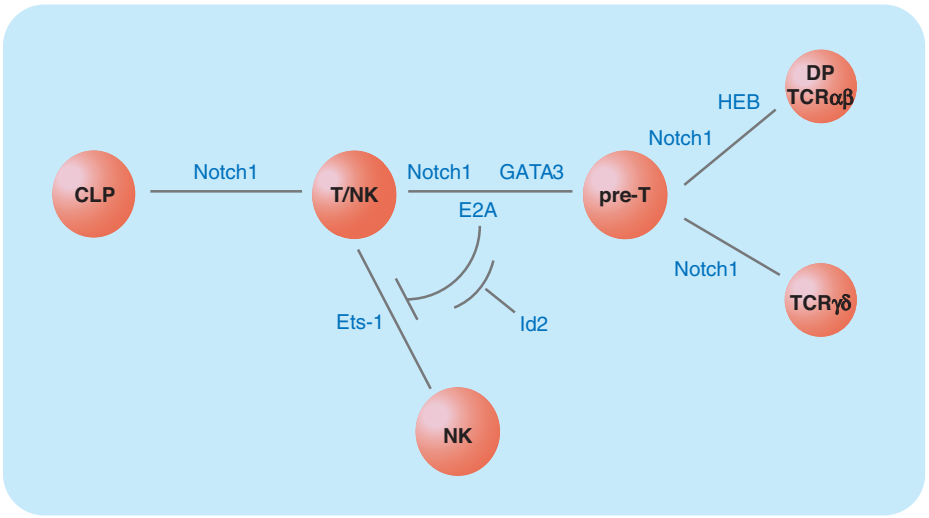


Figure 4

Transcription factors involved in T and NK cell development. The model assumes that NK cells develop from common T/NK cell precursors. It is possible, as indicated in **Figure 2**, that NK cells can also develop from NK/DC precursors that are unable to develop into T cells. E2A (i.e., E47) blocks NK cell development. Because Id2 sequesters E47, ablation of Id2 leads to inhibition of NK cell development, which can be neutralized by ablation of E47 (B. Kee, personal communication).

strong Notch1 signal may promote TCRγδ at the expense of TCRαβ development (59, 60). These data contrast with earlier findings in the mouse system that indicate the reverse, that diminished Notch1 signaling favors TCRγδ above TCRαβ development (61). However, De Smedt et al. (59) found that $CD34^+$-expressing icNotch1 developed into TCRαβ cells in the bone marrow, similarly to the effect found in the mouse, indicating that in an in vivo setting there is no preferential TCRγδ development from icNotch1 overexpressing $CD34^+$ precursor cells. Thus, the role of Notch1 in TCRαβ and γδ diversification in humans remains to be established.

Other transcription factors that have been implicated in human T cell development are members of the basic helix-loop-helix (bHLH) subfamily of E-box binding (E) proteins (**Figure 4**). There are four E proteins, E12, E47, HEB, and E2-2, all of which are involved in both T and/or B cell development in the mouse (62). Ectopic expression of natural antagonists of the E proteins, the Id (inhibitors of DNA binding)-2 and Id3, in human $CD34^+$ cells inhibits T cell development and promotes NK cell development in a FTOC (63, 64). Stimulation of NK cells by Id2 and Id3 overexpression is consistent with the fact that $Id2^{-/-}$ mice have few NK cells (65), and together these data indicate that the balance of Id and E proteins plays a role in T/NK lineage diversification both in humans and mice. Interestingly, introduction of Id3 in CD4 ISP cells results in their inhibition of TCRαβ development, but not of TCRγδ development, in a FTOC (64), which could be reversed by coexpressing HEB (R. Schotte, Y. Yasuda, and H. Spits, manuscript submitted). Studies in the mouse have shown that HEB controls pTα expression (66, 67). These data, combined with the demonstration in mice that β-selection commits precursor cells to the TCRαβ lineage (68), suggest that HEB stimulates TCRαβ development by control of pTα expression (**Figure 4**).

Another transcription factor that has been associated with various stages of T cell development is GATA3 (69). Taghon et al. (70) observed that overexpression of GATA3 in human $CD34^+$ thymic precursors resulted initially in stimulation of development into $CD4^+CD8^+$ cells in a FTOC, consistent with a role for GATA3 in early development. However, further development of GATA3-overexpressing cells was compromised, as the absolute numbers of TCR$\alpha\beta$ cells were severely reduced at later time points. Taghon et al. (70) also found a strongly reduced expression of TCRβ protein by GATA3 overexpression and suggested that deregulated expression of GATA3 interferes with appropriate TCRβ rearrangement or translation. However, these findings are difficult to reconcile with observations of conditional GATA3-deficient mice that suggested that GATA3 is required for TCRβ expression and pre-TCR signaling (69). Experiments, yet to be performed, to test the effect of GATA3 knockdown on T cell development should reveal the role of this transcription factor in human T cell development.

In summary, the role of Notch1 in human T cell development has been established. In addition, it is clear that E proteins are required for early T cell development. It remains to be investigated how Notch1 and E proteins are linked in the control of human T cell development. Also, it has yet to be established that GATA3 is essential for early T cell development in humans.

DEVELOPMENT OF NATURAL KILLER (NK) CELLS

Upon their discovery, NK cells were operationally defined as leukocytes able to kill transformed cells without prior sensitization. Today, NK cells are known to play essential roles in the innate immune response, as well as in the generation of an adaptive immune response. Initially, investigators believed that NK cells were related to other cells of the innate immune system, such as monocytes; however, work in the 1990s established that NK cells are more closely related to T cells, with whom they can share a common precursor. NK cells are functionally similar in particular to CTL, as both types of cells have the capacity to kill other cells and to produce cytokines such as interferon (IFN)-γ. NK cells probably coevolved with other cell types of the lymphoid system, in particular with T cells, because both of these lymphocytes recognize conventional and nonconventional major histocompatibility complex (MHC) molecules. Functional MHC molecules are present in cartilaginous fish, but not in more primitive species. Similarly, NK cells, as they are currently defined, have not been identified in species lower than fish (71).

