The Development of T and Non-T Cell Lineages from CD34⁺ Human Thymic Precursors Can Be Traced by the Differential Expression of CD44

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Summary

In addition to T-lineage cells, a small proportion of hematopoietic non-T cells are present in the human postnatal thymus. However, the origin of this minor non-T cell thymic compartment is presently unknown. In this study we have analyzed the developmental potential of the earliest human intrathymic precursors, characterized as CD34⁺ cells expressing intermediate levels of CD44. We show that these CD34⁺CD44int thymocytes cultured with interleukin 7 were able to develop simultaneously into both T- and non-T (monocytes and dendritic cells) -lineage cells. Both developmental pathways progress through a CD1⁺CD4⁺ intermediate stage, currently believed to be the immediate precursor of double positive thymocytes. However, separate progenitors for either T or non-T cells could be characterized within CD1⁺CD4⁺ thymocytes by their opposite expression of CD44. Downregulated levels of CD44 identified CD1⁻CD4⁺ T-lineage precursors, whereas CD44 upregulation occurred on CD1⁺CD4⁺ intermediates that later differentiated into non-T cells. Therefore, commitment of human early intrathymic precursors to either T or non-T cell lineages can be traced by the differential expression of the CD44 receptor.

Bone marrow-derived hematopoietic precursors homing to the human thymus have been identified as CD3⁻CD4⁻CD8⁻ triple negative (TN)1 thymocytes (1, 2). The T cell precursor potential of TN thymocytes is confined to cells that express CD34 (3), a marker of pluripotent hematopoietic stem cells (4-6). A major question is whether these CD34⁺ early thymic immigrants are already fully committed to the T cell lineage, or whether they represent primitive precursors that can also give rise to the small percentage (<1%) of hematopoietic non-T cells normally present in the human postnatal thymus (7-11). The last possibility is consistent with the reported presence of myeloid progenitors in immature populations of both fetal and neonatal thymocytes (1, 12, 13). It is further supported by very recent results showing that the human fetal thymus is permissive for the development of both lymphoid and myeloid hematopoietic lineages derived from fetal liver progenitors (13). However, direct evidence that human thymic non-T cells are derived from primitive progenitors seeding the thymus is still lacking.

In contrast, recent studies in mice have shown that the first cells in the adult thymus are able to give rise to non-T cell lineages. Early postnatal thymocytes (14) were shown to differentiate into T cells and dendritic cells (DC) after intrathymic injection (15) and had the capacity to form B and NK1.1⁺ cells in the appropriate environment (16, 17). These data suggest that the most immature intrathymic precursors represent an intermediate stage between the multipotential stem cells in the bone marrow and later unipotential T cell-committed thymocytes and support their dual potential to develop into both lymphoid and nonlymphoid lineages (15). At the present time, however, separate intrathymic precursors for these two developmental pathways remain to be identified (14-17).

Identification of the equivalent precursors in the human thymus has been hampered by the lack of in vitro assays to assess both differentiation options. Since IL-7 is the only known factor that does not disrupt the differentiation potential of early thymocytes in vitro (18, 19), while maintaining their viability (18-21), this study was aimed at investigating the capacity of the earliest CD34⁺ human intrathymic precursors to develop into T and non-T cell lineages when cultured in the presence of IL-7.

Materials and Methods

Isolation of CD34⁺ Precursors from the Human Postnatal Thymus.

Normal human thymocytes were obtained from thymus fragments removed during corrective cardiac surgery of patients aged 1 mo
to 3 yr. Intrathymic CD3− CD4− CD8− TN precursors were iso-
lated as previously described (22). Briefly, cells were treated with
anti-CD4 (HP2/6), and -CD8 (B9.4) mAbs plus rabbit comple-
ment (Sera-Lab Ltd., Sussex, UK). Anti-CD3 was not included
in this treatment to avoid the possibility of CD3 capping. After
Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) centrifugation, re-
covered viable cells were treated with a cocktail of anti-CD4− CD8−
and -CD3 (SPV-T3b) mAbs followed by two rounds of immuno-
magnetic bead depletion (Dynabeads; Dynal, Oslo, Norway). In
some experiments, when a high proportion of mature thymic B
and NK cells was detected, depletion with anti-CD19 and -CD56
(Becton Dickinson & Co., San Jose, CA) mAbs was also performed.
CD3+ cells were then purified from adherent cell-depleted TN
thymocytes through positive selection with CD34-coated immuno-
magnetic beads (Dynal).

