

Human bone marrow and umbilical cord blood cells generate CD4⁺ and CD8⁺ single-positive T cells in murine fetal thymus organ culture

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ABSTRACT Murine fetal thymus lobes isolated from both normal and *scid/scid* mice can be colonized by donor cells from either human bone marrow or human umbilical cord blood *in vitro*. Subsequent organ culture results in a transient production of a few CD4⁺ CD8⁺ (double-positive) cells and then the accumulation of CD4⁺ or CD8⁺ (single-positive) T cells. A significant number of immature T-cell intermediates (e.g., CD8^{low}, CD3^{-/low} cells) were present in early organ cultures, suggesting that these were progenitors of the mature CD3^{+/high} single-positive T cells that dominated late cultures. Depletion of mature T cells from the donor-cell populations did not affect their ability to colonize thymus lobes. However, colonization depended on the presence of CD7⁺ progenitor T cells. Limiting dilution experiments using mature T-cell populations (human peripheral blood leukocytes, human bone marrow cells, and human umbilical cord blood cells) suggested that thymic organ culture supports the growth of progenitor T cells but does not support the growth of mature human T cells. Each of these donor populations produced single-positive populations with different CD4/CD8 ratios, suggesting that precursor cells from different sources differ qualitatively in their capacity to differentiate into T cells.

In recent years, much has been learned about the cellular interactions involved in murine T-cell ontogeny. The development of the human immune system is less clearly defined, and the signals that direct human T-cell tolerance and repertoire selection remain to be elucidated. Murine fetal thymus organ culture (FTOC) retains the architecture of the thymic microenvironment and can support the development of phenotypically and functionally mature murine T cells (1–3). FTOC can be used to study the effects of specific antibodies (4, 5), cytokines (6), or cell types (7) on T-cell differentiation and repertoire selection. Human CD4⁻ CD8⁻ double-negative (DN) thymocytes will differentiate into CD4⁺ CD8⁺ double-positive (DP) intermediate T cells, as well as small numbers of CD4⁺ single-positive (SP) and CD8⁺ SP phenotypically mature T cells when seeded into murine fetal thymus lobes (8, 9).

Human umbilical cord blood (HUCB) is a rich source of hematopoietic progenitor cells, comparable to human bone marrow (HBM) (10). We show here that both HUCB and HBM cells can colonize murine fetal thymus lobes and generate mature human T cells. These data suggest that HUCB cells should be a good candidate for human transplantation. The rapid development of many mature SP human T cells by both HBM and HUCB, compared to similar studies using human thymocytes as precursors (8, 9), suggests that

these sources of progenitor T cells differ significantly in their ability to generate human T cells.

MATERIALS AND METHODS

Human Tissues. HBM was obtained from cadaver vertebra body sections and used after cryopreservation in ≈0.6 ml of dimethyl sulfoxide medium. The cells were stored in liquid nitrogen and thawed as described (11). Fresh HBM and human peripheral blood leukocytes (HPBLC) were obtained from healthy volunteers. HUCB samples were obtained from normal full-term vaginal deliveries at the University Medical Center, University of Arizona. Mononuclear cells were purified from HBM, HPBLC, and HUCB by Ficoll-Hypaque density gradient (Sigma) separation.

Mice. Timed pregnant C.B-17 *scid/scid* [severe combined immunodeficiency (SCID)] mice were obtained from L. Shultz (The Jackson Laboratory). Timed pregnant C3H/HeN, C3H/HeJ, AKR/J, or C57BL/6 mice were from Simonsen Laboratories (Gilroy, CA) and The Jackson Laboratory.