In humans, there are two major subsets of NK cells: one expressing high levels of CD56 and low or no CD16 ($CD56^{hi}CD16^{+/-}$), and a second that is $CD56^+CD16^{hi}$ (72, 73). The distribution of the cells and 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 (72, 73). As discussed below, the developmental relationship between these populations has yet to be resolved.

Sites of NK Cell Development

NK cells can develop at multiple sites. In the fetus, NK cell precursors have been found in the bone marrow (6), liver (74), thymus (75), spleen, lymph nodes (T. Cupedo & H. Spits, manuscript in preparation), and possibly in the intestine (76). However, identification of an NK precursor does not prove that NK cells actively develop in those organs. Such proof would require in situ identification of committed NK cell precursors and other downstream intermediate stages. Indirect evidence suggests that the population of fetal liver $CD34^+CD38^+$ cells contains committed NK cell precursors

because these cells have robust NK cell precursor activity but are unable to develop into T cells (74). The fetal thymus contains an immature $CD34^{-}CD5^{-}CD56^{-}$ population with clonogenic NK cell potential (75), as well as bipotential T/NK precursors (77). Together, these data suggest that the fetal liver and thymus are sites for NK cell development. The biological function of fetal NK cells is currently unknown. The fact that patients deficient in γc, most of whom lack NK cells at birth, are born at full term suggests that these cells are not essential for the fetus during pregnancy. Whether the thymus is required for the development of human fetal NK cells is also questionable because infants with DiGeorge syndrome have normal numbers of NK cells (78). The presence of NK cells in the thymus may be a consequence of the fact that some bipotential T/NK precursors, which form obligatory intermediates in the development of multipotent precursors of T cells, by chance develop into NK cells and that these cells have no function in the thymus.

The general consensus is that the bone marrow is the site for NK cell development in children and adults. One study in the mouse has identified a committed NK cell precursor, unable to develop into other lymphoid cells, in the mouse bone marrow (79), but the exact phenotype of the human bone marrow equivalent has yet to be determined. Whether NK cells fully develop and mature in the bone marrow before entering the circulation is unclear. A recent study suggests that human NK cells can develop and mature via another route. A $CD34^{lo}CD45RA^{hi}$ cell that expresses high levels of the integrin $\alpha4\beta7$ was identified in adult peripheral blood and in lymph nodes (80). These cells express c-kit, CD2, low levels of CD7, L-selectin, and IL-2Rα, but were $CD10^{-}$ and were able to develop into $CD56^{hi}CD16^{dim/-}$ NK cells with high efficiency in vitro. Because of their location in the lymph nodes, these cells likely represent committed NK cell precursors, which implies that $CD34^{lo}CD45RA^{hi}$ cells lack T cell-, pDC-, or B cell–differentiating activities, although this was not directly verified in this study. Therefore, proof that these cells are NK lineage–restricted is lacking (80). In the lymph nodes, $CD34^{lo}CD45RA^{hi}\alpha4\beta7^{+}$ cells are located within T cell–rich regions, where $CD56^{hi}$ cells also reside (81). Because $CD34^{lo}CD45RA^{hi}$ lymph node cells could develop into $CD56^{hi}$ NK cells when cultured in IL-2, it was speculated that in vivo T cell activation in the lymph nodes may promote the development of new NK cells from these precursors (80). The study by Freud et al. (80) left unresolved the question of where the $CD56^{+}CD16^{hi}$ NK cells develop because the committed NK precursors in the lymph nodes did not give rise to $CD56^{+}CD16^{hi}$ precursors. However, results of another study suggest that highly cytolytic $CD56^{+}CD16^{+}$ lymph node NK cells arise from IL-2-activated noncytolytic $CD56^{hi}CD16^{+/-}$ cells (82). In addition, two research groups have reported that IL-21, in concert with Flt3-L, SCF, and IL-15, stimulated the generation of $CD56^{+}CD16^{+}$ NK cells in vitro (83, 84), but whether this is a reflection of what happens in vivo is unknown.

Early Stages in NK Cell Development

Experiments both in mouse and human systems have established that T and NK cells share a common precursor (75, 85–89; reviewed in 27, 90). Despite the strong evidence for a common origin of T and NK cells, there are data suggesting that NK cells can be descendants of myeloid precursors. Marquez et al. (91) demonstrated that $CD34^{+}CD33^{lo}$ thymocyte precursors, which are probably identical to ETP, have the capacity of developing into $CD34^{+}CD44^{hi}CD33^{+}$ myeloid precursors. These cells were unable to develop into T cells but contained bipotent NK/DC precursors (91). Furthermore, Perez et al. (92) recently reported that a

$CD14^+CD11b^+CD13^+CD33^+$ cell isolated from cord blood can develop into cytolytic $CD56^+$ NK cells after incubation with Flt3-L and IL-15. The frequency of NK precursors within the $CD14^+$ cord blood population was low, however (1/50) (92), raising the possibility that the $CD14^+$ cells were contaminated with $CD56^-$ NK precursors. The idea that NK cells can derive from myeloid precursors is not supported by studies that examined the lymphoid and myeloid precursor potential of CLP, common myeloid precursors (CMP), and granulocyte/monocyte precursors (GMP) both in humans and in mice. Rather, these studies concluded that NK cells develop predominantly from lymphoid precursors (7, 9, 16, 93). However, as putative human lymphoid precursors retain some GM potential and lack erythrocyte precursor activity (10), the issue of whether in humans there are bipotential precursors able to develop into NK cells and monocytes and/or DC, but not into T cells, remains to be settled.