**Immunofluorescence and Flow Cytometry.** mAbs against the fol-
lowing antigens were used: CD1a (T6-RD1), CD2 (T11-RD1),
CD11b (M01-FITC), and CD14 (MO2-FITC) from Coulter Clone
(Hialeah, FL); CD3 (Leu-4-PE) from Becton Dickinson & Co.;
CD4 (Leu-3a-PE or OKT4A-FITC) from Becton Dickinson & Co.
and Ortho Pharmaceuticals (Raritan, NJ), respectively; CD8 (Leu-
2a-FITC and OKT8-FITC) from Becton Dickinson & Co. and
Ortho Pharmaceuticals, respectively; CD7 (Leu-9-FITC), CD13
(Leu-M7-PE), CD16 (Leu-11c-PE), CD19 (Leu-12-FITC), CD22
(Leu-14-PE), CD33 (Leu-M9-PE), CD34 (HPCA-2-PE or HPCA-
2-FITC), CD44 (Leu-44-FITC), CD56 (Leu-19-PE), B7 (BBI), and
HLA-DR (anti-HLA-DR-PE) from Becton Dickinson & Co.; CD21
(B-E5-FITC) from Serotec (Oxford, UK); and CD23 (I088-
FIC from Immunotech (Marseille, France). Anti-CD25 (MAR108-
FITC) and -CD45 (GAP8.3-FITC) have been previously described
(23). Anti-CD40 (mAb89) was kindly provided by Dr. J. Ban-
chereau (Scherer-Plough, Dardilly, France), and anti-HLA-1P and
-HLA-DQ were the kind gift of Dr. J. Bodmer (Imperial Cancer
Research Fund, London, UK).

Single- and two-color immunofluorescence studies were per-
formed as described elsewhere (23) after incubation of the cells with
PBS/EDTA buffer for 15 min. Stained cells were analyzed in an
flow cytometer (EPICS Profile; Coulter Electronics Inc., Hialeah,
FL). Data were collected on 2− 5 × 10^4 viable cells as determined
by electronic gating on forward scatter and side scatter light
parameters. Isootype-matched irrelevant antibodies were used to define
background fluorescence.

**Cell Cultures.** Positively isolated CD34+ thymocytes (1− 10
^4 ml^-1) were cultured at 37°C in 24-well macroplates (Costar,
Cambridge, MA), in RPMI 1640 (Gibco Laboratories, Paisley, UK),
10% FCS (Gibco) medium either alone or supplemented with 250
U/ml of recombinant human IL7 (chrIL-7, 10^5 U/μg) (ammpoule
code 90/530; National Institute for Biological Standards and
Control, Hertfordshire, UK). Cultures set up either in the presence
or absence of IL-7 were scored at different times for viable cells
by trypan blue dye exclusion.

**MLR Assay.** Cells recovered after 16 d of culture in the presence
of rhlIL-7 (>95% large CD44^hi cells) were irradiated (3,500 rads)
and used at different numbers (30 cells to 2 × 10^9 cells) as stimu-
lators for resting allogeneic T cells (2 × 10^9) isolated from
peripheral blood. After 5 d of culture in round-bottomed 96-well tissue
culture plates (Costar) in RPMI 1640 10% FCS, proliferation was
measured in triplicate cultures by [^3]H]thymidine incorporation
([^3]H]Tdr uptake) after addition of 1 μCi per well of [^3]H]Tdr
(Amersham Corp., Amersham, UK) for the last 4 h.

**Scanning Electron Microscopy.** CD34+ thymocytes cultured with
rhlIL-7 during 12 d were recovered and cultured on glass coverslips
for an additional 2 d. Adherent cells were fixed with 3% glutaralde-
hyde in 0.1 M cacodylate buffer, pH 7.4, washed in the same buffer,
and postfixed with 2% OsO4, as previously described (8). Samples
were then dehydrated with graded ethanol series, coated with gold,
and examined with a scanning electron microscope (model XL-30;
Philips, Eindhoven, The Netherlands).

**Results**

**Antigenic Phenotype of Human CD34^+ Postnatal Thymocytes.** To
investigate the antigenic phenotype of the first hematopoietic
precursors present in the human postnatal thymus, CD34^+ cells were purified from preparations of thymocytes depleted of cells expressing CD3, CD4, or CD8 antigens (TN thymocytes). As displayed in Fig. 1 A, TN thymocytes were highly enriched (>90%) in cells showing a heterogeneous (low-to-bright) expression of the CD34 antigen. CD34^+ TN thymocytes displayed a low CD45 expression, typical for early progenitor cells (6). A 10-fold higher expression of CD45 was detected in a minor fraction (<10%) of CD34^− TN thymocytes, which included mature CD7− CD22^+ B cells and CD7+ CD56^− NK cells (Fig. 1 A and data not shown), normally present in the human postnatal thymus. In contrast to CD34^+ hematopoietic stem cells in the bone marrow (6, 24), intrathymic CD34^+ precursors coexpressed the CD7 molecule. As described previously (3, 20), expression of the CD1 thymic differentiation marker (3, 6) subdivided CD34^+ thymocytes into two different subsets. The majority of CD34^+ thymocytes (75% in this particular experiment) coexpressed CD1, but CD34^+ CD1^− cells were also consistently detected. According to previous data (3), we observed that expression of CD34 on the CD1^− CD34^+ cells was higher than on CD1^+ CD34^+ thymocytes (Fig. 1 A). Depletion of TN thymocyte suspensions to eliminate B and NK cells, followed by immunomagnetic selection of CD34^+ thymocytes, rendered a highly enriched population of cells (0.05−0.1% of all thymocytes, >98% pure) that displayed high-surface density CD34 (Fig. 1 B). This isolation technique was found inefficient for selecting CD34^null thymocytes; therefore, we consistently recovered cells expressing high levels of CD34 (10−15% of total CD34^+ TN thymocytes in different experiments). As shown in Fig. 1 B, these CD34^+ thymocytes lacked specific markers of mature T (CD3, CD4, and CD8) and myeloid (CD14, CD11b) cells. Expression of B- (CD19, CD22) and NK-lineage (CD56) antigens was negative as well (not shown). Positively selected CD34^+ thymocytes displayed high-surface density CD7 and coexpressed CD2 molecules. However, expression of CD1 was barely detectable in a minor proportion (8% in this experiment) of CD34^+ CD7^− CD2^− thymocytes (Fig. 1 B), supporting the finding that expression of high levels of CD34 is mainly confined to CD1^+ thymocytes, considered to be the most immature intrathymic precursors (3).

