Characterization of Prothymocytes With Cloning Capacity in Human Bone Marrow


The identity of human bone marrow (BM)-derived T cell precursors with colony forming capacity has led to controversy because of contamination with mature clonogenic T cells. We achieved 2.0 Log elimination of mature T cells from BM using a cocktail of monoclonal antibodies: CD2, CD3, CD4, CD6, and CD8 followed by two successive baby rabbit C' treatment. T cell depleted BM can generate colonies of CD2, CD3, Ti, mostly CD4, in the presence of PHA, rIL2, and a prothymocyte differentiating activity derived from phytohemagglutinin (PHA) induced mononuclear cells. These precursors could be enriched three- to sixfold by percoll gradient centrifugation and then significantly bypass the number of contaminant mature T cells as shown by limiting dilution analysis. Colony generation by marrow precursors was inhibited by the addition of autologous T cells. This inhibition was mostly caused by the T8 subset. CFU-TL growth was dramatically inhibited by eliminating CD7 cells suggesting their positivity for this surface marker. These precursors needed major histocompatibility complex (MHC) II-positive cells for optimal growth but lack DR themselves.

Although various functional assays were described for prothymocyte evaluation, no direct method has been available to enumerate and directly quantify these precursors in human bone marrow (BM). The low number of such progenitors in normal adult marrow and their contamination with mature post-thymic T cells make it difficult to quantify the induction of lymphoid functions following in vitro differentiation of these pre-T cells. Thus, the elimination of mature T cells from BM appears to be a prerequisite before in vitro studies can be performed on the prothymocytic features of these cells.

Preliminary results from in vitro cloning of T cell depleted fresh marrow as well as BM cells maintained in long-term liquid cultures pointed to the possibility that an E-rosette negative (E) CD2, CD3, BM population is capable of generating colonies with mature T cell markers (designated as CFU-TL). Other observations also indicated that at their earliest stage of development, "prothymic" or intra-thymic precursors or corresponding leukemic cells may lack surface expression of T cell receptor for antigen (T) and T3 complex as well as most surface molecules detectable on mature T cells including CD1a, 2, 4, 5, 6, and 8 antigens. Furley et al. documented the presence of T3 polypeptides in the perinuclear region of immature thymocytes despite the absence of such molecules on their surface. There is one membrane antigen, CD7 (p40) molecules, however, which is expressed by these cells, and this represents one of the earliest markers of T cell lineage during fetal development. Although CD7 expression is not fully restricted to T cell lineage, this matter might be useful for prothymocyte identification as well as for the positive enrichment of these cells from human BM.

In this report, we attempted to establish the conditions by which T cell clones can be isolated from mature T cell-depleted BM. Subsequently, we studied the effect of anti-CD7 + C' treatment on the growth frequency obtained. Other monoclonal antibodies (MoAbs) known to recognize BM progenitor cells were also studied.

The results support earlier studies showing high growth ability of T cell-depleted BM. These CFU-TLs showed higher colony forming capacity than mature T lymphocytes as well as total BM. The growth of these precursor cells is dose dependent and inhibited by E' cells, especially by the T8 subset. The optimal growth of CFU-TL requires DR' cells, PHA, IL2, and a conditioned media contained prothymocyte differentiating activity (PTDA). Finally these cells seem to express CD7 and MY10 (CD34) antigens on their surface but lack class II membrane antigen expression.

Materials and Methods

Marrow cells. Normal human BM cells were obtained from trochanter BM fragments of patients undergoing hip replacement as well as BM samples of allogeneic transplant donors. All samples were obtained with the consent of the patients. Mononuclear cells were isolated by Ficoll-Hypaque gradient. Adherent cells were removed by plastic surface adherence.

Separation of BM cell subsets. Non-adherent BM cells were fractionated into E' and E' subsets using AET (2-Aminoethylthiouronium bromide hydrobromide)-treated sheep RBCs. BM cells or their E' subset were then treated by one or a combination of the following rabbit-complement-fixing MoAbs: OKT11A (CD2), OKT3 (CD3), OKT4 (CD4) (Ortho Pharmaceuticals, Raritan, NJ), B4 (CD19), Leu 1 (CD5), MY7, HNK1 (Coulter Immunology, Hialeah, FL), RFT12 (CD6), RFT2 (CD7), RFT8 (CD8), and RHFLA-DR. For removing MY10 (a gift from Dr Civin) cells we used "panning" as described below because MY10 did not fix rabbit complement (C'). These MoAbs were previously tested for the absence of nonspecific toxicity on human T cell colony precursors. Therefore, the cells (10^6 cells/mL) were incubated for 30 minutes at room temperature with appropriate dilution of each MoAb. Thereafter, an equal volume of young rabbit C' was added, and the cells were incubated for 30 minutes at 37°C. They were then washed and resuspended in Hank's balanced salt solution (HBSS) + 5% FCS, and the treatment with C' repeated once. Dead cells were then