Purification of Progenitor Cells. HBM was depleted of T cells, as described (12). Antibody-plus-complement treatment of fresh HBM resulted in 63 ± 5.8% recovery of total cells with 78.5 ± 5.9% viability. Similar treatment of surgically resected HBM resulted in 64% recovery of total cells after Ficoll separation to remove dead cells. This tissue gave a 42–100% recovery of CFU-C and BFU-E progenitor cells but no functional T cells in 10⁵ total cells (12). T and B cells were removed from HUCB by panning with kits from Applied Immune Systems (Menlo Park, CA). CD4⁺ or CD8⁺ cells were <1% in all tissues by flow cytometry (FC). CD34⁺ and CD34⁺/CD7⁺ cells were isolated from nonadherent mononuclear HBM and HUCB cells by positive indirect adherence (panning) using anti-CD34 (13) and then anti-CD7 (Becton Dickinson Immunocytometry Systems).

FTOC. Seven to 10 thymus lobes dissected from 13- to 15-day gestation fetal mice were organ-cultured (1–4) in medium/20% fetal bovine serum (HyClone). Thymuses were colonized with ≈10⁵–10⁶ cells per lobe, by directly applying cell pellets to the lobes in 0.2-μl aliquots. In some experiments, lobes were treated with 2-deoxyguanosine (dGuo, 1.35 mM) for 5 days at 37°C to deplete resident lymphocytes before reconstitution (7). The cultures were grown in a fully humidified incubator and 5% CO₂/95% air at 37°C.

Cell-Yield Assessment. The cell yield from cultures was determined by placing lobes in collagenase at 0.4 mg/ml

Abbreviations: DN, double negative; DP, double positive; mAb, monoclonal antibody; SP, single positive; HUCB, human umbilical cord blood; HBM, human bone marrow; HPBLC, human peripheral blood leukocyte(s); FTOC, fetal thymus organ culture; FC, flow cytometry; SCID, severe combined immunodeficiency; TCR, T-cell receptor.

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Table 1. Reconstitution of murine FTOC with HBM and HUCB cells

Donor	Host	n	Cells per lobe, no. × 10 ⁻⁴ (% ± SEM)		
			DP	CD4 ⁺	CD8 ⁺
Fresh HBM	FT:dGuo	2	0.7 ± 0.4 (6.7 ± 3.6)	4.3 ± 0.8 (42.4 ± 1.6)	2.4 ± 1.1 (22.6 ± 8.5)
Fresh HBM	FT:SCID	3	1.0 ± 0.06 (1.9 ± 0.36)	17.5 ± 4.8 (31.8 ± 3.7)	8.1 ± 2.7 (14.4 ± 2.5)
Frozen HBM	FT:SCID	3	1.9 ± 0.06 (4.0 ± 0.80)	14.6 ± 3.5 (30.5 ± 5.1)	1.3 ± 0.2 (3.0 ± 0.70)
Fresh HUCB	FT:SCID	4	1.3 ± 0.5 (4.5 ± 0.4)	15.4 ± 6.1 (59.5 ± 3.3)	3.3 ± 2.0 (10.0 ± 1.9)
Fresh HUCB	FT:B6	9	0.9 ± 0.5 (3.1 ± 0.9)	12.9 ± 4.2 (48.3 ± 7.4)	1.8 ± 0.4 (7.3 ± 1.2)
Fresh HPBLC	FT:SCID	5	7.7 ± 4.2 (7.0 ± 2.7)	47.0 ± 20.6 (51.4 ± 10.2)	1.2 ± 0.6 (1.2 ± 0.3)

CB17 *scid/scid* (SCID) and C57BL/6 (B6) untreated fetal thymus (FT) lobes, or C3H/HeJ lobes treated for 5 days with 1.35 mM dGuo were cultured with >10⁵ donor cells per lobe for 12 days. Cells were analyzed by FC.

(Sigma)/0.2 M phosphate buffer/EDTA at 0.2 mg/ml at 37°C for 30 min. This treatment extracts most lymphoid cells from the tissue and yet allows for staining such surface markers as CD4 and CD8. To obtain the rest of the cells, 0.12–0.25% trypsin (Sigma) in EDTA/phosphate buffer was used for a further 15–30 min at 37°C. After washing in Hanks' balanced salt solution/5% fetal bovine serum to prevent further enzyme action, cell viability (>95%) and recovery in both the collagenase- and trypsin-extracted samples was determined. Results are expressed as total recovery in both collagenase and trypsin fractions per lobe.

