Development of Human T and Natural Killer Cells

By Hergen Spits, Lewis L. Lanier, and Joseph H. Phillips

Cells belonging to the lymphoid lineages are instrumental in protecting their hosts against an enormous diversity of foreign agents. Of these, T and B cells are most extensively studied. The way these cells recognize foreign antigens through the T-cell receptor (TCR) and Igs is now well understood. These receptors are generated by gene rearrangements, providing T and B cells with an almost unlimited capacity to recognize potential pathogens. Several mechanisms exist to ensure that T and B cells stay indifferently towards self antigens and insight into how this is achieved is increasing. Cells belonging to the third lymphoid lineage, natural killer (NK) cells, are able to kill a wide variety of target cells, including tumor cells and cells infected with bacteria and viruses. The physiologic role of these cells is not fully understood, but it is generally believed that these cells form a first line of defense towards infections by cytolytic activity or the secretion of certain cytokines. NK cells do not possess rearranged receptor genes, but recent work has established that NK cells do have the capacity to recognize major histocompatibility complex (MHC) class I antigens in vitro and in vivo. However, in contrast to T cells that are generally activated by the interaction between MHC/peptide complexes and TCR, NK cells are turned off by interaction of their specific receptors with MHC class I antigens.

The developmental relationship of the three lymphoid lineages is not precisely known. Like all hematopoietic cells, the lymphoid lineages are derived from pluripotent stem cells, operationally defined only by their capacity to give rise to blood cells but also to self-renew. A popular model of lymphoid differentiation postulates that when stem cells differentiate to mature lymphoid cells they pass through a common lymphoid progenitor cell. This still elusive cell type should be committed to the lymphoid lineages and should have lost the capacity to differentiate into any of the other hematopoietic cell lineages. Evidence for a separate regulation of lymphoid and myeloid/erythroid development is provided by the observation that mice functionally deficient for IL-2 receptors have myeloid and erythroid cells but lack T, B, and NK cells. However, there is some evidence from studies with hematopoietic tumors that pre-B cells have not completely lost their capacity to develop into myeloid cells. As an alternative to the idea of a fixed sequence of branchpoints involving irreversible differentia-

Information about the cell surface antigen phenotype of intermediate cell types in development of stem cells to mature lymphoid cells has accumulated from both human and animal studies. The human system has in principle the potential to be very informative for defining minute intermediate cell types in lymphoid development because of the availability of monoclonal antibodies against many more cell surface antigens than in the mouse. However, until recently, the lack of assays that could assess the capacity of putative progenitor cells to differentiate into mature lymphoid cells was a serious drawback of studies in human compared with animal models. Establishment of the SCID-hu mouse, originally described by McCune et al., has now paved the way for assays that measure the capacity of primitive hematopoietic progenitor cells to develop to T and B cells. Moreover, in vitro fetal thymic organ culture (FTOC) systems have been devised that permit analysis of human T and NK development from primitive hematopoietic progenitor cells. Finally CD34 progenitor cells can differentiate into NK cells in vitro when cultured in the presence of cytokines with or without stromal cells. Application of these assays in combination with detailed phenotypic analyses have resulted in a rapid accumulation of information with regard to T and NK development in humans. In this review we will discuss the state of our understanding of human T and NK development. When appropriate, we will review relevant, related information from mouse models as well. Comprehensive reviews about murine T-cell development have been published elsewhere.

Development of Stem Cells to Early T-Cell Progenitors

The thymic environment. For a long time the thymus has been recognized as the major site for T-cell differentiation. However, the thymus is not the only place where T cells develop. T cells are also formed in the gut, although those T cells have different characteristics that those maturing in the thymus. The thymic nonlymphoid microenvironment supporting T-cell development consists of epithelial components and of mesenchymal cells present in the thymic capsule, vessels, and interlobular septae. Epithelial cells are derived from the endoderm of the third pharyngeal pouch and the ectoderm of the brachial cleft, whereas the mesenchymal cells are derived from embryonic mesoderm. As elegantly shown by Anderson et al. in the mouse, both mesenchymal and epithelial cells are required for T-cell development. These epithelial and mesenchymal elements may

From the Division of Immunology of the Netherlands Cancer Institute, Amsterdam, The Netherlands; and the Department of Human Immunology of the DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA.

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Address reprint requests to Hergen Spits, PhD, Department of Immunology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands.

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not only provide an architectural structure for thymocyte development but also may provide cytokines and/or cell surface molecules supporting T-cell development. The thymic primordia in human are formed at week 7 of gestation.17

The first waves of T-cell progenitors start to populate the thymic rudiment at 7 to 8 weeks of gestation. The progenitor cells that colonize the thymus are initially derived from the yolk sac and/or the liver, which is the major hematopoietic organ in the human fetus from week 7 to 22 of gestation. The human thymus is not differentiated fully before the week 15 of gestation, when the corticomediullary junction becomes visible. At that time, Hassal's corpuscles become visible in the cortex. The fact that full differentiation of the thymic organ occurs 7 to 8 weeks after colonization of the thymic primordium may suggest that the developing T cells contribute to the development of the thymic microenvironment. Indeed, in cases in which T-cell development is blocked in an early stage the thymus consists only of a cortex. Mice with defects in the recombination machinery and therefore lacking T cells and a thymic medulla show development of a medulla after repopulation of these mice with normal wild-type bone marrow cells, supporting the concept that a dialogue between developing T cells andstromal cells is required for thymopoiesis.21

There is considerable debate concerning whether the thymus is colonized and fed by a pluripotent stem cell, a multipotent progenitor cell, or a progenitor cell that is already committed to the T-cell lineage. This question has been approached along two lines. First, it has been explored whether the blood forming organs contain progenitor cells already committed to the T-cell lineage. Second, the earliest thymic progenitors have been analyzed for their capacity to develop into hematopoietic cells other than T cells. In the next sections we will discuss evidence for and against the notion that the thymus is colonized by uncommitted progenitor cells or by cells already committed to the T-cell lineage.

Are there committed T-cell progenitors in the fetal liver and bone marrow? Studies in both humans and mice have shown that pluripotent stem cells can develop into T cells when artificially introduced in the thymus by injection. Human CD34+Cd7+Thy-1+ cells are highly enriched for pluripotent stem cells.3,32,33 These cells develop into T cells in fetal thymic organs transplanted into SCID mice.8 CD34+Cd7+Thy-1+ cells also develop into T cells when introduced into mouse fetal thymic lobes followed by culture in vitro (P. Res, E. Martínez Cáceres, A. C. Jaleco, K. Weyer, and H. Spits, manuscript submitted). Similarly, several groups have found in the mouse that highly enriched stem cells are able to populate a thymus after intrathymic injection in vivo.74-76 These results reflect the expected capacity of stem cells to develop into T cells but do not necessarily imply that the thymus is colonized by pluripotent stem cells during the normal development process. Whether pro-thymocytes are already committed to the T-cell lineage has been addressed by searching for committed T-cell progenitors in fetal liver and fetal and adult bone marrow. Based on the idea of fixed branchpoints in development of stem cells into T cells, it was reasoned that detection of committed T-cell progenitors outside the thymus would support the notion that these cells migrate to the thymus.