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removed by Ficoll centrifugation. The C' used in these studies was previously tested and did not show any nonspecific toxicity on either myeloid or lymphoid colony-forming cells. For CFU-TL assessment, only cell preparations with <0.3% viable T cells, as assessed by their positivity for CD2 + CD4 + CD5 + CD8 with a negativity for simultaneous staining with ethidium bromide, were used. As previously reported, no increase of T cell markers was seen following 24-hour incubation of these preparations with culture medium alone.

For positive cell selection, immunoaffinity (panning) method was used. Briefly, 50 mL plastic flasks were treated overnight with appropriate MoAb dilution at 4°C; the flasks were then washed twice with HBSS. Non-adherent BM subsets were added (106 cells per flask) to the flasks and incubated for 90 minutes at 4°C. Negative cells were removed by gentle washing and positive cells were collected using a sterile rubber policeman. This procedure led to 92% viable cell recovery (range 85% to 97%) of both positive and negative fractions.

Enrichment of T cell precursors by Percoll gradients. Differential centrifugation of marrow cells on discontinuous Percoll gradients was performed as described elsewhere. Briefly, 9 volumes of percoll (Pharmacia, Uppsala, Sweden) were mixed with 1 volume of 1.5 phosphate-buffered saline (PBS). This solution with an osmolarity of 300 mOsm/L is referred to as Percoll 100%. The 100% Percoll was then diluted with 0.15 mol/L PBS to obtain, 40% (d = 1.057), 45% (d = 1.063), 50% (d = 1.069), and 60% (d = 1.081) solutions; 2 mL of 60% Percoll were added to 15-mm diameter (12 mL) plastic tubes (Greiner, Bischwiller, France) and overlayed with 1.5 mL of stepwise Percoll dilutions. Then, 3 x 107 T cell-depleted marrow cells in 1 mL HBSS were layered on top of the gradient, which was centrifuged for 15 minutes at 2,000 g and 4°C. The cell layers thus obtained were collected, washed twice in HBSS, and cell viability (<96%) was determined by trypan blue exclusion. Following this stepwise Percoll dilutions. Then, 3 x 107 T cell-depleted marrow cells were added to each well. The plates tubes and overlayered with 1.5 mL of 50% (d = 1.081) solutions; 2 mL of 60% Percoll were added to 15-mm diameter (12 mL) plastic tubes (Greiner, Bischwiller, France) and overlayed with 1.5 mL of stepwise Percoll dilutions. Then, 3 x 107 T cell-depleted marrow cells were layered on top of the gradient, which was centrifuged for 15 minutes at 2,000 g and 4°C. The cell layers thus obtained were collected, washed twice in HBSS, and cell viability (<96%) was determined by trypan blue exclusion. Following this procedure, immature CFU-TLs were enriched three- to sixfold in this procedure, immature CFU-TLs were enriched three- to sixfold in this procedure, immature CFU-TLs were enriched three- to sixfold in this procedure, immature CFU-TLs were enriched three- to sixfold in this procedure, immature CFU-TLs were enriched three- to sixfold in this procedure.

Plating of BM cells. Unfractionated marrow cells or their various subsets were plated in the upper layer of a micro agar culture as described previously. Briefly, 106 cells were resuspended in 1 mL of 9 volume modified Mac Coy's 5A medium supplemented with 15% AB-sera and one volume of 3% agar. Forty microliters of this suspension (4.106 cells) were layered onto 200 uL underlayers containing medium supplemented with 25% conditioned media and 100 uM phytohemagglutinin-M (PHA-M) (Difco Laboratories, Detroit). For two step cultures, the cells were first incubated in RPMI medium containing various conditioned medium for 24 hours. Thereafter, the cells were washed and cultured both in agar as above or in liquid culture. For liquid culture, the cells were incubated (5 x 104 cells per well) in 96 well microtiter in medium containing 20% human AB serum and various supernatants. Trinitized thymidine was incorporated at day 5 and the radioactivity uptake measured 24 hours later. Preliminary results showed us that at day 6 of second step, maximum response can be obtained in both agar and liquid cultures.