FC Analysis. Single-cell suspensions from collagenase-treated tissues were labeled for two- or three-color fluorescence with directly conjugated antibodies, fixed in 0.1% ρ -formaldehyde, and analyzed by using a Becton Dickinson FACStar^{Plus}, a FACScan, or a Coulter Counter model 753. Lymphocytes were identified from their forward- and side-

scatter profiles; between 1 × 10⁴ and 5 × 10⁴ events were collected per sample.

RESULTS

Colonization of Murine Fetal Thymus with HBM. Approximately 10⁵ unfractionated HBM cells were used to reconstitute 14-day gestation dGuo-treated normal fetal thymus lobes or untreated SCID fetal thymus lobes. After 12 days of culture, thymocytes were recovered and counted; human T cells were detected by staining with anti-human CD4 or CD8 monoclonal antibody (mAb) (Table 1). Seventy percent of cells in HBM/dGuo-treated murine thymus lobes were of human origin, whereas 30% of the cells were of mouse origin as determined by Thy-1.2 staining. Staining with mAb WT-31, specific for T-cell receptor (TCR) $\alpha\beta$ expression, and mAb TCR-DELTA-1, specific for human TCR $\gamma\delta$, gave values of 77.1 ± 9.5% and 3.1 ± 1.3%, respectively. The use