Although early cellular stages in human T and B cell development have been reasonably well defined, our knowledge about early stages of human NK development is very limited. Roughly, three stages can be defined in NK cell development: lineage commitment, NK receptor repertoire selection, and functional maturation (94, 95). Only very recently has a putative committed NK precursor within the $CD34^+$ cell compartment been identified (80). These $CD34^{lo}$ $CD45RA^+\alpha4\beta7^{hi}CD7^{+/-}CD10^-$ NK precursors that give rise to $CD56^{hi}$ $CD16^-$ NK cells in vitro may be downstream from $CD34^+CD45RA^+CD7^+CD10^-$ (10) or $CD34^+CD45RA^+CD7^+CD10^+$ (15) lymphocyte precursors. The immature NK cells developing from committed NK cell precursors are defined by expression of CD161 (NKR-P1) (96). These cells do not express CD56 or CD16. Immature NK cells can be induced to express 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 (97) or Flt3-L (98). **Figure 5** shows a hypothetical model of NK cell development that is deduced from phenotypic and functional analyses of small populations of immature NK cells isolated from different sources and from in vitro experiments (74, 98, 99; reviewed in 94). As discussed in the previous paragraph, the model, which is adapted

CMP: common myeloid precursor

GMP: granulocyte/monocyte precursor

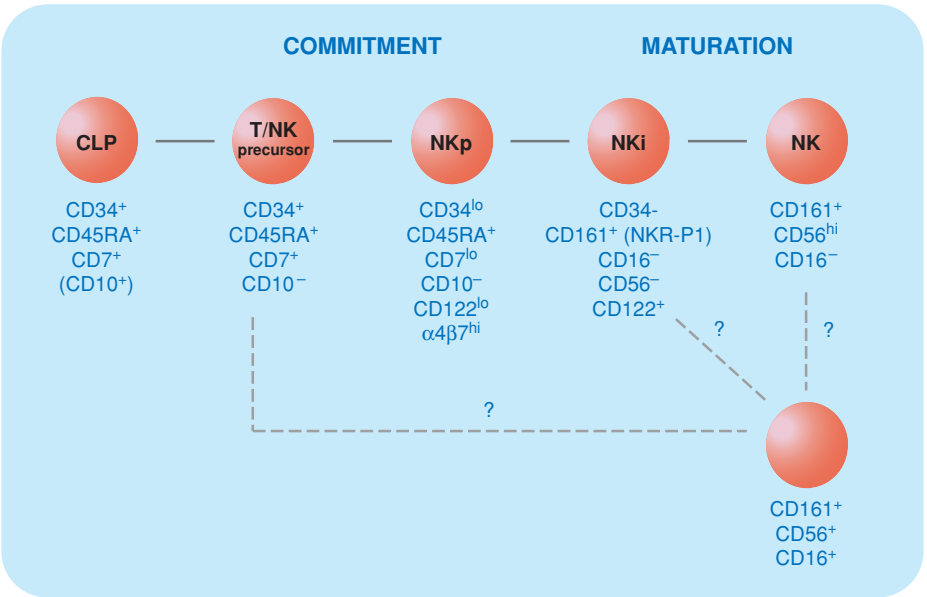


Figure 5

Model of NK cell development modified from Reference 94. Committed NK precursors develop into $CD56^{hi}CD16^-$ NK cells (80), but the immediate precursor of the $CD56^+CD16^+$ NK cells is not yet known. The dashed lines indicate the various possibilities.

from Reference 94, does not resolve the developmental pathway of $CD56^{hi}CD16^{-/+}$ and $CD56^{+}CD16^{hi}$ subsets. There is no consensus whether $CD56^{hi}$ NK cells are precursors of $CD56^{+}CD16^{hi}$ NK cells. These subsets may arise from different precursors, but so far no evidence supports this speculation.

Cytokines Involved in Human NK Cell Development

A number of cytokines have been identified that can support development of human NK cells from $CD34^{+}$ cells in vitro, notably SCF, Flt3-L, IL-7, IL-2, and IL-15. Data from mouse experiments indicate that SCF and Flt3-L may act on early lymphoid precursors, causing their development and thereby promoting NK cell development (reviewed in 94). However, the exact roles of SCF and Flt3-L in human NK cell development have not been determined. IL-2 and IL-15 were previously proposed to be the most relevant factors for human NK cell development. There is now consensus that IL-15, and not IL-2, is the critical NK cell factor, a finding that is supported by the strongly reduced NK cell numbers in mice deficient in IL-15 or in its receptor components, IL-15Rα, β, and γc. SCID patients suffering γc deficiencies lack NK cells (43), whereas IL-2-deficient (100) and IL-2Rα-deficient patients have normal numbers of NK cells (94, 101). One SCID patient, who presented with an absence of NK cells and reduced T cell numbers, was reported to have a strongly reduced expression of the IL-2/15Rβ chain and a marked decrease of signaling through this receptor (102). Data in the mouse suggest that IL-15 is not a differentiation factor but serves to maintain the viability and support proliferation of developing NK cells because NK cell precursors are present in normal numbers in mice deficient for IL-15 or its receptor components, IL-15Rα, β, and γc (103). It is unknown whether NK precursors are present in γc-deficient patients, partly because our knowledge of early NK cell precursors in humans is limited. However, the limited availability of the patient's tissues and the scarcity of NK precursors are more important roadblocks to resolving these questions.

Another cytokine implicated in NK cell development and function is IL-21 (83), but this cytokine acts only in concert with others, including SCF, Flt3-L, and IL-15, and may play a role in later stages of NK development (84). Mice lacking IL-21 develop normal numbers of NK cells (104), which is consistent with the notion that this cytokine may be affecting the function, not development, of NK cells.