Apart from the fact that this premise is not necessarily correct, most of the studies addressing this question have led to ambiguous conclusions, because there are presently no cell surface markers to conclusively distinguish T- from NK-cell progenitors. Cells expressing CD3 antigens in the cytoplasm and/or CD7 are present in the fetal thorax,37,38 fetal liver,37,39-42 and adult bone marrow.34,42 These cells were considered by most groups to represent committed T-cell progenitors based on the fact that CD2, cyCD3, CD5, and CD7 are coexpressed on all mature T but not on all mature B cells. However, these antigens are also expressed on fetal NK cells43,44 or CD34+ NK-cell progenitors from the fetal thymus,13 indicating that these antigens are not specific for early T cells. In addition, CD7 is also present on B-cell45 and myeloid progenitors.46,47 Besides the fact that (co)expression of CD2, cyCD3, CD5, and CD7 does not define committed T-cell progenitors, there is presently no functional evidence for T-cell commitment in the fetal liver and fetal and adult bone marrow. A number of studies have shown that cyCD3+ and/or CD7+ fetal liver or bone marrow cells are able to develop into mature T cells in vitro.37,40,42,47 However, the capacity of these cells to develop into other hematopoietic cells was not investigated, precluding a conclusion with regard to T-cell commitment of these populations. In one study that examined both the myeloid and T-cell potential of CD7+ fetal liver cells, it was found that the CD7+CD34+ fetal liver cell population, which develops into T cells in an FTOC, also contains primitive myeloid precursors (high proliferative potential [HPP]—colony-forming units [CFU]).14 These findings support the notion that expression of CD7 on CD34+ cells does not define T-cell commitment and raise the possibility that the thymus is colonized by an uncommitted multipotent progenitor cell.

It is possible that, although pro-thymocyte cells are multipotential, these cells have a restricted differentiating capacity compared with stem cells. Galy et al characterized a CD34+CD45RA+ lineage marker (lin)- Thy-1+ cell in the human adult bone marrow able to differentiate into T and B cells in a SCID-hu mouse and to NK cells in the presence of IL-2, but unable to differentiate into myeloid cells in vitro CFU assays (A. Galy, presented at the European Federation of Immunology Societies, Barcelona, Spain 1994). In contrast, CD34+Thy-1+ cells differentiate both to lymphoid and myeloid cells.6 The studies of Galy et al present the first evidence in the human that the myeloid and lymphoid developing potential of adult bone marrow cells can be separated and support the notion that stem cells are differentiated towards a more committed progenitor cell before homing to the thymus.

Recent studies in the mouse are consistent with the idea that pro-thymocytes are distinct from pluripotent stem cells but are still multipotent. Antica et al found a cell in the adult bone marrow with a phenotype lin–Sca-1+ that, in contrast to pluripotent stem cells, expresses Sca-2. These cells are multipotent and are capable of differentiating into lympoid and myeloid cells, but the expansion potential was only 2% of that of Sca1 + Sca-2+ stem cells. The Sca-2+ bone marrow progenitors could be the precursor of a thymic progenitor cell with a similar phenotype and the potential to
develop into T, B, and dendritic cells but not into myeloid cells. However, one other study found evidence for T-cell commitment of pro-thymocytes in the mouse. Rodewald et al. analyzed pro-thymocytes in the peripheral blood of mouse embryos at day 15.5 of gestation. At that time the thymus is colonized, but the cells in the thymus have not progressed beyond the CD4+CD8+ stage. A population was identified expressing both Thy-1 and low levels of c-kit that could develop into T cells but not into B or myeloid cells, whereas another population in the fetal peripheral blood (Thy-1+c-kit+68) possesses the capacity to develop into T-, myeloid-, and B-cell lineages. A small percentage of Thy-1+ c-kit+ cells had undergone Dβ to Jβ rearrangements, indicating T-cell commitment. Although Rodewald et al. appreciate that the thymus contains primitive, noncommitted, hematopoietic progenitor cells, these investigators argue that the majority of the early thymic immigrants are already committed to the T-cell lineage before entrance into the thymus. However, it is possible that most of those cells are bipotential T/NK progenitors, not completely committed to the T-cell lineage.

In summary, no definitive evidence has been found for the existence of T-cell-committed progenitor cells in the fetal liver or the bone marrow. However, the analysis of mouse fetal blood raises the possibility that after progenitor cells leave the fetal liver or the bone marrow some of them may undergo T-cell commitment before they enter the thymus.

Early hematopoietic progenitor cells in the thymus. Analysis of hematopoietic potential of progenitor cells in the human and mouse thymus supports the concept that most early CD34+ are not committed to the T-cell lineage. We recently observed CD34+CD38+ cells in the fetal thymus, representing less than 0.01% of total thymocytes. CD34+CD38+CD45RA- cells from the fetal liver and fetal bone marrow population have been shown to be enriched for early stem cells. However, in contrast to these stem cells, the CD34+CD38+ fetal thymocytes do not express Thy-1 (P. Res, E. Martinez Cáceres, A.C. Jaleco, K. Weyer, and H. Spits, manuscript submitted) and are uniformly positive for CD45RA. Furthermore, we detected primitive myeloid progenitor cells (HPP-CFU) in CD3+CD4+CD8+ fetal thymocytes, although at a very low frequency compared with the fetal liver. CD3+CD4+ myeloid progenitors were found in the postnatal thymus, but myeloid progenitors were not present in the CD34+CD7+ thymocytes. However, a proportion of the CD34+CD7+ thymocytes contain bipotential T/NK progenitors (Fig 1, discussed below). These observations indicate that the most primitive CD34+ progenitors are multipotential but are distinct from stem cells.

Similar conclusions can be derived from studies of the mouse. A cell population was found in the adult mouse thymus expressing Sca-1, Thy-1.1+, CD44+, and c-kit but no lineage markers, similar to pluripotent stem cells. This population contains precursors for T and B cells, NK cells, and dendritic cells. In contrast to pluripotent stem cells, these thymic progenitor cells do not have the capacity to develop into macrophages and erythrocytes. Because the developmental potential of early thymic progenitors has not been investigated in clonal assays, it is possible that CD4+ c-kit+ Thy-1.1+ murine thymic progenitors are a mixture of committed T-, B-, NK-, and dendritic-cell precursors. On the other hand, no heterogeneity was observed in phenotype of these cells using a panel of monoclonal antibodies, supporting the idea that CD4+ c-kit+ cells represent a progenitor cell population that is not yet committed to a single-cell lineage.

