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 $CD34^+CD44^{int}$ 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

[1] *Abbreviations used in this paper:* DC, dendritic cells; DP, double positive; TN, triple negative.

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to 3 yr. Intrathymic $CD3^-CD4^-CD8^-$ TN precursors were isolated as previously described (22). Briefly, cells were treated with anti-CD4 (HP2/6), and -CD8 (B9.4) mAbs plus rabbit complement (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, recovered viable cells were treated with a cocktail of anti-CD4, -CD8, and -CD3 (SPV-T3b) mAbs followed by two rounds of immunomagnetic 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. $CD34^+$ cells were then purified from adherent cell-depleted TN thymocytes through positive selection with CD34-coated immunomagnetic beads (Dynal).

Immunofluorescence and Flow Cytometry. mAbs against the following antigens were used: CD1a (T6-RD1), CD2 (T11-RD1), CD11b (MO1-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 (BB1), and HLA-DR (anti-HLA-DR-PE) from Becton Dickinson & Co.; CD21 (B-E5-FITC) from Serotec (Oxford, UK); and CD23 (IOB8) 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. Banchereau (Schering-Plough, Dardilly, France), and anti-HLA-DP and -HLA-DQ were the kind gift of Dr. J. Bodmer (Imperial Cancer Research Fund, London, UK).

Single- and two-color immunofluorescence studies were performed 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 $2-5 \times 10^4$ viable cells as determined by electronic gating on forward scatter and side scatter light parameters. Isotype-matched irrelevant antibodies were used to define background fluorescence.

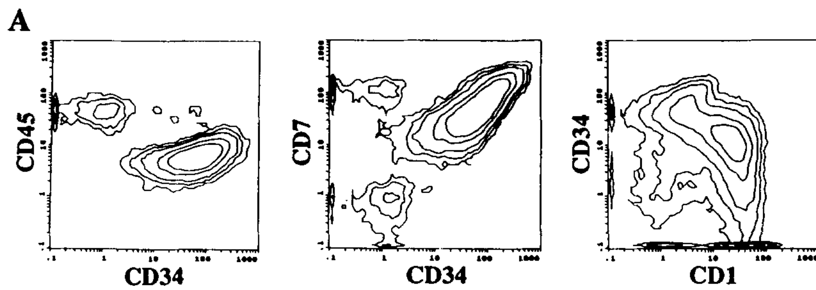
Cell Cultures. Positively isolated $CD34^+$ thymocytes ($1-2 \times 10^6$ ml^{-1}) were cultured at 37°C in 24-well macroplates (Costar, Cambridge, MA), in RPMI 1640 (Gibco Laboratories, Paisley, UK), 10% FCS (Gibco) medium either alone or supplemented with 250 U/ml of recombinant human IL-7 (rhIL-7, 10^5 U/μg) (ampoule 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 rhIL-7 (>95% large $CD44^{bright}$) were irradiated (3,500 rads) and used at different numbers (30 cells to 2×10^4 cells) as stimulators for resting allogeneic T cells (2×10^5) 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 rhIL-7 during 12 d were recovered and cultured on glass coverslips for an additional 2 d. Adherent cells were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, washed in the same buffer, and postfixed with 2% OsO_4, 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 particularly 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 $CD34^{dull}$ 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, 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