Transcription Factors Involved in NK Cell Development

Our knowledge of transcription factors that are specifically involved in NK cell development is very limited compared with what we know about their roles in T and B cell development. Overexpression of Id2 and Id3 in human $CD34^{+}$ cells blocks their development into T cells, B cells, and pDCs but by contrast promotes NK cell development (**Figure 4**). As discussed above, Id2 and Id3 are natural antagonists of E proteins. Id2 also controls NK development in mice: Id2-deficient mice lack NK cells and NK cell precursors (105). However, Id2 is not a transcription factor but rather sequesters E proteins, thereby preventing their activity (62). Thus, the findings that Id2 overexpression stimulates NK cell development and that Id2 ablation prevents this indicate that one or more of the E proteins inhibit NK cell development. Indeed, overexpression of E12 and E47 inhibits NK cell development in vitro (R. Schotte & H. Spits, manuscript in preparation). Moreover, introduction of E47 deficiency into $Id2^{-/-}$ mice rescues NK cell development (B. Kee, personal communication), indicating that Id2 stimulates NK cell development by sequestering the inhibitory factor E47. E47 is an example of a factor that is required for the development of one lineage

(B cells) but that inhibits alternative lineage choices. Another example is Spi-B, which is required for pDC development yet inhibits the development of NK cells (106). The role of Notch1, which drives T cell commitment, is complicated in NK cell development. Triggering Notch by DL1 drives human $CD34^+$ cord blood cells to T/NK cell precursors (52), but inhibition of Notch signaling in a FTOC system that allows for both T and NK cell development inhibits T and stimulates NK cell development (26a). A similar observation has been made in experiments that examined the effect of γ-secretase inhibitor on rat thymus development (107). Thus, although Notch signaling may be required for the early steps in the development of HSCs into NK cells, it may not be required in later stages.

A master transcription factor for NK cells has not been identified. One possible candidate is Ets-1, which is predominantly expressed in lymphoid cells (108). Ets-1-deficient mice have reduced numbers of NK cells, and the few NK cells present in these mice are nonfunctional (109). No follow-up studies have been reported, and there is no information about the stage in which NK development is inhibited. Moreover, the role of Ets-1 in human NK cell development has not been investigated.

DEVELOPMENT OF DENDRITIC CELLS

Several types of DCs, including interstitial DCs, Langerhans DCs, blood DCs, and pDCs, have been identified that, depending on their local microenvironment, mediate different types of immune responses (110, 111). The remarkable versatility and flexibility of DCs, instructed by the priming signals from microbial and tissue-derived factors, distinguish them from other cell types in the immune system that exhibit more limited functions and suggests that DCs may play a central role in integrating the innate and adaptive aspects of various immune responses. In the mouse, there are three main DC subsets: $CD8\alpha^-$ DCs, $CD8\alpha^+$ DCs, and $B220^+Ly6C^+$ pDCs. The relationship between the $CD8\alpha^+$ and $CD8\alpha^-$ subsets is unclear, but for the sake of simplicity these cells are often categorized as conventional DCs (cDCs). The $B220^+Ly6C^+$ pDCs, also referred to as IFN-producing cells (IPCs), are clearly a distinct subset. Human cDCs are $HLA\text{-}DR^+$ cells that express high levels of CD11c and consist of a major $BDCA3^-$ and a minor $BDCA3^+$ population (112). Human $HLA\text{-}DR^+$ pDCs are defined by absence of CD11c expression and by high levels of CD123 (the IL-3Rα chain) and BDCA2 (111). The $CD11c^+HLA\text{-}DR^+BDCA3^-$ population can be further subdivided into $CD16^+$ and $CD16^-$ populations (112). The developmental and functional relationships of the multiple $HLA\text{-}DR^+$ DC populations remain unclear. For the remainder of this review, we use the terms cDC and pDC to denote populations expressing CD11c and CD123, respectively.

cDC: conventional dendritic cell

Developmental Origin of cDCs and pDCs

Galy and coworkers (6) showed that a fraction of bone marrow $CD34^+CD45RA^+CD10^+$ lymphoid progenitors could develop into $CD1a^+CD14^-$ DCs. Later work of the Galy group showed that the in vitro conditions that induced development of $CD1a^+CD14^-$ DCs failed to induce differentiation of myeloid cells into cDCs, suggesting that development of DCs from lymphoid or myeloid precursors has different signaling requirements (113). The underlying mechanisms have not been elucidated. Other groups have shown that lymphoid-restricted progenitors from cord blood ($CD34^+CD38^-CD45RA^+CD7^+$) when cultured in standard conditions (SCF/Flt3-L/GM-CSF/TNF-α) (114) or lymphoid conditions (S17 with IL-3, IL-6, c-kit, Flt3-L) (15) were able to give rise to a similar type of DC ($CD1a^+CD14^-$). Because some of these $CD1a^+$ DCs expressed S100 and low levels of CD207/Langerin,

Canque et al. (114) suggested that they are Langerhans DCs. More recently, Manz and coworkers (16) tested cord blood CLP, CMP ($CD34^+CD38^+CD123^+CD45RA^-$), and GMP ($CD34^+CD38^+CD123^+CD45RA^+$) for cDC precursor activity and confirmed that cDCs ($CD11c^+CD123^{lo}$) can arise from CLP, CMP, and GMP. However, they also noted that the frequency of cDC precursors in CMP and GMP was higher than in CLP (16). As is discussed below, this conclusion is valid only if one assumes that the tested populations are homogeneous.