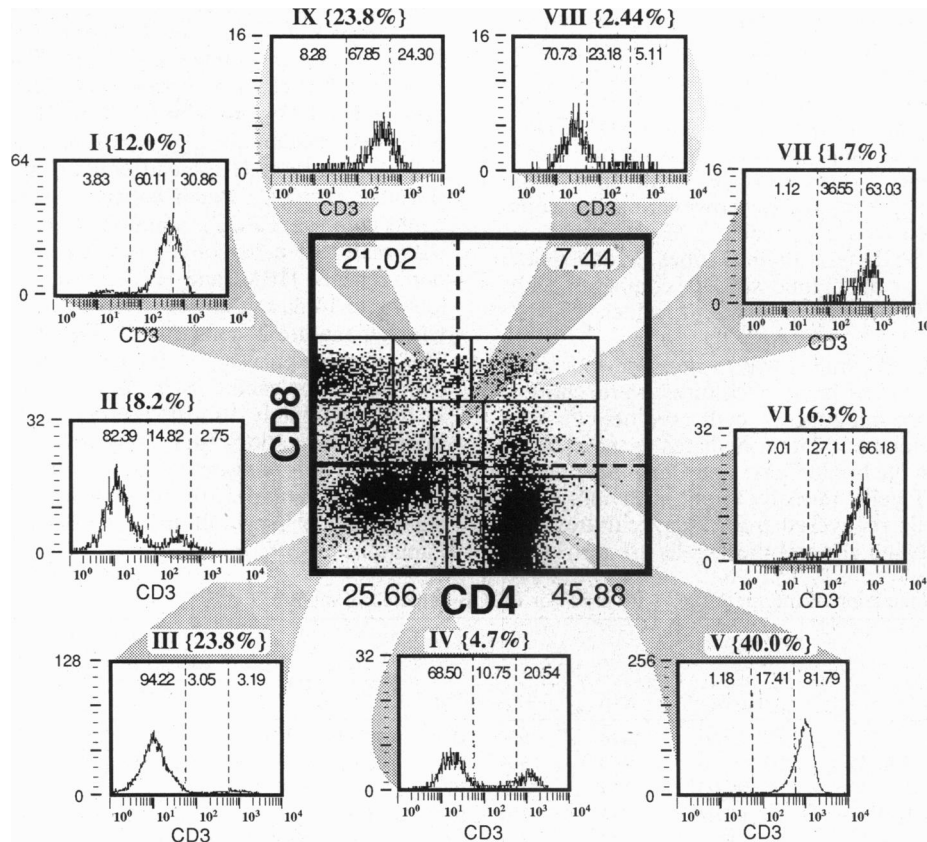


FIG. 1. FC of HUCB/SCID murine FTOC. HUCB/SCID 14-day gestation fetal thymus lobes organ-cultured for 7 days. Cells were stained with anti-human CD4 (Tri-Color; Caltag, South San Francisco, CA), anti-human CD8 fluorescein isothiocyanate, and anti-human CD3 phycoerythrin. Negative controls consisted of mouse IgG conjugated with either Tri-Color, fluorescein isothiocyanate, or phycoerythrin. DN, DP, CD8, and CD4 cells (defined by dashed lines and percentages) were electronically gated into nine subpopulations (solid boxes), and the CD3-staining intensity was determined. Distributions of CD3⁻, CD3^{low}, and CD3^{high} cells from subpopulations I–IX are shown; note shading from CD3 populations to center box.

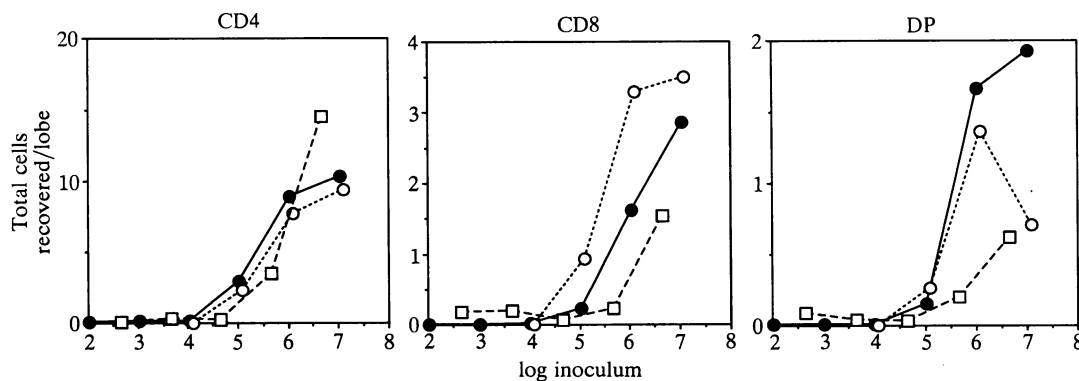


Fig. 2. Titration of HPBLC, HBM, and HUCB cells into SCID FTOC. Lobes were seeded with graded numbers of cells and cultured for 12 days before FC phenotypic analysis. Total recoveries of CD4⁺ SP cells, CD8⁺ SP cells, and DP cells are shown ($\times 10^{-4}$ for all subpopulations of HUCB and HBM, $\times 10^{-4}$ for HPBLC in CD8⁺, and $\times 10^{-5}$ for HPBLC in CD4⁺ and DP populations). ●, HPBLC; ○, HBM; and □, HUCB.

of SCID tissue increased the absolute number of human T cells compared with dGuo-treated recipients, while the overall percentage of human T cells decreased. Presumably, this decrease was due to the presence of SCID murine T cells ($40.0 \pm 9.2\%$ Thy-1.2 cells) and stromal cells that otherwise would be destroyed in dGuo cultures. CD4⁺ SP T cells predominated, although CD8⁺ SP cells and a small population of DP cells also appeared. The CD4⁺/CD8⁺ ratio was $\approx 2:1$ in both dGuo and SCID cultures. Frozen cadaver HBM/SCID chimeras produced similar results as fresh HBM, except that CD8⁺ cell frequency was lower (CD4⁺/CD8⁺ ratio was 10:1).

Colonization of Murine Fetal Thymus with HUCB and HPBLC. HUCB cells were used to colonize untreated 14-day gestation SCID and C57BL/6 untreated fetal thymus lobes (Table 1). Approximately 10^5 HUCB cells gave rise to CD4⁺ and CD8⁺ SP T cells when placed into both SCID and C57BL/6 recipient thymus lobes (Table 1). The CD4⁺/CD8⁺ ratios for these cultures were skewed in favor of CD4⁺ ($\approx 6:1$). HUCB cultures expressed TCR, with most cells expressing $\alpha\beta$ TCR ($57.9 \pm 1.2\%$); however, some cells expressed $\gamma\delta$ TCR ($8.1 \pm 1.4\%$). Table 1 also shows how HPBLC repopulates SCID fetal thymus lobes. Frequencies and total recoveries for all thymic subsets compared with those of HBM and HUCB, except for CD8⁺ values (CD4⁺/CD8⁺ ratio $> 40:1$).