Culture of BM cells in limiting dilution. BM cell preparations were cultured in limiting dilution method that described by Moreau and Miller. Briefly, CFU-TL preparations were suspended in RPMI media containing 20% AB sera, 100 uM phospho-M, 20% PTDA, and IL2 50 U/mL. Forty microliters aliquots containing ten to 120 BM cells were transferred into terasaki microwells (100 wells per titration point) and 105 irradiated (2,500 rad) autologous T cell depleted BM were added to each well. The plates were then incubated at 37°C in a humidified atmosphere of 7% CO2 in air. Colony presence within individual wells were scored using an inverted microscope at day 9 of cultures. Growing wells contained colonies (>100 cells) were scored as non-growing wells (disparate dead cells or no cells).

Estimated frequency was obtained by fitting the experimental data according to the statistical method described by Porter and Berry. Using the optimal culture conditions described above, a fit to limiting dilution theory was obtained.

Sources of T cell colony promoting activity. Conditioned media used in these experiments were three-day supernatants of PHA-stimulated PBL (PBL-CM) or their nonadherent non-T (B+null) subset. We have previously described that these cells release prothymocyte differentiating activity (PTDA) with distinct physicochemical properties. PHA removal from these supernatants was achieved by adsorption of chicken RBCs, according to Alvarez et al.

Human placenta conditioned media were used as a source of colony stimulating factor. This CM promoted optimal growth of granulomonocytic progenitors from human BM at 10% final dilution tested as previously described. Recombinant IL2 (rIL2) IL1a, and IL1b were a gift from Biogen SA (Geneva, Switzerland). rIL2 contained 1.34 x 109 U/mg as tested on CTLII 2 cell line, rIL6, and rIL6 had 1.3 x 109 U/mg as assayed on murine thymocyte proliferation. WEHI-3 supernatant was used as irrelevant CM in our study. This supernatant promoted optimal IL3-dependant cell line proliferation at 10% final dilution.

Cell markers. Marrow cell subsets, intact colonies, or pooled colony cells were tested for their activity with various MoAbs by indirect immunofluorescence. Individual colonies were also transferred on glass slides, air dried, fixed with formalin, and stained with the anti-Leu-2a FITC and anti-Leu-3a PE double reagent (Becton-Dickinson, Mountain View, CA). In the latter equipment, E- and E+ mononuclear cells were used as negative and positive controls, respectively.

Statistics. The plating efficiency of marrow cell subsets was analyzed and compared using the Student's t test for paired data.

**RESULTS**

**T colonies generated from mature T cell-depleted BM.** T colony forming capacity of total BM, E- subset and T cell depleted fraction was assessed. Mature T cells were eliminated from BM by rosetting with sheep erythrocytes and treatment with a cocktail of the following MoAbs: CD2, CD3, CD4, CD6, and CD8. Two cycles of treatment with baby rabbit's complement were applied. Using this procedure, we had a mean of 2 Log elimination of mature T cells: only mature cells with <0.3% residual T cells (CD2-, CD4-, CD8-) were used in this study (range, 0.01% to 0.27%) (see Materials and Methods).

As shown in Table 1, T cell depleted BM still able to generate significant number of colonies is referred to herein as "CFU-TL." The colony number obtained from CFU-TL

<table>
<thead>
<tr>
<th>Table 1. T Cell Colony Formation by Various Marrow Fractions</th>
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<td>BM Fraction</td>
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<tr>
<td>Colomes/4.104 cells</td>
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<tr>
<td>*p cell-depleted, non-adherent BM (CD2-4-8-) 1 cells.</td>
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<tr>
<td>**Cells were cultured on underlayers containing 100 uM/mL PHA-M, rIL2 50 U/mL and 20% PTDA preparations (see methods). Mean ± SE from eight experiments.</td>
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<tr>
<td>*CFU-TL have significantly higher colony forming ability than E- cells (p &lt; .005).</td>
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preparations was higher than the total BM (P < .05) or the E- fraction of the same BM samples (P < .005).