Information about the early stages in development of human HSCs into pDCs is accumulating. We have postulated that the direct progenitor of pDCs is contained within the $CD34^{lo}$ compartment of cord blood, fetal liver, and bone marrow. These progenitors coexpress CD45RA, CD4, and high levels of CD123, and so we have denoted these cells pro-pDCs (for progenitor of pDCs) based on their phenotypic and functional similarities with pDCs (115). The pro-pDCs may be restricted to the pDC lineage, but whether the pro-pDCs lack potential to develop into other lineages has not formally been tested. In early studies, several research groups found that pDCs express lymphoid-related genes, including CD2, CD5, CD7, pTα, Spi-B, and λ5; contain IgH D-J rearrangements (116–120); and lack myeloid-associated markers, including CD11c, CD11b, CD13, CD33, and Mannose receptor (121). On the basis of these findings, it was postulated that pDCs derive from lymphoid progenitors. This idea was tested recently by Chicha et al. (16). They observed that single cells among $CD34^+CD7^+$ and/or $CD34^+CD10^+$ cells from cord blood considered to be lymphoid precursors were able to develop into pDCs, cDCs ($CD11c^+CD123^{lo}$), and B cells (16). Cord blood–derived CMP and GMP (or $CD34^+CD38^+$ $CD123^+$ CMP/GMP together when tested in parallel with CLP) also gave rise to both pDCs and cDCs but not to B cells (16). By using a limiting dilution assay, Chicha et al. observed that single cells with pDC and cDC potential are more frequent within CMP/GMP (16) than within CLP. These results were taken as evidence that human pDCs are predominantly myeloid derived (16). However, in this study (16) lymphoid precursors were not purified on the basis of CD45RA expression, ignoring that a proportion of $CD34^+CD7^+$ cord blood cells lacks expression of CD45RA and is unable to develop into NK cells (114). Therefore, these $CD34^+CD7^+CD45RA^-$ cells cannot qualify as lymphoid precursors. Nonetheless, it seems likely that, akin to cDCs, human pDCs can develop from both lymphoid- and myeloid-restricted precursors, which is consistent with what was observed earlier in mouse models (9, 16, 122–124). Whether lymphoid-derived cDCs and pDCs are identical to their myeloid-derived counterparts remains unclear. Relative differences in levels of expression of pDC surface antigens (114) and pTα and Spi-B transcripts (16) have been observed, but none of these markers was discriminating. There are data indicating that different subsets of pDCs and cDCs exist in vivo. Differences between thymic and peripheral pDCs have been noted in expression of CD2, CD5, and CD7 (125, 126). Mutually exclusive pDC subsets were identified in Flt3-L-treated healthy volunteers (127). In their blood, $CD7^+CD56^-$ and $CD7^-CD56^-$ pDCs express pTα, whereas $CD56^+CD7^-$ pDCs lack pTα expression (127). In addition, several distinct cDC subsets have been described (112). Whether the various cDC and pDC subsets, most of which are poorly characterized, have different developmental origins is, however, unclear.

Growth Factors Involved in pDC Development

It has now been well established that Flt3-L is important for human, mouse, and rhesus monkey DC development in vitro (115, 128, 129) and in vivo (130–134). Mouse pDCs can be generated only from $Flt3^+$ CLP or $Flt3^+$ CMP (135, 136). In humans, Flt3 mRNA has

been observed in both CLP and CMP populations (9, 16). Moreover, $CD34^+CD45RA^-$ early progenitors are responsive to Flt3-L alone, without feeder cells to support pDC development, confirming Flt3 cell surface expression on at least some of these cells (115). The observation that Flt3-L-deficient mice were severely depleted of all DC subsets underscores the significance of Flt3-L in DC development (137). Moreover, mice deficient for STAT3, which mediates the checkpoint of Flt3-L but not GM-CSF-induced DC development, and mice lacking Gfi1, a zinc finger repressor molecule that regulates levels of STAT3, demonstrate a severe block in DC development (138, 139). Development of cDCs and pDCs can be differentially regulated by various cytokines. For example, cytokines that promote cDC development, including GM-CSF and TNF-α, have a strong inhibitory effect on the Flt3-L-induced generation of both human and mouse pDCs (115, 128). Thrombopoietin, which alone does not induce pDC development, enhances the effect of Flt3-L on human pDC development (140). Another cytokine, G-CSF, significantly increased the number of blood pDCs when injected into healthy volunteers (134). Because G-CSF alone did not induce pDC development from HSCs (115), these data suggest that it increases pDC levels by stimulating mobilization of pDC or its progenitors from the bone marrow.

Transcriptional Control of Human DC Development

Several transcription factors involved in differentiation of DCs have now been identified. These include Ikaros (141) and members of the Rel family of transcription factors (142). Information about transcription factors in development of cDCs in humans is scarce. Wu and collaborators (141) observed that mice homozygous for an Ikaros dominant-negative mutation that lack all cells of lymphoid origin, including T, B, and NK cells, also show a defect in development of a subset of cDCs, presumably lymphoid-derived cDCs. Consistent with these observations, Galy et al. (113) reported that ectopic expression of this dominant-negative form of Ikaros interfered with development of lymphoid but not of myeloid cDCs. These findings suggest that Ikaros is involved in development of lymphoid-derived cDCs in both humans and mice.

More is known about the role of transcription factors in human pDC development (**Figure 6**). $CD34^+$ hematopoietic precursor cells ectopically expressing either Id2 or Id3 are blocked in their development into pDCs but not into myeloid cells (126). These findings are in line with observations in mice lacking Id2, which have increased numbers of pDCs (143). As discussed above, Id proteins are not transcription factors but act by sequestering E proteins. It remains to be elucidated, however, which member(s) of the bHLH family is (are) required for pDC development. We recently found that the Ets family member Spi-B is expressed in pDCs and its mature progeny but not in cDCs (119, 144), a finding that has been confirmed in the mouse (118). Moreover Spi-B transcripts have been observed in in vitro CLP-/CMP-derived pDC, but not in other DC subsets (16). Spi-B is involved in development of human pDCs because downregulation of Spi-B by RNA interference in human $CD34^+$ progenitor cells compromises their development into pDCs in vitro and in vivo (145). In contrast, knockdown of Spi-B stimulates myeloid development (145). RNA interference experiments further indicate that another Ets family transcription factor, PU.1, which is highly homologous to Spi-B, is needed for development of both pDCs and $CD14^+$ myeloid cells (145). These data suggest that PU.1 acts at an earlier stage of pDC development than does Spi-B, which is consistent with data in the mouse that show that PU.1 is required for development and survival of myeloid and lymphoid precursors (146) (**Figure 6**).