Further analysis of these postnatal CD34^+ thymocytes revealed an antigenic phenotype very similar to that of CD34^+ early precursors present in the fetal thymus (13), including a variable expression of low levels of CD33 (20% in this particular experiment) and the expression of HLA-DR by most (82%) CD34^+ cells (Fig. 1 B). In addition, CD34^+ thymocytes
Figure 1. Phenotypic characterization of CD34+ TN postnatal thymocytes. (A) Freshly isolated postnatal TN thymocytes were stained with PE- or FITC-coupled anti-CD34 mAbs, and with either FITC-labeled anti-CD45, anti-CD7, or PE-labeled anti-CD1a mAbs. (B) Positively selected CD34+ cells from the TN thymocyte preparation shown in A were reanalyzed for the expression of the indicated molecules by use of the mAbs described in Materials and Methods. Background fluorescence values were set by use of isotype-matched irrelevant mAbs. Results are representative of four independent experiments. (a.u.) Arbitrary units.

CD34+ CD44int Thymic Precursors Differentiate into Two Distinct CD34− Cell Populations, One CD44high, the Other CD44−, in the Presence of IL-7. The developmental potential of early CD34+ postnatal thymocytes was analyzed by phenotypic studies performed on CD34+ cells cultured in the presence of rhIL-7. CD34+ thymocytes were shown to proliferate in an IL-7-dependent manner during the first 3-4 d of culture. Results of one representative experiment (out of three) displayed in Fig. 2 show that viable cell numbers increased about threefold over the initial 3-d culture period and was maintained from day 3 to day 6 (cell viability >99%) in the presence of IL-7. Thereafter, cellular recovery decreased steadily, although a small proportion of viable cells could be maintained for up to 2-3 wk. In contrast, cell viability dropped rapidly without IL-7. The proliferative phase of culture was accompanied by the appearance of colonies containing >50 cells/colony. Nonaggregated smaller round cells were detected as well. The size of the colonies decreased throughout culture. This was paralleled by a gradual increase in large, irregularly shaped cells within the colonies. To study the phenotypic profile of cells undergoing these morphological changes, IL-7–cultured CD34+ thymocytes were then analyzed by flow cytometry. Since differentiation of mouse CD44+ thymic progenitors into mature T cells has been shown to proceed through an intermediate CD44− stage (19), we investi-
tigated whether changes in CD44 expression could also define control points in the development of human CD34+ thymic precursors. As shown in Fig. 3, no significant changes in CD34 and CD44 expression were detected during the initial (3 d) proliferating phase of culture, indicating that IL-7 is a growth factor for CD34+CD44int human thymocytes. From day 3 on, however, CD34 was gradually lost, suggesting progression through further developmental stages (3, 6, 24, 27). Interestingly, the decrease in CD34 was accompanied by the segregation of CD44int cells into two different cell populations showing opposite CD44 expression. Up- or downregulation of CD44 was evident in CD34+ cells by day 6 of culture, and resulted in the appearance of two separate subsets of CD34− cells, one CD44high, the other CD44−, by day 8 (Fig. 3).

Both CD44− and CD44high Subpopulations Acquire the CD1 and CD4 T Lineage-associated Antigens. Light-scattering analysis of 3-d-cultured CD34+CD44int thymocytes revealed a predominant population of intermediate-sized cells that segregated into two separate populations of either small or large CD1+CD4+ thymocytes, respectively. Two-color flow cytometric studies were performed at the indicated days of culture with FITC-labeled anti-CD44 and either anti-CD1 or anti-CD4 PE-coupled mAbs. Based on forward and side scatter light parameters, a single electronic gate was placed in the discrete population of intermediate sized cells recovered after 3 or 6 d of culture, whereas two different populations of either large (L) or small (S) cells were electronically gated and analyzed independently at days 8 and 10. The numbers of total viable cells recovered within the small and large subsets were $3.1 \times 10^6$ and $0.72 \times 10^6$, respectively, at day 8, and $2.3 \times 10^6$ and $0.5 \times 10^6$, respectively, at day 10. Results are representative of six independent experiments.