These studies, taken together, suggest that the thymus is colonized by multipotential hematopoietic progenitor cells distinct from stem cells. This raises the possibility that these progenitor cells can also develop into hematopoietic non-T cells within the thymus. This notion is supported by the finding that the human thymic microenvironment is permissive for development not only of T cells but also of B, NK, dendritic, and myeloid cells. A similar observation has been made by Spangrude and Scollay, who found that the adult mouse thymus can support myeloid development when injected in vivo with stem cell.

**DIFFERENTIATION OF PRE-T CELLS TO MATURE T CELLS IN THE THYMUS**

Transitions of pre-T cells to CD3+CD4+CD8- cells. The phenotype of early thymocytes has been investigated in a large number of studies allowing a detailed picture of the changes occurring during the early stages of human T-cell development. The analysis of these stages has been facilitated by the demonstration that T-cell progenitors in the thymus are defined by the expression of CD34. As shown by Terstappen et al., Galy et al., and DiGiusto et al., expression of CD34 decreases when cells progress during development. Thus, the relative expression of CD34 on thymocyte subsets is useful as a marker for defining different stages of thymic development. As discussed in the previous section, the earliest thymic progenitors are CD34+c-kit+ murine thymic progenitors are a mixture of committed T- and NK cells. The earliest committed T-cell progenitor in the thymus expresses CD1, CD5, and CD28. In addition, these cells express CD33, previously believed to define commitment to the myeloid lineage. This latter notion is incorrect because CD34+CD33+ thymocytes have the capacity to develop into T and NK cells. The earliest committed T-cell progenitor in the thymus expresses CD1, CD2, CD5, and c-kit. These cells acquire CD4 before CD8, which contrasts with the situation in rats and many mouse strains, in which CD3+CD4+CD8- cells first acquire CD8 and then CD4. In some mouse strains, CD3+CD4+CD8- cells appear before the CD4+CD8- double-negative (DN) cells, similar to humans. Like the mouse CD3+CD4+CD8- thymocytes, most human CD3+CD4+CD8- cells are cycling, suggesting that immature thymocytes received a growth-promoting signal before acquisition of CD4. It should be noted here that some CD3+CD4+CD8- cells in the thymus have no relationship with T cells but resemble dendritic cells. The CD8 molecule is a heterodimer consisting of two chains (α and β) encoded by two different genes. These two chains are differentially expressed during T-cell differentiation in the human thymus.
Recent data from mouse studies have clarified some of the molecular events involved in expansion of immature thymocytes and acquisition of CD4 and CD8. It has been established that transition of CD3-CD4-CD8- (triple negative [TN]) to CD4+CD8+DP thymocytes requires the TCRβ chain.\(^\text{24,69}\) The TCR β gene rearranges before the TCR α gene.\(^\text{70,71}\) After completion of a productive rearrangement, the TCR β protein is expressed on the TN cells in conjunction with CD3 proteins (pre-T-cell receptor). It has been reported that TCR β associates with an invariant chain with a molecular weight of 33 kD (gp33).\(^\text{72}\) A cDNA encoding this molecule has recently been cloned.\(^\text{73}\) Mutant mice, deficient for the TCR β gene\(^\text{74,75}\) or unable to rearrange their TCR genes because they have a defect in DNA repair (SCID) or lack recombination-activating genes (RAG), have reduced numbers of DP cells or lack DP cells altogether.\(^\text{76-78}\) Introduction of a β transgene in these mice results in the expansion of thymocytes and the appearance of DP cells.\(^\text{75,79-81}\) The pre-T-cell receptor complex can deliver signals to these early T cells resulting in expansion of immature thymocytes and appearance of DP cells.\(^\text{82-84}\) Signal transduction via this complex may involve the tyrosine kinase p56\(^\text{85}\) because mice expressing a dominant negative mutant of this enzyme\(^\text{86}\) or that are p56\(^\text{85}\) deficient have a block in transition from TN to DP cells.\(^\text{86}\) Moreover, overexpression of an active mutant of p56\(^\text{85}\) in RAG-1 \(-/-\) mice results in the expansion of thymocytes and the appearance of DP cells.\(^\text{87}\) The observations that introduction of a TCR β chain in the germline of normal mice\(^\text{88}\) and that disturbance of p56\(^\text{85}\) leads to continuation of rearrangement at the TCR β locus\(^\text{49}\) indicate that both the TCR β and p56\(^\text{85}\) are required for prevention of further rearrangements of the TCR β genes. Abrogation of TCR β gene rearrangements ensures that almost all DP cells have only one productively rearranged TCR β gene (allelic exclusion). By contrast, because DP thymocytes can undergo multiple productive TCR α gene rearrangements,\(^\text{89}\) a T-cell can have more than one rearranged TCR α gene. Padovan et al\(^\text{19}\) have estimated that around one third of mature human T cells express two TCR α chains on their cell surface.

As yet, there is no information about expression of the pre-T-cell receptor complex in the human thymus. We found recently that approximately 50% and 5% of the CD34+TN thymocytes have rearranged TCR β genes and TCR α genes, respectively (T. Breit, B. Blom, J. van Dongen, and H. Spits, unpublished data). Thus, the human pre-T-cell receptor complex may be expressed on the cell surface just before upregulation of CD4. As in the mouse,\(^\text{20,71}\) the human TCR α gene rearranges after TCRβ.\(^\text{90,91}\) The TCR δ locus is nested within the TCR α locus, between the \(\alpha\) and \(\delta\) gene segments. Rearrangement of the TCR α gene deletes the δ gene. It is believed that the event initiating TCR α rearrangement links δREC (upstream of the TCR δ locus)
to pseudo Jα (the first element of the Jα cluster), thereby deleting the TCR δ locus.98 This event has not yet taken place in the CD34+ TN thymocytes (T. Breit, B. Blom, J. Van Dongen, and H. Spits, unpublished data). We suspect that TCR α rearrangement is initiated at the CD33*CD38-Thy-1+ fetal liver cells express CD33. CD33 is not detectable on stem cells when using FITC-labeled anti-CD33. This discrepancy is probably due to the fact that PE is 5- to 10-fold more sensitive than FITC.224