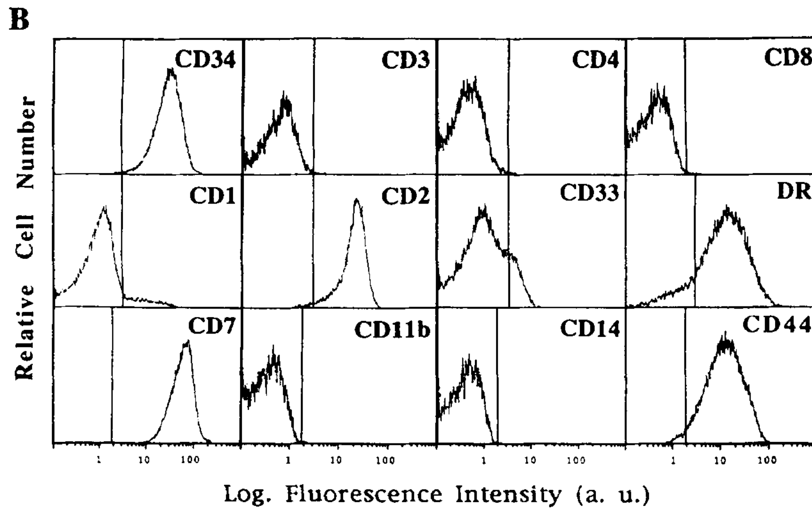


Figure 1. Phenotypic characterization of CD34$^+$ TN postnatal thymocytes. (*A*) Freshly isolated postnatal TN thymocytes were stained with PE- or FITC-coupled anti-CD34 mAbs, and with either FITC-labeled anti-CD45, anti-CD7, or PE-labeled anti-CD1a mAbs. (*B*) Positively selected CD34$^+$ cells from 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.

coexpressed intermediate levels of CD44 (CD44^{int}), a cell adhesion/homing molecule mediating extracellular matrix binding, which is expressed on hematopoietic precursors of different lineages in both humans and mice (1, 19, 25, 26).

CD34$^+$CD44^{int} Thymic Precursors Differentiate into Two Distinct CD34$^-$ Cell Populations, One CD44^{bright}, 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 inves-

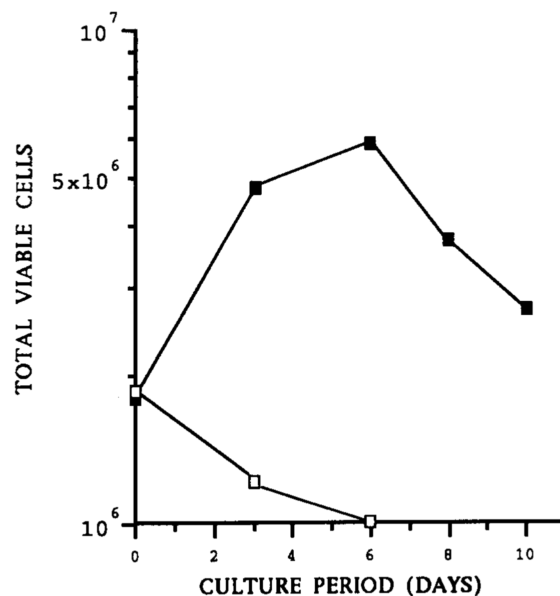


Figure 2. Cellular yield of cultures of CD34$^+$ thymocytes supplemented with rhIL-7. A total of 1.8 × 10^6 CD34$^+$ thymocytes were cultured (10^6 cells/ml) either in the absence (□) or presence (■) of rhIL-7 (250 U/ml). The number of total viable cells recovered at the indicated days was determined by trypan blue dye exclusion. Results are representative of three independent experiments.