Figure 3. CD34+CD44int thymic precursors cultured with IL-7 differentiate simultaneously into two different subsets of either CD44high or CD44− cells. CD34+CD44int thymic precursors cultured with IL-7 as described in Fig. 2 were analyzed by flow cytometry for the correlated expression of CD44 versus CD34 at the indicated days of culture. Staining was performed with PE-labeled anti-CD34 and FITC-labeled anti-CD44 mAbs. Background fluorescence values were set by use of isotype-matched irrelevant mAbs. Results are representative of four independent experiments.

Figure 4. Up- or downregulation of CD44 on CD34+CD44int thymic precursors cultured with IL-7 defines two different populations of either large or small CD1+CD4+ thymocytes, respectively. CD34+CD44int thymocytes cultured with IL-7 were analyzed for the correlated expression of CD44 versus either CD1 or CD4. Two-color flow cytometric studies were performed at the indicated days of culture with FITC-labeled anti-CD44 and either anti-CD1 or anti-CD4 PE-coupled mAbs. Based on forward and side scatter light parameters, a single electronic gate was placed in the discrete population of intermediate sized cells recovered after 3 or 6 d of culture, whereas two different populations of either large (L) or small (S) cells were electronically gated and analyzed independently at days 8 and 10. The numbers of total viable cells recovered within the small and large subsets were $3.1 \times 10^6$ and $0.72 \times 10^6$, respectively, at day 8, and $2.3 \times 10^6$ and $0.5 \times 10^6$, respectively, at day 10. Results are representative of six independent experiments. LFS, logarithm of forward scatter; LSS, logarithm of side scatter.
large cells by day 8 (Fig. 4). A close correlation between CD44 expression and cell size could be established when electronic gates were placed on the small (80–90%) and large (10–20%) subsets. CD44− thymocytes were mainly small cells, whereas CD44bright thymocytes were mostly large cells. Changes in CD44 expression were also accompanied by the sequential acquisition of antigens known to be expressed early during thymic T cell differentiation. As shown in Fig. 4, CD1 was the first molecule expressed on cultured CD34+ thymocytes. At day 3 of culture, expression of CD1 was observed in 19% of CD34+ cells. Low levels of CD4 were also detected at this time on a small cell fraction (3% of total recovered cells) that coexpressed CD1, indicating that, as previously reported (3, 20), CD1+ are the direct precursors of CD4+ thymocytes. In fact, most CD1+ cells acquired CD4 by day 6 (20% CD1+CD4+, 5% CD1+CD4−, in this experiment), CD4+ thymocytes becoming a major population within both the small (60%) and the large (65%) cell subsets by day 8. However, a subset of CD4+ cells lacking CD1 molecules (20%) was detected exclusively within the large CD44bright population by day 8. A similar proportion (21%) of CD44brightCD4+CD1− cells was detected by day 10, when essentially all (96%) CD44bright cells were CD4+. Therefore, CD44bright thymocytes progressed to more mature CD44bright or CD44− cells through a CD1+CD4+ intermediate stage. However, high levels of both CD1 and CD4 molecules were expressed on CD44bright cells, whereas intermediate expression levels of both antigens were consistently found in the CD4− population.

CD44bright but Not CD44− Progeny Derived from CD34+ Precursors Comprise a Mixed Population of Monocytic Cells and Dendritic Cells. To determine the nature of both the large CD44bright and the small CD44− populations derived from CD34+ thymic precursors, cells were stained with a panel of mAbs against distinct lymphoid and myeloid-associated markers (Fig. 5 and Table 1). Both the small and the large cell subsets were shown to keep CD2 expression at variable levels, indicating that they were derived from intrathymic precursors rather than from contaminating peripheral blood stem cells (6, 24). However, each population displayed radically distinct phenotypic profiles (Fig. 5). Small CD44− thymocytes were characterized as T-lineage cells by several criteria: (a) they retained a high CD7 expression; (b) they lacked B cell−, NK cell− (not shown), and myeloid-related antigens, as well as HLA class II molecules; and (c) as expected for T cell precursors, they developed into CD4+CD8− double positive (DP) thymocytes through a CD1+CD4+ transitional subset (Fig. 5). These DP thymocytes expressed cytoplasmic but not membrane CD3 (not shown), suggesting that they represent the immediate precursors of surface CD3+ DP thymocytes. This sequence of maturation events corresponds to that found in vivo for both postnatal and fetal thymic precursors differentiating toward mature T cells (3, 20, 27). However, no transition to CD3+ T cells could be detected under our culture conditions, since cell viability of CD3−DP thymocytes dropped rapidly from day 8 to day 10. This suggests that additional signals to those provided by IL-7, most likely involving the participation of the thymic stromal components, are required for terminal differentiation of T cell-committed precursors.