Development of HUCB cultures was explored further with three-color FC (Fig. 1). Seven-day cultures were chosen because they had more immature T cells (see below). We detected several developmental intermediates in these early cultures, in addition to the bright CD4⁺ and CD8⁺ SP T cells, DP T cells, and DN T cells normally found in thymus. For example, 8.2% of cells recovered from 7-day cultures are CD8^{low} (Fig. 1, population II), and these cells are nearly all

CD3⁻ except for a small percentage of CD3^{low} and CD3^{high} cells. A similar population of CD4^{low} cells (population IV, 4.7% of total population) that were mostly CD3⁻ was also found. Among CD4^{high} cells (population V, 40.0%), CD3 expression was uniformly high. CD8^{high} cells (population I, 12.0%) had a CD3 staining intensity less than the CD4^{high} cells. The CD3 staining of the bright DP cells (population VII), which were only 1.7% of all cells in the culture, contained nearly 65% CD3^{high} cells, with the balance being CD3^{low}. Three other intermediate populations were found: CD4^{high} CD8^{low} (population VI, 6.3%), which were mostly CD3^{high}; CD4^{low} CD8^{low} (population VIII, 2.4%), which were mostly CD3^{low}; and CD4^{low} CD8^{high} (population IX, 2.2%), which were mostly CD3^{low}. The DN cells (population III) were CD3⁻, except for a few CD3^{high} (presumably $\gamma\delta$) cells.

Mature T Cells Are Not Responsible for Colonization of Murine Fetal Thymus with HBM or HUCB. HUCB contains many cells that are SP CD3⁺ cells, although these are CD3^{low} (14). In addition, HBM can contain many mature T cells. Because HPBLC, which consists of mature T cells, also could colonize FTOC, contamination of HBM and HUCB with mature T cells could have caused the FTOC colonization. HUCB, HBM, and HPBLC were titrated (10^2 – 10^7 per lobe) into 14-day gestation SCID thymus lobes to determine whether mature T cells could colonize SCID fetal thymus. The titration curves for HUCB, HBM, and HPBLC are overlapped for each CD4/CD8 subset for comparison (Fig. 2). Approximately 10^5 – 10^6 HPBLC cells were required for thymic colonization, suggesting that mature T cells cannot survive in this culture system. Unfractionated HBM and HUCB also required 10^5 – 10^6 cells for colonization, suggesting that cells from all three sources were derived from immature precursors.

Table 2. Colonization of murine FTOC with HBM or HUCB depleted of mature T cells

Donor	Host	Exp.	Dose/lobe	Cells per lobe, no. $\times 10^{-4}$ (% of total cells recovered)		
				DP	CD4 ⁺	CD8 ⁺
Frozen BMuf	FT:dGuo	418	9.4×10^5	0.14 (0.37)	0.35 (9.48)	0.76 (20.66)
Frozen BM(-2,5,n,7) ⁻	FT:dGuo	418	9.4×10^5	0.002 (0.1)	0.07 (3.46)	0.002 (0.12)
Frozen BMuf	FT:SCID	454	1.2×10^6	1.25 (3.0)	11.2 (27.2)	1.32 (3.2)
Frozen BM(-2,5,n) ⁻	FT:SCID	454	7.0×10^5	1.53 (6.6)	6.9 (29.7)	0.51 (2.2)
HUCB(-3,4,8) ⁻	FT:B6	523	1.0×10^6	0.62 (4.5)	10.1 (73.1)	1.41 (10.2)
HUCB(L) ⁻	FT:B6	520	4.5×10^6	3.3 (14.7)	11.5 (51.2)	0.85 (3.8)