Studies in mice have shown that pDC development also depends on members of the

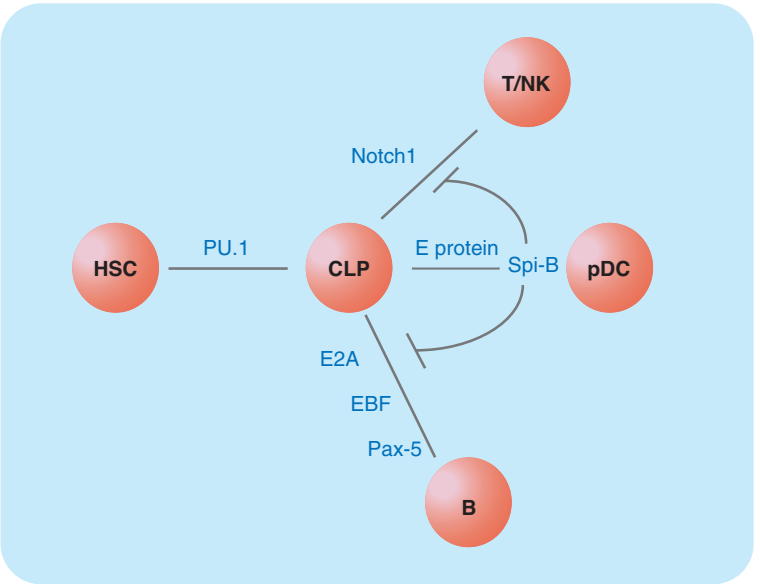


Figure 6

Transcription factors involved in pDC development. The Ets transcription factor Spi-B is involved in development of human pDCs, as shown by the fact that its ablation results in inhibition of human pDCs both in vivo and in vitro (145). Ectopic expression of Spi-B results in inhibition of T, B, and NK cell development. Knocking down Spi-B leads to an increase in B cell and monocyte development. These findings suggest that Spi-B can also play a role in regulation of pDC/monocyte diversification. Based on inhibition of pDC development by Id2 or Id3 (126), an E protein should be involved in pDC development, but the nature of this E protein is unknown. (Abbreviation: EBF, early B cell factor.)

IRF: IFN regulatory factor

IFN regulatory factor (IRF) family, including IRF-8 [or IFN consensus sequence-binding protein (ICSBP)] and to a lesser degree IRF-4 (147, 148). To date no studies have been performed to determine their role in human pDC development. Interestingly, IRF-8, in addition to binding to other members of the IRF family, can also bind to Spi-B and to members of the bHLH family to form transcriptional complexes apparently critical for the regulation of the immune system (149–151). Therefore, it is tempting to speculate that pDC development is driven by a complex of IRF-8, Spi-B, and a bHLH protein.

Recently, the role of Notch signaling in development of pDCs was investigated. In agreement with an observation by Kincade's group that interaction of DL1 with Notch inhibited pDC development from murine bone marrow precursor cells in vitro (118), we found that DL1 inhibited pDC development both from thymic and fetal liver CD34$^+$ cell precursors (55a). Inhibition of pDC development of thymic precursors by Notch signaling could be overcome by overexpressing Spi-B. Thus, Notch, in concert with Spi-B, may regulate T/pDC lineage diversification in the thymus.

DEVELOPMENT OF B CELLS

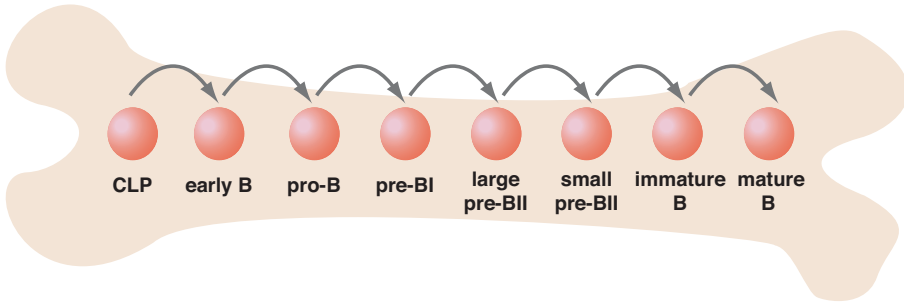
Most hematopoietic cell lineages, including B cells, are believed to originate in the bone marrow. As in mice, all cellular stages of early B cell development in humans can be detected in fetal bone marrow as well as in bone marrow of young and aged adults (see below), indicating that B cell development proceeds throughout life (152). Early in ontogeny, B cell development may take place

in the omentum (153) and fetal liver. The presence of early B cell–biased precursors in cord blood was recently demonstrated (10, 154, 155), and, even after birth, B cells may develop at sites other than the bone marrow. Notably, pre-B cells and mature B cells have been shown to be present in the thymus of young children (26).

Early Stages of B Cell Development

In the early 1990s, Hardy et al. (156) introduced a differentiation pathway for $B220^+$ progenitors in mouse bone marrow. They showed, on the basis of differential surface expression of CD43, BP-1, and HSA, that $B220^+$ progenitors develop via pre-pro-B, early pro-B, late pro-B, and pre-B into B cells. Functionally similar populations of early, pro-, and pre-B cells have been identified in humans, but the transition of common lymphocytes into B cell–restricted or –committed B cell precursors is as yet poorly defined. The current consensus is that human B lineage–restricted cells pass through a $CD34^+CD19^-CD10^+$ early B, $CD34^+CD19^+CD10^+$ pro-B, large $CD34^+CD19^+CD10^+$ pre-BI, large $CD34^-CD19^+CD10^+$ pre-BII, small $CD34^-CD19^+CD10^+$ pre-BII cell development pathway (**Figure 7**). As discussed above, human CLP appear to be present in $CD34^+CD45RA^+$ cells that express CXCR4 and that also express CD10 or CD7, or both (6, 10, 13–15). The $CD34^+CD45RA^+CD10^+CD7^-$ cells express IL-7Rα, and although these cells, found in bone marrow as well as cord blood, contain precursors able to develop into other lymphoid lineages, they are biased to develop into B cells (8, 10). Other studies have documented that early B cells, which express CD34, CD10, and IL-7Rα but lack CD19, have initiated DJ_H rearrangements (157) and express cytoplasmic CD79a (158) and Vpre-B (159). Although these studies suggest the initiation of B cell commitment in a $CD34^+CD45RA^+CD10^+$IL-7Rα^+ cell population, other studies have found cells with B cell characteristics in a small fraction of bone marrow and cord blood, $CD34^+$ cells that lacked CD10 and CD19 but that expressed CD79a transcripts (3.3%) and protein (0.4%) (154, 155). In addition, upon in vitro culture of $CD34^+CD10^-CD19^-$ cord blood progenitor cells with mouse stromal cells and cytokines (SCF, IL-2, IL-15), these cells first acquire CD79a and IL-7Rα before CD10 and CD19 (154). These $CD34^+CD79a^+$IL-7R$\alpha^+CD10^-CD19^-$ cells had DJ_H but no VDJ_H rearrangements and faintly expressed Pax-5 transcripts, which is considered the hallmark of B cell commitment in the mouse (160). Although these findings suggest that $CD79a^+$IL-7Rα^+ cells are committed to the B cell lineage, in vitro–generated $CD79a^+$IL-7Rα^+ pro-B cells were not restricted to the B cell lineage but could still differentiate into macrophages, NK cells, and some T cells in vitro (154). The developmental potential of freshly purified $CD34^+CD79a^+CD10^-CD19^-$ cord blood cells remains to be assessed. However, considering that the possibly more differentiated $CD34^+CD19^-CD10^+$ cord blood cells still have B/NK potential and even retained some myeloid-erythroid potential (15), it seems fair to assume that $CD34^+CD79a^+CD10^-CD19^-$ cells are multipotential.