Table 1. Antigen Expression at Early Stages of T-Cell Development

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<th>Stem Cells</th>
<th>Multipotential Thymic Progenitors</th>
<th>Bipotential T/NK Progenitors</th>
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* The presence of CD33 on stem cells is controversial.19,222 Using PE-labeled anti-CD33, we found that CD34*CD38-Thy-1+ fetal liver cells express CD33. CD33 is not detectable on these cells when using FITC-labeled anti-CD33. This discrepancy is probably due to the fact that PE is 5- to 10-fold more sensitive than FITC.224

Positive and negative selection in the human thymus. Over the past years a wealth of information has provided insight into differentiation of CD4+CD8+ thymocytes into mature CD4+ and CD8+ single-positive (SP) T cells. It has become clear that the TCR repertoire is shaped by positive and negative selection events. Positive selection is required to induce maturation of DP cells into SP T cells that have the capacity to migrate out of the thymus. Negative selection ensures that those T cells efficiently recognizing self antigens are deleted. Both positive and negative selection involve interactions of the TCR αβ dimer with MHC/peptide complexes. Much of the progress in this field comes from experiments with genetically manipulated mice. The present state of knowledge with respect to mouse thymic development is extensively covered in a number of reviews,22-25,96 and we will restrict ourselves to the evidence for positive and negative selection in the human thymus.

There is extensive information about the changes in antigen expression accompanying differentiation of TN to mature SP thymocytes in human (summarized in Fig 2). The time needed to complete differentiation of immature TN thymocytes into mature SP T cells is 24 to 96 hours, as estimated from kinetic studies of TN cells injected into the human fetal thymus/liver transplant of SCID-hu mice.95 After entrance into the DP stage,97 the thymocytes acquire the CD3/TCR complex.98 The CD3low DP thymocytes are the main target for positive selection. The interaction of the TCR/CD3 with the peptide MHC complex results in upregulation of CD3 and the activation antigen CD69.99 At the CD3high DP stage, the cells acquire CD27.100 In addition to acquisition of some cell surface antigens and loss of others, mRNA of the cytotoxic protein perforin and various cytokine genes are induced as well.101 When these cells further mature into SP thymocytes, losing either CD4 or CD8/β/γ followed by CD31,98,102 they switch their CD45 isotype from CD45RO to CD45RA.102,103 Some DP thymocytes lack CD1.98,104 The developmental status of those CD1+ DP cells is unknown. Mature thymocytes are CD3high, CD4+ or CD8+, CD1+CD27−CD45RA+ when they migrate out of the thymus.

Transplantation of bone marrow or fetal liver cells into SCID patients has provided evidence that the human TCR repertoire is shaped by the MHC antigens expressed in the thymic environment. Particularly informative in this respect are the observations with SCID patients transplanted with completely mismatched fetal liver cells104-109 or with haploidentical bone marrow.110-112 Upon transplantation with allo- genic HLA-mismatched fetal liver, two SCID patients developed a fully reconstituted immune system with T cells of donor and B and myeloid cells of recipient origin. After immunization with tetanus toxoid (TT), TT-specific CD4+ T-cell clones, all of donor origin, were obtained that recognized TT only in the context of the MHC of the recipient.113 In addition, CD8+ CTL were obtained that recognize Epstein-Barr virus (EBV) antigens in the context of recipient but not donor MHC.104,109 T-cell clones specific for TT in the context of recipient MHC were also obtained from SCID patients transplanted with haploidentical maternal bone marrow cells.113,114 Thus, the TT-specific CD4+ T cells and the EBV-specific CD8+ CTL were positively selected on cells, presumably thymic epithelial cells, of recipient origin. Surprisingly, in vitro studies showed a high frequency of T cells present in the peripheral blood of SCID patients, transplanted with MHC-mismatched fetal liver cells, which were cytotoxic for target cells of recipient origin and were specific for HLA antigens of the recipient.104 Recipient-reactive clones were also isolated from peripheral blood mononuclear cells (PBMC) of patients transplanted with haploidentical bone marrow.115 Nonetheless, these patients did not suffer graft-versus-host disease (GVHD), indicating that these recipient-specific CTL were not active in vivo. The mechanism of tolerance in these patients is not clear, but may involve IL-10.116 Importantly, these observations indicate that no clonal deletion occurred in the thymus during development of the donor fetal liver stem cells. These findings could be explained by assuming that the thymus of the reconstituted SCID patients did not contain an accessory cell population of recipient origin able to cause deletion of the recipient-specific T-cell clones.117
This notion is supported by experiments of Vanderkerkhove et al.\textsuperscript{118} and Schols et al.\textsuperscript{119} These investigators transplanted HLA-mismatched fetal liver and fetal thymus under the kidney capsule of a SCID mouse and analyzed the specificity of the mature SP thymocytes.\textsuperscript{118,119} The epithelial cells of the thymus of these chimeric SCID-hu mice were derived from the thymic donor, but the hematopoietic cells, present predominantly in the medulla, were of liver origin.\textsuperscript{120} No T cells were found that recognized the HLA antigens of the fetal liver donor.\textsuperscript{118} It is conceivable that hematopoietic cells of fetal liver origin (presumably dendritic cells) induced deletion of T cells with receptors specific for the MHC of the liver, but it is also possible that such cells were not positively selected because of an absence of epithelial cells of fetal liver origin. T cells specific for HLA of the thymus donor were present in the graft.\textsuperscript{118} These thymus-specific T cells were not autoreactive in vivo and were therefore tolerant towards the HLA antigens of the thymus. Incubation of these T cells with IL-2 in vitro broke the tolerance.\textsuperscript{119} These observations are similar as with the transplanted SCID patients. Recipient-specific T cells were tolerized but not deleted and culture in IL-2 reversed the tolerance of recipient-specific T cells isolated from these patients.\textsuperscript{107} The results from these transplanted SCID patients and SCID-hu mice indicate that the human thymic epithelium can induce nonresponsiveness but not by clonal deletion.

Experiments exposing human thymocytes to superantigens have shown that, as in the mouse, these antigens can delete thymocytes expressing the relevant TCR Vβ. Merkenschlager and Fisher\textsuperscript{121} reported that the addition of Staphylococcal enterotoxin (SE) D to a mouse/human FTOC deleted Vβ 5.2/5.3-positive T cells, whereas two other groups reported that injection of SEB in the thymus of SCID-hu mice removed most of the human thymocytes expressing Vβ chains interacting with this superantigen.\textsuperscript{122,123} These data suggest that the human thymic environment can mediate clonal deletion.