CB17 *scid/scid* (SCID) and C57BL/6 (B6) untreated fetal thymus (FT) lobes, or strain AKR lobes treated for 5 days with 1.35 mM dGuo were cultured with donor cells for 12 days. BMuf, unfractionated HBM; BM(-2,5,n,7)⁻, frozen HBM depleted as described (12) with mAbs versus CD2, CD5, CD7, and a distinctive T-cell determinant, n(15), plus complement; BM(-2,5,n)⁻, frozen HBM depleted without anti-CD7. HUCB(-3,4,8)⁻, HUCB panned with mAbs to CD3, CD4, and CD8. HUCB(L)⁻, HUCB panned with mAbs to CD3, CD4, CD8, MAC-1, CD16, and IgG.

Table 3. Colonization of murine FTOC with HBM or HUCB purified for immature T cells

Donor	Host	Exp.	Dose/lobe	Cells per lobe, no. $\times 10^{-4}$ (% of total cells recovered)		
				DP	CD4 ⁺	CD8 ⁺
HBMuf	FT:dGuo	425	1.3×10^5	0.30 (9.2)	1.35 (41.3)	0.93 (28.6)
HBM CD34 ⁺	FT:dGuo	425	4.4×10^4	0.31 (9.6)	1.34 (41.1)	0.62 (18.9)
HBM CD34 ⁺ /CD7 ⁺	FT:SCID	477	7.2×10^3	1.45 (5.1)	3.51 (12.4)	1.3 (4.6)
HUCBuf	FT:B6	518	1.0×10^6	0.3 (1.7)	11.2 (61.9)	1.3 (7.2)
HUCB CD34 ⁺	FT:B6	518	1.0×10^5	0.1 (0.6)	1.5 (11.7)	0.1 (1.0)
HUCB CD34 ⁺	FT:B6	518	1.0×10^5	0.2 (0.8)	6.7 (28.5)	0.5 (2.2)
HUCB CD34 ⁺ /CD7 ⁺	FT:B6	518	1.0×10^5	0.3 (1.9)	7.7 (52.0)	0.6 (3.9)

CB17 *scid/scid* (SCID) and C57BL/6 (B6) untreated fetal thymus (FT) lobes, or C3H/HeJ lobes treated for 5 days with 1.35 mM dGuo were cultured with donor cells for 12 days. HBMuf, unfractionated HBM. HBM CD34⁺ HBM purified for CD34⁺ cells by panning; HBM CD34⁺/CD7⁺, HBM panned for CD34⁺ cells and then for CD7⁺ cells to give cells that presumably bore both markers. HUCBuf, unfractionated HUCB cells; HUCB CD34⁺, HUCB cells panned for CD34⁺ cells; HUCB CD34⁺/CD7⁺, HUCB cells purified sequentially for CD34⁺ cells and then CD7⁺ cells by panning.

Depletion of mature T cells from HBM with anti-CD2, -CD3, -CD4, -CD5, and -CD8 did not affect its ability to generate CD2⁺ cells after 12 days in culture using dGuo-treated recipient tissue ($68.4 \pm 6.8\%$ with 2.5×10^4 CD2⁺ cells recovered per lobe). However, identical pretreatment that included anti-CD7 mAb abolished colonization of the thymus lobes ($2.6 \pm 2.6\%$ with 0.25×10^4 CD2⁺ cells recovered per lobe). Similar results were found when CD4⁺ CD8⁺ cell production was analyzed (compare experiment 418, Table 2, where CD7⁺ cells were depleted and experiment 454 where CD7⁺ cells were not depleted). In these latter experiments, the donor HBM was depleted by mAb and complement, resulting in HBM populations with <1% phenotypically mature T cells as analyzed by FC or <1/10⁵ cells analyzed functionally (12). Because human T-cell progenitors express the CD7 molecule (16), FTOC appears to support the growth of progenitor but not mature T cells. HUCB depleted of phenotypically mature T cells by panning with CD3, CD4, and CD8 mAbs [HUCB(-3,4,8)-] and HUCB depleted of T-cell, B-cell, natural killer, and macrophage mature cell lineages [HUCB(L)-] could also still develop a phenotypic pattern similar to that of unfractionated HUCB (Table 2).