A developmental pathway of human pre-B cell subpopulations in bone marrow was proposed by Ghia et al. (161), who used single-cell PCR analyses to determine the expression of TdT and RAG (recombination activating gene)-1 transcripts, Vpre-B protein, the cycling status, and the configuration of the IgH and IgL chain alleles in human B lineage subpopulations (reviewed in 162) (**Figure 7**). Human pro-B cells in the bone marrow are a well-characterized population expressing CD34, CD10, and CD19 (163). The vast majority of pro-B cells express TdT (161, 163, 164), some express Vpre-B at the cell surface (159, 161), and V-DJ_H rearrangements are easily detected (157, 165). Only a



CD34	+	+	+	-	-	-	-	-
CD10	+	+	+	+	+	+	+	-
IL-7Rα	+	+	+	-	-	-	-	-
CD19	-	-	+	+	+	+	+	+
CD79a	-	+	+	+	+	+	+	+
TdT	-	-	+	-	-	-	-	-
RAG	-	-	+	+	-	+	+	-
Vpre-B	-	+	+	+	+	-	-	-
μH	-	-	+/-	+	+	+	+	+
pre-BCR	-	-	-	-	+	-	-	-
IgH	GL	DJ_H	V_HDJ_H	V_HDJ_H	V_HDJ_H	V_HDJ_H	V_HDJ_H	V_HDJ_H
κL	GL	GL	GL	GL	GL	V_LJ_L	V_LJ_L	V_LJ_L
cycling	-	-	-	+	+	-	-	-
Pax-5	-	-	+	+	+	+	+	+
sIgM	-	-	-	-	-	-	+	+
sIgD	-	-	-	-	-	-	-	+

Figure 7

Model of early stages of human B cell development. The model is adapted from a model presented in Reference 162.

fraction of the pro-B cells express cytoplasmic μHC (161, 166, 167). Recently, a subpopulation (20%) of $CD34^+CD19^+$ bone marrow cells was described that lack CXCR4 (168), which is the receptor for the chemokine CXCL12 (SDF-1). $CD34^+CD19^+CXCR4^-$ cells rapidly express CXCR4 and differentiate into mature κ or λ positive B cells in vitro (PHA-stimulated PBL–conditioned medium, SDF-1, IL-6). Surprisingly, however, a fraction of these cells (12%) were also able to form myeloid colonies containing granulocytes, macrophages, or erythrocytes (168). If we assume that human CD19 expression, like in the mouse, is dependent on Pax-5 (169, 170), which restricts the developmental options of early progenitors to the B cell pathway (160), these findings suggest that lineage-restricted pro-B cells retain a certain plasticity and may develop into either B cells or myeloid cells, depending on the organism's requirements and subsequent bone marrow microenvironmental cues. Alternatively, the observed myeloid differentiation potential of $CD34^+CD10^+CD19^+$ bone marrow cells in the study by Hou et al. (168) may derive from

cells that lack Pax-5 expression. In line with this, Sanz et al. (155) showed that in cord blood 35% of $CD34^+CD10^+CD19^+$ cells do not express Pax-5. Although this remains to be confirmed in bone marrow cells, it will be interesting to determine how CD19 expression is regulated in the absence of Pax-5 in these cells.

Pro-B cell differentiation into pre-BI cells is characterized by loss of CD34 and TdT and by acquisition of cytoplasmic μHC in more than 95% of the cells (161, 163, 164). Based on cell-cycle analysis, human pre-B cells can be generally subdivided into large proliferating pre-BI cells, large proliferating pre-BII cells, and small resting pre-BII cells (161). Pre-BI cells that bear in-frame productive $(V\text{-}DJ)_H$ rearrangements transport the μH chains to the membrane associated with ΨL (Vpre-B and λ5) and CD79 to form the pre-B cell receptor, which signals several rounds of division. The large cycling pre-BI cells that express functional μH downregulate CD34, TdT, RAG-1, and RAG-2 and maintain expression of ΨL, CD10, and CD19 genes. To allow for Ig κ or λ L gene recombination, RAG-1 and RAG-2 expression are reinduced in small resting $CD34^-CD19^+CD10^+$ pre-BII cells.

Growth Factors Involved in B Cell Development

In mice, the c-kit, Flt3, and IL-7R signaling systems together account for the generation of all B lymphocytes in the bone marrow of adult mice (160, 171). In conspicuous contrast to what is known about the role of cytokines in mouse B cell development, the essential growth factors required for the growth of normal human B cell precursors remain elusive, although in vitro studies do suggest some function of c-kit, Flt3, and IL-7 in proliferation and survival of early pro-B cells (167, 172; reviewed in 162). However, in contrast to the well-documented requirement of IL-7 for mouse B cell development, this cytokine is not needed for human B cell development in vitro (173) or in vivo (39, 40). Data obtained from SCID patients lacking expression of either IL-7Rα (39, 40), γc (37), or Jak3 (42, 48, 174), which are all components involved in the IL-7R signaling pathway, have normal or even increased levels of circulating B cells, although their function is greatly impaired (175). The presence of B cells in IL-7Rα-deficient patients also excludes an important role of TSLP (thymic stromal-derived lymphopoietin) in human B cell development because its receptor consists of the IL-7Rα chain, in addition to a specific γc-homolog, TSLP-R. Moreover, the addition of TSLP has no effect on in vitro development of human $CD34^+$ cells cocultured with the murine stromal cell line S17 (B. Blom, unpublished data).