The T cell nature of the colonies obtained was assessed by cell marker analysis of individual colonies as well as pooled colony cells harvested at day 9 of cultures. These colonies had typical T colony morphology as they were flat, superficial, and lymphblastoid; Table 2 shows that most colonies contained cells expressing T cell markers (range, 90% to 96% of colonies). Three percent to 8% of colonies lacked both T, B cell associated (CD19) or myeloid (OKM1, MY7) markers. These colonies may have contained other hematopoietic cells or myeloid cells that could not be detected by the two latter MoAbs.

The majority of CFU-TL derived colonies were T4+ and very low or no T8+ or mixed T4+ T8+ colonies were observed, whereas among the E- derived colonies both T4+ and T8+ clones were seen and 22% ± 7% of them contained both T4+ and T8+ cells. No cell with double positivity (T4- T8+) was observed in either population.

Cultures of CFU-TL in limiting dilution. We use here the limiting dilution system in order to clarify the aspect of CFU-TL growth as well as their distinction from contaminating mature T cells. CFU-TLs were first enriched by Percoll gradient separation (see Materials and Methods) of T cell depleted BM. Then, these precursors were cultured in the presence of irradiated autologous T cell-depleted BM. The advantage of this technique was the ability to enrich T cell precursors without increasing the percentage of contaminant T cells (<0.3%).

Figure 1 shows the proportion of non-growing cultures v the number of cells cultured. As seen, the growth is dependent on CFU-TL number added (P < .01) and enrichment by Percoll increase CFU-TL from 0.42% (range, 0.21% to 0.61%) to 2.5% (range, 0.9% to 3.7%) cloning frequency. This is significantly higher than the number of contaminant T cell (<0.3%) in the corresponding fractions.

T cell nature of these clones was confirmed by surface markers on pooled cells harvested after growth counting at day 9. More than 90% of the viable cells were CD2+ CD3+ (range, 90% to 97%).

Phenotypical approach to CFU-TL. In order to clarify the surface markers of T colony precursors in human marrow depleted of mature T cells, various MoAbs were added to anti-T cocktail. For positive cells selection, the immunoadherence (panning) procedure was used as described in Materials and Methods. The various BM subsets were then cultured under limiting dilution conditions as in Fig 1. The frequency of growing cells at day 9 was then scored and shown in Table 3. As could be seen, growth and precursor frequency varied with the MoAb added to anti-T cocktail. RFT2 (CD7) appears to abolish CFU-TL growth, and MY10 cell elimination decreased cloning frequency but not as dramatically as CD7. No significant inhibition was observed with the other MoAbs. These results suggest that most growing precursors express CD7 (some of them may express MY10 [CD34]) and that they are negative for HLA-DR, HNK1, MY7, and B4. Phenotypic studies of pooled cells from each treatment following growth (day 9) showed approximately 90% of CD2+ CD3+ cells.

Table 2. Phenotypic Analysis of BM Derived Colonies

<table>
<thead>
<tr>
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<th>Total BM</th>
<th>E- Cells</th>
<th>CFU-TL</th>
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<tbody>
<tr>
<td>CD1</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
</tr>
<tr>
<td>CD2</td>
<td>95 ± 3* (93)†</td>
<td>96 ± 2 (98)</td>
<td>93 ± 3 (90)</td>
</tr>
<tr>
<td>CD3</td>
<td>88 ± 3 (85)</td>
<td>92 ± 5 (89)</td>
<td>86 ± 6 (82)</td>
</tr>
<tr>
<td>CD4</td>
<td>53 ± 10 (60)</td>
<td>41 ± 10 (49)</td>
<td>85 ± 8 (80)</td>
</tr>
<tr>
<td>CD7</td>
<td>95 ± 4 (96)</td>
<td>97 ± 2 (98)</td>
<td>93 ± 5 (92)</td>
</tr>
<tr>
<td>CD8</td>
<td>30 ± 12 (32)</td>
<td>32 ± 8 (39)</td>
<td>3 ± 3 (2)</td>
</tr>
<tr>
<td>CD4 CD8</td>
<td>10 ± 4 (0)</td>
<td>22 ± 7 (1)</td>
<td>2 ± 1 (0)</td>
</tr>
<tr>
<td>CD7+CD8</td>
<td>1 ± 1 (1)</td>
<td>1 ± 1 (1)</td>
<td>-0</td>
</tr>
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</table>

*Percentage of positive colonies (>95% of the cells were positive). Negative colonies had normally <1% T cell markers. For each marker, 100 colonies were analyzed. Mean ± SD from four different experiments was given.