In contrast to CD44− thymocytes, large CD44bright cells lacked the CD8 T cell molecule but expressed very high levels of CD4 (10-fold higher than CD4− DP thymocytes) (Fig. 5). They expressed low CD7 levels, suggesting that CD7 was downregulated throughout culture. Most of these cells showed high levels of the myeloid-related antigens CD11b, CD13, and CD33, as well as of HLA-DR, -DP, and -DQ class II molecules, and many of them were CD14− (Fig. 5 and Table 1). This phenotypic pattern closely resembles that recently found on myelomonocytic cells derived from CD34+ fetal liver precursors in a human fetal thymic organ culture system (13). However, >50% of CD44bright cells shown to express...
Table 1. Phenotypic Profile of Large Cells Generated from Human CD34+ Thymic Precursors Cultured with IL-7

| Surface antigens | Positive cells | Fluorescence intensity |
|------------------|----------------|------------------------|
| T cell antigens  |                |                        |
| CD1a             | >80            | High                   |
| CD2              | >80            | Int.                   |
| CD3              | <5             | Low                    |
| CD4              | >95            | Int.                   |
| CD7              | 5-20           | Low                    |
| CD8              | <5             | Low                    |
| B cell antigens  |                |                        |
| CD19             | <5             | Low                    |
| CD21             | 10-30          | Low                    |
| CD22             | <5             | Low                    |
| CD23             | 20-40          | Low                    |
| CD40             | 40-70          | Int.                   |
| CD80 (B7-BB1)    | 40-60          | Int.                   |
| Myeloid antigens |                |                        |
| CD11b            | >80            | High                   |
| CD13             | >80            | High                   |
| CD14             | 60-70          | Int-High               |
| CD33             | >80            | High                   |
| Class II antigens|                |                        |
| DR               | >95            | High                   |
| DP               | >95            | High                   |
| DQ               | >95            | High                   |
| Other antigens   |                |                        |
| CD16             | <5             | Low                    |
| CD25             | 50-60          | Int.                   |
| CD44             | >95            | High                   |
| CD45             | >95            | High                   |
| CD56             | <5             | Low                    |

Large cells (90-95% of total recovered cells) generated from CD34+ thymocytes cultured in the presence of rHIL-7 during 12-16 d were analyzed by flow cytometry with the indicated mAbs. Mean of fluorescence intensity detected within the second (Low), third (Int.) or fourth (High) amplification decades is indicated for each antigen.

myeloid-related markers also displayed a heterogeneous expression of the CD23, CD40, and B7 (CD80) B-lineage markers (Table 1). Interestingly, variable levels of these molecules have previously been reported in different studies (28-30) to be expressed on peripheral Langerhans/DC. Cells displaying this phenotypic profile could be maintained in culture for up to 2-3 wk.

Morphologic studies of in vitro-derived CD44high large cells revealed a mixed population of adherent and nonadherent cells. Cytospin preparations of nonadherent cells showed the presence of cells with a typical monocyctic morphology, as well as cells displaying lobulated nuclei, dense perinuclear chromatin, and sheet-like processes characteristic of Langerhans/DC (8, 28). The adherent population was also heterogeneous and consisted of cells showing the characteristic

Figure 6. Scanning electron micrograph of a CD44high DC generated from IL-7-cultured CD34+ thymocytes. Scanning electron microscopy studies were performed on adherent cells derived from CD34+ thymocytes. Cells cultured with IL-7 during 14 d were treated as described in Materials and Methods and analyzed in a Philips XL-30 scanning electron microscope. Bar, 20 μm.

Figure 7. Stimulatory capacity of CD44high cells derived from CD34+ thymocytes in an allogeneic MLR. Cells derived from CD34+ thymocytes cultured for 16 d with IL-7 (>95% large CD44high) were irradiated (3,500 rad) and used at different numbers (30 cells to 20 x 10^3 cells) as stimulators for resting allogeneic T cells (2 x 10^5) isolated from peripheral blood. Proliferation after 5 d of culture was measured in triplicate. Results are expressed as mean cpm and are representative of three different experiments. Standard deviations represented <10% of the mean value.
morphology of macrophages and cells displaying dendritic processes. Further examination of these cells by scanning electron microscopy revealed a typical dendritic morphology (8, 28), with a stellate shape and dendritic projections (Fig. 6). Finally, functional assays demonstrated the APC capacity of the CD44bright cells by showing that they were potent stimulators of the allogeneic MLR (29). Proliferation of allogeneic peripheral T cells (2 × 10⁵) was increased 40-fold by 10⁶ CD44bright stimulators (Fig. 7). Therefore, based on morphologic, phenotypic, and functional criteria (8, 28–30), we can conclude that CD44bright progeny derived from CD34⁺ precursors includes DC as well as monocytic cells. These data provide evidence that CD34⁺ thymocytes are able to differentiate into non-T cell lineages in the presence of IL-7, suggesting that lymphoid as well as nonlymphoid cells can be newly generated in the human thymus from intrathymic precursors.