Signal transduction pathways involved in positive and negative selection are not yet completely understood. Tyrosine phosphorylation plays a critical role in signalling...
through the TCR. At least three tyrosine kinases are associated with the TCR: lyn, p56<sup>1ck</sup>, and ZAP 70. Moreover, the tyrosine phosphatase CD45 is critical for TCR signalling by regulating the activity of the tyrosine kinases, p56<sup>1ck</sup>, p59<sup>fyn</sup>, and p59<sup>yn</sup>. lyn seems to be dispensable for TCR-mediated selection in the thymus because p59<sup>yn</sup>−/− mutant mice do not present obvious defects in the number of mature SP T cells in the thymus or in the periphery. However, CD45 is important for positive selection, because mice with a targeted deletion of exon 6 of CD45 (resulting in removal of all CD45 isoforms, although very low levels of CD45 may be still present) have a block in intrathymic T-cell development at the DP stage. Recently, SCID patients have been identified who have mutations in the ZAP-70 gene, resulting in inactivation of this tyrosine kinase. Surprisingly, ZAP-70−/− patients totally lack CD8<sup>+</sup> T cells but have CD4<sup>+</sup> T cells. These CD4<sup>+</sup> T cells are defective in signal transduction, because they could not be activated in vitro through the CD3/TCR complex. These findings indicate a critical role for ZAP-70 in generation of CD8<sup>+</sup> T cells. It is not understood how CD4<sup>+</sup> SP thymocytes develop in ZAP-70−/− deficient patients.

DEVELOPMENT OF NK CELLS

The developmental relationship of NK and T cells. Until recently, the developmental relationship of NK cells with the two other lymphoid lineages was unknown. Previously, it was proposed that NK cells are related to T cells or to myeloid cells or may represent an independent lineage. Some SCID patients have been described lacking T and NK cells but having normal numbers of B and myeloid cells, suggesting a common origin of T and NK cells. SCID mice and mice with targeted deletions of the RAGs lack T and B cells but have normal NK cells. These findings may suggest that T and B cells have a common progenitor in which RAGs are expressed and that NK cells branch at an earlier point in development. However, more recent information indicates the existence of a common T/NK progenitor.

Initially, the idea that T and NK cells are closely related was based on a number of phenotypic and functional characteristics common to T and NK cells but not to B cells (summarized in Table 2). Most remarkable is the expression of CD3 γ, δ, ε, and ζ in human fetal NK cells. Because of their association with TCR proteins and the failure to detect CD3 proteins in mature NK and B cells, it was assumed in the past that CD3 was expressed exclusively by T cells. However, although adult NK cells do not express significant levels of cytoplasmic CD3 protein (except for CD3ζ<sup>135,138</sup>), CD3ε mRNA<sup>141,139</sup> and protein<sup>134</sup> are rapidly upregulated in these cells after activation in the presence of interleukin-2 (IL-2). Recently, Wang et al<sup>140</sup> also found evidence for expression of CD3ε in the mouse NK cells and a possible role for CD3ε in T- and NK-cell development. Transgenic mice carrying 30 or more copies of the human CD3ε gene exhibit an absolute block in T- and NK-cell development, whereas in the B cells, myeloid and erythroid development was unaffected. Moreover, the endogenous mouse CD3ε was detected in IL-2-activated NK cells from normal mice. Taken together, these results suggest that overexpressed human CD3ε acts as a dominant negative, possibly affecting a signal that is necessary for early T and NK development. However, the extracellular domain of CD3ε apparently is not required for NK-cell development because normal NK cells are present in mice deficient in expression of CD3ε (E. Vivier, personal communication, January 1995).

Importantly, the findings of Lanier et al (summarized in reference 141) support the notion that T and NK cells originate from a common precursor that expresses CD3ε and is unable to develop into B cells. Further support for this comes from recent studies of NK cells and their progenitors in the human thymus. It has been appreciated for a long time that the thymus contains NK cells. These NK cells constitute a minor fraction of TN thymocytes. In earlier studies, the existence of NK cells in the thymus was demonstrated indirectly by the ability to expand NK cells from thymocytes cultured in IL-2. Cells emerging from these cultures were NK cells, as defined by a variety of criteria, including expression of CD56 and CD16 (which are predominantly but not exclusively expressed in NK cells) and in vitro cytotoxic activity against NK-sensitive target cells. More recently, we and others cloned NK cells from TN thymocytes. Some of those NK clones expressed cyCD3. Moreover, the presence of CD56<sup>+</sup> thymic NK cells in situ was demonstrated in an analysis of enriched TN thymocytes. These findings raised questions about the origin of these NK cells. Do they arise from a NK progenitor in the thymus or do they merely circulate through the thymus?

Several lines of evidence support the notion that NK cells can indeed develop in the thymus. Sánchez et al<sup>40</sup> identified a population of CD5<sup>−</sup>CD56<sup>−</sup> cells within the TN population that are clonogenic in the presence of feeder cells and IL-2. When first analyzed, many of the emerging clones lack expression of CD56 and CD16 and did not kill NK-sensitive target cells. However, upon further culture, many of these clones acquire CD56 and cytotoxic activity, confirming earlier findings that demonstrated a link between expression of CD56 and the cytotoxic activity of NK cells. None of the CD56<sup>−</sup> clones had pre-T–cell potential because they were unable to develop into T cells in a FTOC (M.J. Sánchez and J.H. Phillips, unpublished data). Moreover, all clones analyzed had their TCR β and γ genes in germline configuration. Interestingly, such clones can also be established from fetal liver. These CD3<sup>−</sup>CD56<sup>−</sup> clones express high levels of cyCD3y, δ, and ε and proliferate in response to IL-2, IL-4, IL-7, and IL-3. Most of the fetal thymic CD3<sup>−</sup>CD56<sup>−</sup> clones also respond to these cytokines (H. Spits, unpublished data). These findings suggest that these CD3<sup>−</sup>CD56<sup>−</sup> clones represent committed NK-cell progenitors.

Recently, the presence of CD34<sup>+</sup> NK progenitors in the thymus was reported. CD34<sup>high</sup> thymocytes lacking CD1, 3, 4, 8, and 56 were able to develop into NK cells when cultured in a combination of stem cell factor (SCF), IL-7, and IL-2 in the presence of stromal feeder cells. To demonstrate that these CD34<sup>high</sup> cells were bipotential, CD34<sup>high</sup> TN cells were single-cell sorted and expanded for a short period of time with SCF, IL-2, and IL-7 on a feeder cell layer of irradiated melanoma cells. After a few cell divisions, half of the cells...
of each clone were cultured further in the cytokine mixture, whereas the other half were pooled and tested for the ability to differentiate into T cells in an FTOC. When cultured in SCF, IL-2, and IL-7, almost 100% of the clones developed into CD56+ NK cells, whereas these same clones developed into T cells in an FTOC.37 These experiments present evidence for the existence of bipotential T/NK progenitors. In contrast to the high frequency of NK progenitors, the frequency of primitive myeloid cells (HPF-CFU) among these CD34+ thymic progenitors was less than 1%. Thus, the majority of these progenitors are not pluripotent. It was not determined whether the CD34+ T/NK-cell precursors also have B-cell developmental potential. But a substantial proportion of these progenitor cells express cyCD3; therefore, it is unlikely that those cyCD3+ cells can develop into B cells. Thus, the findings of Sánchez et al.40 suggest that many of the thymic CD34+ progenitor cells can develop only to T and NK cells and not to any other cell type, underscoring the close relationship of NK with T cells.