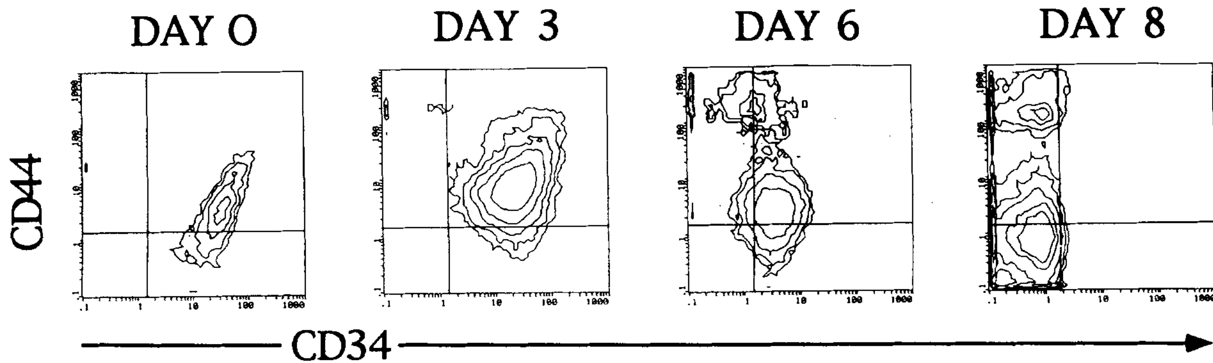


Figure 3. CD34$^+$CD44^{int} thymic precursors cultured with IL-7 differentiate simultaneously into two different subsets of either CD44^{bright} or CD44$^-$ cells. CD34$^+$CD44^{int} thymic precursors cultured with IL-7 as described in Fig. 2 were analyzed by flow cytometry for the correlated expression 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.

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$^+$CD44^{int} 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 CD44^{int} 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 CD44^{bright}, the other CD44$^-$, by day 8 (Fig. 3).

Both CD44$^-$ and CD44^{bright} Subpopulations Acquire the CD1 and CD4 T Lineage–associated Antigens. Light-scattering analysis of 3-d–cultured CD34$^+$CD44^{int} thymocytes revealed a predominant population of intermediate-sized cells that segregated into two separate populations of either small or