Finally, CD34⁺ cells and CD34⁺ CD7⁺ DP cells purified from HBM and HUCB could both develop into mature T cells, even when low doses were used. Thus, 7×10^3 HBM CD34⁺ CD7⁺ cells per lobe was sufficient to colonize SCID recipients, and a 10-times smaller number of HUCB CD34⁺ cells and HUCB CD34⁺ CD7⁺ cells than unfractionated HUCB could colonize B6 recipients (Table 3).

Kinetics of Murine Fetal Thymus Colonization by HUCB Cells Suggests Rapid Development of Mature Human T Cells from Immature Precursors. A small percentage of cells in

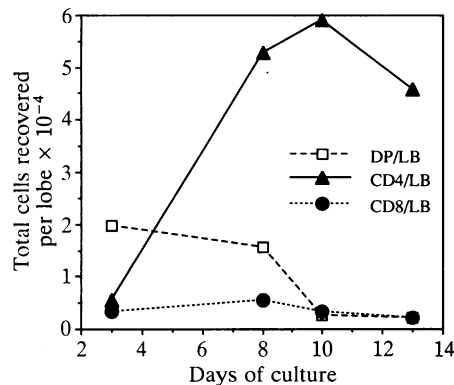


FIG. 3. Kinetics of colonization of SCID FTOC by HUCB cells. Lobes were seeded with $\approx 10^6$ cells per lobe and cultured for various times before FC phenotypic analysis. Number of cells recovered per lobe (LB) for DP cells (□), CD4 cells (▲), and CD8 cells (●) is shown.

12-day cultures had the CD3⁻ CD8⁺ phenotype ($1.4 \pm 0.5\%$ of total cells). This subpopulation has been implicated as an early intermediate in murine T-cell development (17). The frequency of CD3⁻ CD8⁺ cells was much higher in 2-day cultures ($13.8 \pm 4.7\%$), and this value decreased progressively to $6.8 \pm 3.2\%$ at 5 days and $1.7 \pm 0.5\%$ by 10 days of culture. This result would be expected were the mature cells of late cultures derived from CD3⁻ CD8⁺ cells. The progression from CD3⁻ CD8⁺ cells to cells with mature phenotypes suggested that the mature cells at 12 days were derived from immature precursors that go through a CD4⁺ CD8⁺ DP stage. To determine whether a transitional DP phenotype exists in human/mouse chimeric FTOC, the growth kinetics of SCID lobes colonized with HUCB cells was investigated. Approximately 10^6 HUCB cells were seeded per lobe, and the human T-cell phenotype was determined various times after colonization. Fig. 3 shows an experiment in which DP thymocytes were present transiently at the beginning of organ culture, and these DP cells decreased in number as SP cells increased. These data suggest that development of DP cells in this system is rapid and transient, allowing for rapid development of SP cells.

DISCUSSION

Our results suggest that human T cells can rapidly develop into CD4⁺ or CD8⁺ SP cells expressing CD3 in murine FTOC when HBM or HUCB are donor tissues. Deliberate addition of many HPBLC to organ cultures did not increase the frequency of human T-cell precursors compared with HBM and HUCB. HBM and HUCB depleted of mature T cells still provided precursors for T-cell development in the organ culture system. The numbers of T-cell-depleted HBM cells used to colonize murine fetal thymus [Table 2; <1/10⁵ functional T cells for the BM(-2,5,n)- preparation] were also similar to that required for HPBLC, HUCB, and unfractionated HBM. Thus, mature T-cell contaminants in these tissues are probably not responsible for development of the mature cells recovered from these cultures. The ability of HBM to repopulate mouse thymus lobes also depended on CD7⁺ or CD7⁺ CD34⁺ lymphocyte progenitor cells (16). Enrichment of progenitor-cell populations with these markers reduced the cell number required for thymic colonization. The HUCB cells used to colonize organ cultures have little immediate immune-response capacity against allogeneic and xenogeneic stimulation (14), suggesting that the human T cells in these cultures probably did not arise from mature T cells that recognized mouse tissue. Finally, the immature T-cell intermediates detected in early HUCB/SCID cultures support the contention that the human T cells produced by these cultures were derived from immature T-cell precursors.