Rodenwald et al.51 found that cells expressing the Fc receptor (FcγR) II (CD32) and/or FcγR III (CD16) (designated as FcγRII/III-positive cells) present in the mouse thymus at 14 days of gestation can develop into T cells when maintained in a thymic environment. When these cells were removed from the thymus and injected into congenic recipients, NK could be cultured in vitro from the spleens of the injected animals.51 The FcγRIII expression in fetal T-cell progenitors is maximal in the DN stage, preceding TCR V(D)J rearrangement, and FcγRII/III expression disappears during the entry into the DP stage.152 It should be noted here that, although CD16 (FcγRII) is expressed on mature human NK cells, this antigen is not expressed on human T/NK progenitors. Moreover, a subset of human NK cells lacks CD16.149 The developmental relationship of the CD16- and CD16+ NK-cell subsets is presently not clear.43 They may reflect stages of NK-cell maturation or represent different functional subsets.149

Sites of NK-cell development. The demonstration that the thymus contains bipotential T/NK progenitors, committed NK precursors, and mature NK cells and the fact that the human fetal thymic microenvironment is permissive for NK development43-45 indicate that NK differentiation can occur in the thymus. However, the thymus is not required for human NK development because NK cells are already present in the human fetal liver before formation of the thymic primordium.43 Consistent with this finding is the fact that athymic, nude mice have normal numbers of NK cells in the periphery.

It is likely that the bone marrow is the main site for NK-cell development. Studies from several laboratories have shown that human NK cells19,21,154,155 and rat NK cells156 can differentiate from immature bone marrow progenitors in long-term bone marrow cultures. Human NK cells have been generated from either CD34+154 or CD34- bone marrow populations19,20,155 and these progenitors are heterogeneous with respect to expression of HLA-DR and CD7. Stromal cells were required for NK-cell development in certain culture systems19 but not in others.21 This is possibly related to the maturity of the progenitor examined because primitive CD34+CD7- progenitors required contact with stroma for NK differentiation, whereas the more mature CD34+CD7+ progenitor cells could differentiate into NK cells in the absence of contact with stroma.155 Nonetheless, in all experimental models, NK-cell development in vitro required the presence of IL-2. As in other lineages, CD34 expression was lost upon NK-cell differentiation and the cells acquired the expression of CD2, CD16, CD56, and other membrane antigens present on mature peripheral blood NK cells.

Mature NK cells are present before population colonization of the bone with stem cells.43 Moreover, CD34+ cyCD3+ cells that may represent NK progenitors are present in the liver.17 Given the fact that both the fetal liver and adult bone marrow also contain pro- and pre-B cells,157 it is possible that, similar to B cells, NK cells develop in the liver during fetal life and subsequently in the bone marrow after colonization of the bone marrow with stem cells. However, it can presently not be excluded that NK cells can also develop in other organs (eg, spleen, liver, or blood). Resolution

<table>
<thead>
<tr>
<th>Antigen</th>
<th>T Cells</th>
<th>NK Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>++</td>
<td>+ (small subset is CD2-)</td>
<td>-</td>
</tr>
<tr>
<td>CD3γδ, δ</td>
<td>+</td>
<td>Fetal+, adult-</td>
<td>-</td>
</tr>
<tr>
<td>CD3ε</td>
<td>+</td>
<td>Fetal+, adult+ (upon activation)</td>
<td>-</td>
</tr>
<tr>
<td>CD4</td>
<td>Subset</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD5</td>
<td>+</td>
<td>-</td>
<td>Small subset</td>
</tr>
<tr>
<td>CD7</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD8α</td>
<td>Subset</td>
<td>Subset</td>
<td>-</td>
</tr>
<tr>
<td>CD8β</td>
<td>Subset</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD18</td>
<td>Small subset</td>
<td>Subset</td>
<td>-</td>
</tr>
<tr>
<td>CD28</td>
<td>Most</td>
<td>Most fetal+, adult-</td>
<td>Plasma cells?</td>
</tr>
<tr>
<td>CD56</td>
<td>Small subset (activated)</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Function

Cytoxicity | Subset | + | - |
Capacity to produce IFNγ | Subset | + | - |

Table 2. Expression of Some Antigens on T, B, and NK Cells Suggesting That T and NK Cells Are More Related
of the issue on the most prominent site of NK-cell development requires a careful dissection of the developmental stages of early NK cells and localization of these cells in different organs.

Do class I MHC antigens play a role in NK-cell development? Ljungrén and Kärre distinguished first observed that murine NK cells preferentially killed certain tumor cells that had lost expression of MHC class I antigens. The importance of MHC class I recognition for NK cells was further substantiated by the observation that NK cells kill normal lymphoblasts with disrupted β2-microglobulin genes. Moreover, radiation-resistant host NK cells reject bone marrow grafts from syngeneic β2-microglobulin-deficient mice, a finding that may have practical importance in human bone marrow transplantation. These observations imply that NK cells possess specific receptors for MHC class I molecules and that interaction between these receptors and MHC class I molecules on target cells inhibits NK-cell–mediated cytoxicity. In the mouse, Yokohama et al identified an NK-cell receptor (designated Ly-49A) on a subset of NK cells in certain mouse strains that binds to H-2D and thereby prevents lysis of target cells expressing H-2D. Recently, NK-cell receptors for polymorphic HLA-B and HLA-C molecules have also been identified on subsets of human NK cells. NK cells bearing these receptors are unable to kill target cells expressing the appropriate HLA class I alleles. Because NK cells do not lyse normal autologous cells, it is assumed that all NK cells in an individual will express an NK-cell receptor that can recognize at least one self MHC class I allele. These NK-cell receptors may prevent the initiation of cytolysis by transmitting a “negative signal,” but the molecular mechanism of this process is unknown.