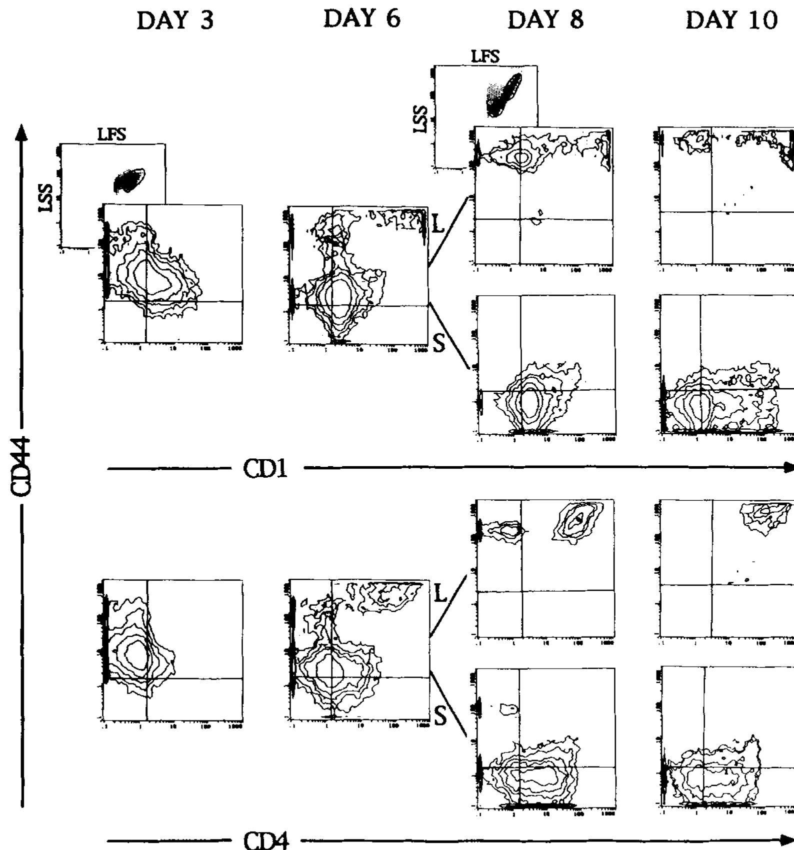


Figure 4. Up- or downregulation of CD44 on CD34$^+$CD44^{int} thymic precursors cultured with IL-7 defines two different populations of either large or small CD1$^+$CD4$^+$ thymocytes, respectively. CD34$^+$CD44^{int} thymocytes cultured with IL-7 were analyzed for the correlated expression of CD44 versus 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-CD1a 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 × 10^6 and 0.72 × 10^6, respectively, at day 8, and 2.3 × 10^6 and 0.5 × 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 CD44^{bright} 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 CD44^{bright} population by day 8. A similar proportion (21%) of CD44^{bright}CD4$^+$CD1$^-$ cells was detected by day 10, when essentially all (96%) CD44^{bright} cells were CD4$^+$. Therefore, CD44^{int} thymocytes progressed to more mature CD44^{bright} or CD44$^-$ cells through a CD1$^+$CD4$^+$ intermediate stage. However, high levels of both CD1 and CD4 molecules were expressed on CD44^{bright} cells, whereas intermediate expression levels of both antigens were consistently found in the CD44$^-$ population.

CD44^{bright} 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 CD44^{bright} 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.

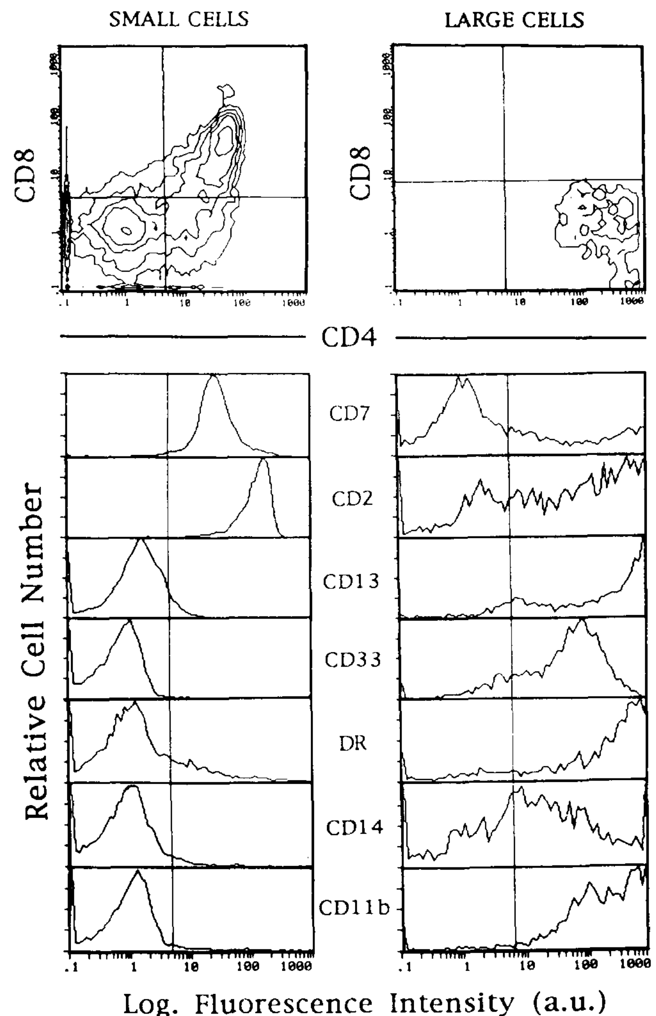


Figure 5. CD34$^+$ thymic precursors give rise to large CD44^{bright} cells, including myelomonocytic cells and DC, and small CD44$^-$ T-lineage cells. Large and small cells generated from CD34$^+$ thymic precursors and gated as shown in Fig. 4, were analyzed by flow cytometry after 10 d of culture in the presence of IL-7. The correlated expression of CD4 versus CD8 was analyzed by direct staining with PE-coupled anti-CD4 and FITC-coupled anti-CD8 mAbs. Single-color flow cytometry was performed by use of FITC-conjugated anti-CD7, anti-CD11b, and anti-CD14 mAbs and PE-labeled anti-CD2, anti-CD13, anti-CD33, and anti-HLA-DR mAbs.