†Marker analysis of colony cells from the same experiments: Colonies from triplicated wells (321 ± 108 colonies were dispersed in a single cell suspension. In each case, 500 cells were analyzed. Shown are the percentage of positive cells.
Cellular control of colony formation by CFU-TL. A slight enrichment (1.3×) of CFU-TL following T cell depletion resulted in a twofold increase of CFU-TL cloning efficiency when compared with total unseparated BM samples (Table I). This finding suggested a negative or suppressive effect of mature T cells on CFU-TL. In order to clarify this point, we have irradiated autologous BM derived E+ cells as well as their T4+ and T8+ subsets and then added them to CFU-TL cultures in an increasing number. A dose dependent inhibition was seen following T cell addition (Fig 2), and the T8+ subset was specially effective in this respect. This phenomenon may explain the poor cloning ability of total BM, as in this tissue the T8+ cells are normally more abundant than T4+ cells.

Triebel et al previously suggested that CFU-TL were DR+ because a loss of colony number was observed when CFU-TL preparations were depleted from major histocompatibility complex (MHC) II positive cells. Our preliminary studies confirmed this point (data not shown), but the use of limiting dilution system showed that CFU-TL growth was not affected by HLA-DR+ cell elimination (Table 3).

Our results in Fig 3 show that DR+ cell elimination from feeder cells decreased colony number, and that feeder cells were necessary for optimal growth. Together, these results suggest a role for DR+ in T cell growth and colony formation from CFU-TL. As this population is very heterogenous, more experiments were needed to find the precise nature of cell populations responsible for this effect.

The growth factors required for CFU-TL. To investigate the growth requirements of CFU-TL, we have tested the effect of IL1, rIL2, IL3 (WEHI-CM), colony stimulating factor, and a prothymocyte differentiating activity (PTDA) recovered from supernatants of PHA-induced PBL as well as their B+ null cells subsets. This differentiating activity was previously shown to be distinct from IL2.

To assay CFU-TL differentiation and/or proliferation, we used a double step culture assay in which CFU-TL preparations were incubated 24 hours with various cytokines and

Table 3. Effect of Various Cytotoxic Treatments on the Frequency of Marrow Precursor Cell Growth

<table>
<thead>
<tr>
<th>BM Treatment*</th>
<th>Cell Recovery Posttreatment</th>
<th>Frequency of Cell Growth†</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>C'</td>
<td>97%</td>
<td>1/21</td>
</tr>
<tr>
<td>Anti T (T11,T4,T12,T8) + C'</td>
<td>73%</td>
<td>1/221</td>
</tr>
<tr>
<td>Anti T + RFT2 + C'</td>
<td>71%</td>
<td>1/1051</td>
</tr>
<tr>
<td>Anti T + C' (MY10)‡</td>
<td>66%</td>
<td>1/534</td>
</tr>
<tr>
<td>Anti T + RFHLA-DR + C'</td>
<td>23%</td>
<td>1/93</td>
</tr>
<tr>
<td>Anti T + HNK1 + C'</td>
<td>66%</td>
<td>1/234</td>
</tr>
<tr>
<td>Anti T + MY7 + C'</td>
<td>56%</td>
<td>1/182</td>
</tr>
<tr>
<td>Anti T + B4 + C'</td>
<td>51%</td>
<td>1/167</td>
</tr>
</tbody>
</table>

*Non adherent BM cells were used for cytotoxic treatment.
†Frequencies were measured by limiting dilution using the procedure of Porter and Berry. Cells were plated (20-120/well) under the same conditions as in Fig 1. Mean of four different experiments.
‡MY10-positive cells were separated using the immunoadherence procedure described in Materials and Methods.
recultured for six days in agar as well as liquid cultures. As shown in Fig 4, only PTDA preparations (B + N) supported significant CFU-TL differentiation. The simultaneous addition of IL2 potentiated this response, although IL2 alone had low, if any effect. Preincubation with PHA showed a slow response which may be due to endogenous production of PTDA by BM. As could also be seen, no other cytokine showed PTDA-like activity. The response to PTDA was less obvious in liquid culture, as compared with PHA. This was due to the high background response to PHA in liquid culture, which is, however, not restricted to T cell lineage and includes other proliferating hematopoietic cells. Nevertheless, the cells emerged in liquid culture following the seven days total incubation period showed the same surface markers (CD2+, CD3+, and mostly CD4+) as those obtained in agar (data not shown). This response is similar to the one step culture assay since CD7 + C' treatment significantly inhibits CFU-TL response in both agar and liquid cultures (Fig 5), suggesting that CFU-TL are indeed the responding cells in both assays.