Mice with disrupted β2-microglobulin genes or TAP-1 genes (encoding proteins that transport peptides from the cytoplasm to the ER for assembly with MHC class I molecules) possess normal numbers of NK cells, despite reduced levels of MHC class I expression in these animals. Therefore, normal expression of self MHC class I molecules is not necessary for the generation of NK cells. However, the cytolytic activity of the NK cells in these mutant mice is substantially reduced compared with normal mice, suggesting that NK-cell maturation may be affected by the diminished expression of MHC class I molecules. Presently, it is unknown when in development NK cells first express these MHC class I receptors and whether self MHC class I alleles influence the acquisition of an “NK-cell-receptor repertoire.” Undoubtedly, these questions will receive considerable attention in the near future.

THE ROLE OF CYTOKINES IN T- AND NK-CELL DEVELOPMENT

Certain combinations of various cytokines including c-kit ligand (SCF), IL-1, IL-3, IL-6, and the colony-stimulating factors (GM-CSF, G-CSF, and M-CSF) induce proliferation and differentiation of stem cells into myeloid cells in vitro. It was therefore suspected that cytokines are also involved in lymphoid development. Early work addressing the possible involvement of cytokines in T-cell development focused on IL-2 and IL-4. However, although these cytokines may induce differentiation of human T-cell progenitors into mature T cells in some in vitro assays, the presence of normal numbers of CD4 and CD8 mature T cells in the circulation of immunodeficient patients whose cells lack the capacity to synthesize IL-2 is consistent with the existence of an IL-2–independent T-cell differentiation pathway in human. Moreover, the presence of a normal thymus and of mature T cells in mice deficient for IL-2, IL-4, or both support the notion that IL-2 and IL-4 are not critical for thymic T-cell development, although an immunodeficiency ensues in IL-2–/– mice. Other cytokines such as IL-6 have also been implicated in T-cell development, but normal intrathymic T-cell development is observed in IL-6–/– mutant mice. The same holds true for mice deficient in the IFNγR, indicating that IFNγ is dispensable for thymic T-cell development as well.

Recently, it was conclusively demonstrated that cytokines are indeed critical for T-cell (and also NK-cell) development when the molecular defect of X-linked severe combined immunodeficiency was identified. These X-SCID patients present a severe block in T- and NK-cell development, whereas normal or even elevated numbers of B cells are present in their circulation. Normal numbers of nonlymphoid hematopoietic cells were present in these patients. The molecular defect in these patients was pinpointed to mutations in the gene encoding the third chain of the IL-2 receptor located on the X chromosome region q13-1. Several mutations of this gene have been identified, all of them resulting in a loss of function of the IL-2 receptor complex. The IL-2R γ chain was originally identified as part of the trimolecular complex that forms the high-affinity IL-2R. It is now known that the IL-2R γ chain is also an element of the IL-4, IL-7, and IL-15 receptors. Based on these observations, the IL-2R γ chain has been designated yc, for common γ chain.

Which of the cytokines that share the yc chain are important for development of T and NK cells? As explained above, it is not likely that IL-2 and/or IL-4 are the ligands of the yc chain that are essential for T- and NK-cell development. A good candidate is IL-7. This cytokine promotes the growth of murine pre-B cells and of immature TN mouse thymocytes. IL-7 also supports growth of immature CD34+ TN human thymocytes and is a powerful growth factor for mature murine and human T cells. Compared with the profound effect of IL-7 on mouse pre-B cells, the efficacy of IL-7 in supporting human pre-B cell proliferation is very modest, but the IL-7 response of human pre-B cells is enhanced by IL-3. IL-7 can also mediate differentiation of human CD34+CD5+ thymic precursors, and bone marrow progenitors in combination with IL-2 and SCF. Evidence that IL-7 is involved in murine T- and B-cell development comes from studies with neutralizing anti–IL-7 antibodies. Anti–IL-7 partly blocks T-cell development of day-14 murine thymocytes in an FTOC. When injected in utero, anti–IL-7 monoclonal antibodies strongly block development of B cells and partially inhibit thymic T cells in vivo. The latter study showed that anti–IL-7 affected all thymic subsets.
Other indications that IL-7 is instrumental in T-cell development come from studies using neutralizing anti-IL-7 receptor antibodies and an analysis of mice with a targeted deletion in the IL-7Rα chain. Whereas an anti-IL-7Rα antibody partially inhibits T-cell development from primitive CD4+ c-kit+ thymic progenitors in an FTOC, a combination of this antibody with an anti-γc antibody strongly blocks in vitro T-cell development. A heterogeneous phenotype with respect to thymic T-cell development is observed in the IL-7Rα−/− mouse. The cellularity of the thymus varies between 0.01% to 10% of the wild-type. Those with only a few thymocytes have relatively more CD4+ CD8+ cells and showed a block at the TN CD25− stage. Thus, transition from CD25+ to CD25− cells and the accompanying cellular expansion is blocked. In cases in which the cellularity of the thymus is 1% or more of the wild-type number, the distribution of CD4+CD8+ cells in the thymus is normal. The cellularity in the spleen is 10% of wild-type in all IL-7Rα−/− mice. The IL-7Rα-deficient mice manifest a near complete block in B-cell development, but these mice were not tested for the presence of NK cells. The findings that fresh thymic stromal cells express IL-7 mRNA and IL-7 protein and human and mouse thymic stromal cell lines express IL-7 mRNA and produce IL-7 protein in vitro are consistent with a role for IL-7 in thymic T-cell development. Recently, mice with a targeted deletion of the γc chain were generated (P. Kränenfort, B. Blom, H. Spits, and A. Berns, unpublished data). These mice have substantially reduced numbers of T and B cells and no detectable NK cells. The deficiency of T and NK cells in the γc−/− mice is consistent with the observations in X-SCID disease. But whereas these patients have normal or even elevated numbers of B cells compared with normal healthy individuals, the γc-deficient mice have reduced numbers of B cells. This finding indicates that human B cells can develop independently of the γc chain. Perhaps in contrast to mouse B cells, human pre-B cells do not require IL-7 in vivo. This possibility is consistent with the rather inefficient in vitro expansion of human pre-B cells in the presence of IL-7 when compared with mouse pre-B cells. Taken together, these findings show that there seems to be strong evidence that IL-7 is involved in T and NK development.