In contrast to CD44$^-$ thymocytes, large CD44^{bright} cells lacked the CD8 T cell molecule but expressed very high levels of CD4 (10-fold higher than CD44$^-$ 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 CD44^{bright} 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 CD44^{bright} large cells revealed a mixed population of adherent and nonadherent cells. Cytospin preparations of nonadherent cells showed the presence of cells with a typical monocytic 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

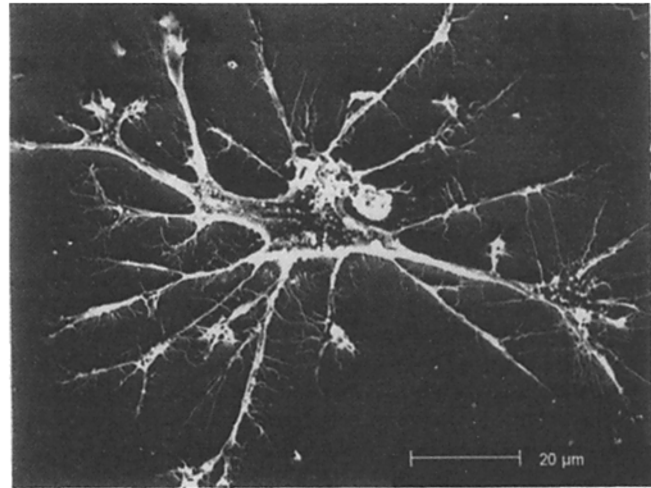


Figure 6. Scanning electron micrograph of a CD44^{bright} DC generated from IL-7-cultured CD34+ thymocytes. Scanning electron microscopy studies were performed on adherent cells derived from CD34+ thymocytes. Cells cultured with IL-7 during 14 d were treated as described in Materials and Methods and analyzed in a Phillips XL-30 scanning electron microscope. Bar, 20 μm.

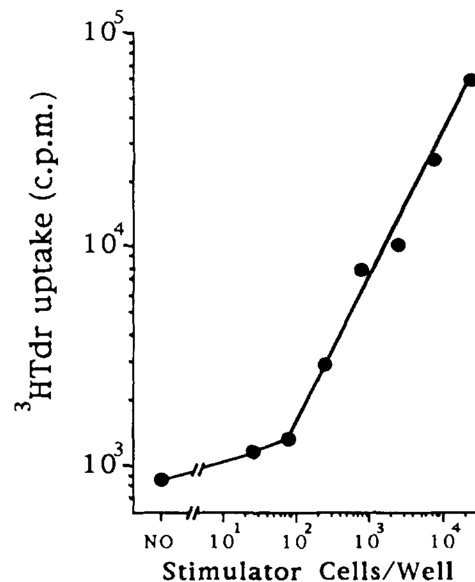


Figure 7. Stimulatory capacity of CD44^{bright} cells derived from CD34+ thymocytes in an allogeneic MLR. Cells derived from CD34+ thymocytes cultured for 16 d with IL-7 (>95% large CD44^{bright}) were irradiated (3,500 rad) and used at different numbers (30 cells to 20 × 10^3 cells) as stimulators for resting allogeneic T cells (2 × 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 CD44^{bright} cells by showing that they were potent stimulators of the allogeneic MLR (29). Proliferation of allogeneic peripheral T cells (2×10^5) was increased 40-fold by 10^4 CD44^{bright} stimulators (Fig. 7). Therefore, based on morphologic, phenotypic, and functional criteria (8, 28–30), we can conclude that CD44^{bright} 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 and either 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 CD34^{bright}CD1$^-$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 CD34^{bright} 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 CD44^{bright}CD4$^+$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 (CD1^{int}CD4^{int} or CD1^{bright} CD4^{bright} 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 CD44^{bright} 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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