**Discussion**

Current in vitro experimental systems for hematopoiesis are hampered by the fact that lineage programs undertaken by multipotential precursors are influenced by the specific growth factors to which they are exposed. However, studies in mice have shown that hematopoietic precursors cultured with IL-7 retain their intrinsic developmental potential (18, 19). In fact, IL-7 appears to be an important regulator of the growth of both T- and B-lymphoid progenitors, (18–21) and, recently, a novel role for IL-7 in early myelopoiesis has been reported (31). In this study, we have analyzed the precursor potential of CD34⁺ human postnatal thymocytes, which are known to represent the earliest intrathymic precursors, when cultured in the presence of IL-7. Our results revealed the capacity of CD34⁺ thymocytes to proliferate in response to IL-7 and develop simultaneously into T-lineage cells, monocytic cells, and DC. Therefore, in addition to its reported involvement in early lymphopoiesis and myelopoiesis (18–21, 31), these data suggest a novel role of IL-7 in the development of DC.

We have shown that IL-7 was able to induce a marked proliferation of CD34⁺ thymocytes (which were ≥98% pure upon reanalysis) during the initial 3 d of culture. As previously reported (20), IL-7 was also able to maintain the viability of CD34⁺ thymocytes at later periods of culture. Phenotypic studies revealed no significant antigenic changes during the proliferating phase of culture, indicating that cells capable of responding to IL-7 were included within the CD34⁺ thymic subset. From day 3 on, however, IL-7–cultured cells sequentially acquired the CD1 and CD4 antigens as well as myeloid/DC-lineage markers or the CD8 T cell molecule, and CD34 was concurrently downregulated. It is thus unlikely that all these phenotypic changes could be explained by a selective death of CD34⁺ thymocytes, or by the outgrowth (from day 3 on of culture) of a minor contaminating CD34⁺ population of already differentiated cells. Supporting our findings, it has been previously shown that proliferation induced by IL-7, as well as differentiation into CD4⁺CD8⁺ DP thymocytes, is restricted to the CD34⁺ thymic subset (3, 32). However, no myeloid or DC precursor potential was detected in a previous study by Schmitt et al. (32) on ex vivo–isolated CD34⁺ thymocytes cultured in the presence of myeloid growth factors. These contradictory results may be explained by the heterogeneity of the CD34⁺ thymic subset. About 75% of the CD34⁺ thymocytes reported by Schmitt et al. expressed CD1, and 30% of them were CD4⁺, whereas our preparations were highly enriched in CD34brightCD1⁻CD4⁻ cells. These data suggest that the myeloid/DC precursor potential of CD34⁺ thymocytes resides in the CD1⁻ subset, while most CD34⁺ CD1⁺ thymocytes may represent T cell–committed precursors. Similarly, it has been recently shown that bipotential T/NK cell progenitors present in the human fetal thymus are confined to the CD1⁻ subset of CD34bright thymocytes (33). It is also possible that IL-7 (which was omitted in the differentiation assay reported by Schmitt et al. [32]) is required to induce the proliferation and maintain the differentiation potential of primitive myeloid/DC intrathymic precursors, whereas specific growth factors may be required to drive their terminal differentiation. This concurs with the observation that IL-7 potently enhances the myelopoietic potential of bone marrow hematopoietic stem cells, whereas it has no effect on committed myeloid progenitors (31). Supporting this possibility, we have observed that the simultaneous addition of IL-7 and GM-CSF to our cultures greatly improved both the proportion and survival of DC, as well as of a CD1⁻ subset of CD44brightCD4⁺CD11b⁺DR⁻ cells, likely representing myelomonocytic precursors (not shown).

Our results provide evidence for a dual potential of human CD34⁺ thymic precursors to develop into either T or non-T (monocytes and DC) cell lineages, but they do not preclude that separate precursors, one for each hematopoietic lineage, may be included within the CD34⁺ thymic population. Therefore, we cannot yet conclude that the CD34⁺ thymic subset displays pluripotent hematopoietic activity. Confirmation of this possibility must await the development of a suitable clonal assay. In our culture conditions, both DC as well as T-lineage cells were shown to develop through a CD1⁺CD4⁺ intermediate stage. However, separate precursors for either the T or the DC lineages could be identified at this early developmental stage by their differential expression of both CD1 and CD4 markers (CD1intCD4int or CD1bright CD4bright for T and DC precursors, respectively). In agreement with our results, Sotzik et al. (9) have recently reported a bright CD4 expression on ex vivo–isolated thymic DC. Intrathymic DC, however, did not express CD1, suggesting that this molecule is lost in more mature differentiation stages in the DC lineage. We have observed that CD34⁺ thymocytes can also give rise to macrophages, although the existence of a CD1⁺CD4⁺ intermediate stage in the development of this myeloid lineage remains to be clarified. It can be speculated that, as proposed for bone marrow–derived macrophages and DC (34), a common bipotential progenitor for these two cell lineages exists in the human thymus.