**DISCUSSION**

This study was focused on the ability of cells in the T cell-depleted BM to differentiate into mature T cells during a seven- to ten-day incubation period under well defined culture conditions. The results show that these BM samples depleted of their mature T cells contain a population of CFU-TL that display a higher growth ability than that seen with total BM samples or with purified E' (CD2) cells (Table 1). The low cloning ability of E' cells was expected because most BM-derived T cells are T8+. Previous studies have already documented that the T8+ population is a poor performer in T cell colony formation (TCFC) and even inhibits mature T4+ colony forming cells (TCFC) obtained from human PBL. This inhibition may explain the low colony number found in total BM in which T cells (especially T8+ cells, Fig 2) repress CFU-TL growth.

The numbers of CFU-TL derived colonies (Table 1) were similar or slightly higher than contaminant mature T cells, which persisted following anti-T cell treatment. This raised doubts that the colony formation was due to resting mature T cells rather than immature T cells in spite of the fact that BM-derived purified T cells had very low clonability. The enrichment of CFU-TL by the Percoll method was therefore the first additional clear indication that this problem might be experimentally solved. In the fraction of $1.063 < d < 1.069 \text{g/mL}$ an increase of CFU-TL was detected by limiting dilution without the concomitant presence of mature T cells. These results (Fig 1) support the hypothesis that CFU-TL is a distinct cell. In addition, we have previously shown that CFU-TL display growth kinetics strictly different from those of mature TCFC. Our studies corroborate earlier reports on murine[35,36] as well as human lymphocytes[3,9] on the ability of BM-derived prothymocytes to differentiate in vitro under special culture conditions that are more demanding than those supporting the growth of mature T cells.[10,34,35] The similarity of these conditions to the intra-thymic microenvironment remains to be investigated. Our study has identified some of the growth requirements for CFU-TL proliferation, including a PTDA (see also reference 10) and the stimulating effect exerted by class II+ cells. These results again support the view that CFU-TL differ from mature TCFC in their growth requirement. In particular, the accessory effect of DR+ cells could be of great significance since the role of class II antigens in the induction of thymocytes has been clearly demonstrated.[3,9] CFU-TL growth requires differentiation factors recovered from B+ null cells derived supernatants,[3,9] which are distinct from other lymphokines studied in this report[25,26] but it is too early to conclude that this growth factor might be a new lymphokine. Studies are now in progress to clarify its molecular characteristics. The ability of such activity to promote CFU-TL differentiation into T4+, T3+, Ti' cells could be of great interest since most helper/inducer cells are comprised in this subset.40

The final question addressed in our study deals with the surface markers of CFU-TL. It is generally accepted that most functional surface molecules are acquired by prothymocytes during their contact with thymic microenvironment.[41,42] Thus, the developmental pathway(s) of early T cells are characterized by the sequential appearance of surface molecules detected by a series of MoAbs.
Among these surface molecules, CD7 (p40) appears very early in T cell ontogeny, even before TCR chain rearrangement.\textsuperscript{14,15} This membrane moiety is expressed by most T cell acute lymphoblastic leukemias as well as fetal or pediatric immature thymocytes.\textsuperscript{12,14,16} The CD7 expression precedes the appearance of CD2, CD5, CD4, and CD8 antigens. On the other hand, BM progenitors including pre-B cells and myeloblasts can be detected by a unique stem-cell associated marker MY10 (CD34).\textsuperscript{20} This antigen is also present on CFU-GEMM.\textsuperscript{20} Our results raise a question as to whether this antigen is present on a fraction of CFU-TL since among other surface markers used, only CD7 + C’ treatment and other surface markers used, including CFU-TLs. The failure of others\textsuperscript{43} to obtain CD7 cells are not accessory cells but include CFU-TLs. CD7 expression precedes T gene rearrangements in precursor T (lymphoblastic) cells and their positive sorting, the identification of minute thymocytes populations and their malignant counterparts might become feasible. Studies are now also in progress to purify and individually characterize CFU-TL. These studies may lead to the clarification of maturation phenomena along the normal T cell pathway in culture systems.

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