Transcriptional Control of Human B Cell Development

From mice studies, a wealth of information is available on the transcriptional control of early B cell commitment and development, which has been extensively reviewed by Busslinger (160). Development of HSCs via lymphoid progenitors into early B cells requires the concerted actions of multiple transcription factors, including Ikaros, PU.1, E2A, early B cell factor (EBF), and Pax-5. PU.1 and Ikaros act in parallel pathways to control transition of HSCs into lymphoid precursors. E2A, EBF, and Pax-5 regulate development of CLP into early B cells. E2A proteins are assumed to control EBF, which in turn regulates Pax-5.

Not surprisingly, the limited information about expression of transcription factors in precursor cell populations and transcriptional control of human B cell development suggests that data obtained in murine systems can be largely extrapolated to the human situation. $CD34^+Lin^-CD10^-CD7^-$ cord blood cells, like mouse progenitor cells (146, 176), express the Ets family member PU.1 (154). Knockdown of PU.1 in human $CD34^+CD38^-$ fetal liver progenitors

results in inhibition of B cell, monocyte, and pDC development, indicating a role for this transcription factor in development of both myeloid and lymphoid precursors (145). Multipotent $CD34^+lin^-CD10^-CD7^-$ cord blood cells lack expression of Pax-5 and EBF (154, 177), whereas early B cells ($CD34^+lin^-CD10^+CD19^-CD7^-$) from adult bone marrow were reported to have markedly upregulated expression of Pax-5 relative to the more primitive $CD34^+lin^-CD10^-CD7^-$ subset or $CD34^+lin^-CD10^-CD7^+$ NK-/T-biased precursors (13) and pro-B cells ($CD34^+CD19^+$) in cord blood (155). These findings correlate well with observations in the mouse, where CLP already express transcripts of various lineage-specific transcription factors, including Pax-5. Indirect evidence for a role for EBF in human B cell development comes from findings that early hematopoietic zinc finger (EHZF, the mouse homolog of Evi3), which inhibits the transcriptional activity of EBF, is highly expressed in $CD34^+$ cells but absent in $CD19^+$ B cells (178). A study by Jaleco et al. (179) strongly suggests that E2A proteins are required for human B cell development because forced expression of one of their antagonists, Id3, strongly inhibits B cell development in vitro. Interestingly, following ectopic expression of Id3 in $CD34^+CD38^-CD10^-$ fetal liver cells, their development into $CD10^+IL\text{-}7R\alpha^+$ cells was almost completely inhibited (179). These data indicate that E2A proteins are required for generation and/or survival of $CD10^+IL\text{-}7R\alpha^+$ B cell–biased precursors (8, 10). These findings are in accord with the observation that expression of another member of the Id family, Id1, is strongly reduced in $CD34^+CD10^+lin^-Pax\text{-}5^+EBF^+$ cord blood progenitor cells (13).

CONCLUDING REMARKS

Our understanding of human lymphocyte development has increased significantly over the past 20 years. In particular, our understanding of human T and B cell development has improved. Nonetheless, there are many gaps in our knowledge, particularly regarding the early stages of development of HSCs into lymphoid-restricted precursors. Much remains to be learned about the phenotype of human CLP and downstream lineage-biased and -restricted precursors. Despite enormous progress in our understanding of the function of NK cells and the mechanisms underlying their activities, our knowledge about the developmental pathways and the mechanisms of development of human NK cells is still surprisingly limited. The same holds true for our knowledge of DC development. One problem is that the different subsets of cDCs and pDCs are still poorly defined. Future studies in the field of human lymphocyte development will undoubtedly make use of recently described mouse strains that support development of all human lymphocyte classes (180–182). The possibility of stably expressing siRNA-encoding DNA fragments in human precursor cells, which is transferred to the mature progeny in an in vivo setting (181), allows for mechanistic studies in the field of human hematopoietic development that were not possible until recently. We may also expect that future investigations in patients with defined deficiencies in lymphocyte development will yield valuable information about the underlying mechanisms.

SUMMARY POINTS

1. CLPs coexpress CD34, CD45RA, and CD7 but are still poorly defined.
2. T cell development in the human thymus occurs via a series of well-defined intermediate stages.

3. T cells require IL-7 and NK cells require IL-15 for their development. The importance of IL-7 in human T cell development may be more stringent than it is in mice.
4. Our understanding of early stages in NK cell development is improving, but the identity of the earliest NK-restricted precursor is not yet firmly established. The developmental relationship of the two major human NK cell subsets, $CD56^{hi}CD16^{-}$ and $CD56^{+}CD16^{hi}$, remains unresolved.
5. cDCs and pDCs can develop from both lymphoid and myeloid precursors. There are multiple subsets of cDCs and pDCs, but the developmental relationship of those subsets is as yet unknown.
6. Development of lymphocytes is driven by transcription factors. The essential role of Notch1 in human T cell development has been demonstrated. bHLH factors belonging to the E protein family control T cell, B cell, and pDC development and negatively regulate NK cell development. Spi-B, probably in concert with other factors, such as IRF-8 and members of the bHLH E protein family, is indispensable for pDC development.
7. We have a clear understanding of early stages of human B cell development. It is completely unknown, however, which cytokine(s) is (are) driving human B cell development. Although IL-7 is essential for B cell development in mice, it is not required for B cell development in humans.

FUTURE ISSUES TO BE RESOLVED

1. We need to develop a uniform definition of human pluripotent stem cells and CLPs and to determine their precursor potential on a clonal level.
2. Improved mouse models are needed to test the developmental potential of human lymphocyte precursors in an vivo setting.
3. We need improved methods that completely knockdown genes in primary hematopoietic precursors to elucidate the roles of key genes in human lymphoid development.
4. The developmental origin and function of various NK cell and DC subsets need to be elucidated.

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