The simultaneous development of both lymphoid (T cells)
and nonlymphoid (monocytes and DC) lineages from CD34+ thymic progenitors allowed us to identify distinctive maturation events associated with lineage choice in the thymus. We have shown that commitment to each alternative developmental pathway can be traced by the differential expression of CD44. Therefore, separate CD1+CD4+ precursors for either DC and T cells can be characterized, respectively, as CD44high or CD44- cells. The observation that both cell populations do exist in the human postnatal thymus (9, and our unpublished results) at a ratio (1-2:10) similar to that found in our in vitro assays provides additional support for their physiological relevance in vivo. Indeed, previous results in mice have shown that transition from CD44+ to CD44- in the thymus is an obligatory step during early T cell differentiation (13). Whether CD44 expression may also be used to identify lymphoid precursors able to give rise to the B and/or NK cell intrathymic compartments is currently under investigation.

In light of these studies, regulation of CD44 expression may be envisaged as an early consequence of the developmental program undertaken by intrathymic precursors. Alternatively, CD44 regulation may play a prominent role in lineage commitment decisions of putative multipotential hematopoietic precursors. Changes in CD44 expression during early thymic development may thus be related to differences in the adhesion requirements to stromal components of distinct thymic precursors and/or the migration patterns of their respective progenies. Finally, the reported expression of CD4 molecules along the differentiation pathway of both T cells and thymic APC (macrophages and DC) has obvious implications for the immunopathological consequences of HIV infection (35). Since either viral carriage by thymic APC or their infection would lead to the induction of tolerance in those T cell clones specific for viral antigens, thus precluding the induction of protective immunity.

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References

1. Haynes, B.F., S.M. Denning, P.T. Le, and K.H. Singer. 1990. Human intrathymic T cell differentiation. Semin. Immunol. 2:67–77.
2. Toribio, M.L., A. de la Hera, J. Borst, M.A.R. Marcos, C. Márquez, J.M. Alonso, A. Bárcena, and C. Martínez-A. 1988. Involvement of the interleukin 2 pathway in the rearrangement and expression of both α/β and γ/δ T cell receptor genes in human T cell precursors. J. Exp. Med. 168:2231–2249.
3. Galy, A., S. Verma, A. Bárcena, and H. Spits. 1993. Precursors of CD3+CD4+CD8- cells in the human thymus are defined by CD34. Delineation of early events in human thymic development. J. Exp. Med. 178:391–401.
4. Civin, C.I., L.C. Strauss, C. Brovall, M.J. Fackler, J.F. Schwartz, and J.H. Shaper. 1984. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J. Immunol. 133:157–165.
5. Andrews, R.G., J.W. Singer, and I.D. Bernstein. 1989. Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of the CD33 and CD34 antigen and light scattering properties. J. Exp. Med. 169:1721–1731.
6. Terstappen, L.W.M.M., S. Huang, and L.J. Picker. 1992. Flow cytometry assessment of human T-cell differentiation in the thymus and bone marrow. Blood. 79:666–677.
7. Kampinga, J., P. Nieuwenhuis, B. Roser, and R. Aspinall. 1990. Differences in turnover between thymic medullary dendritic cells and a subset of cortical macrophages. J. Immunol. 145:1659–1663.
8. Landry, D., M. Lafontaine, H. Barthélémy, N. Paquette, C. Chartrand, M. Pelletier, and S. Montplaisir. 1989. Human thymic dendritic cell-thymocyte association: ultrastructural cell phenotypic analysis. Eur. J. Immunol. 19:1855–1860.
9. Sotzik, F., Y. Rosenberg, A.W. Boyd, M. Honeyman, D. Metcalf, R. Scollay, L. Wu, and K. Shortman. 1994. Assessment of CD4 expression by early T precursor cells and by dendritic cells in the human thymus. J. Immunol. 152:3370–3377.
10. Issacson, P.G., A.J. Norton, and B.J. Addis. 1987. The human thymus contains a novel population of B lymphocytes. Lancet. 2:1488–1491.
11. Lanier, L.L., H. Spits, and J.H. Phillips. 1992. The developmental relationships between NK and T cells. Immunol. Today. 1659–1663.
12. Kurtzberg, J., S.M. Denning, L.M. Nycum, K.H. Singer, and B.F. Haynes. 1989. Immature human thymocytes can be driven to differentiate into nonlymphoid lineages by cytokines from thymic epithelial cells. Proc Natl Acad Sci USA. 86:7575–7579.

13. Bárcena, A., A.H.M. Galy, J. Punnnonen, M.O. Muench, D. Schols, M.G. Roncarolo, J.E. de Vries, and H. Spits. 1994. Lymphoid and myeloid differentiation of fetal liver CD34+ lineage cells in human thymic organ culture. J. Exp. Med. 180:123–132.

14. Wu, L., R. Scollay, M. Egerton, G.J. Spangrude, M. Pearse, M. Antica, G.P. Johnson, T.L. Scollay, and K. Shortman. 1993. CD4 expression on earliest T-lineage precursor cells in the adult murine thymus. Nature (Lond.) 362:761–763.