Two hypotheses have been put forward to explain the action of cytokines in development of hematopoietic cells: the stochastic and inductive models. In the stochastic hypothesis it is argued that multipotent progenitor cells do not require cytokines or other inductive signals to differentiate into lineage-committed cells. Cytokines are necessary to maintain proliferation and/or viability of the progenitor cells. By contrast, the inductive hypothesis is based on the premise that external signals induce a genetic program resulting in lineage commitment and further differentiation. External signals may be provided by cytokines and/or cellular adhesion molecules and the extracellular matrix. A problem with experimental approaches to prove or disprove these hypotheses is that addition of cytokines is required for cell survival. In a recent study this difficulty was overcome by transfecting bcl-2 into progenitor cells. Multipotent progenitor cells, saved from apoptotic death by transfection of DNA encoding bcl-2, differentiate in the absence of cytokines. This result argues in favor of the stochastic hypothesis. Similar experiments have not yet been performed to investigate whether IL-7 acts to promote survival or as an inducer of lymphoid differentiation. However, data both from human and mouse studies suggest that the IL-7 may be an inductive factor. In an analysis of rearrangements of the TCR β locus in X-SCID patients, Dβ to Jβ rearrangements were detected, but the thymocytes of these patients lack Vβ to DJβ rearrangements. Furthermore, it was reported that IL-7 induces V(D)J rearrangement of the TCR β locus in thymocytes from mouse embryos at day 14 of gestation, whereas 15 other factors, including IL-1, IL-2, and IL-4, failed to induce TCR rearrangement. However, IL-7 is only the factor that was able to maintain RAG expression in cultured day-14 thymocytes, raising the possibility that IL-7 allowed gene rearrangements to proceed rather than inducing them. In another study it was found that culturing day-14 mouse fetal liver cells for 7 days in IL-7 induces functionally rearranged TCR γ transcripts. Additionally, IL-7 induces RAG-1 and RAG-2 transcripts in these fetal liver cultures. IL-7-induced TCR γ transcripts were also observed in the presence of the growth-inhibiting agent hydroxyurea, excluding the possibility that IL-7 induces outgrowth of a small population expressing TCR γ transcript. However, none of those studies excludes the possibility that IL-7 does not directly induce TCR gene rearrangement but promotes survival of the cells that rearrange their receptors.

Engagement of the γc chain is required but probably not sufficient for T- and NK-cell development. Recent data from murine studies suggest that SCF is also involved in early stages of T-cell differentiation. The c-kit receptor is expressed on stem cells and early thymic T-cell progenitors. SCF is expressed in the embryonic mouse thymus and enhances proliferation of thymic T-cell progenitors in response to IL-7 both in mouse and human. Antibodies against c-kit inhibit development of mouse fetal liver cells in an FTOC. It is not entirely clear whether c-kit is involved in T-cell development after progenitors have migrated into the thymus.

The role of SCF in human T-cell development has not yet been studied in detail, although some data suggest that SCF augments development of human T and NK cells. SCF is produced by cultured human thymic epithelial cells and increases the response of human CD34+ TN thymocytes to IL-7. Interestingly, the IL-7 response of CD3+ CD4+CD8− immature thymocytes is not enhanced by SCF (H. Spits, unpublished data). Culturing CD34+ thymic precursors in the combination of SCF, IL-7, and IL-2 results in development of NK cells.

CONCLUSION

The developmental pathways of pluripotent stem cells to mature T and NK cells are being elucidated, although many gaps in our understanding of these pathways have yet to be filled. Recent studies have improved our understanding of the developmental relationship of T and NK cells. It is likely that the common T/NK progenitor just upstream of committed T- and NK-cell precursors cannot develop to B cells.
However, the cellular stages between the pluripotent stem cell and the common T/NK progenitor cell are still unknown. The observation that a deficiency in the yc chain leads to severe disturbances in development of all three lymphoid lineages whereas development of other hematopoietic cells is not affected supports the idea that T, B, and NK cells develop from a common progenitor cell. This notion is further substantiated by the lack of T, B, and NK cells in mice deficient in the transcription factor Ikaros. However, the common lymphoid progenitor cell has yet to be precisely defined in terms of expression of cell surface antigens to distinguish these cells from myeloid and erythroid progenitor cells. Characterization of the common lymphoid progenitor and more detailed knowledge of the candidate common thymic T/NK-cell progenitor is required for an understanding of the molecular mechanisms governing T- and NK-cell commitment and will remain a challenge ahead. Compared with T lymphocytes, the differentiation pathways of NK cells with respect to their recognition of self MHC molecules and development of functional subsets are not understood and will undoubtedly be the subject of considerable research efforts. The identification of the IL-7R/yc complex as a critical component in T- and NK-cell development will be the basis for improving our understanding of the role of cytokines in this development and may also lead to the development of methods to expand T/NK-cell progenitors in vitro. These cultured progenitor cells may be used as a therapeutic modality in T-cell replacement therapy in congenital or acquired immunodeficiencies.

Although not discussed in this review, the improved comprehension of different cellular stages in T and NK development will perhaps also provide a more refined staging of T-lineage ALL.

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REFERENCES

44. Horii T, Phillips JH, Duncan B, Lanier L, Spits H: Human fetal liver-derived clones that express CD3 γ, δ, epsilon, and delta, and epsilon and proliferate in response to IL-2, IL-3, IL-4, or IL-7: Implications for the relationship between T and NK cells. Blood 80:1270, 1992
66. Sotzik F, Rosenberg Y, Boyd AW, Honeyman M, Metcalfe...


68. Norton AM, Litman DR: A second subunit of CD8 is expressed in human T cells. EMBO J 7:3433, 1988


75. Mombaerts P, Clarke AR, Hooper ML, Tonegawa S: Mutations in T cell receptor genes α and β block thymocyte development at different stages. Nature 360:225, 1992


80. Groettrup M, Baron A, Griffiths G, Palacios R, von Boehmer H: T cell receptor β chain homodimers on the surface of immature but not mature α, γ and δ chain deficient T cell lines. EMBO J 11:2735, 1992


85. Levin SD, Anderson SJ, Forbush KA, Perlmutt RM: A dominant-negative transgene defines a role for p56lck in thymopoiesis. EMBO J 12:1671, 1993


89. Anderson SJ, Levin SD, Perlmutt RM: Protein tyrosine kinase p56lck controls allelic exclusion of T cell receptor beta genes. Nature 365:552, 1993


132. Hackett J Jr, Bosma GC, Bosma MJ, Bennett M, Kumar V: Transplantable progenitors of natural killer cells are distinct from those of T and B lymphocytes. Proc Natl Acad Sci USA 83:3427, 1986


140. Wang N-P, Biron C, She J, Higgs K, Sunshine M-J, Lacy E, Lonberg N, Terhorst C: A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy

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numbers of the human CD3e gene. Proc Natl Acad Sci USA 91:9401, 1994


177. Pahwa R, Chatila T, Pahwa S, Paradise C, Day NK, Ghe R, Schwarz SA, Slade H, Oyaizu N, Good RA: Recombinant in-


185. Voss SD, Hong R, Sondel PM: Severe combined immunodeficiency, interleukin-2 receptor-2 (IL-2), and the IL-2 receptor: Experiments of nature continue to pave the way. Blood 83:626, 1994


Development of human T and natural killer cells

H Spits, LL Lanier and JH Phillips