Other investigators (8, 9) have reported human T-cell development in murine dGuo FTOC. Development of human T cells in these systems depended upon the presence of an undefined high-density human thymic stromal element(s) (8). In the present study, total cell recovery of human T cells significantly increased when untreated SCID or normal fetal thymus lobes were substituted for dGuo-treated tissue. Cell numbers from dGuo recipients colonized with human thymus-derived precursors, including the undefined thymic stromal elements (8), resembled those obtained here with untreated tissue. Perhaps dGuo treatment is toxic for an important stromal-cell population in the thymus that is required for T-cell development. This cell type, or its precursor, may share some functions with the undefined human stromal elements required for the development of human T cells in earlier reports.

These same workers (8, 9) also found relatively high frequencies of human DP cells after 14–21 days of culture, a result suggesting that the mouse–human organ culture system can support human DP cell production. We found a transient and relatively lower frequency of DP cells. These cells arose faster (3–6 days of culture, with steady decreases up to 21 days of culture) than did the DP cells found by using thymic progenitor T cells (8, 9). It is unlikely that these low numbers of DP cells are from enzymatic down-modulation of CD4 or CD8 during the cell-harvesting process because similarly treated mouse or human thymocytes have readily detectable DP cells (H. Y., D. T. H., and D. D., unpublished work). The low frequency and transient production of DP cells also suggest that organ-cultured progenitor T cells may develop into SP T cells without undergoing a DP developmental stage. This observation is compatible with studies using CD4 or CD8 “knockout” mice (18), showing that murine thymocytes could differentiate into mature T cells without the coexpression of these markers. Perhaps the phenotypic differences in cell types supported by organ cultures using thymocytes, HBM, or HUCB as donor tissue result from qualitative differences in the progenitor T-cell populations used to seed the cultures. One difference may be a lack of the appropriate thymic stromal cell type(s) in murine FTOC. However, we recently found that cultures of human neonatal thymus have high initial numbers of DP cells at 5 days of FTOC, but 12 to 20 days of FTOC results in high numbers of CD4⁺ SP cells and low numbers of DP cells, suggesting that even normal human thymic stroma produce the phenotypes seen in the present study (H. Y., D. T. H., and D. D., unpublished work). Progenitor T-cell populations have been shown to differ qualitatively in proliferative capabilities and TCR gene usage (19, 20). In addition, adult thymocyte precursors produce two to three times more peripheral CD4⁺ T cells than do fetal precursors (21), an observation that may account for the skewed CD4/CD8 ratio of the organ cultured precursor cells seen here. Human dendritic cells derived from the same precursor as human T cells (as in mouse, ref. 22) may present human class II better than class I or human CD4 may interact better with mouse class II than human CD8 can interact with mouse class I. Indeed, Merckenschlager and Fisher (9) found that while blasts of DP and CD4⁺ cells could be found in their cultures, CD8⁺ blasts were undetectable.

Colony-forming assays (for granulocyte–macrophage and erythroid cells) have shown that the numbers of stem cells in HBM and HUCB are comparable and support the use of HUCB as a candidate for hematopoietic transplantation (10). Titration experiments using HBM and HUCB described here showed that these tissues contained similar frequencies of

cells that could colonize organ cultures. However, HPBLC also gave a frequency of T precursors similar to HBM and HUCB in the chimeric organ culture system, unlike colony-forming cell assays (10). HPBLC may contain more precursors for T cells than precursors for other hematopoietic cells.

In conclusion, our results suggest that human T precursor cells can rapidly develop into large numbers of SP cells from readily obtainable tissues such as HBM, HUCB, and HPBLC. Each source of precursors produced SP populations with different CD4/CD8 ratios, suggesting that precursor cells from different sources differ qualitatively in their capacities to differentiate into T cells.

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