15. Ardavin, C., L. Wu, Ch.-L. Li, and K. Shortman. 1993. Thymic dendritic cells and T cells develop simultaneously within the thymus from a common precursor population. Nature (Lond.) 362:761–763.

16. Wu, L., M. Antica, G.R. Johnson, R. Scollay, and K. Shortman. 1991. Developmental potential of the earliest precursor cells from the adult thymus. J. Exp. Med. 174:1617–1627.

17. Matsuzaki, Y., J.-I. Gyotoku, M. Ogawa, S.-I. Nishikawa, Y. Katsura, G. Galechin, and H. Nakauchi. 1993. Characterization of c-kit positive intrathymic stem cells that are restricted to lymphoid differentiation. J. Exp. Med. 178:1283–1292.

18. Suda, T., and A. Zlotnik. 1991. IL-7 maintains the T-cell precursor potential of CD3-CD4+CD8- thymocytes. J. Immunol. 146:3068–3073.

19. Godfrey, D.I., and A. Zlotnik. 1993. Control points in early T-cell development. Immunol. Today. 14:547–553.

20. Hori, T., J. Cupp, N. Wrighton, F. Lee, and H. Spits. 1991. Identification of a novel human thymocyte subset with a phenotype of CD3-CD4+CD8αβ+. Possible progeny of the CD3-CD4+CD8- subset. J. Immunol. 146:4078–4084.

21. Fabbi, M., V. Groh, and J.L. Strominger. 1991. IL-7 induces proliferation of CD3-CD4+CD8- human thymocyte precursors by an IL-2 independent pathway. Int. Immunol. 4:11–5.

22. Törbíö, M.L., J.C. Gutiérrez-Ramos, L. Pezzi, M.A.R. Marcos, and C. Martínez-A. 1989. Interleukin-2-dependent autocrine proliferation in human T-cell development. Nature (Lond.). 342:82–85.

23. Sánchez, M.J., J.C. Gutiérrez-Ramos, E. Fernández, E. Leonardo, J. Lozano, C. Martínez-A., and M.J. Torbíö. 1993. Putative prethymic T-cell precursors within the early human embryonic liver: a molecular and functional analysis. J. Exp. Med. 177:19–33.

24. Terstappen, L.W.M.M., S. Huang, M. Safford, P.M. Lansdorp, and M.R. Loken. 1991. Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+CD38- progenitor cells. Blood. 77:1218–1227.

25. Lesley, J., R. Hyman, and P.W. Kincade. 1993. CD44 and its interaction with extracellular matrix. Adv. Immunol. 54:271–335.

26. A. de la Hera, A. Acevedo, W. Martson, and F. Sánchez-Madrid. 1989. Function of CD44 (Pgp-1) homing receptor in human T cell precursors. Int. Immunol. 1:598–604.

27. Kraft, D.L., I.L. Weissman, and E.K. Waller. 1993. Differentiation of CD3-4-8- human fetal thymocytes in vivo: characterization of a CD3-4-8- intermediate. J. Exp. Med. 178:265–277.

28. Caux, C., C. Deczter-Dambuyant, D. Schmitt, and J. Banchereau. 1992. GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. Nature (Lond.). 360:258–261.

29. O’Doherty, U., R.M. Steinman, M. Peng, P.U. Cameron, S. Gezelter, I. Kopeloff, W.J. Swiggard, M. Pope, and N. Bhardwaj. 1993. Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. J. Exp. Med. 178:1067–1078.

30. Köller, U., and Ch. Peschel. 1989. Cluster report: CD33. In Leucocyte Typing IV. W. Knapp, editor. Oxford University Press, Oxford, UK. 812.

31. Jacobsen, F.W., O.P. Veiby, C. Skjonsberg, and S.E.W. Jacobsen. 1993. Novel role of interleukin 7 in myelopoiesis: stimulation of primitive hematopoietic progenitor cells. J. Exp. Med. 177:1778–1782.

32. Schmitt, Ch., S. Ktorza, S. Sarun, C. Blanc, R. De Jong, and P. Debere. 1993. CD34-expressing thymocyte precursors proliferate in response to interleukin-7 but have lost myeloid differentiation potential. Blood. 82:3675–3685.

33. Sánchez, M.J., M.O. Muench, M.G. Roncarolo, I.L. Lanier, and J.H. Phillips. 1994. Identification of a common T/natural killer cell progenitor in human fetal thymus. J. Exp. Med. 180:569–576.

34. Reid, C.D.L., P.R. Fryer, C. Clifford, A. Kirk, J. Tikerpe, and S.C. Knight. 1990. Identification of hematopoietic progenitors of macrophages and dendritic Langerhans cells (DL-CFU) in human bone marrow and peripheral blood. Blood. 76:1139–1149.

35. Weissman, I.L. 1993. AIDS: The whole body view. Curr. Biol. 3:766–769.